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

21 December 2007
[9-07]

DRAFT ASSESSMENT REPORT

PROPOSAL P306

ADDITION OF INULIN/FOS & GOS TO FOOD*

(*Title of Proposal amended on 8 August 2007)

DEADLINE FOR PUBLIC SUBMISSIONS: 6pm (Canberra time) 22 February 2008
SUBMISSIONS RECEIVED AFTER THIS DEADLINE
WILL NOT BE CONSIDERED
(See 'Invitation for Public Submissions' for details)

For Information on matters relating to this Assessment Report or the assessment process generally, please refer to http://www.foodstandards.gov.au/standardsdevelopment/

Executive Summary

Food Standards Australia New Zealand (FSANZ) has prepared this Proposal to consider the regulatory status of inulin and fructo-oligosaccharide (FOS) added to general foods and inulin, FOS and galacto-oligosaccharide (GOS) added to special purpose foods for infants and young children in the *Australia New Zealand Food Standards Code* (the Code).

For the purposes of this Proposal, special purpose foods comprise: infant and follow-on formula, infant foods and formulated supplementary foods for young children, such as toddler formula.

Inulin is a carbohydrate belonging to a class of compounds known as fructans. But the definition is unfortunately not that simple. Scientists and food manufacturers often use inulin, FOS and GOS generically to refer to a range of substances and there is no widely agreed set of definitions. In this Report, FSANZ uses the terms inulin, long chain inulin, oligofructose, FOS and GOS to describe the substances of interest. The term 'inulin-derived substances' refers to inulin, long chain inulin and oligofructose – the term does not include FOS.

Food manufacturers have added inulin-derived substances and FOS to the general food supply in Australia and New Zealand since the mid 1990s. Manufacturers do this for technological reasons, because these substances emulsify or thicken food, or for nutritional reasons, such as for their prebiotic[1] effect or as dietary fibre. Since 2001, inulin and FOS have appeared in a wide range of foods and are predominantly labelled as dietary fibre.

Manufacturers add prebiotics to infant and follow-on formula to mimic the effects of oligosaccharides that occur naturally in breast milk. These substances are not absorbed in the small intestine and reach the large intestine essentially intact. Breastfed infants generally have softer stools compared with formula-fed infants and this difference may be due in part to the presence of oligo- and polysaccharides in breast milk.

In early 2007, a brand of infant formula products with added long chain inulin and GOS was released onto the Australian and New Zealand markets. These substances are considered to require a pre-market safety assessment and an explicit permission in the Code before they can be added to infant formula. As no permission exists in the Code, the enforcement agencies in Australia and New Zealand took enforcement action against the manufacturer of the infant formula.

An unintended consequence of this action was confusion among the broader food industry about the regulatory status of inulin and FOS when added to general foods which negatively impacts on the efficiency and international competitiveness of the food industry.

To resolve this industry confusion, FSANZ decided, pursuant to section 36 of the *Food Standards Australia New Zealand Act 1991* (FSANZ Act) [in place before 1 July 2007] to omit one round of public consultation on this Proposal prior to making a Draft Assessment. In making this decision, FSANZ was satisfied that the issues raised by this Proposal will not have a significant adverse effect on the interests of anyone.

[1] Prebiotics are defined as '*non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon*' (adapted from Gibson and Roberfroid, 1995).

FSANZ is now inviting submissions on this report to help in the preparation of a Final Assessment.

Purpose

The purpose of the Proposal is therefore to confirm the regulatory position for the food industry of inulin-derived substances and FOS when added to the general food supply, and to consider permissions for the addition of inulin-derived substances, FOS and GOS when added to infant and follow-on formula, infant foods and formulated supplementary foods for young children.

Preferred Approach

In this Draft Assessment, FSANZ's preferred regulatory approach for Proposal 306 is to:

- amend Standard 1.1.1 to state that inulin-derived substances and FOS are taken not to be nutritive substances;

- amend Standard 2.9.1 to permit the voluntary addition of inulin-derived substances to infant formula products up to a total maximum of 110 mg/100 kJ (0.3 g/100 mL), or GOS up to a total maximum of 290 mg/100 kJ (0.8 g/100 mL), or a combination of inulin-derived substances and GOS up to a total maximum of 290 mg/100 kJ (0.8 g/100 mL) where inulin-derived substances do not exceed 110 mg/100 kJ (0.3 g/100 mL); and

- amend Standards 2.9.2 and 2.9.3 Division 4 to permit the voluntary addition of inulin-derived substances and GOS, alone or in combination, to infant foods and formulated supplementary foods for young children up to a total maximum of 0.8 g/100 g and 1.6 g/serve (0.8 g/100 mL), respectively.

FSANZ concludes that the preferred approach provides a net benefit to affected parties because:

General food supply

- There is a history of safe use of inulin-derived substances and FOS in food in Australia and New Zealand, so food manufacturers do not need express permission to add these substances to the general food supply.

- The preferred approach confirms the regulatory position for the food industry by clarifying the status of inulin-derived substances and FOS in the general food supply. This approach removes the potentially negative financial effects for both manufacturers and suppliers and reduces trade barriers.

Special purpose foods for infants and young children

- Based on the scientific evidence, and provided the amounts do not exceed the prescribed maximum levels, FSANZ concludes that infants fed solely on infant formula, and older infants and toddlers fed follow-on-formula, infant foods and formulated supplementary foods for young children containing inulin-derived substances and/or GOS in any ratio, are unlikely to be at risk from these foods.

- There was very little evidence to assess the effects of adding FOS to infant formula products, so FSANZ has not included a recommendation for FOS in this Proposal.

- Inulin-derived substances or FOS are not present in breast milk and GOS is found only in trace amounts. Breast milk contains other oligo- and polysaccharides in amounts up to 25 g/L during the first few weeks following birth but the amounts decline thereafter. The recommended maximum levels proposed to be added to infant formula are based on amounts less than those found in breast milk.

- The preferred approach provides consumers with choice and is likely to maintain their confidence in the safety of infant formula products, infant foods and formulated supplementary foods for young children.

- The preferred approach also confirms the regulatory position for the food industry, thereby reducing potential trade barriers, supporting cost-effective production through harmonisation with overseas regulations, and supporting innovation.

- Furthermore, the preferred approach provides clarity for enforcement agencies in Australia and New Zealand.

FSANZ therefore recommends the proposed draft variation(s) to the Code provided at Attachment 1.

Rationale for preferred drafting approach

FSANZ has, within the context of the Code, previously considered that inulin-derived substances, FOS and GOS when used in foods for infants and young children as regulated under Part 2.9 – Special Purpose Foods, are 'nutritive substances' within the definition of that term in Standard 1.1.1 as a means of requiring pre-market assessment of foods for these vulnerable population groups.

However, for the purposes of this Proposal, FSANZ has drafted the variations to the respective standards in Part 2.9, in a manner that does not adopt a position either way on the status of inulin-derived substances and GOS when added to special purpose foods for infants and young children. FSANZ has taken this interim approach with the draft variations because it plans to undertake a review of the concept of 'nutritive substances' and a review of Standard 2.9.1 – Infant Formula Products.

For the draft variation to Standard 2.9.1, FSANZ has placed the reference to inulin-derived substances and GOS, with corresponding permissions, in a stand-alone provision (new clause 9A) rather than in the Table to clause 7 (which lists a number of permitted nutritive substances). This drafting approach should not be taken to mean that inulin-derived substances and GOS in infant formula products under Standard 2.9.1 are not 'nutritive substances'.

Furthermore, in relation to the proposed draft variation to Standard 1.1.1, inulin-derived substances and FOS are taken to be not nutritive substances (and therefore requiring no pre-market approval) when added to general foods. This is based on a history of safe use in Australia and New Zealand over many years.

This proposed approach also recognises the status of inulin-derived substances and FOS as fulfilling both a technological and nutritional purpose in general foods. This 'taken to be not nutritive' variation to Standard 1.1.1 is to put beyond any doubt the status of inulin-derived substances and FOS when used in general foods. Conversely, and for the purposes of this Proposal, no decision has been taken on GOS when added to general foods in Standard 1.1.1 because there is no history of addition of GOS to these foods.

Consultation

This Proposal will include only one round of public consultation, as permitted under section 36 of the FSANZ Act. However, FSANZ has begun early targeted consultation so stakeholders can provide information to help with the Draft Assessment.

FSANZ held initial discussions with enforcement agencies, Nutricia Australia Pty Ltd., Heinz Wattie's Limited, Wyeth, Orafti Group and the Australian Food and Grocery Council (AFGC) to seek support for the proposed approach before making the public notification of this Proposal on 8 August 2007.

FSANZ has also undertaken targeted consultation with:

- the Infant Formula Manufacturers' Association of Australia (IFMAA) and the New Zealand Infant Formula Marketers' Association (NZIFMA) and their members Nutricia, Heinz Wattie's, Nestle, Wyeth, Bayer and also in New Zealand the Dairy Goat Co-operative NZ Ltd. and Fonterra Cooperative Group Ltd.;

- the AFGC and the New Zealand Food and Grocery Council (NZFGC);

- representatives of Orafti Group; and

- other food manufacturers.

CONTENTS

INVITATION FOR PUBLIC SUBMISSIONS 3

GLOSSARY 4

INTRODUCTION 5

1. BACKGROUND 5
   - *1.1 Section 36 Proposal 5*
   - *1.2 Nutritive substances 6*
   - *1.3 Other relevant FSANZ work 6*
   - *1.4 Terminology 7*
   - *1.5 Ministerial Policy Guidelines 9*
   - *1.6 Domestic and international regulations 9*
   - *1.7 Current use 14*
2. THE PROBLEM 14
3. OBJECTIVES 15
4. KEY RISK ASSESSMENT QUESTIONS 16

RISK ASSESSMENT 16

5. INULIN-DERIVED SUBSTANCES AND FOS ADDED TO THE GENERAL FOOD SUPPLY 17
   - *5.1 Why are inulin-derived substances and FOS added to the general food supply? 17*
   - *5.2 Is there evidence of the safe use of inulin-derived substances and FOS in the general food supply? 18*
6. INULIN-DERIVED SUBSTANCES/FOS AND GOS ADDED TO SPECIAL PURPOSE FOODS FOR INFANTS AND YOUNG CHILDREN 19
   - *6.1 Composition of breast milk compared to infant formula with added inulin-derived substances/FOS and GOS 19*
   - *6.2 Physiological effects of inulin-derived substances/FOS and GOS in infants and young children 20*
   - *6.3 Safety of inulin-derived substances/FOS and GOS in infants and young children 22*
   - *6.4 Current and estimated additional consumption of inulin-derived substances/FOS and GOS in infants and young children 24*
   - *6.5 External advice on safety issues 25*
7. SUMMARY OF RISK ASSESSMENT 26
   - *7.1 Inulin-derived substances and FOS added to the general food supply 26*
   - *7.2 Inulin-derived substances/FOS and GOS added to special purpose foods for infants and young children 26*

RISK MANAGEMENT 27

8. IDENTIFICATION OF RISK MANAGEMENT ISSUES 27
   - *8.1 Inulin-derived substances, FOS and GOS added to food 27*
   - *8.2 Inulin-derived substances and GOS added to special purpose foods for infants and young children 28*
   - *8.3 Labelling requirements 30*
9. REGULATORY OPTIONS 35
   - *9.1 Inulin-derived substances and FOS added to the general food supply 35*
   - *9.2 Inulin-derived substances and GOS added to special purpose foods for infants and young children 35*
10. IMPACT ANALYSIS 36
    - *10.1 Affected Parties 36*
    - *10.2 Benefit Cost Analysis 36*
    - *10.3 Comparison of Options 39*

COMMUNICATION AND CONSULTATION STRATEGY 40

11. COMMUNICATION 40
12. CONSULTATION 40
    - *12.1 Summary of key issues raised from early consultation 41*
    - *12.2 World Trade Organization 42*

CONCLUSION 43

13. CONCLUSION AND PREFERRED APPROACH 43
13.1 General food supply 43
13.2 Special purpose foods for infants and young children 43
14. RATIONALE FOR PREFERRED DRAFTING APPROACH 44
15. IMPLEMENTATION AND REVIEW 45

REFERENCES 45

ATTACHMENT 1 - DRAFT VARIATIONS TO THE *AUSTRALIA NEW ZEALAND FOOD STANDARDS CODE* 46
ATTACHMENT 2 - CHEMICAL AND TECHNOLOGICAL USES ASSESSMENT 51
ATTACHMENT 3 - HUMAN MILK CARBOHYDRATES 59
ATTACHMENT 4 - MICROBIOLOGICAL ASSESSMENT 66
ATTACHMENT 5 - NUTRITION ASSESSMENT 80
ATTACHMENT 6 - SAFETY ASSESSMENT 102
ATTACHMENT 7 - DIETARY INTAKE ASSESSMENT 118

INVITATION FOR PUBLIC SUBMISSIONS

FSANZ invites public comment on this Draft Assessment Report based on regulation impact principles and the draft variations to the Code for the purpose of preparing an amendment to the Code for approval by the FSANZ Board.

Written submissions are invited from interested individuals and organisations to assist FSANZ in preparing the Draft Assessment of this Application. Submissions should, where possible, address the objectives of FSANZ as set out in section 18 of the FSANZ Act. Information providing details of potential costs and benefits of the proposed change to the Code from stakeholders is highly desirable. Claims made in submissions should be supported wherever possible by referencing or including relevant studies, research findings, trials, surveys etc. Technical information should be in sufficient detail to allow independent scientific assessment.

The processes of FSANZ are open to public scrutiny, and any submissions received will ordinarily be placed on the public register of FSANZ and made available for inspection. If you wish any information contained in a submission to remain confidential to FSANZ, you should clearly identify the sensitive information and provide justification for treating it as commercial-in-confidence. Section 39 of the FSANZ Act requires FSANZ to treat in-confidence, trade secrets relating to food and any other information relating to food, the commercial value of which would be, or could reasonably be expected to be, destroyed or diminished by disclosure.

Submissions must be made in writing and should clearly be marked with the word 'Submission' and quote the correct project number and name. Submissions may be sent to one of the following addresses:

Food Standards Australia New Zealand
PO Box 7186
Canberra BC ACT 2610
AUSTRALIA
Tel (02) 6271 2222
www.foodstandards.gov.au

Food Standards Australia New Zealand
PO Box 10559
The Terrace WELLINGTON 6036
NEW ZEALAND
Tel (04) 473 9942
www.foodstandards.govt.nz

Submissions need to be received by FSANZ by 6pm (Canberra time) 22 February 2008.

Submissions received after this date will not be considered, unless agreement for an extension has been given prior to this closing date. Agreement to an extension of time will only be given if extraordinary circumstances warrant an extension to the submission period. Any agreed extension will be notified on the FSANZ website and will apply to all submitters.

While FSANZ accepts submissions in hard copy to our offices, it is more convenient and quicker to receive submissions electronically through the FSANZ website using the Standards Development tab and then through Documents for Public Comment. Questions relating to making submissions or the application process can be directed to the Standards Management Officer at the above address or by emailing standards.management@foodstandards.gov.au.

Assessment reports are available for viewing and downloading from the FSANZ website. Alternatively, requests for paper copies of reports or other general inquiries can be directed to FSANZ's Information Officer at either of the above addresses or by emailing info@foodstandards.gov.au.

GLOSSARY

AFGC	Australian Food and Grocery Council
AI	adequate intake
AOAC	Association of Official Analytical Chemists
CoPoNC	Code of Practice on Nutrient Claims
DP	degree of polymerisation
EFSA	European Food Safety Authority
FOS	fructo-oligosaccharides
FOSHU	foods of specified health use
FSANZ	Food Standards Australia New Zealand
FSFYC	formulated supplementary foods for young children
GOS	galacto-oligosaccharides
GRAS	generally recognized as safe
HMOs	human milk oligosaccharides
ICSAG	Infant and Young Child Scientific Advisory Group
IFMAA	Infant Formula Manufacturers' Association of Australia
NFA	National Food Authority
NHMRC	National Health and Medical Research Council
NNS	national nutrition survey
NZFGC	New Zealand Food and Grocery Council
NZIFMA	New Zealand Infant Formula Marketers' Association
NZMoH	New Zealand Ministry of Health
SCFA	short chain fatty acid
UL	upper level of intake
WTO	World Trade Organization

INTRODUCTION

Food Standards Australia New Zealand (FSANZ) has prepared this Proposal to consider the status of inulin/fructo-oligosaccharide (FOS) added to food and the addition of inulin/FOS and galacto-oligosaccharide (GOS) added to special purpose foods for infants and young children in the *Australia New Zealand Food Standards Code* (the Code).

Inulin/FOS and GOS are often used generically to refer to a range of substances and there is no widely agreed set of definitions. Therefore, in this report, the terms inulin, long chain inulin, oligofructose, FOS and GOS are used to clarify the substances of interest; the term '**inulin-derived substances'** is used to collectively refer to inulin, long chain inulin and oligofructose – it does not include FOS.

This Draft Assessment Report discusses the issues involved in the addition of inulin/FOS and GOS (see Section 1.4 for definitions of these and other terms used throughout this report) to food and seeks comments from stakeholders on the preferred approach and proposed draft variations to the Code to assist FSANZ in making a Final Assessment of this Proposal.

1. Background

In early 2007, one brand of infant formula products with added long chain inulin[2] and GOS was launched on the Australian and New Zealand market. Long chain inulin and GOS are added to infant formula to mimic the effects of oligosaccharides in breast milk. However, the addition of long chain inulin and GOS to infant formula is considered to require a pre-market safety assessment and an explicit permission in the Code. As no expressed permissions exist in the Code the relevant enforcement agencies subsequently took enforcement action against the manufacturer of these infant formula products.

An unintended consequence of this enforcement action was confusion among the broader food industry as to the regulatory status of inulin-derived substances/FOS when added to general foods and food manufacturers were concerned about potential implications for their food products. In response to this concern, FSANZ initiated this Proposal to remove this confusion for the food industry regarding the addition of inulin-derived substances/FOS to the general food supply (and some special purpose foods), but specifically considering the addition of inulin-derived substances/FOS and GOS to infant formula products, infant foods and formulated supplementary foods for young children (FSFYC).

1.1 Section 36 Proposal

In the interest of dealing with this regulatory matter in a timely and responsive manner, FSANZ decided, pursuant to section 36 of the FSANZ Act [in place before 1 July 2007], to omit to invite public submissions in relation to this Proposal prior to making a Draft Assessment. FSANZ made its decision under section 36 because it was satisfied that omitting one round of public consultation prior to making a Draft Assessment would not have significant adverse effects on the interests of anyone. To assist with facilitating consultation, advanced notification of this Proposal and the expected consultation period was publicly announced on 8 August 2007.

[2] Long chain inulin has been referred to by some manufacturers as long chain FOS or high molecular weight FOS.

FSANZ now seeks comment on this Draft Assessment Report to assist FSANZ in progressing this Proposal to Final Assessment.

1.2 Nutritive substances

In the case of the recent enforcement action, the main regulatory issue is that inulin-derived substances/FOS and GOS are not listed as permitted 'nutritive substances' in the Table to clause 7 of Standard 2.9.1. As a result, there has been considerable discussion among the food industry, industry groups and food regulatory agencies as to whether these substances are 'nutritive substances' in the Code. The then National Food Authority (NFA) (a forerunner of FSANZ) stated in writing in the early 1990s that inulin did not require an explicit permission before it could be added to foods. However, in the mid 1990s, an Expert Panel on Infant Formula advised the NFA, as part of the revision of the infant formula standard (Proposal P93), that oligosaccharides should not be permitted to be added to infant formula because there was no demonstration of efficacy and some concerns about safety. This advice was tabled at the 33rd meeting of the NFA in August 1995.

Since that time, the current joint Code was introduced and contains a general prohibition on the addition of 'nutritive substances' to foods unless expressly permitted. This prohibition did not exist before 2002 as the concept of a 'nutritive substance' was introduced for the first time through its inclusion in the new joint Code. A 'nutritive substance' is defined in Standard 1.1.1, clause 2 as

> *a substance not normally consumed as a food in itself and not normally used as an ingredient, but which, after extraction and/or refinement, or synthesis is intentionally added to a food to achieve a nutritional purpose, and includes vitamins, minerals, amino acids, electrolytes and nucleotides.*

Clause 9 of Standard 1.1.1 states that 'nutritive substances' must not be added to food unless expressly permitted in the Code.

It is outside the scope of this section 36 Proposal to review the definition of 'nutritive substance' in the Code to accommodate these and other substances not currently listed in the definition. Therefore, this Proposal has adopted a narrow approach, consistent with a section 36 proposal, to resolve the current uncertainty surrounding the addition of inulin-derived substances/FOS to food (and some special purpose foods) and inulin-derived substances/FOS and GOS to special purpose foods for infants and young children. FSANZ expects to undertake a review of the definition of 'nutritive substance' and its application in the Code at a later date to ensure that further polarity of views and attendant confusion does not arise in the future.

1.3 Other relevant FSANZ work

FSANZ has received three Applications that relate to this Proposal.

An unpaid Application (Application A598) was received from Heinz Wattie's Limited[3] in December 2006 requesting amendments to the Code to allow the addition of FOS and GOS to infant formula products and Nutricia Australia Pty Limited[4] submitted a paid Application (Application A609) in July 2007 requesting amendments to the Code to allow the addition of long chain inulin and GOS to infant formula products and infant foods. Orafti, a manufacturer of inulin and oligofructose, also submitted an unpaid Application (Application A613) in August 2007 seeking to clarify the status of these substances in the general food supply. Work on A609 has begun and the Initial Assessment Report is being released at the same time as this Proposal. Work on the unpaid Applications is due to commence in 2008. This Proposal does, however, deal with aspects of each Application and may therefore satisfy in part, or whole, each of these respective Applications.

1.4 Terminology

1.4.1 Inulin-derived substances and fructo-oligosaccharides

FSANZ acknowledges that there are diverse opinions regarding the description of inulin-derived substances and FOS and that a number of different terms and expressions are used to describe these substances. To ensure that there is clarity in this report about the terminology and identity of these substances, the following terms are used:

- the term '**inulin**' means those fructans[5], with β (2→1) fructosyl-fructose linkages, where the average degree of polymerisation[6] (DP) is equal to or greater than ten;

  - the term '**long chain inulin'** means those fructans with β (2→1) fructosyl-fructose linkages, where the average DP is equal to or greater than 23;

- the term '**oligofructose**' means those fructans, with β (2→1) fructosyl-fructose linkages, where the average DP is less than ten but greater than or equal to four. Oligofructose is derived from inulin. Chicory inulin, for example, contains about 30% oligofructose; and

- the term '**fructo-oligosaccharides'** means those fructose polymers with β (2→1) fructosyl-fructose linkages, where the average DP is less than four and is **typically** produced from enzymic condensation of sucrose.

FSANZ acknowledges that sometimes oligofructose and inulin are referred to as 'FOS' and sometimes FOS is referred to as 'oligofructosyl-saccharose'. In addition, the terms oligofructose and FOS are sometimes used interchangeably. Given the differences in the terminology currently in use, this report uses the terms described above to ensure clarity in the FSANZ assessment process and related consultations. More detail about the basis for these terms is in Attachment 2.

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[3] Heinz Wattie's Application is seeking to add GOS at a maximum of 290 mg/100 kJ or FOS at a maximum of 110 mg/100 kJ or total oligosaccharides of 290 mg/100 kJ to infant formula products. No specifications were provided for these substances, hence until processing of the Application begins, FSANZ is uncertain of the specific substances being requested.

[4] Nutricia'a Application is seeking to add GOS and long chain inulin in the ratio 9:1 and at a level of 0.8 g/100 mL to infant formula and infant foods.

[5] Polymers of fructose.

[6] Degree of polymerisation is the number of fructose or saccharide units.

Fructans are characterised by the range of the Degree of Polymerisation (DP), including the average DP. The DP is a measure of the number of fructose molecules or saccharide units in the substance. The DP ranges can vary for the different fructans with these ranges overlapping for the different substances. For this reason, the terms used above have been described on the basis of the average DP (see Attachment 2 for more information on the DP ranges for particular substances).

Throughout this report the term '**long chain inulin'** is used to describe the processed inulin fraction that is proposed to be added to infant formula, follow-on formula, infant foods and FSFYC. The terms **inulin**, **oligofructose** and **FOS** will be used where appropriate; the term '**inulin-derived substances'** is used to collectively refer to inulin, long chain inulin and oligofructose.

Question to submitters:

FSANZ acknowledges that there are diverse opinions regarding the description of inulin-derived substances and FOS. Do submitters agree with the proposed descriptions of these substances or should alternative expressions be used? If alternative expressions are suggested then could submitters provide documentation to support the alternatives?

1.4.2 Galacto-oligosaccharides

The term '**galacto-oligosaccharides'** (sometimes referred to as oligogalactosyl-lactose) is used consistently to describe those substances comprised of between two and eight saccharide units with one of these units being a terminal glucose and the remaining saccharide units being galactose. While the disaccharide lactose is present in GOS mixtures, it is not regarded as a GOS. GOS is produced from lactose by enzymatic action and is also referred to as 'trans-GOS'.

1.4.3 Oligosaccharides and polysaccharides

The terms oligosaccharide and polysaccharide are used throughout this report.

Oligosaccharides refer to component sugars with a DP range 3-10.

Polysaccharides contain several simple sugars (DP > 10) linked together and are often referred to as complex carbohydrates.

Human milk oligosaccharides (HMOs) is a collective term used throughout this report to refer to the oligosaccharide and polysaccharide content of human breast milk (see Section 6.1.1 and Attachment 3).

1.4.4 Formulated supplementary foods for young children

Formulated supplementary food is defined in Standard 2.9.3 as *a food specifically designed as a supplement to a normal diet to address situations where intakes of energy and nutrients may not be adequate to meet an individual's requirements.*

Formulated supplementary foods for young children is defined in Standard 2.9.3 as *a formulated supplementary food for children aged one to three years.*

Toddler formula is the main type of formulated supplementary foods for young children currently available.

1.4.5 Prebiotics

Prebiotics are defined as *non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon* (adapted from Gibson and Roberfroid, 1995).

1.5 Ministerial Policy Guidelines

When developing and varying food standards, FSANZ must have regard to any written policy guidelines formulated by the Australia and New Zealand Food Regulation Ministerial Council (Ministerial Council). The Ministerial Council is currently developing a policy guideline on the addition of substances other than vitamins and minerals to foods. The addition of inulin/FOS to food is expected to be covered by this policy guideline. However, preliminary indications suggest that the policy will not apply to special purpose foods, such as infant formula products, infant foods and FSFYC.

In the absence of policy guidance, FSANZ has considered only the safety aspects of the addition of these substances to infant formula products, infant foods and FSFYC; potential benefits are not assessed in this report.

1.6 Domestic and international regulations

1.6.1 Domestic Regulations

The addition of inulin/FOS to the general food supply is not regulated domestically unless it is added for a nutritive purpose; in which case it is captured by the operation of Standard 1.1.1 (see below). There are, however, domestic regulations specifically relating to the addition of substances to infant formula products, infant foods and FSFYC.

The Standards in the Code relevant to this Proposal are:

- **Standard 1.1.1 – Preliminary Provisions**, clause 2 defines and clarifies the use of a nutritive substance (see Section 1.2).

Standard 2.9.1 – Infant Formula Products regulates the compositional and labelling requirements for infant formula products. Clause 6 of Standard 2.9.1 prohibits the addition of nutritive substances to infant formula products unless specifically permitted. Clause 7 lists the permitted nutritive substances that may be voluntarily added to infant formula, the form(s) in which they may be added, the minimum amount per 100 kJ for their declaration, and the maximum amount permitted per 100 kJ when the substance is added. The maximum permitted amount applies to the sum of the naturally occurring and added nutritive substance.

Relevant definitions from Standard 2.9.1 are:

> ***infant formula product*** *means a product based on milk or other edible food constituents of animal or plant origin which is nutritionally adequate to serve as the principal liquid source of nourishment for infants.*

infant formula *means an infant formula product represented as a breast milk substitute for infants and which satisfies the nutritional requirements of infants aged up to four to six months.*

follow-on formula *means an infant formula product represented as either a breast milk substitute or replacement for infant formula and which constitutes the principal liquid source of nourishment in a progressively diversified diet for infants aged from six months.*

Standard 2.9.2 – Foods for Infants regulates the compositional and labelling requirements of foods intended and/or represented for use as food for infants. Foods in this Standard are intended to be fed to infants in addition to human milk and/or infant formula products. Clause 2 states that *food for infants must not contain a food additive or nutritive substance unless expressly permitted by this Code.*

Relevant definitions from Standard 2.9.2 are:

Food for infants *means a food that is intended and/or represented for use as a source of nourishment for infants, but does not include –*

(a) infant formula products; and
(b) formulated meal replacements; and
(c) formulated supplementary foods; and
(d) unprocessed fruit and vegetables.

Infant *means a person up to the age of 12 months.*

Standard 2.9.3 – Formulated Meal Replacements and Formulated Supplementary Foods regulates the compositional and labelling requirements of these foods. Division 4 – Formulated Supplementary Foods for Young Children, specifies the required macronutrient composition of these foods and also the addition of at least one ore more permitted vitamins and minerals.

Formulated Supplementary Foods for Young Children *means a formulated supplementary food for children aged one to three years.*

1.6.2 International regulations

Specific international regulations relating to the addition of inulin/FOS to the general food supply do not appear to exist. The Applicant for A613 reported that in most other countries, the status of inulin and FOS as dietary fibre (and therefore as a macronutrient) has been confirmed[7].

There are, however, international regulations relating to the addition of inulin-derived substances/FOS and GOS to infant formula products and foods for infants. The relevant international regulations of which FSANZ is aware of are outlined below.

[7] ORAFTI. Application to amend the *Australia New Zealand Food Standards Code:* Application A613 – Definitions for Nutritive Substance and Nutritive Ingredient.

1.6.2.1 Codex Alimentarius

The recently adopted revised Codex Standard for Infant Formula[8] allows the addition of *optional ingredients* to infant formula. Optional ingredients, in addition to the essential compositional requirements, 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 outcomes of populations of breast-fed babies*. The revised Standard also states that *the suitability for the particular nutritional uses of infants and the safety of these substances shall be scientifically demonstrated* and that *the formula shall contain sufficient amounts of these substances to achieve the intended effect, taking into account levels in human milk.*

The Codex Standards for Canned Baby Foods[9] states that *baby foods may* be *prepared from any suitable nutritive material that is used, recognized or commonly sold as an article or ingredient of food, including spices.* Similarly, the Codex Standard for Processed Cereal-based Foods for Infants and Young Children[10] allows the addition of *other ingredients suitable for infants who are more than six months of age and for young children*.

1.6.2.2 United States of America (U.S.)

Inulin from the root of the chicory plant (*Cichorium intybus*) is 'generally recognized as safe' (GRAS) for use in food as a bulking agent[11]. Of the 43 food categories that are permitted to contain added inulin in varying levels, baby foods and beverages are included, but infant formula is excluded. Based on these food categories, dietary intakes of inulin in the U.S. at the 90th percentile level are estimated to be approximately 6 g per day for infants less than one year of age, approximately 15 g per day for infants older than one year of age, and approximately 20 g per day for the general population (i.e. two years of age and older).

FOS is GRAS for use as a bulking agent in specific foods[12,13]. Infant foods (0-12 months) and toddler foods (12-24 months) are included in the permitted range of foods but infant formula is explicitly excluded.

In addition, a GRAS proposal for BI^{2}MUNO (low molecular weight GOS) was submitted to the US Food and Drug Administration for consideration in March 2007. The decision is pending.

[8] Codex Alimentarius Commission. Alinorm 07/30/26 – Report of the 28th Session of the Codex Committee on Nutrition and Foods for Special Dietary Uses. Appendix II – Draft revised standard for infant formula and formulas for special medical purposes intended for infants.
[9] Codex Standards for Canned Baby Foods. Codex Stan 71-1981.
[10] Codex Standard for Processed Cereal-based Foods for Infants and Young Children. Codex Stan 074-1981, Rev 1-2006.
[11] US Food and Drug Administration. Agency Response Letter GRAS Notice No. GRN 000118, 5 May 2003. Available at: http://www.cfsan.fda.gov/~rdb/opa-g118.html
[12] US Food and Drug Administration. Agency Response Letter GRAS Notice No. GRN 000044, 22 November 2000. Available at: http://www.cfsan.fda.gov/~rdb/opa-g044.html
[13] US Food and Drug Administration. Agency Additional Correspondence Letter GRAS Notice No. GRN 000044, 1 June 2007. Available at: http://www.cfsan.fda.gov/~rdb/opag044a.html

1.6.2.3 European Union

In December 2006, the European Commission published Commission Directive 2006/141/EC on infant formulae and follow-on formulae[14]. The previous directive, Commission Directive 91/321/EEC[15], will be repealed with effect from 1 January 2008.

Infant formula products and follow-on formula products containing added oligosaccharides have been marketed in the European Union for several years. Commission Directive 91/321/EEC provides a general provision for the addition of *other food ingredients* to infant formula and follow-on formula; this includes oligosaccharides[16].

However, at the request of Member States, the European Commission asked the Scientific Committee on Food[17] (SCF) to comment on *the suitability and safety of the resistant short chain carbohydrates, FOS and GOS, in infant formula and follow-on formula*. The SCF released two statements on the above matter in 2001[18,19]. The statements concluded that there were no major concerns about the combination of 90% GOS and 10% *high molecular weight oligofructosyl-saccharose*[20] in infant formula and follow-on formula in total concentrations up to 0.8 g/ 100 mL in the product ready for consumption.

Subsequently, as part of a review of the essential requirements of infant formula and follow-on formula, the SCF was requested to address the content of FOS and GOS in these products. In April 2003, the SCF released a report[21] that reaffirmed their previous statement of December 2001. In addition, the SCF concluded that *fructans other than* [high molecular weight] *oligofructosyl-saccharose should not be included in infant formulae and follow-on formulae*, based on available data at that time.

In 2003, the European Commission requested the European Food Safety Authority (EFSA) to provide a scientific opinion on the safety and suitability for particular nutritional use by infants of oligofructose (referred to as fructo-oligosaccharides by EFSA) at conditions specified by a manufacturer of infant formula and follow-on formula. The conditions specified by the manufacturer were an infant formula supplemented with 1.5 or 3.0 g/L of oligofructose.

On 19 February 2004, the Scientific Panel on Dietetic Products, Nutrition and Allergies of EFSA concluded that *there is no evidence of benefits to infants from the addition of fructo-oligosaccharides to infant formula at the conditions specified by the manufacturer while there are reasons for safety concerns.*

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[14] Commission Directive 2006/141/EC of 22 December 2006 on infant formulae and follow-on formulae and amending Directive 1999/21/EC. *Official Journal of the European Union, L 401/1, 30/12/2006.*

[15] Commission Directive 91/321/EEC of 14 May 1991 on infant formulae and follow-on formulae. *Official Journal of the European Union, L 175, 04/07/1991.*

[16] Personal communication – Health and Consumer Protection Directorate-General, European Commission, June 2007.

[17] Scientific Committee on Food is now known as the European Food Safety Authority (EFSA).

[18] Scientific Committee on Food. Statement on the use of resistant short chain carbohydrates (oligofructose and oligigalactose) in infant formulae and in follow-on formulae, expressed on 26 September 2001.

[19] Scientific Committee on Food. Additional statement on the use of resistant short chain carbohydrates (oligofructosyl-saccharose and oligogalactosyl-lactose) in infant formulae and in follow-on formulae, expressed on 13 December 2001.

[20] *High molecular weight oligofructosyl-saccharose* is referred to as 'long chain inulin' in this report.

[21] Scientific Committee on Food. Report of the Scientific Committee on Food on the revision of essential requirements of infant formulae and follow-on formulae, adopted on 4 April 2003.

This conclusion was based on *an increased prevalence of adverse effects, including loose stools, in infants fed formula with added fructooligosaccharides. As no measures were made to demonstrate satisfactory water balance, the possibility of increased risk of dehydration can not be excluded, raising concerns with respect to the safety of such formulae*.

On 22 December 2006, the European Commission issued a revised Directive on infant formulae and follow-on formulae (Commission Directive 2006/141/EC). In relation to infant formula, this Directive states that:

Fructo-oligosaccharides and galacto-oligosaccharides may be added to infant formulae. In that case their content shall not exceed: 0.8 g/100 mL in a combination of 90% oligogalactosyl-lactose and 10% high molecular weight oligofructosyl-saccharose.

Other combinations and maximum levels of fructo-oligosaccharides and galacto-oligosaccharides may be used in accordance with Article 5.

Article 5 states that:

Infant formulae shall be manufactured from protein sources defined in point 2 of Annex I and other food ingredients, as the case may be, whose suitability for particular nutritional use by infants from birth has been established by generally accepted scientific data.

Such suitability shall be demonstrated through a systematic review of the available data relating to the expected benefits and to safety considerations as well as, where necessary, appropriate studies, performed following generally accepted expert guidance on the design and conduct of such studies.

The Directive also states that similar amounts and ratios may be voluntarily added to follow-on formula. This Directive must be adopted by Member States by 31 December 2007.

1.6.2.4 United Kingdom

The European Commission Directive 2006/141/EC will be implemented by respective regulation in England, Wales, Scotland and Northern Ireland. For example, in England the *Infant Formula and Follow-on Formula (England) Regulations 2007* will implement the Commission Directive 2006/141/EC, and will revoke and replace, in England, the existing *Infant Formula and Follow-on Formula Regulations 1995*.

The Food Standards Agency will develop Guidance Notes to assist stakeholders interpret the provisions of the new Regulations before 1 January 2008.

1.6.2.5 Asian countries

Infant formula products with added inulin, oligofructose and GOS have been marketed in many Asian countries for a number of years. It appears that these countries do not prohibit the addition of these substances to infant formula. However, many Asian countries require products to be registered before they are permitted to be imported into the respective country. In some instances, product registration requires the product to be compliant with the food regulations of the country of origin.

In August 2007, the New Zealand Food Safety Authority published an exemption from the requirements of the Code, in relation to the use of oligosaccharides in the manufacture of dairy based infant formula products for export to specified countries, including China, Malaysia, Indonesia and Republic of Korea[22].

In Japan, oligosaccharides have 'foods of specified health use' (FOSHU) status, relating to a *health function* as a food to *modify gastrointestinal conditions*[23].

1.7 Current use

1.7.1 Presence in food

Inulin/FOS occur naturally in many plant foods including wheat, bananas, onions, garlic, Jerusalem artichoke and chicory. GOS is found in some dairy products, such as lactose-reduced milk and yoghurt.

1.7.2 Added to the general food supply in Australia and New Zealand

Inulin/FOS have been used in the general food supply in Australia and in many other countries for almost 15 years. They are added to a range of foods in Australia and New Zealand including: bread, fruit juice, milk, breakfast bars, biscuits, chocolate, soup, custard, and ice cream. The purpose for their inclusion varies and can include functions such as promoting healthy gut bacteria or for other non-nutritional purposes, for example, as a bulking agent (see Section 5.1).

As far as FSANZ is aware, GOS is not added to the general food supply.

1.7.3 Added to foods for infants and young children - internationally

Infant formula containing long chain inulin and GOS has been available in the United Kingdom and Ireland for several years however, similar products are not available for sale in Canada or the USA.

As mentioned previously, infant formula and follow-on formula containing added oligosaccharides have been marketed in the European Union for several years (see Section 1.6.2.3).

Many Asian countries also sell infant formula and toddler formula products containing inulin, oligofructose and GOS.

2. The Problem

In response to the advice from the then NFA in the early 1990s that explicit permission was not required to add inulin to foods, inulin and the shorter chain FOS have been added to a wide range of foods in Australia and New Zealand.

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[22] New Zealand Food Safety Authority. Animal Products (Exemption from New Zealand Standards – Oligosaccharides in Infant Formula Products) Notice 2007 (3 August 2007). Available at: http://www.nzfsa.govt.nz/dairy/publications/specifications/fos-gos-60b-exemption-notice-2007.pdf

23 Ministry of Health, Labour and Welfare. Available at: http://www.mhlw.go.jp/english/topics/foodsafety/fhc/02.html

They are added for technological or nutritional reasons (see Section 5.1).

However, FSANZ is aware of growing confusion within elements of the food industry concerning the status of added inulin-derived substances and FOS, specifically whether these substances fall within the definition of a nutritive substance. This confusion stems from an apparent polarity of views as to the status of inulin-derived substances and FOS.

First, within the food industry some sectors consider the substances to be nutritive substances while others consider them to be ingredients.

Second, some food regulatory agencies believe that the substances are nutritive substances, whereas some sectors of the food industry remain of the view that they are not of a nutritive nature.

This apparent confusion undermines an efficient and internationally competitive food industry (clause 10(2)(c) of the FSANZ Act).

3. Objectives

The specific objective of this Proposal is to remove the confusing polarity of views within the food industry of the status of inulin-derived substances and FOS when added to the general food supply, and to consider permissions for the addition of inulin-derived substances, FOS and GOS when added to infant and follow-on formula, infant foods and formulated supplementary foods for young children.

In developing or varying a food standard, FSANZ is required by its legislation to meet three primary objectives which are set out in section 18 of the FSANZ Act. These are:

- the protection of public health and safety;
- the provision of adequate information relating to food to enable consumers to make informed choices; and
- the prevention of misleading or deceptive conduct.

In developing and varying standards, FSANZ must also have regard to:

- the need for standards to be based on risk analysis using the best available scientific evidence;
- the promotion of consistency between domestic and international food standards;
- the desirability of an efficient and internationally competitive food industry;
- the promotion of fair trading in food; and
- any written policy guidelines formulated by the Ministerial Council.

4. Key risk assessment questions

To assist in meeting the objectives of this Proposal, the following key risk assessment questions are being addressed as part of the Draft Assessment:

General food supply

1. Why are inulin-derived substances and FOS added to the general food supply?

2. Is there evidence of the safe use of inulin-derived substances and FOS in the general food supply?

Special purpose foods for infants and young children

3. Are inulin-derived substances/FOS and GOS *present in breast milk?* If so:

   - at what levels; and
   - how do these compare with the levels proposed for infant formula?

4. What are the *physiological effects* in infants and young children of consuming inulin-derived substances/FOS and GOS?

5. How do the identified physiological effects compare with those in similarly aged breastfed infants?

6. Do all *forms* of inulin-derived substances/FOS and GOS produce the same identified physiological effects?

7. What are the *potential risks* to infants and young children consuming formula containing added inulin-derived substances/FOS and GOS alone or in combination? If in combination, are there any *safety issues with different proportions*?

8. Is there a *maximum safe intake* above which *adverse effects* may occur?

9. How much inulin-derived substances/FOS and GOS do infants and young children already consume and how much additional inulin-derived substances/FOS and GOS would young children consume if these substances were added to special purpose foods for infants and young children?

RISK ASSESSMENT

This risk assessment addresses the key risk assessment questions and is in two parts. The first part, discusses the technological and nutritional reasons for adding inulin-derived substances and FOS to the general food supply and evidence for their safe use. The second part discusses the nutritional reasons for adding inulin-derived substances/FOS and GOS to special purpose foods for infants and young children and compares their microbiological and physiological effects with that of breastfed infants. In addition, the safety of these substances in special purpose foods for infants and young children has been assessed based on the available evidence. Potential benefits have not been assessed.

Current intakes and additional intakes in infants and young children from added inulin-derived substances/FOS and GOS in general and special purpose foods have been estimated as a guide only; there is no upper reference value with which to compare these.

To further enhance the scientific rigour of the risk assessment, FSANZ discussed the risk assessment with the recently established Infant and Young Child Scientific Advisory Group (ICSAG)[24]. FSANZ convened this Group to provide scientific advice on risk assessment issues relating to Standards 2.9.1, 2.9.2 and 2.9.3. Their input relates to the health, nutritional status and safety of infants and young children consuming food products with added inulin/FOS and GOS. A member of this Group, an expert in paediatric nutrition[25], has peer reviewed the risk assessment relating to infants and young children (see Section 6) and two international experts in the fields of prebiotics[26], and dietary fibre and carbohydrates[27], respectively, peer reviewed the safety assessment (see Attachment 6). A brief summary of comments from the peer reviewers of the safety assessment are provided in Section 6.5. Also in Section 6.5 is commentary on anecdotal evidence regarding safety concerns provided by the New Zealand Food Safety Authority.

5. Inulin-derived substances and FOS added to the general food supply

5.1 Why are inulin-derived substances and FOS added to the general food supply?

Inulin-derived substances and FOS are added to the general food supply for technological (see Section 5.1.1) and nutritional reasons (see Section 5.1.2).

The chemical structure of these substances, and their stability, method of analysis and mode of action is described in greater detail in Attachment 2.

5.1.1 Technological reasons

The technological reasons for adding inulin-derived substances and FOS to foods relate to their capacity to act as fat and sugar replacers as well as emulsifiers, thickeners and stabilisers. These functions vary with the nature of the inulin-derived substance (e.g. chain length), its concentration in a food and the food itself. The technological reasons relate to the dispersing properties of inulin, in particular its ability to mimic fat droplets dispersed in water. These dispersions can then be used in foods to replace fat or to impart textural qualities in foods.

The amount of inulin and inulin-derived substance used for these purposes will vary depending on the technological purpose to be fulfilled.

It has been reported that specific applications for inulin and inulin-derived substances include:

- Beverages (to improve mouth feel or creaminess but mostly for nutritional reasons);

[24] Details of the ICSAG, including membership, will be available on the FSANZ website in the near future.
[25] Dr Clare Wall, Senior Lecturer, Human Nutrition, Auckland University, New Zealand.
[26] Professor Glenn Gibson, Head of Food Microbial Sciences, University of Reading, England
[27] Professor John Cummings, Emeritus Professor of Experimental Gastroenterology, University of Dundee, Scotland

- Bread or cereal products (for fat or sugar replacement or processing benefits and also for nutritional reasons); and

- Dairy products (for fat or sugar replacement or texture improvement).

Some inulin-derived substances (e.g. oligofructose) and FOS are also used as sweeteners with the relative sweetness being dependent on the degree of polymerisation and the proportion of the monosaccharide and disaccharide content in these products.

Inulin-derived substances are generally stable in most food matrices but under acid conditions (e.g. certain beverages when not refrigerated) hydrolysis may occur. FSANZ considers that these substances are likely to have acceptable stability in most dry foods but that inulin type fructose polymers may not be suitable in unrefrigerated low pH liquid foods, as inulin potentially might be hydrolysed to fructose.

FSANZ is unaware of any technological reason for adding GOS to the general food supply. Based on information provided to FSANZ, GOS are represented as having good stability to heat and acid conditions.

5.1.2 Nutritional reasons

In Australia and New Zealand, inulin and FOS can be added to the general food supply as a dietary fibre (see Section 5.2). Dietary fibre is defined in Standard 1.2.8 as:

…that fraction of the edible parts of plants or their extracts, or synthetic analogues, that are resistant to the digestion and absorption in the small intestine, usually with complete or partial fermentation in the large intestine. Dietary fibre includes polysaccharides, oligosaccharides (degree of polymerisation>2) and lignins, and promotes one or more of the following beneficial physiological effects:

(i) laxation
(ii) reduction in blood cholesterol
(iii) modulation of glucose.

For the purposes of making a nutrition claim, inulin and FOS may also be added to general foods as a biologically active substance. A biologically active substance is defined in Standard 1.2.8 to *mean a substance, other than a nutrient, with which health effects are associated*.

FSANZ is not aware of wide use of GOS being added to the general food supply for nutritional reasons but it may be present in low levels in some processed dairy products, such as lactose-reduced milk and yoghurt. Consumption of small amounts has been considered in the dietary intake assessment (see Section 6.4.1).

5.2 Is there evidence of the safe use of inulin-derived substances and FOS in the general food supply?

Inulin-derived substances and FOS have been added to the general food supply since the mid 1990s. FSANZ considered the safety of inulin as part of Application A277 – Inulin & Fructo-oligosaccharides as Dietary Fibre.

Since completion of the Application in 2001, added inulin and FOS may be labelled as dietary fibre. Inulin has been added to a wide range of foods with the aim of increasing the fibre content. As described in Section 5.1.1.1, inulin-derived substances are also used in some foods (e.g. margarine) as a bulking agent.

In humans, inulin-derived substances and FOS are resistant to digestion in the small intestine and pass largely intact into the colon, where they are subject to fermentation by the resident microflora. This fermentation results in the production of gases and short chain fatty acids (SCFAs). The SCFAs are utilised locally as an energy source by the resident microflora, taken up systemically for use as an energy source by the host, or excreted in the faeces.

Up to 20-30 g/day of inulin-derived substances and FOS can be tolerated by most adults, although some individuals may experience increased flatulence at levels of intake around 10 g/day. A high level of consumption of these substances (e.g. over 30 g/day) is likely to result in increased flatulence, and may cause more severe side effects such as stomach cramps and diarrhoea however, these are common effects of over consumption of any dietary fibre. In addition, excessive consumption is likely to be self-limiting due to the unpleasant side effects.

The Adequate Intake (AI) of dietary fibre (from all sources) is 30 g/day among adult males aged 19 years and over and 25 g/day among adult females aged 19 years and over (NHMRC and NZMoH, 2006). The AI is based on the median dietary fibre intake in the 1995 Australian NNS and the 1997 New Zealand NNS. Intakes of inulin-derived substances and FOS would have been fairly small at this time because these substances were only beginning to be added to the food supply. There is no UL set for dietary fibre.

There are two published reports of individual patients with an allergy to inulin (see Attachment 6). Both individuals exhibited allergies to artichoke, and some processed foods containing inulin. However, due to the rarity of these cases, inulin is not recognised as a major allergen in Australia, New Zealand or elsewhere.

In terms of nutrient interactions, data indicate that inulin-derived substances or FOS added to diets in the range of 8-20 g/day does not inhibit the absorption of magnesium, iron, zinc, copper, selenium or calcium along the small intestine. There is some indication that calcium, copper and magnesium absorption is enhanced in the large intestine when inulin-derived substances are included in the diet.

Otherwise, no adverse effects have been reported due to the consumption of foods containing inulin-derived substances or FOS. Thus, FSANZ considers these substances to have a history of safe use in the general food supply.

6. Inulin-derived substances/FOS and GOS added to special purpose foods for infants and young children

6.1 Composition of breast milk compared to infant formula with added inulin-derived substances/FOS and GOS

6.1.1 Are inulin-derived substances/FOS and GOS present in breast milk? If so, at what levels; and how do these compare with the levels proposed for infant formula?

FSANZ describes the carbohydrate composition of human breast milk in Attachment 3.

The carbohydrates in greatest abundance in breast milk are lactose (~7% of the milk) and oligosaccharides (~1-2%). Inulin-derived substances/FOS are not present in breast milk and GOS is found only in trace amounts. Cow's milk, the standard base of infant formula, contains only trace amounts of indigestible oligo- and polysaccharides. Goat's milk contains a low concentration of oligosaccharides, reported to be in the order of 0.25-0.3 g/L (Martinez-Ferez *et al.* 2006). Although soy beans contain indigestible oligosaccharides, infant formula based on soy appears to contain soy protein isolate that is free from oligosaccharides.

There is considerable variation in the composition of human milk oligosaccharides (HMOs) among women throughout the world, due in part to genetic variations. To date over 200 complex oligosaccharides have been identified in breast milk, however, it is thought that the total number may be in the thousands.

The total oligo- and polysaccharide content of breast milk may be up to 25g/L during the first few weeks following birth; declining thereafter. From one to four months the concentration is likely to be around two-thirds of the concentration in early lactation. There is a large variation in the breast milk concentration of oligosaccharides among individual women, as much as a four-fold difference. The effect of maternal diet on the carbohydrate content of breast milk has not been extensively studied.

The oligo- and polysaccharide concentration of breast milk is higher (up to 12 g/L in mature breast milk) than the levels of long chain inulin and GOS proposed to be added to infant formula in the two applications received from Heinz Wattie's and Nutricia; both are seeking to add up to a maximum of 8 g/L of total oligosaccharides.

6.2 Physiological effects of inulin-derived substances/FOS and GOS in infants and young children

6.2.1 What are the physiological effects in infants and young children of consuming inulin-derived substances/FOS and GOS?

Similar to HMOs, inulin-derived substances/FOS and GOS are undigested in the small intestine. However, they influence the ecology of the infant's large intestine including the composition of the intestinal microflora (see Section 6.2.2.1), stool consistency and frequency, stool pH and faecal SCFA profile (see Section 6.2.2.2).

6.2.2 How do the identified physiological effects compare with those in similarly aged breastfed infants?

6.2.2.1 Microbiological function

FSANZ has assessed the evidence on the effect of inulin-derived substances/FOS and GOS in combination or alone as a component in infant formula products on gut microflora (see Attachment 4).

Microbial colonisation of the gastrointestinal tract starts in newborn infants immediately after birth. The initial microorganisms are acquired from the mother through contamination during the birth, breastfeeding and caring, and from the environment. Because of the differences in exposure to the initial bacterial species and population from one individual to another, the exact pattern/distribution of the intestinal microflora in each individual is unique.

Approximately 90% of the intestinal microflora of breastfed infants is *Bifidobacterium* and *Lactobacillus* species. In comparison, the proportion of *Bifidobacterium* and *Lactobacillus* species present in the gastrointestinal tract of formula-fed infants is relatively small, approximately 40-60% of the overall intestinal microflora, and the rest consisting of *Streptococcus*, *Bacteroids*, *Clostridium*, *Staphylococcus* and a few genera belonging to the family of Enterobacteriaceae.

Bifidobacterium and *Lactobacillus* species preferentially metabolise HMOs, resulting in increased dominance of these bacteria in the colon, contributing to a bulking effect that may benefit laxation. The evidence from nine studies indicates that a combination of GOS and long chain inulin at a ratio of 9:1, with a dose not exceeding 10 g/L in infant formula, may also selectively stimulate the growth of colonic bifidobacteria in infants. There was insufficient evidence, however, to indicate if inulin-derived substances alone or GOS alone had a similar effect. The effect of FOS could not be assessed due to insufficient evidence.

Increased dominance of *Bifidobacterium* and *Lactobacillus* species leading to a reduced colonic pH has been claimed to restrict the proliferation of other intestinal microorganisms in the colon. The competition for nutrients available in the colon by *Bifidobacterium* and *Lactobacillus* species is claimed to lead to an exclusion effect on other intestinal microorganisms. These suppressive effects on other intestinal microorganisms, particularly those that are pathogenic to the host, are considered to be probiotic effects that are generally regarded as beneficial to the host, although direct and convincing evidence to support these claims is lacking.

Despite the recognition that oligosaccharides in breast milk promote *Bifidobacterium* and *Lactobacillus* bacteria, this effect also relates to a number of interactive factors including oligosaccharides, lactoferrin, lactose, nucleotides and low concentration of proteins and phosphates present in the breast milk.

6.2.2.2 Stool consistency and frequency, stool pH and faecal SCFA profile

FSANZ has assessed the evidence on the physiological effect of added inulin-derived substances/FOS and GOS in infant formula products and infant foods, in particular, the effect on stool consistency and frequency, stool pH and faecal SCFA profile compared with breastfed infants and/or infants fed unsupplemented formula or foods (see Attachment 5).

Breastfed and formula-fed infants appear to have similar capability to digest complex oligosaccharides into SCFAs in the large intestine. SCFAs are easily absorbed thus preventing the occurrence of osmotic diarrhoea which can be caused by the presence of undigested carbohydrates in the gut. However, breastfed infants generally have softer and more frequent stools than formula-fed infants. This is at least partly due to the oligo- and polysaccharides that are unique to breast milk.

The bulk of the research has been done comparing standard infant formula to formula supplemented with GOS and long chain inulin at a ratio of 9:1 at concentrations of 4-8 g/L of formula, with one study involving formula containing 10 g/L. The majority of these studies reported softer stools, with a lower pH, when infant formulas were supplemented. The one study that examined faecal SCFA also reported that supplemented formula resulted in a SCFA profile similar to that reported for breastfed infants.

The ability of GOS and long chain inulin to increase stool frequency is less well established, possibly because this effect gets smaller with age. The addition of GOS and long chain inulin to infant foods did not influence stool frequency or softness, nor did it significantly lower stool pH.

Sole addition of inulin-derived substances to infant formula products or infant foods may increase stool frequency or stool weight in infants less than 12 months of age. The limited data available suggests they have little effect on stool pH. There appears to be little impact on stool frequency or consistency in young children over 12 months of age.

The one study that examined the addition of GOS to infant formula in isolation, reported higher stool frequency in those infants receiving the GOS supplemented formula. The frequency was similar to that of a breastfed reference group however more data are needed to draw a firm conclusion. Data on the effect of formula supplemented with GOS alone on stool pH and faecal SCFAs is limited and contradictory, thus no conclusion can be made.

FOS have not been sufficiently studied in infants and young children to allow any conclusions about their effects to be made.

6.2.3 Do all forms of inulin-derived substances and GOS produce the same identified physiological effects?

The majority of the evidence is based on infants. Hence the ability to compare physiological effects is restricted to this age group.

The evidence indicates that the addition of GOS and inulin-derived substances at a ratio of 9:1, with a dose not exceeding 10 g/L in infant formula products may selectively stimulate the growth of colonic bifidobacteria and soften stools in infants, similar to that of breastfed infants. Inulin-derived substances alone may increase stool frequency in infants. There was insufficient evidence to indicate if inulin-derived substances alone or GOS alone selectively stimulated colonic bifidobacteria or whether GOS alone results in softer and/or more frequent stools in infants.

These conclusions cannot be extended to FOS, primarily because few studies used the shorter chain FOS.

6.3 Safety of inulin-derived substances/FOS and GOS in infants and young children

6.3.1 What are the potential risks to infants and young children consuming formula containing added inulin-derived substances/FOS and GOS alone or in combination? If in combination, are there any safety issues with different proportions?

FSANZ has assessed the evidence on the potential for inulin-derived substances and GOS to cause adverse effects in infants and young children (see Attachment 6). Limited information is available on the safety of FOS in infant formula and foods for infants and young children, therefore FSANZ did not consider FOS as part of the safety assessment.

Inulin-derived substances and GOS, like HMOs, are not digested to any great extent in the small intestine.

As there is virtually no systemic exposure to these intact oligosaccharides, the only possible adverse effect identified was an increased osmotic potential within the colon, potentially leading to increased water loss and dehydration. This possibility had also previously been considered by the European Scientific Committee on Food.

FSANZ has considered this potential risk. It was concluded that inulin-derived substances and GOS, either alone or in **any** combination, at concentrations up to 8 g/L will contribute to increased osmotic potential in the colon of formula-fed infants; the increase is considered to be no greater than in breastfed infants where undigested HMOs also enter the colon. To reach this conclusion FSANZ has considered the following evidence:

- HMOs are present in colostrum and mature breast milk at levels up to 25 g/L and 15 g/L respectively; these levels are safe for newborn and older infants;
- GOS and long chain inulin preparations (9:1) at 8 g/L are safe for formula-fed infants;
- Oligofructose preparations at 3 g/L are safe for formula-fed infants; and
- *In vitro* evidence that inulin-derived substances and GOS are fermented by colonic microflora to a similar or greater extent than HMOs.

Although it is concluded that up to 8 g/L inulin-derived substance is unlikely to pose a public health and safety risk to infants, evidence from adult studies suggests that some individuals experience increased flatulence and bloating upon consumption of high levels of inulin-derived substances. It is not clear if this would occur in infants due to differences in colonic microflora and overall diet. However, while not usually an endpoint considered in a conventional safety assessment, gastrointestinal discomfort in young infants is undesirable. Therefore it may be prudent to limit the addition of inulin-derived substances to levels which have been shown to be well tolerated in infants i.e. 3 g/L.

Some gastrointestinal discomfort may initially be experienced by young infants changing from breast milk or conventional formula to oligosaccharide-supplemented formula. The phenomenon of changed gastrointestinal effects is not uncommon for infants when their formula is changed. It is anticipated that this effect will be less evident in older infants (e.g. 6 months and over).

Unlike infant formula products, toddler formula and infant foods do not represent the sole source of nutrition for older infants and young children. It follows that if GOS, oligofructose and inulin are safe for newborns and infants, they will be equally safe for older infants and young children.

6.3.2 Is there a maximum safe intake above which adverse effects may occur?

A number of studies on the 9:1 GOS to long chain inulin preparation in infant formula products support the conclusion that 8 g/L of oligosaccharides is unlikely to pose a risk to young infants. This conclusion applies to GOS, inulin and oligofructose at any ratio to a total level of 8 g/L, based on data indicating that these oligosaccharides are fermented to a similar or greater extent than HMOs. The safety of this level (8 g/L) is further supported by the presence of higher levels of indigestible oligosaccharides: up to 25 g/L in colostrum and up to 12 g/L in mature breast milk.

Although HMOs are present in breast milk at higher levels, inulin-derived substances and GOS above 8 g/L have not been thoroughly tested in young infants and FSANZ has not extrapolated further.

6.4 Current and estimated additional consumption of inulin-derived substances/FOS and GOS in infants and young children

6.4.1 How much inulin-derived substances and GOS do infants and young children already consume and how much additional inulin-derived substances and GOS would young children consume if these substances were added to special purpose foods for infants and young children?

A dietary intake assessment was undertaken to estimate the potential intakes from naturally-occurring inulin as well as inulin-derived substances and GOS added to special purpose foods for infants and young children less than three years of age (see Attachment 7). The supplemented special purpose foods comprise infant formula products, infant foods and FSFYC.

The Applications and literature reviewed by FSANZ were used as the basis for assigning concentrations for the addition of inulin-derived substances and GOS in infant formula products, infant foods and FSFYC.

Dietary intakes of inulin-derived substances and GOS were estimated in two ways:

- **'Combined' assessment –** based on a combined concentration of inulin-derived substances and GOS in special purpose foods for infants and young children of 0.8 g/100 mL; and

- **'Separate' assessment –** based on separate concentrations of inulin-derived substances and GOS, respectively at 0.8 g/100 mL in special purpose foods for infants and young children.

Current dietary intakes of inulin-derived substances and GOS were estimated based on natural sources and added sources according to the current market uptake of these substances in processed food products. An additional intake estimate was then calculated to account for potential intakes from infant formula products, infant foods and FSFYC supplemented with inulin-derived substances and GOS.

As food consumption data were not available for children less than two years of age in Australia, model diets were constructed for infants aged three, nine and 12 months. In New Zealand, food consumption data were not available for children less then five years of age, so a model diet was constructed for toddlers aged 1-3 years. These diets included an estimated intake of 800 mL/day of infant formula for three month olds, of 545 mL/day of follow-on formula for nine month olds, of 425 mL/day of toddler formula for 12 month olds, and of 280 mL/day of toddler formula for 1-3 year olds plus intakes from infant foods and other foods. Infants aged three months were assumed to be exclusively fed infant formula.

Estimated mean dietary intakes (g/day) for each of the dietary intake assessments are given in Table 1. More detailed results and additional assumptions underpinning the dietary intake assessment are described in Attachment 7.

Table 1: Estimated mean dietary intake (g/day) of inulin-derived substances/FOS and GOS among infants and toddlers for the combined and separate intake assessments, Australia and New Zealand

	Combined assessment intakes (g/day)			Separate assessment intakes (g/day)					
	Inulin-derived substances and GOS			Inulin-derived substances alone			GOS alone		
Age	Current intakes	Projected intake	Increase	Current intakes	Projected intake	Increase	Current intakes	Projected intake	Increase
Australia									
3 months	0	6	6	0	6	6	0	6	6
9 months	5	9	4	4	8	4	1	5	4
1 year	7	10	3	5	9	4	1	5	4
New Zealand									
1-3 years	17	19	2	12	15	3	5	7	2

Source: FSANZ estimates based on model diets for each age group.

The estimates indicate that following the addition of inulin-derived substances and GOS to infant formula, three month old infants are likely to have the highest increase in mean intakes for all three intake assessments. While the increases for older infants and children are not as great they are still considerable; with up to a 100% increase in mean intakes.

The main source of inulin-derived substances and GOS among Australian and New Zealand infants aged 9 months and 1 year were from supplemented follow-on formula, infant foods and FSFYC. Thus, intakes from naturally-occurring food sources and added sources in processed foods did not make a large contribution to the estimated intakes of inulin-derived substances and GOS for infants 9 months and 1 years of age.

The main source of inulin-derived substances and GOS intakes for New Zealand children (aged 1-3 years) were from yoghurts. Toddler formula was also a major contributor but was lower than yoghurts.

6.5 External advice on safety issues

6.5.1 Responses from peer reviewers on safety assessment

Professor Gibson concurred with FSANZ's recommendation that 8 g/L in any combination of GOS, oligofructose and inulin, either alone or combined was safe. Professor Cummings, was more circumspect. He considered that these substances when added to infant formula would be safe for infants of all ages, however, he highlighted that inulin may cause gastrointestinal discomfort based on studies in adults where some individuals experienced increased flatulence at supplementary intakes of 10 g inulin/day. As a result, Professor Cummings suggested that in the absence of appropriate studies with inulin-derived substances at higher levels it would be justified to limit the addition of these to the levels that have been directly studied in infants (3 g/L). Professor Gibson had no similar concern about gastrointestinal symptoms.

This recommendation was considered by the ICSAG in early December 2007. Members agreed with the recommended upper levels for GOS, oligofructose and inulin; no significant issues were raised. In particular, they supported the recommendation to restrict the upper level of inulin-derived substances added to infant formula products to 3 g/L as suggested by Professor Cummings.

6.5.2 Anecdotal evidence regarding safety concerns of oligosaccharide supplemented infant formulas

The New Zealand Food Safety Authority provided anecdotal evidence (reports by parents) to FSANZ of a small number of adverse events including diarrhoea, and blood and mucous in stools that may be associated with infant formula containing added long chain inulin and GOS. FSANZ sought additional advice from an expert in paediatric nutrition who considered that although the information was suggestive of an adverse effect, the same effects could be due to other causes, such as rotavirus, which is common in infants.

7. Summary of risk assessment

7.1 Inulin-derived substances and FOS added to the general food supply

There is a history of safe use of inulin-derived substances and FOS added to the general food supply. In Australia and New Zealand, the substances have been used since the mid-1990s. Since 2001, inulin and FOS could be labelled as dietary fibre in a wide range of foods.

Although there have been reports of two individual patients with an allergy to inulin, due to the rarity of these cases, it is not recognised as a major allergen.

7.2 Inulin-derived substances/FOS and GOS added to special purpose foods for infants and young children

FSANZ has assessed the evidence on the potential of inulin-derived substances and GOS to cause adverse effects in infants and young children. In particular, the possibility of oligosaccharides increasing osmotic potential in the colon, which could lead to increased water loss and dehydration was considered.

The evidence indicates that inulin-derived substances and GOS, like naturally occurring HMOs, are not digested to any great extent in the small intestine. They reach the large intestine mostly intact and contribute to a small increase in osmotic potential in the colon. However, this slight increase in osmotic potential for GOS and inulin-derived substances is not considered to be undesirable because breast-fed infants also have levels of undigested HMOs present in the colon.

A number of studies on the 9:1 GOS to long chain inulin preparation in infant formula products support the conclusion that 8 g/L of oligosaccharides will not pose a risk to young infants. This conclusion applies to GOS, inulin and oligofructose at any ratio to a total level of 8 g/L, based on data indicating that these oligosaccharides are fermented to a similar or greater extent than HMOs. The safety of this level (8 g/L) is further supported by the presence of higher levels of indigestible oligosaccharides (up to 25 g/L) in human milk.

However, given evidence in adults that increased intakes of inulin-derived substance may lead to gastrointestinal symptoms (e.g. flatulence and bloating), it may be prudent to limit the amount of these substances permitted in formula to those which have been shown to be tolerated by infants i.e. 3 g/L.

For young children, toddler formula and infant foods are not the sole source of nutrition, therefore similar or somewhat higher levels of GOS and oligofructose or inulin in these foods, leading to similar overall intakes of GOS and oligofructose or inulin as evaluated in the available studies, or as seen in young infants consuming oligosaccharide-supplemented infant formula products, is very unlikely to pose a risk to young children.

FSANZ considers inulin and FOS to have a history of safe use as food ingredients in the general food supply.

RISK MANAGEMENT

8. Identification of risk management issues

The risk assessment is aimed to determine the safety of inulin-derived substances and GOS when added to special purpose foods for infants and young children. The use of inulin-derived substances and FOS in the general food supply has also been assessed.

FSANZ has considered the management of any risks identified through the risk assessment and from information provided through consultation with key stakeholders.

8.1 Inulin-derived substances, FOS and GOS added to food

8.1.1 History of safe use

FSANZ's risk assessment indicates that inulin-derived substances and FOS added to food has a history of safe use in the general food supply.

Therefore it is proposed at Draft Assessment that the Code be amended to the effect that inulin and FOS are taken **not** to be nutritive substances and therefore do not require express permission for addition to food.

FSANZ is not aware of the wide use of GOS added to food (other than to infant formula products, infant foods and FSFYC) and intakes from the general food supply from naturally-occurring GOS are likely to be negligible. Therefore FSANZ considers the regulation of GOS in food does not need to be included in the Code, other than for their use in infant formula products, infant foods and FSFYC.

8.2 Inulin-derived substances and GOS added to special purpose foods for infants and young children

8.2.1 Safety

FSANZ has assessed the safety of inulin-derived substances and GOS and their potential to cause adverse effects in infants and young children, in particular, the effect on water balance and potential dehydration in infants fed solely on infant formula containing inulin-derived substances and/or GOS.

FSANZ's risk assessment indicates that the combined addition of inulin-derived substances and GOS in infant formula up to 8 g/L is unlikely to represent a risk to infants and young children up to three years of age.

FSANZ has also assessed the addition of inulin-derived substances or GOS alone to infant formula products, infant foods and FSFYC and concludes that their singular addition is unlikely to represent a risk to infants and young children as long at the maximum permitted level is not exceeded (see Section 8.2.2).

Therefore, FSANZ is proposing to amend the Code to permit the addition of inulin-derived substances and/or GOS to infant formula products, infant foods and FSFYC with limits set as below (see Section 8.2.2).

FSANZ's assessment found very little evidence to support the safe addition of FOS to infant formula products, infant foods and FSFYC. However, on the basis of information provided to FSANZ it appears that many manufacturers who indicate they are adding FOS to these products are actually adding inulin-derived substances, rather than FOS, as defined in this report (refer to Section 1.4).

8.2.2 Levels of addition, permitted form and ratio of GOS and inulin-derived substances

8.2.2.1 Levels in infant formula products, infant foods and FSFYC

The risk assessment concludes that a total level up to 0.8 g/100 mL of inulin-derived substances and GOS added singularly or in combination in any ratio to infant formula products, infant foods and FSFYC is safe.

However, the safety assessment (see Attachment 6) also notes that some adults experience gastrointestinal effects such as increased flatulence and bloating upon consumption of high levels of inulin-derived substances. It is not clear if this would occur in infants due to differences in colonic microflora and overall diet. As a result, however, FSANZ considers that it may be prudent to limit the addition of inulin-derived substances to levels which have been shown to be well tolerated in infants i.e. 3 g/L.

Therefore, at Draft Assessment, FSANZ is proposing the following levels[28]:

[28] The maximum permitted amounts only apply when the substance is added and then apply to the total of the naturally occurring and added substances.

- for infant formula products a permitted maximum level for the singular addition of inulin-derived substances of 110 mg/100 kJ (0.3 g/100 mL) or for the singular addition of GOS of 290 mg/100 kJ (0.8 g/100 mL) , or for the combined addition of these substances up to a maximum of 290 mg/100 kJ (0.8 g/100 mL) with inulin-derived substances not exceeding 110 mg/100 kJ (0.3 g/100 mL) of the total amount;

- for infant foods a permitted maximum level of 0.8 g/100 g for total combined inulin-derived substances and GOS, or for the singular addition of either inulin-derived substances or GOS; and

- for FSFYC a permitted maximum level of 1.6 g/serve[29] (0.8 g/100 mL) for total combined inulin-derived substances and GOS, or for the singular addition of either inulin-derived substances or GOS.

8.2.2.3 Ratio of GOS to inulin-derived substances

The majority of studies considered in the risk assessment were of a combination of GOS and inulin-derived products in the ratio of 9:1. However, FSANZ considers total added GOS and inulin-derived substances, in any ratio up to the maximum level, is safe.

Therefore at Draft Assessment FSANZ does not consider a prescribed ratio requirement is necessary in the Code.

FSANZ notes however, that Nutricia[30] in its Application A609, states that '*Numico*[31] *presently holds an intellectual property position, including patents, in relation to the use of prebiotics GOS and long chain FOS (high molecular weight) in a ratio of 9:1'*.

Consequently, other manufacturers will need to consider this position, including the patents, and make their own enquiries to inform the formulation of their infant formula products.

8.2.3 Specifications for inulin-derived substances and GOS for addition to food

Specifications for GOS are included in the Chemical and Technological Uses Appendix A (Attachment 2) and will be included in Standard 1.3.4 – Identity and Purity of the Code.

Specifications for inulin, long chain inulin and oligofructose will vary in accordance with the wide variety of mixtures available. Therefore, it is not considered practical to develop specific specifications for these substances. However, there is a need to define these substances in the Code to ensure regulatory clarity. FSANZ is proposing that the definitions as described in the draft Variation (see Attachment 1) be inserted into the Code.

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[29] The level of 1.6 g per serve is based on a serving of 200 mL of toddler formula.
[30] Nutricia's Application is seeking to add GOS and long chain FOS in the ratio 9:1 and at a level of 0.8 g/100 mL to infant formula and infant foods.
[31] Numico is an international food manufacturer of specialised food products including the Nutricia brand of infant formula products and infant food.

8.2.4 Changes to infant feeding regimes

FSANZ has also considered potential initial gastrointestinal discomfort that may be experienced by some young infants changing from breast milk or conventional formula to oligosaccharide-supplemented formula.

FSANZ considers changed gastrointestinal effects are not uncommon for infants when their formula is changed and that this effect is likely to be less evident in older infants (e.g. 6 months and over).

FSANZ acknowledges that heath professionals are the most appropriate source of information in guiding parents/carers in infant feeding. Health professionals who are advising those caring for formula-fed infants are likely to include information regarding potential gastrointestinal effects and the management of these, when changes are made to an infants feeding regime.

8.3 Labelling requirements

8.3.1 Foods in the general food supply

Current labelling requirements for the addition of inulin-derived substances and FOS to food reside in Part 1.2 of the Code. Where no nutrition claim is made, added biologically active substances [32] and nutrients that are not already prescribed must be declared as an ingredient and meet the relevant labelling requirements for the nutrition information panel (NIP).

8.3.1.1 Ingredient labelling

Clause 4 of Standard 1.2.4 – Labelling of Ingredients states ingredients must be declared in the statement of ingredients using either the common name of the ingredient or a name that describes the true nature of the ingredient. The Editorial note to clause 4 provides further clarification that the names of ingredients should be sufficiently detailed and accurate to ensure that that they are not false, misleading or deceptive, or likely to mislead or deceive.

Under the current proposal, the addition of inulin-derived substances and FOS to food would require terminology that denotes a common name or the true nature of the ingredient. FSANZ agrees that that the terms already in common usage are appropriate. For example, the generic term 'inulin' might be used in the ingredient list where inulin or long-chain inulin has been added to the food. Oligofructose or fructo-oligosaccharides could be used interchangeably.

These terms are consistent with requirements for ingredient labelling in other countries. In a review of the international legal status of inulin and oligofructose[33], it was stated that 'inulin' is the generally accepted term in order to denote the presence of chicory inulin. It was also noted that the term 'fructo-oligosaccharides' was considered a synonym for oligofructose.

[32] Biologically active substance is defined in Standard 1.2.8 to mean a substance, other than a nutrient, with which health effects are associated.
[33] Coussement, P. A. A., (1999). Inulin and Oligofructose: Safe Intakes and Legal Status. J. Nutr. 129: 1412S-1417S

It was noted in the review that where …*commercial products contain fractions of mono- and disaccharides, these sugars may need separate labelling. Native inulin and standard oligofructose products always contain some sugars, which can be considered as a normal part of the inulin or oligofructose. Therefore, it has been legally accepted that these sugars do not have to be labelled specifically in most practical cases.*

FSANZ supports this approach to ingredient labelling as it is consistent with current requirements in the Code. The definition of dietary fibre in Standard 1.2.8 includes oligosaccharides that have a degree of polymerisation of greater than two, and as such mono- and disaccharides are excluded. Mono- and disaccharides would be classified as available sugars and captured under the definition of carbohydrate. Where a significant proportion of mono- and di-saccharides are present in commercial fructose polymers, these sugars are required to be declared separately in the statement of ingredients.

8.3.1.2 Nutrition information labelling

At present, it remains at the discretion of the food manufacturer whether to treat the addition of inulin-derived substances and FOS as biologically active substances or as specific forms of dietary fibre.

Clause 5 of Standard 1.2.8 – Nutrition Information Requirements states that where a nutrition claim is made for any other nutrient not already mandated, or a biologically active substance, the name and average quantity must be declared in the NIP in accordance with the prescribed format. Health claims for inulin-derived substances and FOS are not permitted by the current regulations.

If the food manufacturer elects to make a dietary fibre nutrition claim for inulin-derived substances or FOS, these fructose polymers would need to be declared in the NIP as a sub-sub-group nutrient, indented and nested under 'total dietary fibre'. Voluntary claim conditions for nutrition claims about dietary fibre are specified in the Code of Practice on Nutrient Claims (CoPoNC). Subparagraph 5(5)(b) of Standard 1.2.8 requires that where a nutrition claim in respect of fibre, any specifically named fibre, sugars, or any other type of carbohydrate, a fibre declaration is then required in the NIP.

In addition, where mono- and disaccharide fractions are present in a significant proportion in commercial fructose polymers, these sugars should be declared separately in the NIP. This approach is consistent with current requirements in the Code, as previously discussed in subsection 8.3.1.1.

Other nutrition labelling requirements, in relation to the NIP, small packages and the form of the food, are covered in Division 2 of Standard 1.2.8 and apply to the substances being considered in this proposal.

Clause 18 of Standard 1.2.8 notes that a declaration of dietary fibre in the NIP must be determined in accordance with the prescribed methods of analysis to determine total dietary fibre and specifically named fibre content of food. Established methods for the food components 'inulin and fructo-oligosaccharide', and 'inulin', are listed in the Table to clause 18(1). It is proposed that these food components are replaced by the group name 'fructans', with inulin-derived substances and FOS provided in brackets for clarity, for example 'fructans (including inulin-derived substances and fructo-oligosaccharides)'.

The term 'fructans' is used in the prescribed methods of analysis and encompasses inulin-derived substances and FOS, as defined in subsection 1.4.1 of this Draft Assessment Report.

Under the current proposal, requirements for nutrition information labelling for food would be essentially unchanged. The positioning of nutrition claims as either a dietary fibre claim or a biologically active claim would remain at the discretion of the food manufacturer.

Under Proposal P293 – Nutrition, health and related claims, FSANZ is currently considering new regulations around nutrition, health and related claims, which will be contained within Standard 1.2.7. It is proposed that for foods in the general food supply, general level health claims would be permitted for inulin-derived substances and FOS, and such foods would need to meet nutrient profiling scoring criteria to be eligible to carry a general level health claim.

8.3.2 Infant-formula products

8.3.2.1 General labelling and packaging requirements

Specific labelling and packaging requirements for infant-formula products are prescribed in Standard 2.9.1. In addition, the general labelling requirements under Part 1.2 of the Code, including Standard 1.2.4 – Labelling of Ingredients, also apply to these products, subject to any specified exemptions.

Under the current proposal, permissions for the addition of inulin-derived substances and GOS to infant formula products will also require specific declarations of the substance in the statement of ingredients (in accordance with definitional requirements set out in the draft Variation to the Code (Attachment 1).

Warning Statements

Clause 14 of Standard 2.9.1 prescribes specific warning statements that must be included in the label on a package of infant formula product. These statements refer to the importance of correctly following instructions for preparation, and for parents to seek advice from an appropriate health professional on whether or not to use the product.

FSANZ has considered the need for a specific warning statement on infant formula products regarding the presence of inulin-derived substances and/or GOS and the possibility of any potential discomfort in some infants.

There is limited evidence that inulin-derived substances and/GOS contribute to gastrointestinal discomfort during adaptation to a new formula, however this is not uncommon for infants who change formulations, and health professionals are likely to provide guidance on the management of any such effects (see section 8.2.4).

Also FSANZ's safety assessment (see Section 6.3) has concluded that the proposed amounts of inulin-derived substances and GOS are safe and that these amounts are less than the amounts found in breast milk. Furthermore, it is proposed that FOS, as defined in the draft Variation to the Code (Attachment 1), will not be permitted for addition to infant formula products.

Therefore, FSANZ considers mandating a specific warning statement regarding the presence of inulin-derived substances and/or GOS to be unnecessary.

Question to submitters:

Should a warning statement be required in the label on a package of infant formula product, if inulin-derived substances and/or GOS have been voluntarily added? (If you believe that a warning statement is warranted, please provide any evidence to support this requirement).

8.3.2.2 Nutrition information labelling

Clause 16 of this Standard regulates the declaration of nutrition information in the label on an infant formula product. FSANZ is proposing that where inulin-derived substances and GOS are added voluntarily to infant formula products, a mandatory declaration of these substances will be required, as for energy, protein, fat, carbohydrate, and permitted vitamins and minerals and other nutritive substances. The statement may be in the form of a table and must contain the average amount of inulin-derived substances and/or GOS expressed as weight per 100 mL. As guidance, the Nutrition Information Table as provided in the 'Guidelines for Infant Formula Products' of Standard 2.9.1 has been amended to reflect how the presence of inulin-derived substances and GOS may be declared in accordance with the nutrition information statement requirements in clause 16.

Question to submitters:

Do you agree with the suggested approach (as included in the Guidelines for Infant Formula Products) for declaring the presence of inulin-derived substances and/or GOS in the nutrition information statement? If you disagree, please describe what guidance should be provided on how this nutrition information is to be declared in the nutrition information statement.

Clause 20 of Standard 2.9.1 provides for claim prohibitions relevant to all infant formula products. Subparagraph 20 (1)(f) prohibits a reference to the presence of a nutrient or nutritive substance except where it relates to the name of a low lactose or lactose free infant formula, or is in the ingredient list or the nutrition information statement. Clause 28 regulates claims on infant formula products formulated for metabolic, immunological, renal, hepatic or malabsorptive conditions. FSANZ is proposing to insert a new sub clause to clause 20. The intention of the sub clause is to clarify that, for the purposes of subparagraph 20 (1)(f), inulin-derived substances and GOS are taken to be a nutrient. The proposed amendment to clause 20 would therefore permit a reference to the presence of inulin-derived substances and GOS only in the statement of ingredients or in a nutrition information statement.

The existing prohibition on claims would therefore extend to inulin-derived substances and GOS. Under Proposal P293 – Nutrition, health and related claims, FSANZ is proposing to retain the prohibition for nutrition content claims and health claims on infant formula products.

8.3.3 Infant foods

8.3.3.1 Ingredient labelling

Under existing Code requirements, Standard 1.2.4 applies to foods for infants as regulated in Standard 2.9.2, with the exception of the requirement to declare compound ingredients.

FSANZ is proposing to permit the addition of inulin-derived substances and GOS to infant foods, where they must be declared in the ingredient list in accordance with the requirements in Standard 1.2.4.

8.3.3.2 Nutrition information labelling

At present, general requirements for nutrition information labelling under Standard 1.2.8 apply to food for infants, notwithstanding exemptions for specific provisions in Standard 1.2.8 that are listed in clause 9 of Standard 2.9.2 – Foods for Infants. Subclause 9(2) prescribes the format for the nutrition information panel. Where there is a direct inconsistency between nutrition labelling requirements in Standard 1.2.8 and in Standard 2.9.2, the latter would prevail to the extent of the inconsistency.

Clause 5 of Standard 2.9.2 contains permission for nutrition claims relating to 'no added sugar' and 'sweetened' in addition to a specific claim condition. These claims are also subject to general labelling provisions in Standard 1.2.8. Clauses 6 and 9 permit nutrition content and function claims for protein and for vitamins and minerals, respectively. Infant foods are prohibited from carrying health claims under the existing requirements in the Code.

The current proposal will permit the addition of inulin-derived substances and GOS and include permission for nutrition claims to be made for these substances on infant foods. General labelling provisions for nutrition claims in Standard 1.2.8 would also apply. The option of whether to treat the addition of inulin-derived substances as a biologically active substance or as a type of dietary fibre will remain.

It is intended that general level health claims for inulin-derived substances and GOS would be permitted under Proposal P293 – Nutrition, Health & Related Claims, although infant foods are to be exempted from the proposed requirement for meeting nutrient profiling scoring criteria.

8.3.4 Formulated supplementary foods for young children

8.3.4.1 Ingredient labelling

Under existing requirements, general labelling provisions contained within Standard 1.2.4 would apply to FSFYC, as regulated in Division 4 of Standard 2.9.3 – Formulated Meal Replacements and Formulated Supplementary Foods.

FSANZ is proposing to permit the addition of inulin-derived substances and GOS to FSFYC, where they must be declared in the ingredient list in accordance with the requirements in Standard 1.2.4.

8.3.4.2 Nutrition information labelling

Current provisions in Division 4 of Standard 2.9.3 regulate nutrition claims for energy, protein and vitamins and minerals on FSFYC. Permission for nutrition content and function claims is located in clause 6 of this Standard. Where there is a direct inconsistency between nutrition labelling requirements in Standard 1.2.8 and in Standard 2.9.3, the latter would prevail to the extent of the inconsistency. FSFYC are not currently permitted to carry health claims.

FSANZ is proposing the same approach for FSFYC as for infant foods, whereby nutrition claims would be permitted for inulin-derived substances and GOS. General labelling provisions for nutrition claims in Standard 1.2.8 would also apply where no conflict exists between Standards. The option of whether to treat the addition of inulin-derived substances as a biologically active substance or as a type of dietary fibre will also remain.

It is intended that general level health claims on FSFYC would be permitted for inulin-derived substances and GOS through Proposal P293. FSFYC would not be required to meet nutrient profiling scoring criteria to be eligible to carry a health claim.

9. Regulatory options

9.1 Inulin-derived substances and FOS added to the general food supply

Two regulatory options have been identified at Draft Assessment.

Option 1 – the *status quo* – maintain the Code whereby there are no explicit permissions for the addition of inulin-derived substances and FOS in food.

Option 2 – amend the Code to insert a clause in Standard 1.1.1 to the effect that inulin-derived substances and FOS are taken to not be nutritive substances, and therefore do not need explicit permissions for addition to food.

9.2 Inulin-derived substances and GOS added to special purpose foods for infants and young children

Two regulatory options have been identified at Draft Assessment.

Option 1 – the *status quo* – maintain the Code whereby there are no explicit permissions for the addition of inulin-derived substances and GOS to infant formula products, infant foods and FSFYC.

Option 2 – amend Standard 2.9.1 to permit the voluntary addition of inulin-derived substances to a maximum of 110 mg/100 kJ (0.3 g/100 mL), or GOS to a maximum of 290 mg/100 kJ (0.8 g/100 g), or a combination of inulin-derived substances and GOS up to a total maximum of 290 mg/100 kJ (0.8 g/100 g) where inulin-derived substances do not exceed 110 mg/100 kJ (0.3 g/100 mL); and amend Standards 2.9.2 and 2.9.3 Division 4 to permit the voluntary addition of inulin-derived substances and GOS, alone or in combination, to infant foods and FSFYC to a total maximum of 0.8 g/100 g and 1.6 g/serve (0.8 g/100 mL), respectively.

10. Impact Analysis

10.1 Affected Parties

The parties likely to be affected by the Application are:

- **consumers** of foods with added inulin-derived substances,, FOS and GOS, including infants and young children who consume infant formula products, infant foods and FSFYC;

- **carers** of infants and toddlers consuming infant formula products, infant foods and FSFYC;

- manufacturers and / or marketers of specialty ingredients for application in foods (with added inulin-derived substances and FOS) and/or infant formula products, infant foods and FSFYC **(industry);**

- manufacturers, importers and exporters of foods with added inulin-derived substances and FOS, and infant formula products, infant foods and FSFYC with added inulin-derived substances and GOS **(industry)**; and

- the **Governments** of Australia and New Zealand.

10.2 Benefit Cost Analysis

10.2.1 Foods in the general food supply

10.2.1.1 Consumers

Status Quo: maintaining the *status quo* for the general food supply is unlikely to have any significant impact on consumers. Based on their history of safe use, foods with added inulin-derived substances or FOS currently available on the market will not present a safety concern for consumers. Any potential advantage consumers may receive from the addition of inulin-derived substances or FOS will continue to be provided. However, if consumers are aware of the current uncertainty regarding the status of these substances in infant formula products, any uncertainty or confusion they may have regarding its use in foods could remain. Also, if the regulatory uncertainty causes manufacturers to reformulate or remove products that currently contain inulin-derived substances or FOS from the market, product choice and any potential benefits to consumers may decrease.

Option 2 would enable foods with added inulin-derived substances or FOS to continue to be manufactured and be available to consumers and, based on their history of safe use, will not present safety concerns for consumers. Any potential benefits consumers may receive from the addition of inulin-derived substances or FOS to foods will continue to be provided.

In addition, clarifying the status of inulin-derived substances or FOS is likely to dispel any uncertainty or concerns consumers may have regarding the safety of these substances in foods.

10.2.1.2 Industry

Status Quo: Maintaining the *status quo* for the general food supply would not confirm the regulatory position for the food industry around the use of inulin-derived substances or FOS in foods. The current uncertainty amongst food manufacturers and suppliers of inulin-derived substances or FOS would continue. Manufacturers adding inulin-derived substances or FOS to foods would continue to be unsure if their products comply with the Code and potential enforcement action could remain a concern. The potential need to make compositional and packaging changes to products, with associated costs would also remain a concern for manufacturers.

Suppliers of inulin-derived substances or FOS would also remain uncertain as to the status of these substances with negative financial impact should manufacturers decide to reformulate their products without adding these substances.

A lack of clarity regarding the status of inulin-derived substances or FOS in general foods and uncertainty regarding compliance with the Code may also result in trade difficulties and create barriers to export in some instances.

Option 2 would confirm the regulatory position for food manufacturers and suppliers of inulin-derived substances or FOS. The manufacture of food products currently containing inulin-derived substances or FOS could continue for both the domestic and overseas market, thus reducing potential barriers to trade. This would also avoid the financial implications of having to reformulate and repackage products, for both food manufacturers and suppliers of inulin-derived substances or FOS. Option 2 would also support product innovation for manufacturers.

10.2.1.3 Government

Maintaining the **status quo** may require enforcement agencies to determine whether they consider manufacturers of foods with added inulin-derived substances or FOS to be in breach of the Code.

10.2.2 Special purpose foods for infants and young children

10.2.2.1 Consumers / carers

Maintaining the ***status quo*** for special purpose foods for infants and young children would not have a significant impact on consumers and their carers as suitable foods for this age group would continue to be available. However, any potential benefit provided by the addition of inulin-derived substances and GOS to these products would not be available to formula-fed infants, and toddlers. Also, carers of infants and toddlers may be confused as to the safety of these substances which could affect confidence and trust in infant formula products, infant foods and FSFYC, and therefore the industry as a whole.

Option 2 would provide consumers and their carers with an additional choice of special purpose foods for infants and young children and enable them to receive any potential advantages from the addition of inulin-derived substances and GOS. Option 2 would also clarify the safety of these products and maintain consumer confidence in infant formula products, infant foods and FSFYC.

These products with added inulin-derived substances and GOS may incur additional costs for consumers as any extra manufacturing costs may be passed on to consumers who purchase the products.

10.2.2.2 Industry

Maintaining the ***status quo*** would not confirm the regulatory position for the food industry around the status of inulin- derived substances and GOS and uncertainty amongst manufacturers would remain.

Maintaining the *status quo* could limit the options to manufacture products which are suitable for both the local and overseas market as regulations would not harmonise with some countries, such as European Union countries. Manufacturers may need to manufacture separate products for internal and external markets affecting economies of scale and therefore manufacturing costs.

In some instances, the status quo could impact on trade opportunities by restricting the ability to export products to countries that permit the addition of inulin-derived substances, FOS and GOS. Industry has noted that some countries will not accept/register products that do not comply with local regulations, for example some Asian countries. This would result in lost markets for those manufacturers with financial implications. Some manufacturers may need to reformulate products to meet overseas requirements causing additional costs.

Also, the status quo would not encourage innovation in product development.

Option 2 would confirm the regulatory position for manufacturers, and for suppliers of inulin-derived substances and GOS, by providing explicit permission (with limits) for the addition of these substances to infant formula products, infant foods and FSFYC.

Alignment with international regulations would allow for the single formulation and manufacture of products for both local and overseas markets maximising production costs and reducing barriers to trade.

In addition, **Option 2** is likely to maintain the trust of consumers in the infant formula and infant food industry.

Option 2 would potentially support product innovation for infant formula manufacturers. However, the intellectual property position held by Numico (see Section 8.2.2.3), including patents in relation to a specified ratio, may limit product innovation. Manufacturers would need to make their own enquiries and consider this in the development of new products.

Question to submitters, in particular, industry submitters:

FSANZ's risk assessment conclusions indicate that up to 0.8 g/100 mL of inulin-derived substances could safely be added to infant formula products. However, FSANZ has decided to take a conservative approach and restrict the maximum to 0.3 g/100 mL based on the potential to cause gastrointestinal discomfort at higher levels than has been studied and because this is the maximum amount that industry has requested to date.

Would industry consider adding more than the proposed 110 mg/100 kJ (0.3 g/100 mL) of inulin-derived substances to infant formula products? If yes, on what basis would a higher level of addition be considered appropriate?

10.2.2.3 Government

Maintaining the ***status quo*** may require enforcement agencies to determine whether manufacturers of special purpose foods for infants and young children are in breach of the Code should they add inulin-derived substances or GOS to these products.

Option 2 would confirm the regulatory position for enforcement agencies.

10.3 Comparison of Options

10.3.1 Foods in the general food supply

A comparison of the Options presented at Draft Assessment indicates that maintaining the ***status quo*** (Option 1) would present no safety concerns for consumers as inulin or FOS have a history of safe use in the general food supply.

However, maintaining the *status quo* would not provide certainty for manufacturers of foods, suppliers of inulin-derived substances and FOS and enforcement agencies. Potential enforcement action, reformulation and repackaging of products and possible trade barriers could result in additional costs for food manufacturers and suppliers.

In comparison, **Option 2** also presents no safety concerns for consumers as inulin-derived substances and FOS have a history of safe use in the general food supply. In addition, Option 2 would confirm the regulatory position for the food industry by clarifying the status of inulin-derived substances and FOS in foods and would avoid any negative financial impacts for both manufactures and suppliers, and reduce any trade barriers.

The analysis of potential impacts, in conjunction with the history of safe use in the general food supply indicates that an overall net-benefit is achieved through **Option 2**.

10.3.2 Special purpose foods for infants and young children

A comparison of the Options presented at Draft Assessment indicates that maintaining the ***status quo*** (Option 1) would present no safety concerns for consumers as appropriate infant formula products, infant foods and FSFYC will remain available for infants and toddlers.

However, maintaining the *status quo* would not clarify the situation for consumers and their carers, or confirm the regulatory position for manufacturers and suppliers of inulin-derived substances and GOS, or enforcement agencies. Uncertainty would remain with negative financial, trade and potential enforcement implications for industry, lack of regulatory clarity regarding enforcement, and reduced choice for consumers. Also there may be a loss of consumer confidence in manufacturers of infant formula products, infant foods and FSFYC.

In comparison, **Option 2** also presents no safety concerns for infants or toddlers.

In addition, Option 2 would provide consumers with choice and clarity and would likely maintain their confidence in the safety of infant formula products, infant foods and FSFYC. Option 2 would reduce potential trade barriers, support cost-effective production through harmonisation with overseas regulations, support product innovation and avoid potential enforcement action. Also, the regulatory position would be confirmed for enforcement agencies regarding the need for any enforcement action in the future.

The analysis of potential impacts, in conjunction with the safety assessment indicates that an overall net-benefit is achieved through Option 2.

COMMUNICATION AND CONSULTATION STRATEGY

11. Communication

The communication strategy for Proposal P306 aims to ensure relevant industry players, consumers, health professionals and jurisdictions are aware of any changes to the Code which may result from this Proposal.

A range of communication channels and activities are available to provide appropriate information.

At Draft Assessment, in addition to the public consultation round (see Section 12), FSANZ will undertake targeted communication on the proposed variation to the Code with key stakeholders including health professionals, the food industry and enforcement agencies. Also, information will be provided on the FSANZ website and for the FSANZ Advice Line, particularly regarding the proposed permissions for addition of inulin-derived substances and GOS to special purpose foods for infants and young children. This will ensure consumers have access to appropriate information.

Following the consultation period, FSANZ will review the nature of the feedback received from submitters at Draft Assessment, and determine whether additional communication strategies are required for the Final Assessment.

12. Consultation

FSANZ decided, pursuant to section 36 of the FSANZ Act to omit to invite public submissions in relation to the Proposal prior to making a Draft Assessment. FSANZ made its decision under section 36 (to omit to invite public submissions prior to making a Draft Assessment) because it was satisfied that the Proposal raised issues that will not have a significant adverse effect on the interests of anyone.

Section 63 of the FSANZ Act [in place before 1 July 2007] provides that, subject to the *Administrative Appeals Tribunal Act 1975*, an application for review of FSANZ's decision to omit to invite public submissions prior to making a Draft Assessment, may be made to the Administrative Appeals Tribunal.

However, FSANZ has undertaken early targeted consultation with key stakeholders to assist in preparing the Draft Assessment.

Initial discussions were held with key stakeholders prior to the public notification of Proposal P306, including the NSW Food Authority, the New Zealand Food Safety Authority, Nutricia, Heinz Wattie's, Wyeth, Orafti Group and the Australian Food and Grocery Council (AFGC). These discussions primarily sought to seek support for the proposed approach to the Proposal.

In addition, FSANZ has undertaken early targeted consultation with key industry stakeholders to enable them to provide additional information to assist with the Draft Assessment. Key stakeholders consulted with are:

- the Infant Formula Manufacturers' Association of Australia (IFMAA) and the New Zealand Infant Formula Marketers' Association (NZIFMA). Joint meetings have been held with members including Nutricia, Heinz Wattie's, Nestle, Wyeth, Bayer and also in New Zealand the Dairy Goat Co-operative NZ Ltd. and Fonterra Cooperative Group Ltd. Individual meetings have also been held with Nutricia, Heinz Wattie's and Wyeth;

- the AFGC and the New Zealand Food and Grocery Council (NZFGC);

- representatives of Orafti Group; and

- other food manufacturers.

In addition, stakeholders have been asked to advise FSANZ of any other parties they consider should provide input.

FSANZ now invites written submissions for the purpose of the Final Assessment under paragraph 17(3)(c) of the FSANZ Act and will have regard to any submissions received.

12.1 Summary of key issues raised from early consultation

Key issues raised during targeted consultation have been addressed where possible within the Draft Assessment Report. Points raised by stakeholders include:

12.1.1 Technological issues

- there is need to clarify the terminology and technological information regarding the substances being added or proposed to be added to foods and/or infant formula products, infant foods and FSFYC. Some manufacturers provided specifications for inulin, FOS[34] and GOS;

- FOS is currently added for functional/physiological or technical reasons rather than to achieve a nutritional purpose;

- permissions to add FOS to infant formula products should not be restricted to one particular type of FOS; and

- consideration of the use of FOS alone as well as in combination with GOS is recommended. It is considered that a standard that allows the addition of FOS alone would maximise industry flexibility.

[34] A reference to FOS in this section reflects terminology as used by submitters.

12.1.2 Safety

- inulin/FOS and GOS have previously been assessed as dietary fibre (Application A277) and are now widely used in the general food supply with a history of safe use;

- there is a need for a different approach between infant formula products and foods in the general food supply, as infant formula products are a sole source of nutrition;

- a full safety and nutritional assessment is necessary for infant formula products including consideration of any adverse reactions such as diarrhoea, allergies and nutrient interactions;

- labelling requirements need to be considered, including the potential need for any advisory or warning statement on infant formula products; and

- the composition of breast milk should also be considered.

12.1.3 Implications for industry

- there are significant cost implications for manufacturers and ingredient suppliers should there be a need to change the composition and packaging of products if the *status quo* remains;

- the current regulatory uncertainty and warnings regarding the use of infant formula products with added inulin/FOS and GOS is impacting on the credibility of formula with added prebiotics exported from Australasia. Some countries will not register products that do not comply with local regulations e.g. some Asian countries. This is having a negative commercial impact on those manufacturers who export to countries where the addition of prebiotics is accepted; and

- not permitting inulin/FOS and GOS to infant formula products, infant foods and FSFYC is inconsistent with overseas standards such as the European Union. Therefore economies of scale are not possible when manufacturing different products for local and export markets.

12.1.4 Definitions

- there is a need to reconsider the definition of a nutritive substance at a later date; and

- nutritional purpose is not defined in the Code.

12.2 World Trade Organization

As members of the World Trade Organization (WTO), Australia and New Zealand are obligated to notify WTO member nations where proposed mandatory regulatory measures are inconsistent with any existing or imminent international standards and the proposed measure may have a significant effect on trade.

As the proposed amendments to the Code would be voluntary permissions, it is expected they will harmonise Australian and New Zealand regulations with relevant current international practices, and therefore will not result in a potential barrier to trade. As such, WTO member nations will not been notified of the proposed amendment to Standards 1.1.1, 2.9.1, 2.9.2 and 2.9.3 under either the Technical Barriers to Trade or Sanitary and Phytosanitary Agreements.

CONCLUSION

13. Conclusion and Preferred Approach

Preferred Approach

In this Draft Assessment, FSANZ's preferred regulatory approach for Proposal 306 is to:

- amend Standard 1.1.1 to state that inulin-derived substances and FOS are taken not to be nutritive substances;

- amend Standard 2.9.1 to permit the voluntary addition of inulin-derived substances to infant formula products up to a total maximum of 110 mg/100 kJ (0.3 g/100 mL), or GOS up to a total maximum of 290 mg/100 kJ (0.8 g/100 mL), or a combination of inulin-derived substances and GOS up to a total maximum of 290 mg/100 kJ (0.8 g/100 mL) where inulin-derived substances do not exceed 110 mg/100 kJ (0.3 g/100 mL); and

- amend Standards 2.9.2 and 2.9.3 Division 4 to permit the voluntary addition of inulin-derived substances and GOS, alone or in combination, to infant foods and formulated supplementary foods for young children up to a total maximum of 0.8 g/100 g and 1.6 g/serve (0.8 g/100 mL), respectively.

FSANZ concludes that the preferred approach provides a net benefit to affected parties because:

13.1 General food supply

- There is a history of safe use of inulin-derived substances and FOS in food in Australia and New Zealand, so food manufacturers do not need express permission to add these substances to the general food supply.

- The preferred approach confirms the regulatory position for the food industry by clarifying the status of inulin-derived substances and FOS in the general food supply. This approach removes the potentially negative financial effects for both manufacturers and suppliers and reduces trade barriers.

13.2 Special purpose foods for infants and young children

- Based on the scientific evidence, and provided the amounts do not exceed the prescribed maximum levels, FSANZ concludes that infants fed solely on infant formula, and older infants and toddlers fed follow-on-formula, infant foods and formulated supplementary foods for young children containing inulin-derived substances and/or GOS in any ratio, are unlikely to be at risk from these foods.

- There was very little evidence to assess the effects of adding FOS to infant formula products, so FSANZ has not included a recommendation for FOS in this Proposal.

- Inulin-derived substances or FOS are not present in breast milk and GOS is found only in trace amounts. Breast milk contains other oligo- and polysaccharides in amounts up to 25 g/L during the first few weeks following birth but the amounts decline thereafter. The recommended maximum levels proposed to be added to infant formula are based on amounts less than those found in breast milk.

- The preferred approach provides consumers with choice and is likely to maintain their confidence in the safety of infant formula products, infant foods and formulated supplementary foods for young children.

- The preferred approach also confirms the regulatory position for the food industry, thereby reducing potential trade barriers, supporting cost-effective production through harmonisation with overseas regulations, and supporting innovation.

- Furthermore, the preferred approach provides clarity for enforcement agencies in Australia and New Zealand.

FSANZ therefore recommends the proposed draft variations to the Code provided at Attachment 1.

14. Rationale for preferred drafting approach

FSANZ has, within the context of the Code, previously considered that inulin-derived substances, FOS and GOS when used in foods for infants and young children as regulated under Part 2.9 – Special Purpose Foods, are 'nutritive substances' within the definition of that term in Standard 1.1.1 as a means of requiring pre-market assessment of foods for these vulnerable population groups.

However, for the purposes of this Proposal, FSANZ has drafted the variations to the respective standards in Part 2.9, in a manner that does not adopt a position either way on the status of inulin-derived substances and GOS when added to special purpose foods for infants and young children. FSANZ has taken this interim approach with the draft variations because it plans to undertake a review of the concept of 'nutritive substances' and a review of Standard 2.9.1 – Infant Formula Products.

For the draft variation to Standard 2.9.1, FSANZ has placed the reference to inulin-derived substances and GOS, with corresponding permissions, in a stand-alone provision (new clause 9A) rather than in the Table to clause 7 (which lists a number of permitted nutritive substances). This drafting approach should not be taken to mean that inulin-derived substances and GOS in infant formula products under Standard 2.9.1 are not 'nutritive substances'.

Furthermore, in relation to the proposed draft variation to Standard 1.1.1, inulin-derived substances and FOS are taken to be not nutritive substances (and therefore requiring no pre-market approval) when added to general foods. This is based on a history of safe use in Australia and New Zealand over many years.

This proposed approach also recognises the status of inulin-derived substances and FOS as fulfilling both a technological and nutritional purpose in general foods. This 'taken to be not nutritive' variation to Standard 1.1.1 is to put beyond any doubt the status of inulin-derived substances and FOS when used in general foods. Conversely, and for the purposes of this Proposal, no decision has been taken on GOS when added to general foods in Standard 1.1.1 because there is no history of addition of GOS to these foods.

15. Implementation and Review

Following the consultation period for this document, a Final Assessment of the Application will be completed and proposed draft variations to the Code considered for approval by the FSANZ Board. The FSANZ Board's resulting decision will then be notified to the Ministerial Council.

Following notification, the proposed draft variation to the Code is expected to come into effect on gazettal, subject to any request from the Ministerial Council for a review of FSANZ's decision.

References

Gibson G.R. and M.B. Roberfroid (1995) Dietary modulation of the human colonic microbiotia: introducing the concept of prebiotics. *J. Nutr* 125: 1401-1412.

Martinez-Ferez, A., Rudloff, S., Guadix, A., Cordula A. Gottfried Pohlentz, H., Boza, J.J., Guadix, E.M., Kunz, C. (2006) Goats' milk as a natural source of lactose-derived oligosaccharides: Isolation by membrane technology. *Int Dairy J* 16: 173-181.

NHMRC and NZMoH (2006). *Nutrient reference values for Australia and New Zealand including recommended dietary intakes*. NHMRC, Canberra.

ATTACHMENTS

1. Draft variations to the *Australia New Zealand Food Standards Code*
2. Chemical and Technological Uses Assessment
3. Human Milk Carbohydrates
4. Microbiological Assessment
5. Nutrition Assessment
6. Safety Assessment
7. Dietary Intake Assessment

Attachment 1

Draft variations to the *Australia New Zealand Food Standards Code*

Standards or variations to standards are considered to be legislative instruments for the purposes of the Legislative Instruments Act (2003) and are not subject to disallowance or sunsetting.

To commence: on gazettal

[1] ***Standard 1.1.1*** *of the Australia New Zealand Food Standards Code is varied by –*

[1.1] *inserting in clause 2 –*

fructans means polymers of fructose with or without a terminal glucose molecule.

fructo-oligosaccharides (FOS) means fructose polymers with β (2→1) fructosyl-fructose linkages, where the average degree of polymerisation is less than four.

galacto-oligosaccharides means a mixture of those substances comprised of between two and eight saccharide units with one of these units being a terminal glucose and the remaining saccharide units being galactose, produced from lactose by enzymatic action.

inulin means fructans, with β (2→1) fructosyl-fructose linkages, where the average degree of polymerisation is equal to or greater than ten.

inulin-derived substances means inulin, long chain inulin or oligofructose.

long chain inulin means those fructans with β (2→1) fructosyl-fructose linkages, where the average degree of polymerisation is equal to or greater than 23.

oligofructose means those fructans, with β (2→1) fructosyl-fructose linkages, where the average degree of polymerisation is less than ten but greater than or equal to four.

[1.2] *inserting after clause 9 –*

9A Certain substances not nutritive substances

Inulin-derived substances and fructo-oligosaccharides are taken not to be nutritive substances.

[2] ***Standard 1.2.8*** *of the Australia New Zealand Food Standards Code is varied by omitting from* Columns 1 *and* 2 *of the* Table to subclause 18(1) *the entries for* Inulin and fructooligosaccharide and Inulin, *substituting –*

Fructans (including inulin-derived substances and fructo-oligosaccharides)	Section 997.08 of the AOAC, 17th Edition (2000); or Section 999.03 of the AOAC, 17th Edition (2000).

[3] ***Standard 1.3.4*** *of the Australia New Zealand Food Standards Code is varied by –*

[3.1] *inserting in the* Schedule

Specification for Galacto-oligosaccharides

Appearance	Syrup, clear, golden colour
Taste	Sweet
pH (10% solution)	pH 3.2-3.8
Ash (sulphated)	Maximum 0.3% on dry weight basis
Galacto-oligosaccharides (glucose linked to between one and seven galactose units)(excluding lactose)	Minimum 57% on dry weight basis
Moisture	24-26%
Lactose	23% maximum (dry weight basis)
Glucose	22% maximum (dry weight basis)
Galactose	0.8% minimum (dry weight basis)

[4] ***Standard 2.9.1*** *of the Australia New Zealand Food Standards Code is varied by –*

[4.1] *inserting after clause 9 –*

9A Permitted inulin-derived substances and galacto-oligosaccharides

(1) Infant formula product may contain no more than –

(a) 110 mg per 100 kJ of inulin-derived substances; or
(b) 290 mg per 100 kJ of galacto-oligosaccharides; or
(c) 290 mg per 100 kJ of combined inulin-derived substances and galacto-oligosaccharides, where the inulin-derived substances is no more than 110 mg per 100 kJ.

(2) For subclause (1) the maximum permitted amount only applies when the substances are added. In that case the maximum permitted amount then applies to the sum of the naturally occurring and the added substances.

[4.2] *omitting paragraph 16(1)(c), substituting –*

(c) the average amount of each vitamin, mineral and any other nutritive substance permitted by this Standard expressed in weight per 100 mL; and

(d) the average amount of –

(i) a combination of inulin derived substances and galacto-oligosaccharides; or
(ii) inulin derived substances; or
(iii) galacto-oligosaccharides

whether naturally occurring or added expressed in weight per 100 mL.

[4.3] *omitting paragraph 16(2)(d), substituting –*

(d) a declaration –

(i) of the weight of one scoop in the case of powdered infant formula; and
(ii) of the proportion of powder or concentrate required to reconstitute the formula according to directions; and

(e) the average amount of –

(i) a combination of inulin derived substances and galacto-oligosaccharides; or
(ii) inulin derived substances; or
(iii) galacto-oligosaccharides

whether naturally occurring or added expressed in weight per 100 mL.

[4.4] *omitting clause 20, substituting –*

(1) The label on a package of infant formula product must not contain –

(a) a picture of an infant; or
(b) a picture that idealises the use of infant formula product; or
(c) the word 'humanised' or 'maternalised' or any word or words having the same or similar effect; or
(d) words claiming that the formula is suitable for all infants; or
(e) information relating to the nutritional content of human milk; or
(f) subject to clause 28, a reference to the presence of any nutrient or nutritive substance, except for a reference to a nutrient or nutritive substance in -

(i) the name of a lactose free formula or a low lactose formula; or
(ii) a statement of ingredients; or
(iii) a nutrition information statement; or

(g) subject to Division 3, a representation that the food is suitable for a particular condition, disease or disorder.

(2) Subject to clause 28, the label on a package of infant formula product must not contain a reference to inulin-derived substances or galacto-oligosaccharides except for a reference to either substances in –

(a) a statement of ingredients; or
(c) the nutrition information statement.

[4.5] *omitting the* Nutrition Information table *in the* Guidelines for Infant Formula Products, *substituting –*

NUTRITION INFORMATION

	Average amount per 100 mL made up formula *1	**Average amount per 100 g of powder (or per 100 mL for liquid concentrate)** *2
Energy	kJ	kJ
Protein	g	g
Fat	g	g
Carbohydrate	g	g
Vitamin A	µg	µg
Vitamin B_6	µg	µg
Vitamin B_{12}	µg	µg
Vitamin C	mg	mg
Vitamin D	µg	µg
Vitamin E	µg	µg
Vitamin K	µg	µg
Biotin	µg	µg
Niacin	mg	mg
Folate	µg	µg
Pantothenic acid	µg	µg
Riboflavin	µg	µg
Thiamin	µg	µg
Calcium	mg	mg
Copper	µg	µg
Iodine	µg	µg
Iron	mg	mg
Magnesium	mg	mg
Manganese	µg	µg
Phosphorus	mg	mg
Selenium	µg	µg
Zinc	mg	mg
Chloride	mg	mg
Potassium	mg	mg
Sodium	mg	mg
(insert any other nutritive substance or inulin-derived substances and galacto-oligosaccharides to be declared)	g, mg, µg	g, mg, µg

***1** – Delete the words 'made up formula' in the case of formulas sold in 'ready to drink' form.
***2** – Delete this column in the case of formulas sold in 'ready to drink' form.

[4.6] *deleting the Note at the end of the Nutrition Information table in the Guidelines for Infant Formula Products.*

[5] ***Standard 2.9.2*** *of the Australia New Zealand Food Standards Code is varied by* omitting paragraph 2(2)(b), substituting –

(b) lactic acid producing cultures; and

(c) either singularly or in combination, no more than 0.8g/ 100 g of inulin-derived substances and galacto-oligosaccharides.

[6] ***Standard 2.9.3*** *of the Australia New Zealand Food Standards Code is varied by –*

[6.1] *inserting in clause 6 –*

(4) Formulated supplementary foods for young children may contain singularly or in combination, no more than 1.6 g of inulin-derived substances and galacto-oligosaccharides per serving.

Attachment 2

Chemical and Technological Uses Assessment

Summary

Galacto-oligosaccharides

The term 'galacto-oligosaccharide' is used consistently to describe those substances comprised of between two and eight saccharide units with one of these units being a terminal glucose and the remaining saccharide units being galactose. While the disaccharide lactose is present in 'galacto-oligosaccharide' mixtures, it is not regarded as a galacto-oligosaccharide. Galacto-oligosaccharides are produced from lactose by enzymatic action and are also referred to as 'trans-galacto-oligosaccharides'.

Galacto-oligosaccharides are added to infant formula, infant foods and formulated supplementary foods for young children for nutritional reasons. FSANZ is unaware of any technological reason for the addition of galacto-oligosaccharides to other foods. For this reason, FSANZ has not considered the technological aspects of the addition of galacto-oligosaccharides to foods.

Fructose polymers (inulin/oligofructose/fructo-oligosaccharides)

The following terms are used in this report:

- the term '**inulin'** is used to describe those fructose polymers with β (2→1) fructosyl-fructose linkages, where the average Degree of Polymerisation (DP)[35] is equal to or greater than ten;

- the term '**long chain inulin'** is used to describe those fructose polymers with β (2→1) fructosyl-fructose linkages, where the average DP is equal to or greater than 23;

- the term '**oligofructose**' is used to describe those fructose polymers with β (2→1) fructosyl-fructose linkages, derived from inulin, where the average DP is less than ten; and

- the term '**fructo-oligosaccharide'** is used to describe those fructose polymers with β (2→1) fructosyl-fructose linkages, where the average DP is less than four and is typically produced from enzymic condensation of sucrose. FOS could be regarded as a subset of oligofructose because it contains some similar compounds as oligofructose. However, it is not typically produced from inulin and is therefore not considered to be derived from inulin.

The substance that has been used overseas in infant formula, infant foods and formulated supplementary foods for young children has been represented as 'high molecular weight fructosyl-saccharose', 'long chain fructo-oligosaccharides', 'long chain inulin' or 'high molecular weight inulin'.

[35] Degree of polymerisation is the number of fructose or saccharide units in a substance.

Based on the terms described above, this substance is considered to be a fraction of processed 'inulin'. This is supported by the specifications for these substances that have been provided to FSANZ. Throughout this report the term 'long chain inulin' will be used to describe the processed inulin fraction that has been added or has been proposed to be added to infant formula, infant foods and formulated supplementary foods for young children.

Inulin type fructose polymers may be added to foods for technological reasons or nutritional reasons (e.g. dietary fibre). The technological reasons for the addition of inulin, oligofructose or fructo-oligosaccharides to foods include as an emulsifier, thickener, stabiliser and sweetener. These functions vary with the nature of the inulin derived mixture (e.g. chain length), its concentration in a food and the food itself. These technological purposes relate to the gelling properties of inulin, in particular its ability to form stable gels with water to replace fat or to impart textural qualities in foods. Some inulin derived products (e.g. oligofructose) and fructo-oligosaccharides are used as sweeteners.

Specifications

Substances added to foods must comply with the requirements in Standard 1.3.4 – Identity and Purity of the *Australia New Zealand Food Standards Code* (the Code), where applicable. These requirements include specifications for specific substances where appropriate.

Based on the 'galacto-oligosaccharide' proposed to be added to infant formula, infant foods and formulated supplementary foods for young children, a proposed specification for this substance is included in Appendix A.

The specifications for inulin, oligofructose, and ingredients derived from inulin will vary in accordance with the variety of different compositions that are available. It is therefore impractical to develop specific specifications for these substances. However, the general provisions in Standard 1.3.4 would still be applicable to these substances.

Stability data

Inulin type fructose polymers are generally stable in most food matrices but under acid conditions (e.g. certain beverages when not refrigerated) hydrolysis may occur. Based on information provided to FSANZ galacto-oligosaccharides are represented as having good stability to heat and acid conditions. FSANZ considers that these substances are likely to have acceptable stability in most dry foods but that inulin type fructose polymers may not be suitable in low pH liquid foods, as inulin potentially might be hydrolysed to fructose.

Compliance with any limits

FSANZ considers that suitable methods exist for compliance purposes and these methods have been published, including by the Association of Official Analytical Chemists.

1. Structure of Inulin, Oligofructose, Fructo-oligosaccharides (FOS) and Galacto-oligosaccharides (GOS)

In general terms an 'oligosaccharide' is a carbohydrate that consists of a relatively small number of linked monosaccharide units, typically between three and ten units. The term 'oligosaccharide' has been further characterised by adjectives used to describe specific inulin fractions e.g. Long chain oligosaccharides.

1.1 Inulin/Oligofructose/Fructo-oligosaccharides

To clarify the use of the terms 'inulin', 'oligofructose' and 'fructo-oligosaccharides', the following information is provided to define the terms for use throughout this report. The information and terms below are based upon terms used by Carabin and Flamm36.

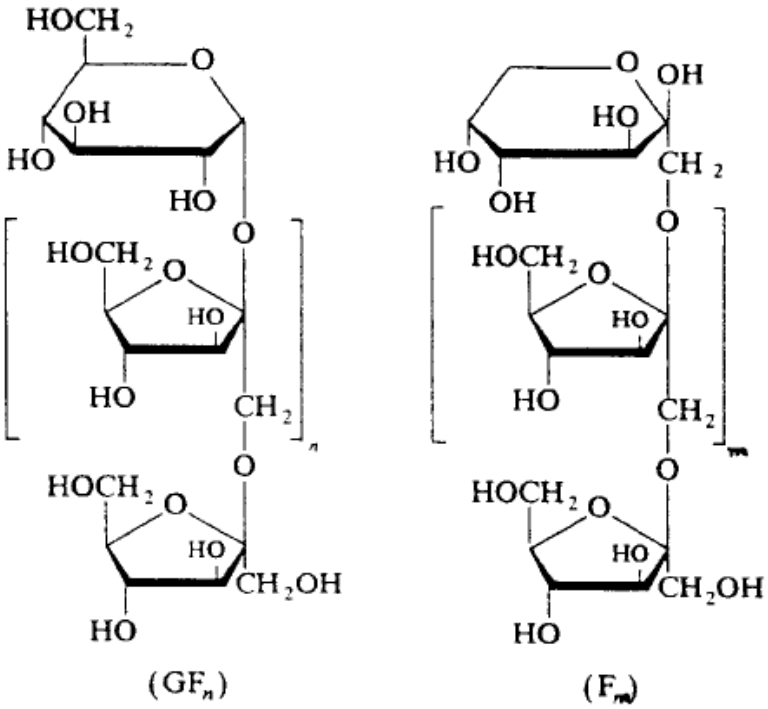
Polymers of fructose are referred to as 'fructans' with the fructose units primarily joined by two types of molecular linkages, inulin type β (2→1) and levan type β (2→6). These linkages refer to the numbering of the carbon atoms on the six carbon fructose molecule.

Inulin is a mixture of carbohydrate polymers (polysaccharides) that are extracted from plant material. It occurs naturally in many plants and depending on the degree of refinement, the substance will vary in terms of its physical and chemical characteristics. In general terms, it is a substance composed of molecules of fructose units of differing lengths or degrees of polymerisation (DP), typically with a terminal glucose molecule. The differences between the terms used to describe fructose polymers are characterised by the range of the DP, including the average DP. The DP is a measure of the number of fructose molecules or saccharide units in the chain and the degree of polymerisation is therefore a key compositional component in characterising fructose polymers. The proportion of monosaccharides and disaccharides present in a mixture of substances are also considered to be characterising aspects of these ingredient mixtures.

Standard inulin contains a small amount of mono (glucose & fructose), di (sucrose) and other longer chain saccharides. Inulin can also contain branched or linear chains of fructose units, which may or may not be terminated with a glucose molecule. Inulin extracted from most plant sources typically has only a small degree of branching and is predominantly composed of linear chains of fructose, the blue agave being an exception. The major commercial source of inulin is chicory and the DP is between two and seventy fructose units with an average DP above ten. Refining of standard inulin can result in partial removal of the shorter chain molecules and increase the average DP to approximately 25.

The term 'oligofructose' has been used to describe those mixtures of substances that are produced from the partial enzymic hydrolysis of inulin, typically with a degree of polymerisation in the range of two to eight, although a range of three to nine has also been quoted. The term 'fructo-oligosaccharide' has been used to mainly describe those mixtures of substances with a degree of polymerisation in the range of three to five, although this term has also been used to describe oligofructose like substances and inulin.

[36] Carabin, I and Flamm, G. Evaluation of Safety of Inulin and Oligofructose as Dietary Fiber. Regulatory Toxicology and Pharmacology 30, 268-282 1999.



Structure of inulin[37] where G =glucose, F=fructose and where *n* and *m* represent the number of fructose units

The number of saccharide units in inulin and other polysaccharide mixtures is not defined specifically, although the DP for the substances in inulin are described as between 2 and seventy. Terms such as 'oligofructose' and 'fructo-oligosaccharides' are generally used to describe substances where the average DP is less than ten. The term 'fructo-oligosaccharides' also appears to be used more commonly (but not exclusively) to describe those short chain fructose polymers (usually with a glucose terminating molecule) that are prepared from enzymic condensation of sucrose and fructose. The term 'fructo-oligosaccharides' has also been used to describe all inulin type fructans, both long and short chain.

To ensure that there is clarity about the substances, this report will use the terms 'inulin' to describe those fructans, with β (2→1) fructosyl-fructose linkages, where the average DP is equal to or greater than ten. The term 'oligofructose' will be used to describe those fructans, with β (2→1) fructosyl-fructose linkages, where the average DP is less than ten. Consistent with the view of Carabin and Flamm, the term 'fructo-oligosaccharide' will only be used to describe those fructans, with β (2→1) fructosyl-fructose linkages, where the average DP is less than four. In accordance with this terminology and on the basis of the information available to FSANZ, 'fructo-oligosaccharides' do not appear to be the substances that are proposed to be added or are being added to infant formula or infant foods as 'FOS'.

[37] A Franck, De Leenheer, ORAFTI,

The substances used overseas in infant formula, infant foods and formulated supplementary foods for young children have been represented as 'high molecular weight fructosyl-saccharose', 'long chain fructo-oligosaccharides' or 'high molecular weight inulin'. Based on the terms described above, this substance is considered to be a fraction of processed 'inulin'. This is supported by the specifications for these substances that have been provided to FSANZ. Throughout this report the term 'long chain inulin' has been used to describe the processed inulin fraction that has been added or is proposed to be added to infant formula, infant foods and formulated supplementary foods for young children.

Substance	Degree of Polymerisation	Average Degree of Polymerisation
Inulin	2 - 70	≥10
Oligofructose	2 – 10	< 10
Long Chain Inulin	10 - 70	≥ 23
Fructo-oligosaccharide	3 - 5	< 4

1.2 Galacto-oligosaccharides

The term 'galacto-oligosaccharide' is used more consistently to describe those substances comprised of between two and eight saccharide units with one of these units being a terminal glucose and the remaining saccharide units being galactose. Galacto-oligosaccharides produced from lactose by enzymatic action and are also referred to as 'trans-galacto-oligosaccharides'. This mixture of substances is available as a syrup or powder and the lactose content (disaccharide) is typically regarded as part of the 'galacto-oligosaccharide' mixture.

1.3 Specifications

The specifications for inulin, oligofructose and inulin derived ingredients will vary in accordance with the wide variety of proprietary mixtures that are available. This variation makes it impractical to ascribe a particular specification for these mixtures. However, these substances would still need to comply with the general requirements in Standard 1.3.4 – Identity and Purity.

In relation to infant formula, infant foods and formulated supplementary foods for young children, and based on information provided to FSANZ, the syrup form of galacto-oligosaccharides is proposed to be added to infant formula, infant foods and formulated supplementary foods for young children. This is characterised with a general specification as indicated in Appendix A.

1.4 Stability

1.4.1 Fructose polymers (inulin/oligofructose/fructo-oligosaccharides)

Inulin type fructose polymers are generally stable in most food matrices but under acid conditions (certain beverages if not refrigerated) hydrolysis can occur. FSANZ considers that these substances are likely to have acceptable stability in most dry foods but that inulin type fructose polymers may not be suitable in low pH unrefrigerated liquid foods. The significance of this unsuitability would depend on the purpose for the addition and whether any breakdown substantially reduced the capability of the inulin type fructose polymers to fulfil the technological or nutritional purpose.

In such cases, inulin type fructose polymers may be partially hydrolysed to oligofructose.

1.4.2 Galacto-oligosaccharides

Based on information provided to FSANZ galacto-oligosaccharides are represented as having good stability to heat and acid conditions. FSANZ considers that these substances are likely to have acceptable stability in most foods.

1.5 Analysis

The Association of Official Analytical Chemists have published methods for determining the amount of inulin and certain 'oligosaccharides' in food. These methods are primarily based on the saccharide analysis of products following selective enzyme action. FSANZ regards these methods as suitable for compliance purposes in a range of foods.

1.6 Mode of Action

1.6.1 Fructose polymers (inulin/oligofructose/fructo-oligosaccharides)

Inulin, inulin-derived substances and fructo-oligosaccharides may be added to foods for technological reasons or nutritional reasons.

In the case of infant formula, infant foods and formulated supplementary foods for young children, from the data available to FSANZ, the use of inulin or inulin-derived substances is for nutritional reasons and not for technological reasons associated with the food.

For this reason, this report will focus on technological uses for inulin, inulin-derived substances and fructo-oligosaccharides in foods other than infant formula, infant foods and formulated supplementary foods for young children.

The technological purposes for the addition of these substances to foods include as an emulsifier, thickener and stabiliser as well as fat and sugar replacement ingredients. These purposes vary with the nature of the inulin derived mixture (e.g. chain length), its concentration in a food and the food itself. These technological purposes relate to the dispersing properties of inulin, in particular its ability to mimic fat droplets dispersed in water. These dispersions can then be used in foods to replace fat or to impart textural qualities in foods.

The amount of these inulin-derived ingredients used to fulfil these purposes will vary in the food application as it will be necessary to specifically consider the technological aspects in each situation, given the varying composition of the foods to which it will be added and the precise technological purpose to be fulfilled. Therefore different levels of inulin-derived ingredients will be added to foods to achieve these functions.

It has been reported that specific applications for inulin and inulin derived products include:

- Beverages (including nutritional purposes);
- Bread or cereal products for fat or sugar replacement or processing benefits (also for nutritional purposes); and

- Dairy products for fat or sugar replacement or texture improvement.

Some inulin derived products (e.g. oligofructose) and fructo-oligosaccharides are also used as sweeteners with the relative sweetness being dependent on the degree of polymerisation and the proportion of the monosaccharide and disaccharide content in these products.

While inulin and inulin derived products may be refined to fulfil specific technological characteristics, the substances in these products occur naturally in many foods. While some foods would not be expected to contain inulin or inulin derived products or substances, foods containing these substances would be part of the diet of most if not all people.

1.6.2 Galacto-oligosaccharides

From the information available to FSANZ the addition of galacto-oligosaccharides to infant formula, infant foods and formulated supplementary foods for young children is for nutritional reasons and not for any technological purpose in the foods. In addition, FSANZ is unaware of any technological reason for the addition of galacto-oligosaccharides to other foods.

For this reason, FSANZ has not considered the technological aspects of the addition of galacto-oligosaccharides to foods.

References

Carabin, I and Flamm, G. Evaluation of Safety of Inulin and Oligofructose as Dietary Fiber. Regulatory Toxicology and Pharmacology 30, 268-282 1999.

Coussement, P.A.A. Inulin and Oligofructose: Safe Intakes and Legal Status. *Journal of Nutrition* 1999;129:1412S-1417S.

Franck, A. and De Leenheer, L. Inulin, *Biopolymers*, Vol. 6 (A. Stenbuchel, ed.),VCH Weinheim, Germany, 2002, p 439.

IUB-IUPAC Joint Commission of Biochemical Nomenclature Abbreviated Terminology of oligosaccharide chains. Journal of Biological Chemistry. 1982; 257:3347-3351.

Niness, K. Inulin and Oligofructose: What Are They? *Journal of Nutrition* 1999;129:1402S-1406S.

Appendix A

Specific Compositional Characteristics of 'Galacto-oligosaccharides'
(Galacto-oligosaccharides proposed to be added to infant formula, infant foods and formulated supplementary foods for young children)

Parameter	Specification
Description	Mixture of those substances comprised of between two and eight saccharide units with one of these units being a terminal glucose and the remaining saccharide units being galactose, produced from lactose by enzymatic action.
Appearance	Syrup, clear, golden colour
Taste	Sweet
pH (10% solution)	pH 3.2-3.8
Ash (sulphated)	Maximum 0.3% on dry weight basis
Galacto-oligosaccharides (glucose linked to between one and seven galactose units)(excluding lactose)	Minimum 57% on dry weight basis
Moisture	24-26%
Lactose	23% maximum (dry weight basis)
Glucose	22% maximum (dry weight basis)
Galactose	0.8% minimum (dry weight basis)

Attachment 3

Human Milk Carbohydrates

Summary

Human breast milk contains a carbohydrate component that is composed of mixed saccharide monomers connected by bonds that are resistant to hydrolysis in the small intestine. The structures range from simple tri-saccharides to complex multiply-branched polysaccharides. These oligosaccharides with a degree of polymerization (DP) of 3-10 and polysaccharides with a DP > 10 are comprised of neutral and acidic compounds, of which the neutral fraction is the major component. The number of structurally different oligo- and polysaccharides may exceed 1,000. Some of the structures in breast milk are determined by maternal genetics and regional differences in breast milk composition have been found.

The total oligo- and polysaccharide content of human milk may be up to 25 g/L during the first few weeks following birth. From one to four months the concentration is likely to be around two-thirds of the concentration in early lactation and the concentration may continue to decline with time. Neutral oligo- and polysaccharides have been found in concentrations of up to 15 g/L in Italian women over the first 3 months of lactation. There is a large variation in the breast milk concentration of oligosaccharides among individual women, as much as a four-fold difference. The effect of maternal diet on the carbohydrate content of breast milk has not been extensively studied. Between and within country comparisons indicate similar lactose concentrations in breast milk independent of the mother's state of nourishment or economic status.

1. Structure

The carbohydrates in greatest abundance in human milk comprise lactose (~7% of the milk) and oligosaccharides (1-2%). Polysaccharides are described as being in low abundance (Stahl *et al*., 1994). The monomers found in oligo- and polysaccharides include galactose, fucose, N-acetyl-glucosamine, N-acetyl-galactosamine, and N-acetyl-neuraminic acid (NeuAc; sialic acid). These sugar bases are all hexoses and fucose should not be confused with the fruit sugar fructose, a pentose that is not present in human milk (Huisman *et al*., 1996). The oligo- and polysaccharide fraction contains a complex mixture of compounds. Citing a number of articles, Ninonuevo and colleagues estimate that more than 200 oligo- and polysaccharides have been identified (Ninonuevo *et al*., 2006). However, due the occurrence of isomeric structures that are not easily identified from each other, the number of different oligo- and polysaccharides may exceed 1000 (Boehm, 2005).

Oligo- and polysaccharides are synthesized in the mammary gland by monomer-specific enzymes (transferases). The disaccharide lactose is used as a starting substance to which sugar monomers are added to form larger linear or branched structures. A simple tri-saccharide in high abundance is 2'-fucosyllactose. Other major components of human milk are 'core' oligosaccharides. These structures are formed from the elongation of lactose with N-acetyl-glucosamine and galactose. Two of these core structures, lacto-N-tetraose and lacto-N-neo-tetraose are in high abundance in human milk; the structure of lacto-N-tetraose is shown in **Figure 1**. In addition to their presence in human milk, core oligosaccharides also represent the starting structures of more complex oligosaccharides.

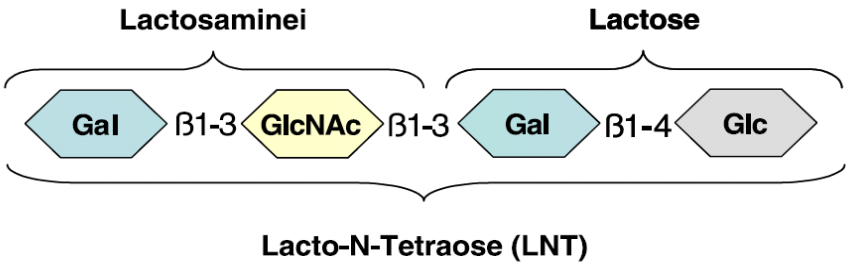


Figure 1: Lacto-N-tetraose (source: Kunz & Randolf, 2006)

Branching and elongation of the core oligosaccharides with additional monosaccharides creates longer oligosaccharides and polysaccharides with a degree of polymerization of 50 or more. If one or more fucose molecules are added to lactose or to a core oligosaccharide the resulting compound is classified as a fucosyl-oligosaccharide. Similarly, if sialic acid is added to lactose or to a core oligosaccharide it is classified as a sialyl-oligosaccharide. Compounds containing both fucose and sialic acid have been identified (Smith *et al*., 1987). Human milk oligosaccharides are sometimes classified on the basis of charge according to the presence of sialic acid into neutral and acidic (sialylated) compounds. The majority fraction is comprised of neutral compounds with the sialylated acidic compounds comprising 16% of the total oligo- and polysaccharides found in the breast milk of five milk-bank donors (Ninonuevo *et al*., 2006). The proportion of acidic compounds is not fixed and varied from 3% to 36% among the five samples of breast milk. The galacto-oligosaccharide 6'-galactosyl-lactose has been found in human milk at a low concentration of around 0.003 g/L (Yamashita & Kobata, 1974), and 3'- galactosyl-lactose has been identified (Donald, 1988). Some of the oligosaccharides found in human milk in relatively high concentrations are shown in **Table 1**.

Table 1: Some of the major oligosaccharides in human milk sampled 30 days postpartum (Coppa *et al*., 1999)

Classification	Sub-classification	Oligosaccharide	Concentration (g/L)
Neutral	Fucosyl-oligosaccharide	2'-fucosyl-lactose	2.78 (0.94)
		3-fucosyl-lactose	0.28 (0.07)
		Trifucosyl-lacto-N-hexaose	3.10 (1.40)
		Difucosyl-lacto-N-hexaose	2.62 (0.82)
		Lacto-N-fucopentaose I	0.99 (0.25)
	Core oligosaccharide	Lacto-N-tetraose	0.71 (0.21)
		Lacto-N-neo-tetraose	1.40 (0.55)
Acidic	Sialyl-oligosaccharide	6'-sialyl-lactose	0.44 (0.14)
		Sialyl-lacto-N-tetraose c	0.21 (0.08)
		Disialyl-lacto-N-tetraose	0.67 (0.57)

The expression of enzymes that facilitate the addition of fucose, the fucosyltransferases, are influenced by the Secretor and Lewis blood group genotype of the lactating woman. Hence the structures of some of the fucosylated compounds in human milk are genetically determined. The majority of Caucasian women (~70 - 80%) are secretors of A and B antigenic material and their milk contains lacto-N-fucopentaose I. In non-secretors, the milk oligosaccharide is Lewis gene dependent and lacto-N-fucopentaose II is the major form (**Figure 2**). Other forms may be present dependent upon maternal genetics.

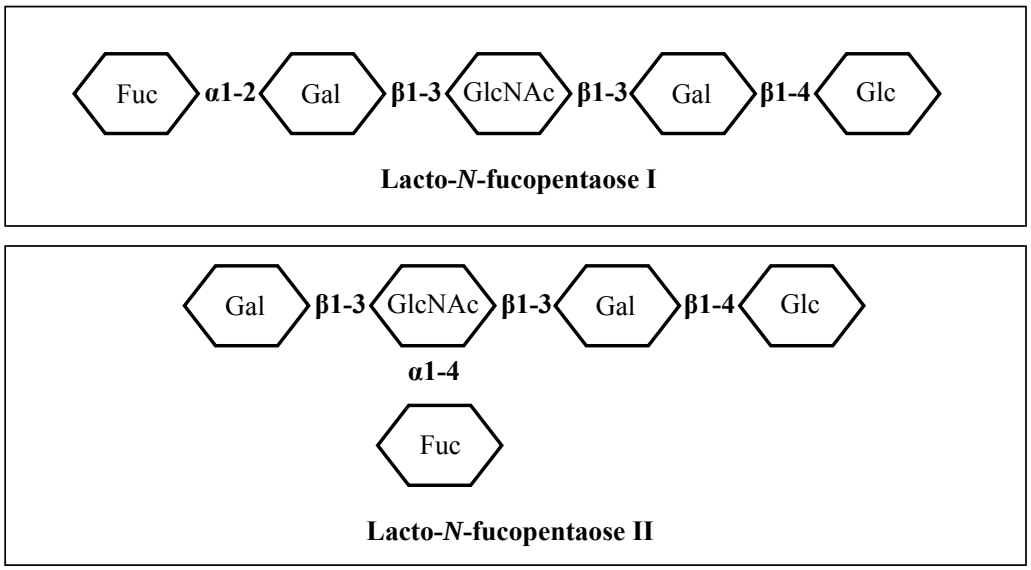


Figure 2: Genetically-determined structures of two lacto-N-fucopentaoses

Regional variation in breast milk oligosaccharide composition has been found (Erney *et al*., 2000). The presence and concentration of nine neutral oligosaccharides were determined in the breast milk of women living in 10 countries. Results of two of the major oligosaccharides, 2'-fucosyl-lactose and 3-fucosyl-lactose, are shown in **Table 2**. The breast milk of almost all of the women in all countries contained 3-fucosyl-lactose. The proportion of women whose breast milk contained 2'-fucosyl-lactose varied over a range of 46 to 100%.

Table 2: **Percentage of women whose breast milk contains 2'-fucosyl- and 3-fucosyl-lactose**

Country	n	2'-fucosyl-lactose (% of women)	3-fucosyl-lactose (% of women)
Chile	44	84	100
China	32	78	100
France	22	91	89
Germany	18	83	100
Italy	29	86	100
Mexico	156	100	100
Philippines	22	46	96

Country	n	2'-fucosyl-lactose (% of women)	3-fucosyl-lactose (% of women)
Singapore	26	72	92
Sweden	7	100	Not determined
United States	79	68	98

These data demonstrate the diversity in the composition of human milk oligosaccharides that differ with respect to size, sequence, charge and abundance (Ninonuevo *et al*., 2006). The number of structurally different oligo- and polysaccharides may exceed 1000. The structures range from simple tri-saccharides to complex multiply-branched polysaccharides. Some of the structures are genetically determined.

2. Concentration

The oligosaccharide concentration of breast milk obtained from 46 Italian women was measured over a period of four months postpartum and the results are shown in **Table 3** (Coppa *et al*., 1993).

Table 3: Mean (SD) oligosaccharide concentration in the breast milk of 46 women

Day after delivery	Oligosaccharide concentration (g/L)
4	20.9 (4.81)
10	20.1 (3.95)
30	15.5 (3.44)
60	12.9 (2.60)
90	12.4 (2.80)
120	12.9 (3.30)

In a later study by the same group involving 18 women delivering term infants, oligosaccharide fractions (fucosyl-, sialyl- and core oligosaccharides) were reported separately as shown in **Table 4** (Coppa *et al*., 1999).

Table 4: Mean oligosaccharide concentration in the breast milk of 18 women.

Day after delivery	Oligosaccharide concentration (g/L)		
	Fucosyl-	Core	Sialyl-
4	13.88	3.13	3.30
10	12.60	2.71	2.70
30	11.59	2.26	2.14
60	12.13	2.73	1.79
90	11.45	3.1	1.58

The total oligosaccharide concentration at 4 days was in excess of 20 g/L followed by a decline of around 20% at 30 days and relatively constant thereafter. The long-term neutral oligosaccharide concentration (excluding sialyl-oligosaccharides) was around 14-15 g/L. Among the fractions, the concentration of fucosyl-oligosaccharides tended to decrease slightly from early to late lactation, core oligosaccharides remained relatively constant, and the sialyl-oligosaccharide fraction fell by approximately half from day 4 to day 90. Within each fraction, the concentrations of individual oligosaccharides were not necessarily constant over time; for example, the concentration of 2'-fucosyl-lactose decreased over the first month of lactation after which the concentration rose again.

The mean concentration of oligosaccharides in the milk of 15 French women whose milk was sampled from day 2 to day 8 postpartum ranged from 13-17 g/L (Viverge *et al*., 1990). These values may be compared with neutral oligosaccharide concentrations found in the breast milk of 12 North American women (Chaturvedi *et al*., 2001). The mean total neutral oligosaccharide concentration during the first 14 weeks of lactation was 9 g/L, somewhat lower than concentrations found in other studies. Chaturvedi *et al*. suggested that this may be due to different populations or as a consequence of their analytical method that did not measure oligo- and polysaccharides with a degree of polymerization of 9 or more. Samples of breast milk were taken over a period of one year and the concentration declined from the concentration found at 14 weeks to less than half at one year. Variability among women was large, the neutral oligosaccharide concentration averaged throughout the year for individual women differed four-fold.

Coppa et al. have found that the oligo- and polysaccharide concentration in the colostrum of mothers delivering pre-term infants is higher than that of mothers of term infants (Coppa *et al*. 1997). From 26 mothers delivering pre-term infants, the mean (SD) oligo- and polysaccharide concentration of their milk at four days postpartum was 25.61 (5.19) g/L, higher than the concentration of 20.9 (4.81) g/L found in a study of term infants ($P < 0.001$) (Coppa *et al*., 1993). Differences did not persist beyond the 4-day sample.

These data indicate that the total oligo- and polysaccharide content of human milk may be up to 25 g/L during the first few weeks following birth. From one to four months the concentration is likely to be around two-thirds of the concentration in early lactation and the concentration may continue to decline with time. The total oligo- and polysaccharide content of human milk is comprised of neutral and acidic compounds, of which the neutral fraction is the major component. The neutral oligo- and polysaccharide content of human milk has been found in concentrations of up to 15 g/L over the first 3 months of lactation.

3. Maternal dietary influence

The oligosaccharides found in human milk are produced in the mammary gland from lactose, which itself is synthesized principally from plasma glucose with some *de novo* generation of glucose and galactose in the breast (Sunehag *et al*., 2002). Whether maternal diet affects the oligosaccharide content or composition of breast milk has not been extensively studied. A comparison of milk sampled from women living in a number of countries variously described as being 'well' or 'poorly' nourished found little difference in the lactose content of milk among countries (Jelliffe & Jelliffe, 1978). Similarly, within a country there was little difference in lactose concentration between healthy and malnourished Egyptian women (6.65 *cf*. 6.48 g/100 mL) or between Brazilian women of low or high economic status (6.5 *cf*. 6.8 g/100 mL).

References

Boehm G, Stahl B, Jelinek J, Knol J, Miniello V, Moro GE. (2005) Prebiotic carbohydrates in human milk and formulas. *Acta Paediatr Suppl*. 94(449):18-21.

Chaturvedi P, Warren CD, Altaye M, Morrow AL, Ruiz-Palacios G, Pickering LK, Newburg DS. (2001) Fucosylated human milk oligosaccharides vary between individuals and over the course of lactation. *Glycobiology*. 11(5):365-72.

Coppa GV, Pierani P, Zampini L, Carloni I, Carlucci A, Gabrielli O. (1999) Oligosaccharides in human milk during different phases of lactation. *Acta Paediatr Suppl*. 88(430):89-94.

Coppa GV, Pierani P, Zampini L, Gabrielli O, Carlucci A, Catassi C, Giorgi PL. (1997) Lactose, oligosaccharide and monosaccharide content of milk from mothers delivering preterm newborns over the first month of lactation. *Minerva Pediatr*. 49(10):471-5.

Coppa GV, Gabrielli O, Pierani P, Catassi C, Carlucci A, Giorgi PL. (1993) Changes in carbohydrate composition in human milk over 4 months of lactation. *Pediatrics.* 91(3):637-41.

Donald AS, Feeney J. (1988) Separation of human milk oligosaccharides by recycling chromatography. First isolation of lacto-N-neo-difucohexaose II and 3'-Galactosyllactose from this source. *Carbohydr Res*. 178:79-91.

Erney RM, Malone WT, Skelding MB, Marcon AA, Kleman-Leyer KM, O'Ryan ML, Ruiz-Palacios G, Hilty MD, Pickering LK, Prieto PA. (2000) Variability of human milk neutral oligosaccharides in a diverse population. *J Pediatr Gastroenterol Nutr*. 30(2):181-92.

Huisman M, van Beusekom CM, Lanting CI, Nijeboer HJ, Muskiet FA, Boersma ER. (1996) Triglycerides, fatty acids, sterols, mono- and disaccharides and sugar alcohols in human milk and current types of infant formula milk. *Eur J Clin Nutr*. 50(4):255-60.

Jelliffe DB, Jelliffe EFP. (1978) The volume and composition of human milk in poorly nourished communities. *Am J Clin Nutr* 31:492-515.

Ninonuevo MR, Park Y, Yin H, Zhang J, Ward RE, Clowers BH, German JB, Freeman SL, Killeen K, Grimm R, Lebrilla CB. (2006) A strategy for annotating the human milk glycome. *J Agric Food Chem*. 54(20):7471-80.

Smith DF, Prieto PA, McCrumb DK, Wang WC. (1987) A novel sialylfucopentaose in human milk. Presence of this oligosaccharide is not dependent on expression of the secretor or Lewis fucosyltransferases. *J Biol Chem*. 262(25):12040-7.

Stahl B, Thurl S, Zeng J, Karas M, Hillenkamp F, Steup M, Sawatzki G. (1994) Oligosaccharides from human milk as revealed by matrix-assisted laser desorption/ionization mass spectrometry. *Anal Biochem*. 223(2):218-26.

Stahl B, Thurl S, Henker J, Siegel M, Finke B, Sawatzki G. (2001) Detection of four human milk groups with respect to Lewis-blood-group-dependent oligosaccharides by serologic and chromatographic analysis. *Adv Exp Med Biol*. 501:299-306.

Sunehag AL, Louie K, Bier JL, Tigas S, Haymond MW. (2002) Hexoneogenesis in the human breast during lactation. *J Clin Endocrinol Metab* 87:297-301.

Viverge D, Grimmonprez L, Cassanas G, Bardet L, Solere M. (1990) Variations in oligosaccharides and lactose in human milk during the first week of lactation. *J Pediatr Gastroenterol Nutr*. 11(3):361-4.

Yamashita K & Kobata A. (1974) Oligosaccharides of human milk. V. Isolation and characterization of a new trisaccharide, 6'-galactosyllactose. *Arch. Biochem. Biophys*. 161:164-70.

Attachment 4

Microbiological assessment

Summary

The microbiological assessment evaluates the effect of inulin-derived substances and galacto-oligosaccharides (GOS) in combination or alone as a component in infant formula and infant formula products on gut microflora.

The dominance of *Bifidobacterium* and *Lactobacillus* species in the intestinal tract of breast-fed infants and their associated beneficial effects to the host appear to be the result of a combination of factors present in human breast milk. Oligosaccharides in human breast milk promote *Bifidobacterium* and *Lactobacillus* dominated intestinal microflora, however the effect results from a number of interactive factors including the presence of oligosaccharides, lactoferrin, lactose, nucleotides and low concentration of proteins and phosphates in the human breast milk.

There is partial evidence to suggest that supplementation of infant formulae with GOS and inulin-derived substances at a ratio of 9:1, with a dose not exceeding 10 g/L, may stimulate selectively the growth of the colon bifidobacteria population in infants.

There is a lack of persuasive evidence to support the suggestion that the supplementation of GOS, or inulin-derived substances, used in isolation, in infant or toddler formulae would selectively stimulate the growth of intestinal *Bifidobacterium* and *Lactobacillus* in infants. Many of the published studies summarised in this report have small sample sizes and variable supplementation methodology and hence it is difficult to draw firm conclusions from the data currently available.

There has been no research conducted or evidence found associated with the addition of fructo-oligosaccharide (FOS) to infant formula products and hence these substances have not been considered in this report.

1. Biology of gut microflora in infants and young children

1.1 Distribution of microflora in human intestinal tract

The number of microorganisms inhabiting the human gastrointestinal tract is estimated in the order of 10^{14} cells per person, about 10 times the total cell number making up the entire human body (Bengmark, 1998). Microorganisms in the gastrointestinal tract are distributed as an increasing gradient from the stomach to the colon. This is largely the result of the low pH of the luminal content in the upper digestive tract, the digestive fluids produced by the gallbladder and the pancreas, and the phasic propulsive motor activity of the small intestinal tract, rendering an environment inhospitable to bacteria (Guarner and Malagelade, 2003). With reduced availability of oxygen in the intestinal tract from stomach to the colon, this gradient of intestinal microflora becomes increasingly dominated by obligate anaerobes towards the colon.

Helicobacter pylori is the only recognised bacterium capable of survival in the gastro-stomach environment due to its unique survival attributes, this organism can invade the epithelial cells of the stomach and may cause stomach and duodenum ulcers (Suerbaum and Michetti, 2002). The gastrointestinal tract from the duodenum to the caecum contains a few species of microorganisms including Gram-positive, acid tolerant lactic acid bacteria like *Streptococcus* and *Lactobacillus*. Their concentration is usually in the order of 10^3 organisms/ml of lumen. According to Marteau *et al.*, (2001) and Ramakrishna (2007), lactic acid bacteria like *Lactobacillus*, *Streptococcus* and *Enterococcus*, and facultative anaerobes like *Escherichia coli* can reach very high densities in the caecum, up to 10^8 bacteria/g of the content. Beyond the caecum, and into the ascending colon, strict anaerobic bacteria become dominant. The ratio of anaerobes to aerobes in the colon is estimated in the order of 100:1 to 1000:1 (Simon and Gorbach, 1984, and Salminen *et al.*, 1998). The predominant anaerobes found in the colon include *Bacteroides*, *Bifidobacterium*, *Eubacterium*, *Clostridium*, *Peptococcus*, *Peptostreptococcus* and *Ruminococcus*. Table 1 lists the major bacteria present in the human colon. Bacterial cell density reaches approximately 10^{12}/g of faeces in the colon (Ramakrishna, 2007). Such microbial biomass contributes to around 60% of the faecal solids (Stephen and Cummings, 1980).

Table 1: Major bacteria present in human colon (Ramakrishna, 2007)

Oxygen requirement	Gram stain and shape	Genus	Estimated concentration (log/gram faeces)
Strict anaerobes	Gram-negative bacilli	*Bacteroids*	11
	Gram-negative bacilli	*Fusobacterium*	9
	Gram-positive bacilli	*Eubacterium*	10
		Bifidobacterium	10
		Clostridium	10
	Gram-negative cocci	*Ruminococcus*	10
		Peptostreptococcus	NA
Facultative anaerobes	Gram-negative rods	*E. coli, Citrobacter, Enterobacter, Proteus, Klebsiella* etc	NA
Aerotolerant	Gram-positive bacilli	*Lactobacillus*	4-8
	Gram-positive cocci	*Streptococcus*	NA

NA: Estimation not available

1.2 Process of microbial colonisation of human intestinal tract

Microbial colonisation of the human gastrointestinal tract starts immediately after birth. The initial microorganisms are acquired from the mother through contamination during the birth, as a result of breast feeding and caring, and from the environment. Birth delivery methods of newborns strongly influence the composition of the intestinal microflora in the very early days of the infant (Bezirtzoglou, 1997, and Coppa *et al.*, 2004a). It is generally accepted that the critical stage of bacterial colonisation of the colon occurs after birth and during weaning. During the early days and weeks of infant life, bacterial colonisation of the colon is a symbiotic process where the stimulation caused by the presence of the bacteria and their activities contribute to the formation of the immune system of the host (Edwards and Parrett, 2002; Gibson and Roberfroid, 1995). The interactive process between the bacteria and the human intestine results in gradually formed recognition, tolerance and coexistence of the specific intestinal bacteria population. Because of the differences in exposure to the initial bacterial species and population from one individual to another, the exact pattern/distribution of the intestinal microflora is unique per individual (Guarner and Malagelade, 2003; Ramakrishna, 2007).

1.3 Bifidogenic effect

Approximately 90% of the intestinal microflora of breast-fed infants is made up of *Bifidobacterium* and *Lactobacillus* species, collectively referred to in the literature as **bifidogenic flora** (Coppa *et al.*, 2004a, and Edwards and Parrett, 2002). In comparison, the proportion of *Bifidobacterium* present in the gastrointestinal tract of formula-fed infants is relatively small, approximately 40-60% of the overall intestinal microflora, and the rest is shared by *Streptococcus*, *Bacteroids*, *Clostridium*, *Staphylococcus* and a few genera belonging to the family of Enterobacteriaceae (Coppa *et al.*, 2004a, and Edwards and Parrett, 2002). The intestinal microflora of mixed-fed (breast and formula fed) infants is different from, and between those found in the breast-fed and formula-fed infants (Edwards and Parrett, 2002). Figure 1 below illustrates the difference in intestinal microflora in infants according to their food intake, and the evolution of intestinal microflora as food intake changes over time. The dominance of *Bifidobacterium* in the intestinal tract of breast-fed infants is described as the **bifidogenic effect** (Coppa *et al.*, 2004a).

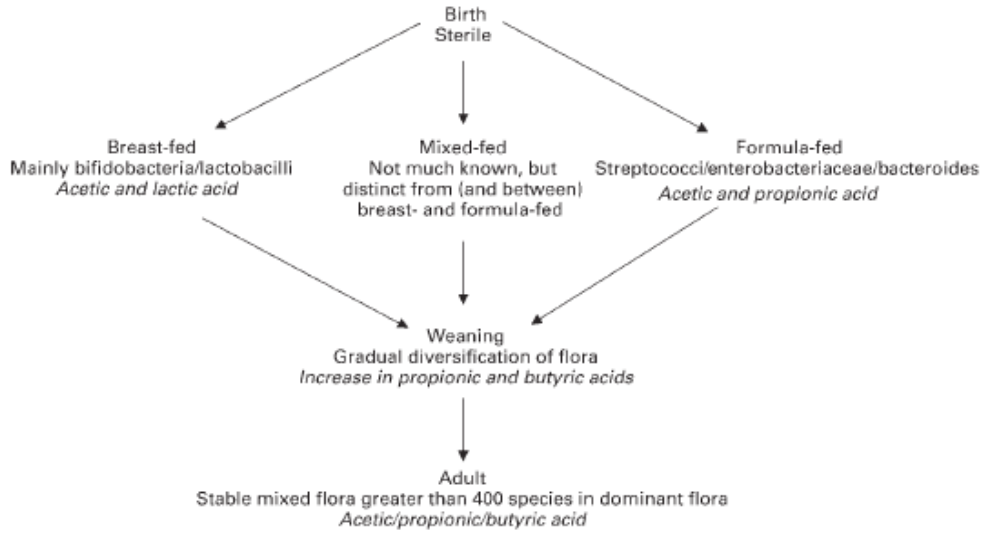


Figure 1: Development of intestinal microflora in infants (Edwards and Parrett, 2002)

During the weaning stage, the intestinal microflora becomes gradually more diverse. By the second year of life, the intestinal microflora of a child becomes increasingly similar to the stable mixed intestinal microflora found in adults where greater than 400 different species are present and 30-40 of them are in a dominant state (Edwards and Parrett, 2002; Guarner and Malagelade, 2003, and Ramakrishna, 2007).

1.4 Effect of gut microflora on the host

Gastrointestinal bacteria and their activities play important roles in host physiology including in nutrition, health and wellbeing. It is increasingly recognised that intestinal bacteria and their host intestinal environment share a mutual and symbiotic relationship that affects the nutritional and physiological status of the host (Backhed *et al.*, 2005, Hooper and Gordon, 2001, and Sears, 2005). The fermentative activities of the intestinal bacteria in the colon result in the breakdown of complex carbohydrates and proteins leading to recovery of nutrients and energy that otherwise would not be available to the human body because the human digestive system alone cannot breakdown these complex molecules.

Animal experimental data indicate that without the activities of the intestinal bacteria, an additional 30% more calories is required to enable sterile animals (animal free of intestinal microorganisms) to maintain the same body mass as that of a normal animal where intestinal bacteria are active (Wostmann *et al.*, 1983).

Gastro-intestinal bacteria and their activities contribute also to the formation and function of the host immune system, the protection of the host against infection by pathogenic microorganisms, the synthesis of vitamins and the absorption of some minerals (O'Hara and Shanahan, 2006), and also certain physiological disorders of the host, for example colon cancer and inflammatory bowel diseases (Guarner and Malagelada, 2003).

Among the intestinal microflora, the *Bifidobacterium* and *Lactobacillus* are recognised as the fraction whose presence/population and activities largely benefit the host (Edwards and Parrett, 2002, Guarner and Malagelade, 2003, and Ramakrishna, 2007). This is not to say that other factions of the intestinal microflora exert only a negative effect on host health and wellbeing. Metabolic activities of key *Bacteroides* species, such as *B. thetaiotaomicron*, have been found to be beneficial to the host (Sears, 2005). It is apparent that there is a significant knowledge gap about the complex interactions between the human host and major anaerobes of the gastrointestinal tract, such as *Bacteroides*, *Clostridium* and *Eubacterium*.

Bifidobacterium and *Lactobacillus* are recognised for their ability to ferment oligosaccharides present in human breast milk that are non-digestible by the host. Short chain fatty acids (SCFA) produced by these bacteria in the metabolism of oligosaccharides modulates the formation of the immune structure of the host, through their stimulation of the host intestinal epithelial cells (Binder *et al.*, 1994). These stimulations facilitate the development and differentiation of the host epithelial cells. SCFA also promotes the absorption of sodium and chloride by the host and promotes host lipid metabolism (Binder *et al.*, 1994, Ramakrishna, 2007). Another beneficial role played by *Bifidobacterium* and *Lactobacillus* results from their preferential metabolism of human milk oligosaccharides, which includes the increased dominance of this population in the colon that contributes to a bulking effect that may benefit the laxation of the host. Increased dominance of *Bifidobacterium* and *Lactobacillus* leads to a reduced pH of the colon content, and this has been claimed to restrict the proliferation of other intestinal microorganisms in the colon. The competition for nutrients available in the colon by *Bifidobacterium* and *Lactobacillus* is also claimed to lead to an exclusion effect on other intestinal microorganisms (Moro and Arslanoglu, 2005). These suppressive effects on other intestinal microorganisms, particularly those that are pathogenic to the host, are attributed as probiotic effects that are generally regarded as beneficial to the host, although direct and convincing evidence to support these claims is lacking.

2. Human breast milk oligosaccharides and their effect on gut microflora

2.1 Prebiotic and probiotic approach to food for young children

Because of the perceived health promoting effects of *Bifidobacterium* and *Lactobacillus* dominated intestinal microflora, attempts have been made to increase the proportion and population levels of these gut bacteria in infants and toddlers fed with infant and toddler formulae. Three approaches have been developed to increase the number and activity of these bacteria in the intestinal tract for young children who are fed by infant/toddler formulae according to Coppa *et al.*, (2004a).

The first approach is a direct ingestion of live probiotic bacteria[38]. Organisms considered to be probiotic include selected *Lactobacillus* and *Bifidobacterium* species. The second approach is to incorporate prebiotics[39] into the foods for young children. The third approach incorporates both probiotics and prebiotics into foods for young children.

2.2 Oligosaccharides from human breast milk

Oligosaccharides with a degree of polymerization (DP) of 3-10 are found in human milk (FAO/WHO, 1998). These human milk oligosaccharides (HMOs) account for the third largest component of human milk, with the peak concentration of around 25g/L in the colostrum during the first few weeks following birth and decline there after in normal human milk (Coppa *et al.*, 1994, and Coppa *et al.*, 1999).

Human milk oligosaccharides are synthesized by the glycosyltransferase in the mammary gland by adding sequentially monosaccharide units (galactose, fucose, N-acetyl-glucosamine, sialic acid) to lactose. Human milk oligosaccharides are resistant to enzymes present in the infant digestive system, and most human milk oligosaccharides pass to the colon of the infants undigested. These oligosaccharides facilitate selective proliferation of *Bifidobacterium* and *Lactobacillus* through fermentative metabolism by these bacteria and production of SCFA leading to the beneficial effect to the host as outlined above (Coppa et al, 2004a).

The amount of oligosaccharides in normal infant formulae is considerably less than those found in human breast milk, and cow's milk contains <1 g oligosaccharides/L (Coppa *et al.*, 2004a). Deliberate fortification of infant formula with oligosaccharides is part of the approach to simulate the composition of human breast milk.

2.3 Prebiotic effect of human breast milk oligosaccharides

Human breast milk oligosaccharides are recognised prebiotics, because of their resistance to digestion by host enzymes and their ability to selectively promote the growth of *Bifidobacterium* and *Lactobacillus* in the colon (Coppa *et al.*, 2004a). The overall prebiotic effect of the human oligosaccharides in young children is a combination of the roles played by *Bifidobacterium* and *Lactobacillus* in the intestinal tract and the roles played by oligosaccharides as dietary fibres.

Despite the recognition that HMOs promote *Bifidobacterium* and *Lactobacillus* dominated intestinal microflora, this is most likely the result of a number of complex and interactive factors present in the human milk including the lactoferrin, lactose, nucleotides and low concentration of proteins and phosphates present in the human breast milk (Coppa *et al.*, 2006). The exact role of these other factors found in human breast milk on the formation of *Bifidobacterium* and *Lactobacillus* dominated intestinal microflora is not clear at this stage.

[38] Probiotic bacteria are "*Live microorganisms which when administered in adequate amounts confer a health benefit on the host*" (FAO/WHO, 2001).
[39] Prebiotic are defined as "*non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves host health*" (Gibson and Roberfroid, 1995).

2.4 Effect of inulin-derived substances and GOS on gut microflora

2.4.1 Carbohydrates simulating human breast milk oligosaccharides

Researchers have used commercially available carbohydrates in an attempt to mimic the prebiotic qualities of human milk oligosaccharides (HMOs). The most frequently studied include inulin-derived substances including inulin, long chain inulin and oligofructose (as defined in attachment 2) as well as Galacto-oligosaccharides (GOS). Other oligosaccharides tested for their potential prebiotic effect include lactulose, soybean oligosaccharides, lactosucrose, isomalto-oligosaccharides, gluco-oligosaccharides and xylo-oligosaccharides.

In clinic trial studies, inulin preparations, with degree of polymerisation greater than 10 (defined as Inulin) are used to simulate the high molecular fraction of the human breast milk oligosaccharides.

2.4.2 Effect of Inulin-derived substances and GOS in combination on gut microflora

A clinical trial using a combination of a GOS preparation and a high molecular weight fraction of inulin preparation at the ratio of 9:1 supplemented into an infant formula demonstrated a significant stimulation to the growth of bifidobacteria and lactobacilli (Moro *et al.*, 2002). Table 2 shows a summary of the changes in the population of *Bifidobacterium* and *Lactobacillus* bacteria over the course of the trial. The trial involved 90 full term infants, and the infant formula was fortified at 4 g/L and 8 g/L respectively. The results indicated that *Lactobacillus* and *Bifidobacterium* cell numbers in infant colons were positively correlated to the presence of GOS and the inulin preparation. While changes to the *Bifidobacterium* population appeared to correlate to the inulin-derived substance (defined as high molecular weight inulin) and GOS concentration present in the infant formula, this wasn't the case for *Lactobacillus* population. The authors did not attempt to interpret such differences encountered in the experiment. Due to the lack of information about microbiological analysis and how the original data was treated, it is difficult to ascertain the variations of the parameters presented in the publication.

The same trial conducted with 42 preterm infants (Boehm *et al.*, 2002) found approximately a 3 log increase of *Bifidobacterium* in infants fed with 10 g/L of GOS and high molecular mass inulin (defined as high molecular mass FOS in the reference) supplemented infant formula against a control where less than 1 log increase of *Bifidobacterium* was observed. The authors in this study found supplementation of the GOS and inulin preparation showed no effect on the population of faecal *Lactobacillus* and no effect on the population of faecal *Bacteroids*, *Clostridium*, *E. coli*, *Enterococcus*, *Citrobacter*, *Proteus*, *Klebsiella* and *Candida*.

Table 2: Summary of data presented of the effect of supplemented GOS and inulin-derived (defined as FOS in the table) preparations on the population of bifidogenic bacteria in full term infants (Moro *et al.*, 2002)

	Bifidobacterium $\log_{10}$ CFU/g wet faeces (mean)			*Lactobacillus* $\log_{10}$ CFU/g wet faeces (mean)		
	Control*	GOS:FOS 4 g/L	GOS:FOS 8 g/L	Control	GOS:FOS 4 g/L	GOS:FOS 8 g/L
Day 1	8.8	8.5	7.7	3.4	3.3	3.4
Day 28	7.2	9.3	9.7	3.4	5.9	5.6
Difference	- 1.4	+ 0.8	+ 2.0	0	+ 2.6	+ 2.2

* Control refers to infants been fed with control infant formula.

Bakker-Zierikzee *et al.* (2005) assessed the effect of a GOS and inulin (Raftiline HP, supplied by Orafti; now redefined at Beneo HP) supplementation (ratio at 9:1) during the first 4 months of life of infants and compared the analysis with controls and breast-fed infants. The study involved 91 infants and another 101 breast fed infants as the reference group, and the GOS and inulin combination was supplemented at 6 g/L. Their study found that the combination of GOS and inulin supplemented infant formula stimulated the growth of lactobacilli and 6.1% in the total colon bacteria at the end of feeding trial was lactobacilli. This was comparable to the lactobacilli counts in infants fed with human breast milk. Supplementation of GOS alone to the infant formula or infant formula without supplementation of either inulin or GOS did not stimulate colon lactobacilli growth.

A summary of the above studies together with several other human trials using GOS and inulin-derived substances in combination are presented in Table A of the Appendix to this report. The available information suggests that supplementation of infant formula with a combination of GOS and inulin-derived substances at a specific ratio of 9:1, with a dose not exceeding 10 g/L, may stimulate selectively the growth of colon bifidobacteria in infants. Future studies employing consistent methodology, for example, inclusive of both the proportion and actual counts of faecal bifidobacteria, and increased frequencies in faecal sampling and microbiological analysis may assist to generate consistent information about the extent of the selective growth stimulation of bifidobacteria by GOS and inulin-derived substances in combination.

2.4.3 Effect of inulin-derived substances alone on gut microflora

In a study involving 20 young children attending a day care centre aged between 7 and 19 months, consumption of inulin-derived oligofructose (Beneo® P95 supplied by Orafti) at 2 g/day was found to only slightly stimulate the growth of bifidobacteria, with the population increasing by 0.4 log (from 9.1-9.4 log CFU/g) over a period of 20 days (Waligora-Dupriet *et al.* 2007). In a separate report of the same study, Waligora-Dupriet *et al.* (2005) presented data that indicates that while there was a small increase in the levels of faecal bifidobacteria over the trial period the differences of bifidobacteria counts between the infants fed with oligofructose supplemented formula and the control were not significant.

Kapiki *et al.* (2007) reported that bifidobacteria in 36 infants fed with inulin (4 g/L) supplemented formula increased above those in the control group (20 infants).

Euler *et al.* (2005) assessed the effect of inulin (Raftilose®95 supplied by Orafti; now redefined at Beneo P95) on full term healthy infants. Inulin was supplemented in infant formula at 1.5 g/L or 3.0 g/L using human breast milk fed infants as control, and the study involved a total of 72 subjects. The study found that inulin supplemented at 1.5 or 3.0 g/L had minimal effect on the intestinal microflora including bifidobacteria, lactobacilli, bacteroids, clostridia and enterococci.

In a study involving 14 infants aged at an average of 12.4 weeks, where inulin was tested for prebiotic effect, the authors claimed that inulin (Frutafit® IQ supplied by Sensus, defined in the study as native inulin) fed at 1.5 g/day stimulated both bifidobacteria and lactobacilli growth (Kim *et al.*, 2007). The author claimed that a significant stimulation to these intestinal microorganisms by exposure to inulin is associated with a lower initial bifidobacteria population in the colon.

Data presented in the report provides insufficient detail to enable appropriate peer review of how the conclusion was derived.

A summary of these studies is presented in Table A of the Appendix to this report. In general, consistent information on the effect of inulin-derived substances alone on the growth and activities of intestinal bifidobacteria and lactobacilli in infants is lacking.

2.4.4 Effect of GOS alone on gut microflora

As reported earlier, Bakker-Zierikzee *et al.* (2005) found supplementation of GOS alone (at 6g/L for a period of 12 weeks) to the infant formula did not stimulate *Lactobacillus* growth in newborn infants. The authors did not examine the effect of GOS supplementation on the growth and activity of bifidobacteria.

Ben *et al.* (2004) reported that GOS supplemented at 2.4 g/L stimulated selectively the growth of bifidobacteria and lactobacilli and the concentrations of faecal bifidobacteria and lactobacilli at 3 months and 6 months were similar to those found in subjects fed with human breast milk.

Further research employing consistent methodology in the future may assist to elucidate the role of GOS alone on the growth and activity of intestinal microflora.

References

Backhed F., R.E. Ley, J.L. Sonnenburg, D.A. Paterson and J.I. Gordon (2005) Host-bacterial mutualism in the human intestine. *Science* 30: 1915-1920

Bakker-Zierikzee A.M., A.S. Alles, J.Knol, F.J. Kok, J.J. Tolboom and J.G. Bindels (2005) Effects of infant formula containing a mixture of galacto- and fructo-oligosaccharides or viable *Bifidobacterium animalis* on the intestinal microflora during the first 4-monts of life. *British J Nutri* 94: 783-790

Bakker-Zierikzee A., M. Alles, J. Bindels, and J. Knol, (2005) The effects of infant formulas containing either prebiotics or probiotics on the faecal *Lactobacillus* population. ESPGHAN 38th Annual Meeting. *J Pediatr Gastroenterol Nutr* 40(5): PN1-22

Ben X, X. Zhou, W. Zhao, W. Yu, W. Zhang, S-M Wu, C. M. Van Beusekom and A. Schaafsma (2004) Supplementation of milk formula with galacto-oligosaccharides improves intestinal micro-flora and fermentation in term infants. *Chin Med J* 117(6): 927-931

Bengamrk S. (1998) Ecological control of the gastrointestinal tract: the role of probiotic flora. *Gut* 42:2-7

Bezirtzoglou E. (1997) The intestinal microflora during the first weeks of life. *Anaerobe* 3: 173-177

Binder H.J., J.H. Cummings and K.H. Soergel (editors) (1994) Short Chain Fatty Acids, Lancaster: Kluwer Academic Publishers

Boehm G., M. Lidestri, P. Casetta, J. Jelinek, F. Negretti, B. Stahl and A. Marini (2002) Supplementation of a bovine milk formula with an oligosaccharide mixture increases counts of faecal bifidobacteria in preterm infants. *Arch Dis Child Fetal Neonatal* Ed 86: F178-F181

Brunser O., M. Gotteland, S. Cruchet, G. Figueroa, D. Garrido and P. Steenhout (2007) Effect of a milk formula with prebiotics on the intestinal microbiota of infants after an antibiotic treatment. *Pediatric Research* 59(3) 451-456

Coppa G.V., O. Gabrielli, P. Pierani, C. Catassi, A. Carlucci and P.L. Giorgi (1993) Changes in carbohydrate composition in human milk over 4 month of lactation. *Pediatrics* 9: 637-641

Coppa G.V., P. Pierani, L. Zampini, I. Carloni, A. Carlucci and O. Gabrielli (1999) Oligosaccharides in human milk during different phases of lactation. *Acta Paediatr*. (Suppl 430): 89-94

Coppa, G.V., S. Bruni, L. Morelli, S. Soldi and O. Gabrielli (2004a) The first prebiotics in humans: human milk oligosaccharides. *J Clin Gastroenterol* 38 (Suppl 2): S80-S83

Coppa, G.V., L. Zampini, T. Galeazzi and O. Gabrielli (2004b) Prebiotics in human milk: a review. *Dig Liver Dis* 38 (suppl 2): S291-294

Edwards, C.A. and A.M. Parrett (2002) Intestinal flora during the first months of life: new perspectives. *British J. Nutrit* 88 (suppl.) 1: S11-S18

EFSA (2004) Opinion of the scientific panel on dietetic products, nutrition and allergies on a request from the Commission relating to the safety and suitability for particular nutritional use by infants of fructo-oligosaccharides in infant formulae and follow-on formulae. *The EFSA Journal* 31: 1-11

Euler, A.R., Mitchell, D.K., Kline, R. and Pickering, L.K. (2005) Prebiotic effect of fructo-oligosaccharide supplemented term infant formula at two concentrations compared with unsupplemented formula and human milk. *J Pediatr Gastroenterol Nutr* 40:157-164.

Fanaro S. J. Jelinek, B. Stahl, G. Boehm, R. Kock and V. Vigi (2005) Acidic oligosaccharides from pectin hydrolysate as new component for infant formulae: effect on intestinal flora, stoll characteristics, and pH. *J Pediatr Gastroenterol Nutr* 41: 186-190

FAO/WHO (1998) Carbohydrates in human nutrition, http://www.fao.org/docrep/x2650T/x2650t02.htm#TopOfPage Accessed 11 September 2007

FAO/WHO (2001) Health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria. Report of a Joint FAO/WHO Expert Consultation on Evaluation of Health and Nutritional Properties of Probiotics in Food Including Powder Milk with Live Lactic Acid Bacteria.

Gibson G.R. and M.B. Roberfroid (1995) Dietary modulation of the human colonic microbiotia: introducing the concept of prebiotics. *J. Nutr* 125: 1401-1412

Gibson G.R. (2004a) Fibre and effects on probiotics (the prebiotic concept). *Clin Nutri. Suppl* 1: 25-31

Gibson G.R., H.M. Probett, J.V. Loo, R.A. Rastall and M.B. Roberfroid (2004b) Dietary modulation of the human colonic microbiota: updating the concept of prebiotics. *Nutri Res Rev* 17: 259-275

Guarner, F. and J-R Malagelada (2003) Gut flora in health and disease (Review). *The Lancet* 361(9356): 512-519

Hooper L.V. and J.L. Gordon (2001) Commensal host-bacterial relationships in the gut. *Science* 292: 1115-1118

Kapiki A., C. Costalos, C. Oikonomidou, A. Triantafyllidou, E. Loukatou and V. Pertrohilou (2007) The effect of a fructo-oligosaccharide supplemented formula on gut flora of preterm infants. *Early Human Dev* 83: 335-339

Kim S-H, D.H. Lee and D. Meyer (2007) Supplementation of infant formula with native inulin has a prebiotic effect in formula-fed babies. *Asia Pac J Clin Nutr* 16: 172-177

Knol J., P. Scholtens, C. Kafka, J. Steenbakkers, S. Groß, K. Helm, M. Klarczyk, H. Schöpfer, H.-M.Bröckler and J. Wells (2005) Colon microflora in infants fed formula with galacto- and fructo-oligosaccharides: more like breast-fed infants. *J Pediatr Gastroentol Nutr* 40: 36-42

Loo J.V., P. Coussement, L.D. Leenheer, H. Hoebregs and G. Smits (1995) On the presence of inulin and oligofructose as natural ingredients in the Western diet. *Crit. Rev in Food Sci and Nutr* 35(6): 525-552

Moro G., S. Arslanoglu, B. Stahl, J. Jelinek, U. Wahn and G. Boehm (2006) A mixture of prebiotic oligosaccharides reduces the incidence of atopic dermatitis during the first six months of age. *Arch Dis Child* 91: 814-819

Moro, G.E. and Arslanoglu, S. (2005) Reproducing the bifidogenic effect of human milk in formula-fed infants: Why and how? *Acta Paediatrica* 94:14-17.

Moro G., I. minoli, M. Mosca, S. Fanaro, J. Jelink, B. Stahl and AG. Boehm (2002) Dosage-related bifidogenic effects of galacto- and fructo-oligosaccharides in formula-fed term infants. *J Pediatr Gastroenterol* 34: 291-295

O'Hara A.M. and F. Shanahan (2006) The gut flora as a forgotten organ. *EMBP Reports* 7: 688-693

Ramakrishna B.S. (2007) The normal bacterial flora of the human intestine and its regulation. *J Clin Gastroenterol* 41(suppl):S2-S6

Salminen S., C. Bouley, M.C. Bouton-Renault, J.H. Cummings, A. Franck, G.R. Gibson, E. Isolauri, M.C. Moreau, M. Roberfroid and I. Rowland (1982) Functional food science and gastrointestinal physiology and function. *Br. J Nutr* 80(suppl): S147-S171

Schmelzle H., S Wirth, H. Skopnik, M. Radke, J. Knol, H-M. Böckler, A. Brönstrup, J. Wells and C. Fusch (2003) Randomized double-blind study of the nutritional efficacy and bifidogenicity of a new infant formula containing partially hydrolysed protein, a high ß-palmitic acid level, and nondigestible oligosaccharides. *J Pediatr Gastroenterol Nutr* 36: 343-351

Scholtens P.A.M., M.S. Alles, J.G. Bindels, E.G.M. van der Linde, J.J.M. Tolboom and J. Knol (2006) Bifidogenic effects of solid weaning foods with added prebiotic oligosaccharides: a randomised controlled clinical trial. *J Pediatr Gastroenterol Nutr* 42: 553-559

Scientific Committee on Food (2003) Report of the scientific committee on food on the revision of essential requirement of infant formulae and follow-on formulae. http://ec.europa.eu/food/fs/sc/scf/out199_en.pdf accessed 8 August 2007

Sears, C.L. (2005) A dynamic partnership: celebrating our gut flora. *Anaerobes* 11: 247-251

Simon G.L. and S.L. Gorbach (1984) Intestinal flora in health and disease. *Gastroenterology* 86: 174-193

Stephen A.M. and J.H. Cummings (1980) The microbial contribution to human faecal mass. *J. Med Microbiol* 13: 45-56

Suerbaum S and Michetti P (2002) Helicobacter pylori infection. *N Engl J Med* 347 (15) :1175–1186

Thurl S., B. Muler-Werner, and G. Sawatzki (1996) Quantification of individual oligosaccharide compounds from human milk using high-pH anion exchange chromatography. *Anal Biochem* 235: 202-206

Thurl S., J. Henker, M. Siegel, K. Tovar and G. Sawatzki (1997) Detection of four human milk groups with respect to Lewis blood group dependent oligosaccharides. *Glycoconj* 14: 795-799

Waligora-Dupriet, A-J., F. Campeotto, I. Nicolis, A. Bonet, P. Soulaines, C. Dupont and M-J Butel (2007) Effect of oligofructose supplementation on gut microflora and well-being in young children attending a day care centre. *Intern J Food Microbiol* 113: 108-113

Waligora-Dupriet, A.J., F. Campeotto, A. Bonet, P. Soulaines, I. Nicolis, C. Dupoint, and M.J. Butel, *et al.*, (2005) Effects of oligofructose supplementation in infants 6 to 24 months of age on gut microflora and well-being: A double-blend, placebo-controlled study. ESPGHAN 38th Annual Meeting. *J Pediatr Gastroenterol Nutr* 40(5): PN1-19

Wostmann, B.S., C. Larkin, A. Morairty and E. Bruckner-Kardoss (1983) Dietary intake, energy metabolism, and excretory losses of adult male germfree Wistar rates. *Lab Anim Sci* 33: 46-50

Zarate S., M.H. and Lopez-Leiva (1990) Oligosaccharide formation during enzymatic lactose hydrolysis: a literature review. *J Food Protect* 53: 262-268

Table A: Studies on the Effect of Inulin-derived and Galacto-oligosaccharide (GOS) on Intestinal Microflora

Dose	Subject Details	Study Duration	Effect on gut microflora	References
GOS and Inulin-derived substances in combination				
6 g/L GOS : Inulin at 9:1 (Raftiline HP) Or GOS alone	91 + 101 - breast fed reference (infants of average age 5 days)	12 weeks	GOS and Inulin combination supplemented infant formula stimulated the faecal lactobacilli, with its proportion matching that found in breast-fed infants. No stimulation of lactobacilli was observed with standard infant formula, or after the addition of 6/L GOS to the standard infant formula.	Bakker-Zierikzee *et al.*, (2005)
10 g/L GOS : Inulin at 9:1 (high molecular mass inulin)	30 + 12 - breast fed reference (preterm infants, average age 8 days)	28 days	Supplementation of GOS and Inulin in combination stimulated the growth of bifidobacteria, but had no effect on the growth of *Lactobacillus*, *Bacteroids*, *Clostridium*, *E. coli*, *Enterococcus*, *Citrobacter*, *Proteus*, *Klebsiella* and *Candida*.	Boehm *et al.*, (2002)
4 g/L GOS : Inulin at 9:1 (high molecular weight fraction of inulin)	140 (infants of average age 5 days old)	6 weeks	Proportion of faecal bifidobacteria increased significantly (5.36% at week 0 to 39.69% at week 6) in GOS and Inulin supplemented infants whereas the increase was small in the control infants (8.04% at week 0 to 14.87% at week 6).	Costalos C. *et al.*, (2007)
6 g/L GOS :Inulin at 9:1 (high molecular mass inulin) 2 g/L of acidic oligosaccharides	46 (infants with average of 3 days old)	6 weeks	Counts of faecal bifidobacteria of infants fed with GOS and Inulin supplemented formula (in the presence of acidic oligosaccharides (AOS) derived from enzyme hydrolysed citrus pectin at week 6 was approximately 1.5 log higher than those found in the infants fed with control formula or control formula with AOS.	Fanaro *et al.*, (2005)
8 g/L GOS : Inulin-derived substance at 9:1 (Assumed Inulin described as FOS in the study, no further definition given)	53 (infants age at 7 to 8 weeks)	6 weeks	Proportion of faecal bifidobacteria increased from 45.2% (week 0) to 59.5% (week 6) in infants fed with GOS and Inulin supplemented formula, and those in the control changed from 47.3% to 49.5%. Proportion of bifidobacteria at week 6 (59.5%) in infants fed with GOS and Inulin supplemented infant formula is comparable to that (67.7%) in breast-fed infants.	Knol *et al.* (2005)

Dose	Subject Details	Study Duration	Effect on gut microflora	References
8 g/L GOS : Inulin-derived substance at 9:1 (long chain FOS)	206 (infants approximately 6 weeks old)	6 months	Significant increases in the number of faecal bifidobacteria were found with infants fed with infant formula supplemented with GOS and Inulin. Supplementation of infant formula with GOS and Inulin resulted in no significant influence on the faecal lactobacilli counts as comparing with the control subjects.	Moro *et al.*, (2006)
4 – 8 g/L GOS : Inulin at 9:1 (high molecular weight inulin)	90 (infants average age at 7 days)	28 days	Supplementation of GOS and Inulin in combination stimulated the growth of bifidobacteria and lactobacilli. The growth stimulation was Inulin and GOS dose dependent for bifidobacteria, but no for lactobacilli.	Moro *et al.*, (2002)
8 g/L GOS : Inulin-derived substance at 9:1 (FOS with a reduced proportion of DP<10)	102 (infants < 2 weeks)	10 weeks	The number of faecal bifidobacteria in infants fed with GOS and Inulin-derived substance supplemented formula increased from 3.87 x 10^9 to 10.3 x 10^9/g from day 0 to week 6 whereas those in the control increased from 3.50 x 10^9 to 5.60 x 10^9/g.	Schmelzle *et al.*, (2003)
4.5 g/day GOS : Inulin at 9:1 (Raftiline HP)	35 (infants 4 to 6 months)	6 weeks	Inclusion of GOS and Inulin in solid infant food induced an increase in the faecal proportion of bifidobacteria of fully formula fed infants.	Scholtens *et al.*, (2006)
			GOS Only	

Bakker-Zierikzee *et al.*, (2005) above

	271	6 months	Faecal bifidobacteria and lactobacilli counts of infants fed with GOS supplemented formula were similar to those found in infants fed with breast milk at 3 months and 6 months. These counts were approximately 2-log higher than those found in infants fed with control formula.	Ben, (2004)

Dose	Subject Details	Study Duration	Effect on gut microflora	References
Inulin-derived substances Only				
1.5 – 3.0 g/L Oligofructose (RaftiloseP95®)	72 (2 – 6 weeks old infants)	5	Supplementation of oligofructose in infant formula had minimal effect on faecal counts of *Bifidobacterium*, *Lactobacillus*, *Enterococcus*, *Bacteroids* and *Clostridium*. Faecal counts of these faecal bacteria did not change significantly in infants fed with human milk.	Euler *et al.*, (2005)
4 g/L Inulin	56 (0-14 days preterm infants)	14	Faecal counts of bifidobacteria of infants fed with Inulin supplemented formula increased from day 1 to day 7 by 0.5 log and were 0.5 log higher than those from control infants at day 7. Faecal *E. coli* and enterococci counts apparently reduced by 1.8 to 0.8 log from day 1 to day 7 in infants fed with Inulin supplemented formula.	Kapiki *et al.*, (2007)
2 g/day Oligofructose (RaftiloseP95®)	20 (7-19 months)	42	Bifidobacteria increased slightly in infants fed with oligofructose supplemented formula. While there was a small increase in the levels of faecal bifidobacteria over the trial period, the differences between the infants fed with oligofructose supplemented formula and the control infants were insignificant. *Lactobacilli* species were seldom identified from both the treatment and control groups.	Waligora-Dupriet *et al.*, (2007) Waligora-Dupriet *et al.*, (2005)
1.5 g/day Native inulin (Frutafit® IQ)	14 (infant of average age at 12.6 weeks)	3	Faecal counts of bifidobacteria and lactobacilli of infants fed with inulin supplemented formula were approximately 0.5 log higher than those found in control. A large scale of growth stimulation on bifidobacteria is associated with a lower initial population.	Kim *et al.*, (2007)
Inulin-derived substances in combination				
4.5 g/L (minimum 500 ml/day) Oligofructose (RaftiloseP95®) and Inulin (Raftiline)	140 (12-24 month infants)	28	Faecal population of *Bifidobacterium* increased in oligofructose and inulin supplemented infants by 0.37 log and reduced in control infants by 0.27 log at day 7. Faecal population of *Lactobacillus* and *Enterococcus* increased in oligofructose and inulin treated infant by 0.67 log and reduced in control by 0.10 log at day 7. No observable changes with *Bacteroides*, *E. coli*, *Clostridium lituseburense* and *C. histolyticum*.	Brunser *et al.* (2006)

Attachment 5

Nutrition Assessment

Summary

Breastfed infants generally have softer and more frequent stools than those given infant formula. They also have a lower stool pH and a different faecal short chain fatty acid (SCFA) profile; one in which acetate and lactate predominate. This is at least partly due to the oligo- and polysaccharides that are unique to breast milk. The range of oligo- and polysaccharides in human milk is large. It is not possible for infant formula product manufacturers to copy this diversity, but attempts have been made to achieve the same result through the addition of commercially available oligo- and polysaccharides. The most frequently studied have been long chain inulin, inulin, oligofructose and galacto-oligosaccharides (GOS).

Combined Addition of Galacto-oligosaccharides and Long Chain Inulin

The bulk of research has been done comparing standard infant formula to formula supplemented with GOS and long chain inulin at a ratio of 9:1 and concentrations of 4-10 g/L of formula. The majority of these studies reported softer stools, with a lower pH, when infant formulas were supplemented. The one study that examined faecal SCFA also reported that supplemented formula resulted in a SCFA profile similar to that reported for breastfed infants. The ability of GOS and long chain inulin to increase stool frequency is less well established, possibly because this effect gets smaller with age. The addition of GOS and long chain inulin to infant foods did not influence stool frequency or softness, nor did it significantly lower stool pH.

Single Addition of Inulin-derived Substances or Galacto-oligosaccharides

Sole addition of derived substances to infant formula products or infant foods has sometimes been shown to increase stool frequency or stool weight in infants less than 12 months of age. The limited data available suggests they have little effect on stool pH. There appears to be little impact on stool frequency or consistency in infants over 12 months of age.

The one study that examined the addition of GOS to infant formula in isolation, i.e. without also adding other poly- or oligosaccharides, reported higher stool frequency in GOS-fed infants. The frequency was similar to that of a breastfed reference group. More data are needed to draw a firm conclusion. Data on the effect of formula supplemented with GOS alone on stool pH and faecal SCFA is limited and contradictory, thus no conclusion can be made.

Single Addition of Fructo-oligosaccharides

It should be noted that fructo-oligosaccharides, as defined in the Draft Assessment Report, have not been sufficiently studied to draw any conclusions about their effect on stool characteristics in infants or young children.

Study Limitations

When considering these results it is important to keep in mind that many of the studies done in this area had one or more of the following limitations: loss of a large proportion of participants, small sample size, and industry sponsorship. Stool consistency was measured simply by rating the stool on a scale that often varied across studies; it therefore has an element of subjectivity.

Mineral Interaction

In adults there does not appear to be any interference of mineral absorption from the small intestine when fructo-oligosaccharides (FOS), inulin-derived substances are added to diets in amounts typically in the range of 8-20 g/d. There is evidence to suggest that mineral absorption from the large intestine may be increased due to the presence of added fermentable fructan polymers, the evidence being particularly strong for an enhanced effect on calcium absorption.

1. Inulin, Long Chain Inulin, FOS and GOS in Infant Formula Products, Infant Food, and Formulated Supplementary Foods for Young Children – Effect on Stool Frequency and Consistency

1.1 Stool Frequency and Consistency

A recognised difference between formula-fed and breastfed infants is that the latter have softer and more frequent stools (Quinlan *et al*, 1995). One of the potential functions of inulin-derived substances and/or GOS added to infant formula and infant food is to promote stool frequency and consistency similar to that observed in breastfed infants. The other, and perhaps related, proposed function of these compounds is to modify the microflora of the colon, but this aspect is discussed further in section 2 below, and in detail in Attachment 4. The laxative properties of inulin-derived substances, FOS and GOS in adults are discussed in Attachment 7.

1.1.1 Search Strategy

An electronic literature search was conducted via pubmed (www.ncbi.nlm.nih.gov/sites/entrez/) using the following search terms:

- (galacto-oligosaccharide OR galactooligosaccharide OR fructo-oligosaccharide OR fructooligosaccharide OR inulin OR fructan) AND (infant OR formula OR stool)
- Limited to: Human, English, German, All Infant (0-23 months), Clinical Trial, Meta-Analysis, Review, Randomized Controlled Trial

Particularly relevant journals were also searched individually including the American Journal of Clinical Nutrition and the Journal of Pediatric Gastroenterology and Nutrition. Terms used were *galactooligosaccharide* and *fructooligosaccharide* and the results examined manually without further electronic filtering.

References were also provided by Nutricia Ltd and H.J. Heinz Co Ltd, including abstracts from conference proceedings published in peer reviewed journals. Where an apparently relevant abstract was identified an author search was conducted on pubmed to identify if the research had since been published as a full paper. The reference list of key papers was also consulted to identify other potentially relevant research.

1.1.2 Summary of Identified Studies

Seventeen randomised controlled trials comparing oligo- and/or polysaccharide supplemented foods for infants with an unsupplemented control, and in some cases breast milk, were identified in the peer reviewed literature. A further two studies published in conference proceedings as abstracts were also identified and included. Breastfed reference groups were not randomised.

The studies are summarised in the tables and text below. Where authors provided the trade name or other details about the oligosaccharide(s) tested and/or the commercial formula used this is included. If no such information was provided the generic term for the oligosaccharide(s) is given. Formula that was made up for the study from generic recipes are listed as *experimental*. Table 1 provides a basic guide to the products tested. The sample sizes that are reported here are based on the number in each treatment group at the end of the study.

Table 1: Guide to Branded Oligosaccharides Used in Identified Studies

Manufacturer	Brand Name	Description	Generic Term
Orafti	Raftiline® HP	Inulin with no mono- or disaccharides	Long chain inulin
	Raftilose® P95/ Beneo® P95	Hydrolysed inulin from chicory; 5% di- and monosaccharides	Oligofructose
Sensus	Frutafit IQ	Instantised inulin from chicory	Inulin
Friesland Foods Domo	Vivinal	Galacto-oligosaccharide	Galacto-oligosaccharide

1.1.3 Study Limitations

Studying infants is arguably a difficult undertaking and many of the studies had one or more of the following limitations:

- small sample size,
- loss of a large proportion of subjects to follow-up,
- incomplete descriptions of the poly- and/or oligosaccharides used, i.e. no data on brand name, manufacturer or descriptors such as the degrees of polymerisation;
- no indication that an intention to treat analysis was conducted/failure to conduct an intention to treat analysis;

A further problem is that none of the studies that used maltodextrin as a control specified its characteristics. As maltodextrin refers to a range of products including some resistant to digestion, this may not have been a neutral control. It is reasonable to assume that digestible maltodextrins were used. However, *in vitro* evidence indicates that if maltodextrins were to reach the colon they would be fermented by colonic bacteria (Probert *et al*, 2002).

Further, it has been demonstrated, at least *in vitro*, that maltodextrin administration can lead to increased numbers of specific bacterial groups including *bifidobacteria* and *lactobacilli* (Olano-Martin *et* al, 2000; Rycroft *et al*, 2001). Thus it is possible that some studies underestimate the effect of the test formula.

Many studies were funded by industries with financial interest in the outcome and conducted by industry employees.

1.1.4 Measuring Stool Frequency and Consistency

Stool frequency has been measured in terms of number of defecations per day usually reported by parents or other care givers. Stool consistency has been assessed by observation and graded using a numeric scale of 1-4 or 1-5. In some studies the lower numbers indicate softer stools; in others the higher numbers indicate softer stools. Most studies compare average scores but some compare the number or proportion of soft stools in each study group. The lack of a single consistent grading method makes numeric comparisons between studies impractical, results are therefore reported as either (statistically significantly) different or similar.

1.2 Inulin-derived Substances or Fructo-oligosaccharide Alone in Infant Formula Products, Weaning Foods, and Formulated Supplementary Foods for Young Children

The main details of the studies in this section, including age at entry, study design, sample size and outcome in terms of stool characteristics, are presented in Table 2.

Euler *et al* (2005) compared stool frequency and consistency before and after infants received infant formula supplemented with 1.5 or 3.0 g/L oligofructose in a crossover design. Stool frequency increased and consistency softened when infants received 3.0 g/L. Conversely, frequency decreased and consistency stayed the same relative to pre-supplementation when infants were only given 1.5 g/L. Kapiki *et al* (2006) reported more frequent stools but similar consistency with inulin supplemented pre-term formula compared to unsupplemented control formula. Kim *et al* (2007) reported no difference in stool frequency or consistency between supplemented and control formula-fed infants. The group did report greater stool weight in infants given inulin supplemented infant formula; this measure was not reported by the other studies. Stool weight was measured by weighing nappies and therefore without indication that the urinary content of the nappy was accounted for.

Waligora-Dupriet *et al* (2007) examined the effect of mixing oligofructose at 2 g/d with food and drink in 7-19 month olds and observed no difference in stool frequency or consistency between supplemented and unsupplemented groups. Brunser *et al* (2006) compared a formulated supplementary food for young children with and without inulin at 4.5 g/L in 12-24 month olds after treatment with amoxicillin for acute bronchitis, and observed no difference in stool frequency or consistency. However, 16-46 week olds given infant cereal supplemented with FOS, at an average FOS intake of 0.74 g/d , had a higher stool frequency and more stools described as soft than those fed formula containing the same amount of maltodextrin (Moore *et al*, 2003).

Collectively, these studies suggest that the addition of inulin-derived substances or FOS, at approximately 3 g/L in infant formula or 0.74 g/d in infant foods, can increase stool frequency and/or stool weight in infants less than 12 months of age. There appears to be little impact on stool frequency or consistency in infants 7-24 months of age when they are given up to 4.5 g/d of inulin.

1.3 Combination of Galacto-oligosaccharides and Long Chain Inulin at a Ratio of 9:1 added to Infant Formula Products and Infant Foods

Eleven studies have reported stool frequency and/or consistency in infants receiving formula supplemented with long chain inulin in combination with GOS. All but one of these studies clearly identified the ratio as 1:9; Moro *et al* (2006) are unclear though the use of this ratio is implied by referencing earlier work that used a 1:9 ratio. Key study details including age at entry, study design, sample size and outcome in terms of stool characteristics are provided in Table 3.

Five studies published as six papers have looked at the addition of long chain inulin and GOS to infant formula without also adding other components that may influence stool characteristics (Boehm *et al*, 2002; Boehm *et al*, 2003; Moro *et al*, 2002; Moro *et al*, 2006; Litov *et al*, 2006; Costalos *et al*, 2007). The two largest studies reported greater stool frequency with supplemented formula at 4 and 8 g/L (Moro *et al*, 2006; Costalos *et al*, 2007). Only the small short duration study by Litov *et al* (2006) reported lower stool frequency at 4 g/L. The relative increase in stool frequency has been shown to be maintained at least until the age of six months, but the magnitude of the difference reduces over time (Moro *et al*, 2006). All four studies that reported stool consistency observed softer stools in supplemented groups (Boehm *et al*, 2002; Boehm *et al*, 2003; Moro *et al*, 2002; Moro *et al*, 2006; Costalos *et al*, 2007). Only 8 g/L of oligosaccharide mix resulted in similar stool consistency in supplemented and breastfed groups (Moro *et al*, 2002).

Three studies tested long chain inulin and GOS in combination with high concentration β-palmitic acid in infant formula (Schmelzle *et al,* 2003; Bongers *et al*, 2007; Fuentes *et al*, 2005). All three studies report a greater proportion of soft stools in those receiving supplemented formula. Only Fuentes *et al* (2005) observed a greater of frequency of defecation with supplemented formula. They were the only group to include breastfed infants a further comparison; stool characteristics were similar between supplemented formula-fed and breastfed infants. When evaluating the outcome of these studies, it must be considered that β-palmitic acid added to infant formula alone has previously been shown to result in softer stools (Kennedy *et al*, 1999). As it was not also tested in isolation in these three studies, it is unclear what contribution the GOS and long chain inulin made to the reported findings.

Formula supplemented with long chain inulin and GOS in combination with *Bifidobacterium longum* BL999 resulted in softer and more frequent stools (Puccio et al, 2007). However, as there was no comparison group that received Bifidobacterium longum BL999 alone, it is not clear what if any impact the oligosaccharides had.

Fanaro et al (2005) sought to mimic the oligosaccharide composition of breast milk more closely by adding an acidic carbohydrate, i.e. hydrolysed pectin, as well as the neutral long chain inulin and GOS.

Hydrolysed pectin alone and in combination long chain inulin and GOS resulted in softer stools. Hydrolysed pectin alone did not alter stool frequency relative to control formula. Supplementation of weaning foods with long chain inulin and GOS at up to 4.5 g/d made no difference to stool frequency or softness (Scholtens et al, 2006).

Despite some variation in findings and confounding in several studies, it appears likely that inclusion of long chain inulin and GOS at a ratio 9:1 in infant formula products leads to a softening of stools similar to those of breastfed infants. The impact on stool frequency is less clear with many studies reporting no statistically significant difference between fortified and unfortified formula-fed infants. The available evidence suggests that any increase in frequency declines with age. Supplementation at 8-10 g/L was more likely to result in softer and/or more frequent stools than 4 g/L.

With only one small study examining long chain inulin and GOS in weaning foods at up to 4.5 g/d, no conclusion on its impact can be made.

1.4 Galacto-oligosaccharides Alone Added to Infant Formula

Using a six month parallel study design, Ben *et al* (2004) compared infants receiving standard formula supplemented with GOS at 2.4 g/L (n=69) with those given unsupplemented control formula (n=52) or breast milk (n=26). Stool frequency was the same in breastfed infants and those receiving supplemented formula, but higher in both these groups than in infants receiving control. This result somewhat inconsistent with the findings of studies using a combination of GOS and long chain inulin, which suggest only a small, and sometimes non-significant impact on stool consistency with 4 g/L of GOS and long chain inulin at a ratio of 9:1, i.e. 3.6 g/L of GOS. Further studies examining the sole addition of GOS to formula are needed before firm conclusions can be made.

1.5 Galacto-oligosaccharides in Combination with Non-inulin Oligo- and Polysaccharides added to Infant Formula

Ziegler et al (2007) studied an alternative combination of oligosaccharides also using a parallel design, but with a study duration of four months. Infants received standard formula (n=58), the same formula supplemented with polydextrose and GOS in a one-to-one ratio at 4 g/L (n=58), or polydextrose, GOS and lactulose in a ratio of 50:33:17 at 8 g/L (n=48). Both treatment groups had softer stools than control infants at 30, 60 and 90 days of age. Only those receiving the polydextrose, GOS and lactulose supplemented formula had more frequent stools than controls and only at 30 days of age. This work serves to highlight the possibility that a range of oligo- and/or polysaccharides may help to soften stools in infants.

1.6 Conclusion

The available evidence indicates that the addition of GOS and long chain inulin at a ratio of 9:1 and concentration 4-10 g/L to infant formula is likely to result in softer stools. The ability of this mixture to increase stool frequency is less well established, possibly because this effect gets smaller with age.

Sole addition of inulin-derived substances to infant formula products or infant foods has sometimes been shown to increase stool frequency or stool weight in infants less than 12 months of age. The limited data available suggests they have little effect on stool pH. There appears to be little impact on stool frequency or consistency in infants over 12 months of age.

The one study that examined the addition of GOS to infant formula in isolation, i.e. without also adding other poly- or oligosaccharides, reported higher stool frequency in GOS-fed infants. The frequency was similar to that of a breastfed reference group. More data are needed to draw a firm conclusion. Data on the effect of formula supplemented with GOS alone on stool pH and faecal SCFA is limited and contradictory, thus no conclusion can be made.

2. Long Chain Inulin and GOS in Infant Formula: Impact on Stool pH and Short Chain Fatty Acid Profile

2.1 Colonic/Faecal pH and Short Chain Fatty Acids

The microflora of breastfed infants tends to be more heavily dominated by lactic acid bacteria, particularly *Bifidobacteria* and *Lactobacilli*, than that of formula-fed infants, which is more diverse (Edwards and Parrett, 2002). As a result of the metabolic activity of the lactic acid bacteria dominated microflora, breastfed infants have a lower colonic pH and a different, acetate and lactate dominated, short chain fatty acid (SCFA) profile than those fed formula (Coppa *et al*, 2004; Edwards and Parrett, 2002).

Such a lactic acid dominated microflora is considered to be beneficial to the infant and has been at least partially attributed to the influence of oligo- and polysaccharides inherent to human milk (Edwards and Parrett, 2002). Supplementation of infant formula with inulin-derived substances and/or GOS is seen as a potential way to simulate human milk's influence on colonic pH and the SCFA profile (Boehm *et al*, 2005). As it is not practical to measure colonic conditions directly, stool pH and faecal SCFA are used as a surrogate.

For a more detailed description of the microflora of both breastfed and formula-fed infants see Attachment 4.

2.1.1 Search Strategy, Study Limitations

The search strategy and study limitations were the same as those previously described in sections 1.1.1 and 1.1.3 respectively.

2.2 Effect of GOS and Long Chain Inulin and at a Ratio of 9:1 in Infant Formula or Infant Foods on Stool pH and Faecal Short Chain Fatty Acids

Five studies comparing stool pH in infants fed unsupplemented formula or formula supplemented with GOS and long chain inulin at a 9:1 ratio were identified (Bakker-Zierikzee *et al*, 2005; Costalos *et al*, 2007; Fuentes *et al*, 2005; Moro *et al*, 2002; Scholtens *et al*, 2006). The main details of these studies, including age at entry, study design, sample size and outcome in terms of stool characteristics, are presented in Table 3.

Bakker-Zierikzee *et al* (2005) and Fuentes *et al* (2005) were the only researchers to include a breastfed control group. Both reported a lower stool pH in breastfed infants and those receiving formula supplemented with either 6 or 8 g/L oligosaccharide than in infants receiving unsupplemented formula. However, Bakker-Zierikzee *et al* (2005) reported that stool pH is lowest in breastfed infants, and that the faecal SCFA profile was similar in breastfed and supplemented formula-fed infants; i.e. both groups had higher proportions of lactate and lower proportions of butyrate and propionate than unsupplemented formula-fed infants. The study by Fuentes *et al* (2005) was only available as an abstract; it is not clear if this group found a difference in stool pH between breastfed and supplemented formula-fed infants, or if they measured faecal SCFA.

Infants given formula supplemented with 4 g/L of GOS and long chain inulin mixture were reported to have a similar stool pH to those on standard formula (Costalos *et al*, 2007). An earlier study also reported no difference in stool pH between infants fed unsupplemented formula or formula supplemented with 4 g/L of GOS and long chain inulin mixture, but did report a lower stool pH at a concentration of 8 g/L (Moro *et al*, 2002).

Scholtens *et al* (2006) were the only group to compare the effect of GOS and long chain inulin when added to weaning food. Stool pH in infants fed up to 4.5 g of mixture was similar to those not given GOS and long chain inulin supplemented foods.

Collectively these findings suggest that the addition of a 9:1 mixture of GOS and long chain inulin to infant formula, at a concentration above of 6-8 g/L, would be expected to lower stool pH. The very limited available data suggest that such a formula may also promote a faecal SCFA profile more akin to that commonly observed in breastfed infants.

2.3 Effect of GOS Alone in Infant Formula or Infant Foods on Stool pH

Ben *et al* (2004) compared infants receiving standard formula supplemented with GOS at 2.4 g/L (n=69) with those given unsupplemented control formula (n=52) or breast milk (n=26) using a six month parallel study design. Infants receiving GOS supplemented formula or breast milk were reported to have had lower stool pH and higher faecal acetate concentrations than those on unsupplemented formula. Conversely, Bakker-Zierikzee *et al* (2005) report similar stool pH and faecal SCFA profile in infants receiving either unsupplemented formula (n=17) or formula supplemented with GOS at 6 g/L over a sixteen week period. The group also reported that stool pH was lower and faecal SCFA profile different in breastfed controls (n=unreported). It should be noted that this study was only available as an abstract from a conference proceeding.

With two studies conflicting so completely, no conclusion can be reached at this time.

2.4 Effect of Inulin Alone in Infant Formula or Infant Foods on Stool pH

Kim *et al* (2007) reported similar stool pH in infants fed either unsupplemented formula or formula supplemented so that each infant received 0.25 g/kg of inulin per day. Further details of the study can be found in Table 2.

2.5 Conclusion

The available evidence indicates that the addition of GOS and long chain inulin at a ratio of 9:1 and concentration 6-8 g/L, but not 4 g/L makes infant formula more similar to human milk than unsupplemented formula with respect to stool pH and faecal SCFA profile. This was not observed when weaning foods were supplemented.

The influence of sole addition of GOS or inulin-derived substances to infant formula is unclear.

Table 2: Inulin or Oligofructose Alone in Infant Formula Products, Weaning Foods, and Formulated Supplementary Foods for Young Children

Reference	Study Participants	Comparisons	Oligosaccharide(s)	Concentration	Duration & Design	Outcome(s)
Brunser *et al* 2006	12-24 month old term infants after treatment with amoxicillin for acute bronchitis	test formula - Prebio 1 (Nestle de Chile) n=57 control formula - as per test without oligosaccharide n=56	Raftilose 95® & Raftiline at 7:3	4.5 g/L (2.25 g/d)	21 day parallel	No difference in stool frequency or consistency between groups
Euler *et al* 2005	2-6 week old term infants	test formula - S26® Gold (Wyeth Nutrition, Collegeville, PA) + oligosaccharide vs. no oligosaccharide n=58 breast milk n=14	Raftilose 95®	1.5 or 3.0 g/L	5 week total, but 1 week per treatment + 1 week washout	Greater stool frequency with either concentration, softer stools with higher concentration of oligosaccharide relative to no oligosaccharide. Softer and more frequent stools in the breastfed infants.
Kapiki *et al* 2006	preterm infants with maximum gestation age of 36 weeks	test - standard preterm formula + oligosaccharide n=36 control - standard preterm formula + maltodextrin n=20	inulin	4 g/L	14- days, measurements at 7 days	Greater stool frequency with oligosaccharide
Kim *et al* 2007	5-24 week old orphan term infants	test: standard formula + inulin n=14 control: standard formula n=14	Frutafit IQ®	0.25 g/kg/d	3 week cross-over	Greater faecal weight with oligosaccharide
Moore *et al* 2003	16-46 week old term infants	test: Nestle Carnation Premium Baby Cereal ® (Nestle, USA) + oligosaccharide n=27 control: Nestle Carnation Premium Baby Cereal ® + maltodextrin n= 29	*Fructo-oligosaccharide* no further details provided	0.75 g per 25 g serving of cereal	28 days	Greater stool frequency with oligosaccharide Stools were more likely to be soft with oligosaccharide cereal
Waligora-Dupriet *et al* 2007	7-19 month old term infants in day care	test: food & drink + with oligosaccharide n=9 control: food & drink + maltodextrin n=10	Beneo P95®	2 g/d	21 days parallel	Frequency & consistency of the stools were similar between groups

Table 3 : Combination of GOS and Long Chain Inulin at a Ratio of 9:1 Added to Infant Formula Products and Infant Foods

Reference	Study Participants	Comparisons	Oligosaccharide(s)	Concentration	Duration & Design	Outcome(s)
Bakker-Zierikzee *et al* 2005	~ 1 week old term infants	test 1: Nutrilon I (Nutricia, Zoetermeer, The Netherlands) + oligosaccharides n=19 test 2: Nutrilon I + Bifidobacterium animalis Bb-12 n=19 control: Nutrilon I; Nutricia, Zoetermeer, The Netherlands) n=19 breastfed n=63	90% Vivinal® & 10% Raftiline HP®	6 g/L	16 week parallel	Lowest stool pH in breastfed infants. Oligosaccharide supplemented formula-fed infants had lower stool pH than standard formula-fed infants. No difference in total SCFA. Proportions of SCFA were similar in breast- and supplemented formula-fed infants with higher proportions of lactate and lower proportions of butyrate & propionate than in standard formula-fed infants.
Boehm *et al* 2002/3	preterm infants	test: experimental formula + oligosaccharides n=12 control: experimental formula + maltodextrin n=15 breastfed n=15	90% galacto-oligosaccharide & 10% long chain inulin	10 g/L	28 days parallel	Breastfed and supplemented formula-fed infants had similar stool consistency, both had softer stools than control formula-fed infants
Bongers *et al* 2007*	constipated infants aged 3-20 weeks	test: Nutrilon Omneo (Nutricia, Zoetermeer, The Netherlands) n=12 control: 75% Nutrilon 1 & 25% Aptamil HA I (Nutricia, Zoetermeer, The Netherlands) n=12	90% galacto-oligosaccharide & 10% long chain inulin	8 g/L	3 weeks cross-over	No difference in stool frequency, but oligosaccharide fed infants had more soft stools.
Costalos *et al* 2007	0-14 day old term infants	test: standard formula + oligosaccharides (Numico Research, the Netherlands) n=70control: standard formula n=70	90% galacto-oligosaccharide & 10% long chain inulin	4 g/L	12 week parallel	Softer and more frequent stools with oligosaccharides. Similar stool pH between groups.

Reference	Study Participants	Comparisons	Oligosaccharide(s)	Concentration	Duration & Design	Outcome(s)
Moro *et al* 2002	~7 day old term infants	test: experimental formula + oligosaccharides n=30 for lower concentration & n=27 for higher concentration control: experimental formula + maltodextrin n=33	90% galacto-oligosaccharide & 10% long chain inulin	4 & 8 g/L	28 day parallel	Same stool frequency across groups, softer stools with 8 g/L relative to control and similar to breast milk. Lower stool pH in those on supplemented formula with a dose response.
Moro *et al* 2006†	0-14 day old term infants	test: experimental formula + oligosaccharides n=102 control: experimental formula + maltodextrin n=104	90% galacto-oligosaccharide & 10% long chain inulin	8 g/L	~26 week parallel	Softer and more frequent stools with oligosaccharides
Schmelzle *et al* 2003†	0-14 day old term infants	test: experimental formula + oligosaccharides n=76 control: Pre-Aptamil with Milupan (Milupa, Friedrichsdorf, Germany) n=78	90% galacto-oligosaccharide & 10% long chain inulin	8 g /L	12 week parallel	More soft stools with oligosaccharide
Scholtens *et al* 2006	4-6 month old formula fed term infants being weaned	test: food with added oligosaccharides n=9 control: unsupplemented food n=7	90% Vivinal® & 10% Raftiline HP®	4.5 g/day	6 week parallel	Similar stool consistency and frequency between groups. Similar stool pH and faecal SCFA across groups.
Puccio *et al* 2007	0-14 day old term infants	test: Nan (Nestlé, Vevey, Switzerland) + oligosaccharides + *Bifidobacterium longum* BL999 n=49 control: Nan n=55	90% galacto-oligosaccharide & 10% long chain inulin	4 g/L	112 day parallel	Higher stool frequency with fortified formula, more yellow/fewer green stools with symbiotic, lower risk of constipation
Litov *et al* 2006†‡	~ 2 month old infants	test: experimental + oligosaccharides control: experimental	90% galacto-oligosaccharide & 10 & long chain inulin	4 g/L	14 +/- 3 day crossover	Less frequent stools with oligosaccharide
Fuentes *et al* 2005*‡	term infants	test: Nutrilon Omneo n=25 control: Nutrilon Premium n=23 breastfed n=20	90% galacto-oligosaccharide & 10 & long chain inulin	8 g/L	6 months	More frequent and softer stools with oligosaccharides, similar faecal appearance as in breastfed infants. Lower stool pH in breast- and oligosaccharide supplemented formula-

Reference	Study Participants	Comparisons	Oligosaccharide(s)	Concentration	Duration & Design	Outcome(s)
						fed infants than in standard formula fed infants.

* Test formula also contains a high concentration of β-palmitic acid
† Test formula contains hydrolysed whey protein
‡ Study only available as an abstract

3. Nutrient interactions

Fibre intakes in the form of wheaten wholemeal or thickening agents made from soluble fibre have been found to interfere with mineral absorption (Reinhold et al., 1976; Bosscher et al., 2001). Although inulin is often added to food in the form of a purified extract it shares some of the characteristics of intact dietary fibre suggestive of the potential for nutrient interaction.

3.1 Magnesium, iron, zinc, copper and selenium

A positive effect of oligofructose consumption on magnesium absorption has been reported (Tahiri et al., 2001). Using a two-way crossover design, 14 healthy postmenopausal women were randomized to receive 10 g/d (5 g at lunch and 5 g at dinner) oligofructose (Actilight®; Eridania Beghin-Say, Vilvoord, Belgium) or sucrose for 35-days on each treatment. A stable isotope was given on day 28 after which faeces were collected for 5 to 7 days. Apparent absorption was defined as the difference in isotope intake and excretion expressed as a percentage of intake. Mean (SD) percentage intestinal absorption of isotopic magnesium increased from 30.2 (5.0)% to 33.9 (7.2)% after the placebo and oligofructose periods, respectively. Using the same protocol and isotopes of copper, zinc and selenium, the apparent absorption of copper was increased during the oligofructose intervention period compared with the control period ($P = 0.042$) whereas there was no difference in the absorption of zinc and selenium between treatments (Ducros et al., 2005). Iron absorption, as assessed by stable iron isotope enrichment in erythrocytes, was not different among treatments when 12 men took part in a randomized multi-crossover study involving a control diet or diets supplemented with 15 g/d inulin, FOS or GOS, each diet consumed for 21 days (van den Heuval et al., 1998).

An indirect way to assess mineral absorption along the small intestine is to measure the mineral content in the effluent of people with ileostomies, a surgical procedure in which an opening is created at the end of the small intestine that bypasses the colon. In a three-way crossover study, 10 people with ileostomies were randomized to receive a standard diet supplemented either with 17 g inulin (Raftiline ST; Orafti, Tienen, Belgium) 17 g oligofructose (Raftilose P95; Orafti, Tienen, Belgium), or 7 g sucrose as a control. The inulin or oligofructose were added to meals throughout the day and each diet was given for a period of three days (Ellegard et al., 1997). The excretion of magnesium, zinc, iron and calcium were not different among treatments indicative that 17 g inulin or 17 g oligofructose did not affect the absorption of these minerals along the small intestine compared with the sucrose control. A similar study design was used in healthy adults in which faeces were collected for the determination of mineral content (Coudray et al., 1997). In a three-way crossover study, nine young men were randomized to a control diet or to the same diet supplemented with 40 g/d inulin extracted from chicory roots (Agro Industries, Compiegne, France). Each experimental period lasted 28 days during which there was a progressive increase in inulin intake followed by 12 days at full dose. Sixty percent of the daily inulin intake was added to bread and 40% to beverages. The apparent absorptions assessed as mineral intake minus the mineral content of the faeces were not different between the inulin and control diet for magnesium, iron or zinc, but were increased for calcium ($P < 0.01$). These data suggest that the addition of inulin or oligofructose to food does not interfere with the absorption of magnesium, iron or zinc along the small intestine.

3.2 Calcium

In adults, positive differences in calcium absorption whilst consuming inulin supplemented diets with respect to controls has been shown using 15 g/d partially hydrolyzed inulin (Raftilose P95; Orafti, Tienen, Belgium) and 10 g/d of a 1:1 mix of short:long chain inulin (Synergy 1; Orafti, Tienen, Belgium) (van den Heuvel et al., 1999; Holloway et al.,2007).

Calcium absorption measured using a double stable isotope technique was assessed in 60 female adolescents randomized in a crossover design to receive 8 g/d oligofructose (Raftilose; Orafti, Tienen, Belgium) or 8 g/d of a mix of inulin and oligofructose (Synergy 1; Orafti, Tienen, Belgium) or a control diet, each for a period of 3-weeks (Griffin et al., 2002). Calcium absorption was not different following the control and the oligofructose supplemented dietary periods but there were both fractional and absolute increases in calcium absorption following the inulin and oligofructose (Synergy 1) period. A clinical consequence of increased calcium absorption may be to increase bone mineralization. Results of a randomized controlled trial in which 100 adolescents were assigned to receive 8 g/d inulin and oligofructose (Synergy 1; Orafti, Tienen, Belgium) or maltodextrin for 1-year showed greater changes from baseline in bone mineral content and bone mineral density in the inulin-treated group (Abrams et al., 2005).

An enhancement in calcium absorption has not been found in all studies. In a doubly-labelled stable isotope study in 12 young men, there was no difference in calcium absorption assessed using 24-hour urinary calcium excretion between a control diet and diets containing 15 g/d inulin or fructo-oligosaccharides (van den Heuvel et al., 1998). The authors suggested that a 24-hour period may have been too short to observe an effect for an enhancement in calcium absorption occurring in the large intestine. Fourteen postmenopausal women were randomized to diets for 5-weeks containing 10 g/d FOS or a control (Tahiri et al., 2003). Calcium absorption was assessed by difference, intake less excretion, and by the amount of calcium isotope in plasma and urine. There was no difference in any of the parameters when comparing the control diet with the diet containing added FOS. The authors suggested that the prolonged ingestion of FOS might have down-regulated active calcium absorption in the small intestine, thus offsetting a possible increase in calcium absorption in the large intestine, although this could not be confirmed using this study design. Calcium absorption in 15 young adults was estimated using a doubly-labelled technique in a short-term study in which FOS (Ebro-Puleva, Spain) were added to semi-skimmed milk at a concentration of 5 g/L (Lopez-Huertas et al., 2006). Following consumption of a test drink containing approximately 1 g FOS, calcium absorption was not different when compared with a control. The lack of effect may have been because the amount FOS was small or that the short-term nature of the study did not allow enough time for intestinal changes conducive to calcium enhancement to occur. An acute study in which inulin was added to cheese or to a calcium supplement did not show differences in serum ionized calcium postprandially over 8 hours or calcium in urine collected for up to 24 hours (Teuri et al., 1999). Preterm infants were randomized to receive a 9:1 mix of GOS and long chain inulin added to formula in an amount that equated to a concentration of 10 g/L in the made-up formula, or a control formula containing maltodextrine (Lidestri et al., 2003). After 4 weeks, mean serum and spot urinary calcium concentrations were not different between the groups. No difference in fasting urinary calcium excretion was found in 10 wheelchair-bound adults randomised to receive 15 g/d inulin added to thickened drinks for 3-weeks (Dahl et al., 2005).

Variability in results among studies might be expected given diversities in the population groups, the amount and type of oligosaccharide, duration of studies, and methods of determining calcium absorption, some of the details of which are shown in Table 4. Nevertheless, although an enhancement in calcium absorption has not been found in all studies, in no case has a negative effect of added inulin-derived substances or FOS on calcium absorption been reported.

Table 4: Calcium absorption in randomized controlled trials with a crossover design, unless otherwise indicated

Reference	Treatment[1,2]	Amount (g/d)	n and duration	Main outcome
Ellegard et al., 1997	Inulin (Raftiline ST) Oligofructose (Raftilose P95)	17 17	10 people with ileostomies, 3 days	Ca excretion 29.8 mmol (inulin); 31.2 mmol (oligofructose); *cf*. 29.3 mmol (control) (P > 0.05)
Coudray et al., 1997	Inulin (Agro Industries)	40	9 young men, 28 days	Apparent Ca absorption on treatment 33.7% *cf*. 21.3% on control (P < 0.01)
van den Heuvel et al., 1998	Inulin Oligofructose (Raftilose P95)	15 15	12 young men, 21 days	Fractional Ca absorption 25.8% (inulin); 26.3% (oligofructose); *cf*. 28.1% (control) (P > 0.05)
van den Heuvel et al., 1999	Oligofructose (Raftilose)	15	12 male adolescents, 9 days	Fractional Ca absorption on treatment 60.1% *cf.* 47.8% on control (P < 0.05)
Teuri et al., 1999	Inulin (Raftiline)	15	15 young women, acute study (1 day)	Inulin in cheese: Urinary Ca on treatment 1.60 *cf*. 1.45 mmol on control (NS). Serum ionized Ca 8-h area under the curve on treatment 0.010 *cf*. 0.002 mmol/L on control (NS). Inulin in a Ca supplement: Urinary Ca on treatment 1.47 *cf*. 1.46 mmol on control (NS). Serum ionized Ca 8-h area under the curve on treatment 0.011 *cf*. 0.009 mmol/L on control (NS).

Reference	Treatment[1,2]	Amount (g/d)	n and duration	Main outcome
Griffin et al., 2002	Oligofructose (Raftilose P95)	8	60 female adolescents, 21 days	Fractional Ca absorption on oligofructose 31.8% *cf.* 31.8% on control (P = 0.75)
	Inulin oligo- and polysaccharides (Synergy 1)	8		Ca absorption on inulin and oligofructose 38.2% *cf.* 32.3% on control (P= 0.007)
Tahiri et al., 2003	Short-chain FOS	10	14 young women, 5 weeks	True Ca absorption on treatment 36.6% *cf*. 35.6% on control (P > 0.05)
Lidestri et al., 2003	9:1 mix galacto- and fructo-oligosaccharides	2.7 + 0.3	30 healthy pre-term infants, 4 weeks	Urinary Ca on treatment 1.63 *cf*. 0.93 mmol/L on control (P = 0.055)
Abrams et al., 2005[3]	Inulin oligo- and polysaccharides (Synergy 1)	8	100 adolescents, 1 year	Fractional Ca absorption 37.7% on treatment *cf*. 31.7% on control (P = 0.04)
Dahl et al., 2005	Inulin (Frutafit IQ)	15	15 wheelchair-bound adults, 3 wk	Urinary Ca on inulin 0.53 *cf*. 0.55 nM/mM Cr on control (P > 0.05)
Lopez-Huertas et al., 2006	Short-chain FOS (Ebro-Puleva)	1	15 young adults, acute	Fractional Ca absorption on treatment 25.6% *cf*. 24.5% on control (P > 0.05)
Holloway et al.,2007	Inulin oligo- and polysaccharides (Synergy 1)	10	15 post-menopausal women, 6-wk	Change in true absorption +5.1% on treatment *cf*. -3.3% on control (P < 0.05)

[1] The following branded products were used:

Orafti Raftiline ST – Inulin as extracted from the Chicory plant
Raftiline HP – Long chain inulin - inulin with the oligosaccharide fraction removed
Raftilose P95 – Partially hydrolyzed inulin (5% monosaccharides, 95% oligosaccharides)
Synergy1 – A 1:1 mix of long chain inulin and partially hydrolyzed inulin

Sensus Frutafit IQ - Instantised powdered inulin

Ebro-Puleva FOS – Fructo-oligosaccharide enzymatically synthesized from sucrose

[2] Other products:

Coudray et al - Agro Industries Research and Development – Inulin extracted from Chicory roots

Tahiri et al – No manufacturers details. Possibly Actilight FOS synthesized from sugar as this product was used by this group in another study.

Lidestri et al – The fructan part of the mix was described as inulin with a reduced amount of low molecular mass fraction; and as fructo-oligosaccharides with a degree of polymerization greater than 10. This description matches that of Raftiline HP.

[3] Parallel study design

The variety of products used, differences in the amounts used, and the small number of studies would make comparisons of effect among the products difficult to assess. However, the data strongly suggest that inulin-derived substances and FOS added to diets do not inhibit the absorption of magnesium, iron, zinc, copper, selenium or calcium along the small intestine. In addition, some of the data is indicative of an enhancement of calcium, copper and magnesium absorption in the large intestine when inulin-derived substances are included in the diet.

References

Abrams SA, Griffin IJ, Hawthorne KM, Liang L, Gunn SK, Darlington G, Ellis KJ. A combination of prebiotic short- and long-chain inulin-type fructans enhances calcium absorption and bone mineralization in young adolescents. Am J Clin Nutr. 2005 Aug;82(2):471-6.

Alliet, P., Scholtens, P., Raes, M., Hensen, K., Jongen, H., Rummens, J.L., Boehm, G., Vandenplas, Y. (2007) Effect of prebiotic galacto-oligosaccharide, long-chain fructo-oligosaccharide infant formula on serum cholesterol and triacylglycerol levels. *Nutrition,* 23(10):719-23. Epub 2007 Jul 30.

Bakker-Zierikzee, A.M., Alles, M.S., Knol, J. Kok, F.J., Tolboom, J.M. Bindels, J.G. (2005) Effects of infant formula containing a mixture of galacto- and fructo-oligosaccharides or viable Bifidobacterium animalis on the intestinal microflora during the first 4 months of life. *Br. J. Nutr.*, 94:783-90.

Bakker-Zierikzee, A.M., Alles, M.S., Knol, J. Kok, F.J., Tolboom, J.M. Bindels, J.G. (2005) Prebiotic oligosaccharides in infant nutrition: addition of only galacto-oligosaccharides does not induce faecal acidic pH and SCFA-spectrum typical for breast fed infants. *J. Pediatr. Gastroenterol. Nutr.,* 40:693. Abstract - ESPGHAN 38th Annual Meeting

Ben, X.M., Zhou, X.Y., Zhao, W.H., Yu, W.L., Pan, W., Zhang, W.L., Wu, S.M., Van Beusekom, C.M., Schaafsma, A. (2004) Supplementation of milk formula with galacto-oligosaccharides improves intestinal micro-flora and fermentation in term infants.
Chin. Med. J.,117(6):927-31.

Boehm, G., Stahl, B., Jelinek, J., Knol, J., Miniello, V., Moro, G.E. (2005) Prebiotic carbohydrates in human milk and formulas. *Acta Pædiatrica*, 94(Suppl 449):18–21

Boehm, G., Lidestri, M., Casetta, P., Jelinek, J., Negretti, F., Stahl, B., Marini, A. (2002) Supplementation of a bovine milk formula with an oligosaccharide mixture increases counts of faecal bifidobacteria in preterm infants. *Arch. Dis. Child Fetal Neonatal Ed.*, 86(3):F178-81.

Bongers, M.E., de Lorijn F., Reitsma, J.B., Groeneweg, M., Taminiau, J.A., Benninga, M.A. (2007) The clinical effect of a new infant formula in term infants with constipation: a double-blind, randomized cross-over trial. *Nutr. J.,* 11;6:8.

Bosscher D, Van Caillie-Bertrand M, Deelstra H. Effect of thickening agents, based on soluble dietary fiber, on the availability of calcium, iron, and zinc from infant formulas. Nutrition. 2001 Jul-Aug;17(7-8):614-8.

Brunser, O., Gotteland, M., Cruchet, S., Figueroa, G., Garrido, D., Steenhout, P. (2006) Effect of a milk formula with prebiotics on the intestinal microbiota of infants after an antibiotic treatment. *Pediatr. Res..*,59(3):451-6.

Coppa, G.V., Bruni, S., Morelli, L., Soldi, S., Gabrielli, O. (2004) The first prebiotics in humans: human milk oligosaccharides. *J. Clin. Gastroenterol.*, 38 (Suppl 2): S80-S83

Coppa G.V., Gabrielli, O., Pierani, P., Catassi, C., Carlucci, A., Giorgi, P.L (1993). Changes in carbohydrate composition in human milk over 4 months of lactation. *Pediatrics*, 91(3):637-41.

Costalos, C., Kapiki, A., Apostolou, M., Papathoma, E. (2007) The effect of a prebiotic supplemented formula on growth and stool microbiology of term infants.
Early Hum. Dev. Apr 10; [Epub ahead of print]

Coudray C, Bellanger J, Castiglia-Delavaud C, Remesy C, Vermorel M, Rayssignuier Y. Effect of soluble or partly soluble dietary fibres supplementation on absorption and balance of calcium, magnesium, iron and zinc in healthy young men. Eur J Clin Nutr. 1997 Jun;51(6):375-80.

Dahl WJ, Whiting SJ, Isaac TM, Weeks SJ, Arnold CJ. Effects of thickened beverages fortified with inulin on beverage acceptance, gastrointestinal function, and bone resorption in institutionalized adults. Nutrition. 2005 Mar;21(3):308-11.

Ducros V, Arnaud J, Tahiri M, Coudray C, Bornet F, Bouteloup-Demange C, Brouns F, Rayssiguier Y, Roussel AM. Influence of short-chain fructo-oligosaccharides (sc-FOS) on absorption of Cu, Zn, and Se in healthy postmenopausal women. J Am Coll Nutr. 2005 Feb;24(1):30-7.

Edwards, C.A., Pratt, A.M. (2002) Intestinal flora during the first months of life: new perspectives. *British J. Nutr.,* 88 (suppl.) 1: S11-S18

Ellegard L, Andersson H, Bosaeus I. Inulin and oligofructose do not influence the absorption of cholesterol, or the excretion of cholesterol, Ca, Mg, Zn, Fe, or bile acids but increases energy excretion in ileostomy subjects. Eur J Clin Nutr. 1997 Jan;51(1):1-5.

Euler, A.R., Mitchell, D.K., Kline, R., Pickering, L.K. (2005) Prebiotic effect of fructo-oligosaccharide supplemented term infant formula at two concentrations compared with unsupplemented formula and human milk. *J. Pediatr. Gastroenterol. Nutr*., 40(2):157-64.

Fanaro, S., Jelinek, J., Stahl, B., Boehm, G., Kock, R., Vigi, V. (2005) Acidic oligosaccharides from pectin hydrolysate as new component for infant formulae: effect on intestinal flora, stool characteristics, and pH. *J. Pediatr. Gastroenterol. Nutr.*, 41(2):186-90.

Fuentes, D., Fuentes, L.A., López, C., Suárez, L., Escobar, H. (2005) Clinical effect of a prebiotic-supplemented formula in healthy infants during the first six months of life. . *J. Pediatr. Gastroenterol. Nutr*., 40(5):660 (Conference Proceeding Abstract)

Griffin IJ, Davila PM, Abrams SA. Non-digestible oligosaccharides and calcium absorption in girls with adequate calcium intakes. Br J Nutr. 2002 May;87 Suppl 2:S187-91.

Holloway L, Moynihan S, Abrams SA, Kent K, Hsu AR, Friedlander AL. Effects of oligofructose-enriched inulin on intestinal absorption of calcium and magnesium and bone turnover markers in postmenopausal women. Br J Nutr. 2007 Feb;97(2):365-72.

Kapiki, A., Costalos, C., Oikonomidou, C., Triantafyllidou, A., Loukatou, E., Pertrohilou, V. (2007) The effect of a fructo-oligosaccharide supplemented formula on gut flora of preterm infants. *Early. Hum. Dev.,* 83(5):335-9.

Kim, S.H., Lee da, H., Meyer, D. (2007) Supplementation of baby formula with native inulin has a prebiotic effect in formula-fed babies. *Asia Pac. J. Clin. Nutr.*;16(1):172-7.

Knol, J., Boehm, G., Lidestri, M., Negretti, F., Jelinek, J., Agosti, M., Stah,l B., Marini, A., Mosca, F. (2005) Increase of faecal bifidobacteria due to dietary oligosaccharides induces a reduction of clinically relevant pathogen germs in the faeces of formula-fed preterm infants. *Acta Paediatr.* Suppl, 94(449):31-3.

Kunz C & Rudloff,S. Health promoting aspects of milk oligosaccharides. Internat. Dairy J. 2006;16:1341-46.

Lidestri, M., Agosti, M., Marini, A., Boehm, G. (2003) Oligosaccharides might stimulate calcium absorption in formula-fed preterm infants. *Acta Paediatr.* Suppl, 91(441):91-2.

Litov, R., Moore, N., Sun, S. (2006) Evaluation of prebiotic tolerance in pre-weaning infants. *J. Pediatr. Gastroenterol. Nutr.*, 43(4):E64 (Conference Proceeding Abstract).

López-Huertas E, Teucher B, Boza JJ, Martínez-Férez A, Majsak-Newman G, Baró L, Carrero JJ, González-Santiago M, Fonollá J, Fairweather-Tait S. Absorption of calcium from milks enriched with fructo-oligosaccharides, caseinophosphopeptides, tricalcium phosphate, and milk solids. Am J Clin Nutr. 2006 Feb;83(2):310-6.

Marini, A., Negretti, F., Boehm, G., Li Destri, M., Clerici-Bagozzi, D., Mosca, F., Agosti, M. (2003) Pro- and pre-biotics administration in preterm infants: colonization and influence on faecal flora. *Acta Paediatr.* Suppl., 91(441):80-1.

Marini, A., Boehm, G., Negretti, F., Agosti, M. (2005) Probiotics, prebiotics, or both in a very low birth weight infant. *Pediatrics*, 116(2):522-3.

Moore, N., Chao, C., Yang, L-P., Storm, H., Oliva-Hemker, M., Saavedra, J.M. (2003) Effects of fructo-oligosaccharide-supplemented infant cereal: a double-blind, randomized trial. *Br. J. Nutr.*, 90:581-587.

Moro, G., Minoli, I., Mosca, M., Fanaro, S., Jelinek, J., Stahl, B., Boehm, G. (2002) Dosage-related bifidogenic effects of galacto- and fructo-oligosaccharides in formula-fed term infants. *J. Pediatr. Gastroenterol. Nutr.*, 34(3):291-5.

Moro, G.E., Mosca, F., Miniello, V., Fanaro, S., Jelinek, J., Stahl, B., Boehm, G. (2003) Effects of a new mixture of prebiotics on faecal flora and stools in term infants. *Acta Paediatr.* Suppl., 91(441):77-9.

Moro, G.E., Stahl, B., Fanaro, S., Jelinek, J., Boehm, G., Coppa G.V. (2005) Dietary prebiotic oligosaccharides are detectable in the faeces of formula-fed infants. *Acta Paediatr.* Suppl., 94(449):27-30.

Moro, G., Arslanoglu, S., Stah, l. B., Jelinek, J., Wahn, U., Boehm, G. (2006) A mixture of prebiotic oligosaccharides reduces the incidence of atopic dermatitis during the first six months of age. *Arch. Dis. Child.*, 91(10):814-9.

Olano-Martin, E., Mountzouris, K.C., Gibson, G.R., Rastall, R.A. (2000) In vitro fermentability of dextran, oligodextran and maltodextrin by human gut bacteria. *Br. J. Nutr.*, 83(3):247-55.

Probert, H.M., Gibson, G.R. (2002) Investigating the prebiotic and gas-generating effects of selected carbohydrates on the human colonic microflora. *Lett. Appl. Microbiol.*, 35(6):473-80.

Puccio, G., Cajozzo, C., Meli, F., Rochat, F., Grathwohl, D., Steenhout, P. (2007) Clinical evaluation of a new starter formula for infants containing live Bifidobacterium longum BL999 and prebiotics. *Nutrition*, 23(1):1-8.

Quinlan, P.T., Lockton S., Irwin J., Lucas, A.L. (1995) The relationship between stool hardness and stool composition in breast- and formula-fed infants. *J. Pediatr. Gastroenterol. Nutr.*, 20:81-90.

Reinhold JG, Faradji B, Abadi P, Ismail-Beigi F. Decreased absorption of calcium, magnesium, zinc and phosphorus by humans due to increased fiber and phosphorus consumption as wheat bread. J Nutr. 1976 Apr;106(4):493-503.

Rycroft, C.E., Jones, M.R., Gibson, G.R., Rastall, R.A. (2001) Fermentation properties of gentio-oligosaccharides. *Lett. Appl. Microbiol.*, 32(3):156-61.

Stahl B, Thurl S, Zeng J, Karas M, Hillenkamp F, Steup M, Sawatzki G. Oligosaccharides from human milk as revealed by matrix-assisted laser desorption/ionization mass spectrometry. Anal Biochem. 1994 Dec;223(2):218-26.

Schmelzle, H., Wirth, S., Skopnik, H., Radke, M., Knol, J., Bockler, H.M., Bronstrup, A., Wells, J., Fusch, C. (2003) Randomized double-blind study of the nutritional efficacy and bifidogenicity of a new infant formula containing partially hydrolyzed protein, a high beta-palmitic acid level, and nondigestible oligosaccharides. *J. Pediatr. Gastroenterol. Nutr.*, 36(3):343-51.

Scholtens, P.A., Alles, M.S., Bindels, J.G., van der Linde, E.G., Tolboom, J.J., Knol, J. (2006) bifidogenic effects of solid weaning foods with added prebiotic oligosaccharides: a randomised controlled clinical trial. *J. Pediatr. Gastroenterol Nutr.,* 42(5):553-9.

Stepans, M.B., Wilhelm, S.L., Hertzog, M., Rodehorst, T.K., Blaney, S., Clemens, B., Polak, J.J., Newburg, D.S. (2006) Early consumption of human milk oligosaccharides is inversely related to subsequent risk of respiratory and enteric disease in infants. *Breastfeed. Med*., 1:207-15

Tahiri M, Tressol JC, Arnaud J, Bornet FR, Bouteloup-Demange C, Feillet-Coudray C, Brandolini M, Ducros V, Pepin D, Brouns F, Roussel AM, Rayssiguier Y, Coudray C. Effect of short-chain fructo-oligosaccharides on intestinal calcium absorption and calcium status in postmenopausal women: a stable-isotope study. Am J Clin Nutr. 2003 Feb;77(2):449-57.

Tahiri M, Tressol JC, Arnaud J, Bornet F, Bouteloup-Demange C, Feillet-Coudray C, Ducros V, Pepin D, Brouns F, Rayssiguier AM, Coudray C. Five-week intake of short-chain fructo-oligosaccharides increases intestinal absorption and status of magnesium in postmenopausal women. J Bone Miner Res. 2001 Nov;16(11):2152-60.

Teuri U, Karkkainen M, Lamberg-Allardt C, Korpela R. Addition of inulin to breakfast does not acutely affect serum ionized calcium and parathyroid hormone concentrations. Ann Nutr Metab. 1999;43(6):356-64.

van den Heuvel EG, Muys T, van Dokkum W, Schaafsma G. Oligofructose stimulates calcium absorption in adolescents. Am J Clin Nutr. 1999 Mar;69(3):544-8.

van den Heuvel EG, Schaafsma G, Muys T, van Dokkum W. Nondigestible oligosaccharides do not interfere with calcium and nonheme-iron absorption in young, healthy men. Am J Clin Nutr. 1998 Mar;67(3):445-51.

Waligora-Dupriet, A.J., Campeotto, F., Nicolis, I., Bonet, A., Soulaines, P., Dupont, C., Butel, M.J. (2007) Effect of inulin polysaccharides supplementation on gut microflora and well-being in young children attending a day care centre. *Int. J. Food Microbiol.*, 1;113(1):108-13.

Ziegler, E., Vanderhoof, J.A., Petschow, B., Mitmesser, S.H., Stolz, S.I., Harris, C.L., Berseth, C.L. (2007) Term infants fed formula supplemented with selected blends of prebiotics grow normally and have soft stools similar to those reported for breastfed infants.
J Pediatr Gastroenterol Nutr., 44(3):359-64.

Attachment 6

Safety Assessment

Summary

Inulin (average Degree of Polymerisation ≥10), oligofructose (average DP <10) and fructo-oligosaccharides (FOS, average DP<4) have been used in the general food supply for over ten years without any reported adverse effects. This Proposal (P306) has been raised to consider whether oligofructose, inulin and galacto-oligosaccharides (GOS, average DP 2-8) ought to be permitted to be voluntarily added to infant formula products and infant foods with the purpose of providing a prebiotic effect for formula-fed infants. This assessment aims to determine the safety of these indigestible oligosaccharides when added to infant formula products (including infant formula and follow-on formula), toddler formula and infant foods. For the purpose of this report the term 'inulin-derived substances' refers to oligofructose and inulin.

Inulin-derived substances, composed primarily of fructose, are not present in human milk. Trace amounts of GOS (composed primarily of galactose) may be present. Human milk contains a mixture of complex oligo- and polysaccharides (referred to in this report as human milk oligosaccharides) at levels up to 25 g/L in colostrum and somewhat less in mature milk. These human milk oligosaccharides (HMOs) are not digested in the upper gastrointestinal tract but enter the colon where they are partially fermented by the colonic microflora to short chain volatile fatty acids (SCFAs). These SCFAs may be absorbed, fermented further to form carbon dioxide, or excreted in the faeces.

FSANZ has assessed the evidence on the potential for inulin-derived substances and GOS to cause adverse effects in infants and young children. These soluble oligosaccharides, like naturally occurring HMOs, are not digested to any great extent in the small intestine, and reach the large intestine intact where they are also fermented by colonic bacteria to SCFAs and carbon dioxide. There is virtually no systemic exposure to these intact oligosaccharides, therefore the only possible adverse effect identified was an increased osmotic potential within the colon, potentially leading to increased water loss and dehydration. This possibility had also previously been considered by the European Scientific Committee on Food.

FSANZ has considered this potential risk. It was concluded that inulin-derived substances and GOS either alone or in combination at concentrations up to 8 g/L will beneficially contribute to increased osmotic potential in the colon of infant formula-fed infants; the increase is considered to be no greater than in breast-fed infants where undigested HMOs also enter the colon. To reach this conclusion FSANZ has considered the following evidence:

- HMOs in breast milk at levels up to 25 g/L are safe for breast-fed infants;
- GOS and long chain inulin (9:1) preparations at 8 g/L are safe for formula-fed infants;
- Oligofructose preparations at 3 g/L are safe for formula-fed infants; and
- *In vitro* evidence that inulin-derived substances and GOS are fermented by colonic microflora to a similar or greater extent than HMOs.

Although it is concluded that up to 8 g/L inulin-derived substance is unlikely to pose a public health and safety risk to infants, evidence from adult studies suggests that some individuals experience increased flatulence and bloating upon consumption of high levels of inulin-derived substances. It is not clear if this would occur in infants due to differences in colonic microflora and overall diet. However, while not usually an endpoint considered in a conventional safety assessment, gastrointestinal discomfort in young infants is undesirable. Therefore it may be prudent to limit the addition of inulin-derived substances to levels which have been shown to be well tolerated in infants i.e. 3 g/L.

Some gastrointestinal discomfort may initially be experienced by young infants changing from breast milk or conventional formula to oligosaccharide-supplemented formula. The phenomenon of changed gastrointestinal effects is not uncommon for infants when their formula is changed. It is anticipated that this effect will be less evident in older infants (e.g. 6 months and over).

Unlike infant formula products, toddler formula and infant foods do not represent the sole source of nutrition for young children. It follows that if GOS, oligofructose and inulin are safe for newborns and infants, they will be equally safe for older infants and young children.

Limited information is available on the safety of FOS in infant formula and foods for young children, therefore FSANZ did not consider FOS for infants as part of this Proposal.

1. Safety assessment of inulin-derived substances and GOS

FSANZ has not previously assessed the safety of inulin-derived substances or GOS in infant formula products, formulated supplementary foods for young children (FSFYC, e.g. toddler formula), and infant foods. Therefore, the aim of the current assessment was to review the published data on the chemical characteristics, use and effects of inulin-derived substances and GOS to determine their safety in these products. This covers the age group zero to three years. The use of inulin-derived substances as ingredients in the general food supply has also been considered.

1.1 Human milk oligosaccharides – Chemistry and metabolism

In human milk, a mixture of complex oligosaccharides is present. Complex oligosaccharides are branched oligosaccharides with a range of monosaccharides. To date over 200 complex oligosaccharides have been identified in breast milk, however, it is thought that the total number may be in the thousands (Boehm *et al*., 2005; Ninonuevo *et al*., 2006). The total oligo- and polysaccharide content of human milk is reported to be up to 25 g/L during the first few weeks following birth (Coppa *et al*., 1993; Coppa *et al*., 1997). Of this, up to 15 g/L are neutral oligo- and polysaccharides. It appears that levels are usually highest in colostrum, with lower levels reported in mature milk. These oligosaccharides generally have a lactose unit at their reducing end, linked to other monosaccharides by β1-3 or β1-6 linkages, although other β-glycosidic and α-glycosidic bonds may also be present. The saccharides present include glucose, galactose, fucose and N-acetylglucosamine. The degree of polymerisation of these oligosaccharides has been reported to be predominantly up to 8, but polysaccharides are present with DPs of greater than 20 (Boehm *et al*., 2003). Other sources reports the DP to range between three and thirteen (Stahl *et al.*, 2005) and over 50 (Attachment 3 – Breast milk composition).

Fructose and inulin-derived substances are not found in human milk; however the galacto-oligosaccharides 6'-galactosyl-lactose and 3'- galactosyl-lactose have been found at trace levels. Further information on the types and concentrations of oligo- and polysaccharides in human milk is in Attachment 3 – Breast milk composition.

It appears that human milk oligosaccharides (HMOs) are resistant to hydrolysis in the upper gastrointestinal tract. HMOs have been shown to be resistant to *in vitro* degradation by enzymes present in human and porcine brush border membranes, and human duodenal aspirates (Engfer *et al.,* 2000). Gnoth et al. also demonstrated that HMOs are resistant to acidic hydrolysis *in vitro* and are not digested by human salivary amylase or porcine pancreatic amylase (Gnoth *et al*., 2000).

Trace amounts of HMOs have been detected in preterm breast-fed infants' urine (Rudloff *et al*., 1996), supporting the evidence that they are resistant to digestion by amylases and glycosidases in the small intestine. Intact oligosaccharides are generally not absorbed by adults; the presence of HMOs in infant urine may be due to the higher intrinsic permeability of the infants' gut, however the mechanism by which absorption occurs is not known (Engfer *et al.,* 2000).

In the large intestine, HMOs are fermented by colonic microflora (Engfer *et al*., 2000) where they are converted to short chain volatile fatty acids (predominantly acetate, propionate and butyrate) which are mostly absorbed, preventing osmotic diarrhoea (Parrett and Edwards, 1997). The exact fermentation end products will vary depending on the genera of micro-organisms principally involved in their breakdown. In one-month old breast-fed infants, fermentation of HMOs is only partial, with around 40-50% of those ingested being excreted in the faeces (Coppa *et al*., 1999; Coppa *et al*., 2001). After one month of lactation the HMO concentration in breast milk is reported to be around 16 g/L (See Table 3, Attachment 3).

1.2 Inulin-derived substances, FOS and GOS – Chemistry and metabolism

Inulin is a polydisperse β2-1 fructan, with an average chain length of greater than 10 fructose units. The fructose units are linear, typically with a glucose unit at one end linked by an α1-2 bond, although glucose is not always present (Niness, 1999; Flickinger *et al*., 2003; Gibson *et al*., 2005). Inulin has high solubility in water and alcohol, and is not detected by traditional dietary fibre analyses (Flickinger *et al.,* 2003). Oligofructose is defined as having a chain length of less than 10 fructose units, while FOS has a chain length of less than 4 fructose units.

GOS is comprised of a glucose residue joined to galactose by a β1-4 glycosidic bond, to form lactose. Additional galactose units are joined to the first galactose by predominantly β1-4 or β1-6 glycosidic bonds (Schmelzle *et al*., 2003). β1-3 glycosidic bonds may also be present. The chain length of GOS is generally between 2-8 monosaccharide residues.

The β -glycosidic linkages present in inulin-derived substances and GOS (as well as HMOs) are broken down by different enzymes to those that break down α -glycosidic bonds. In general, β-glycosidic bonds are not digested by human gastrointestinal enzymes, whereas α -glycosidic bonds (such as those in starch) are quickly hydrolysed. For this reason, inulin-derived substances and GOS will not be hydrolysed to any great extent in the upper gastrointestinal tract; rather they will be transported mostly intact to the colon where bacterial fermentation occurs.

This was confirmed in a study on the *in vitro* digestibility of GOS by human intestinal enzymes. It was shown that small intestinal β-galactosidase activity on GOS was less than 10% of its activity on lactose (Boehm *et al.,* 2005). Similarly, for inulin, studies with ileostomists have shown on average 86-89% of ingested inulin is not digested in the small intestine and can be recovered from the terminal ileum (Cummings *et al*., 2001b).

Inulin and GOS have been detected in the faeces of infants fed formula containing 8 g/L of the 9:1 (GOS:inulin) formulation (Moro *et al*., 2005). However, the amount detected was not quantified so it is unclear what proportion this represents. Another study similarly detected inulin and GOS in the faeces of 4-week old, pre-term infants fed formula containing the 9:1 preparation. The authors concluded that inulin-derived substances and GOS are at least partially unfermented, however, again the amount of oligosaccharides in the faeces was not quantified (Moro *et al*., 2004). In an *in vitro* study colonic bacteria in the faeces of breast-fed and formula-fed infants were capable of fermenting GOS and inulin (9:1) into the short chain volatile fatty acids acetate, propionate and butyrate (Boehm *et al*., 2004).

In human adults, inulin is completely fermented in the large intestine (Cherbut, 2002). No fructans were detected in the faeces of 6 healthy adults given 20 g FOS/day for 11 days (Molis *et al*., 1996), 24 volunteers given 5 and 15 g FOS/day for seven days (Alles *et al*., 1996) or three volunteers given 50 g inulin/day for 16 days (Castiglia-Delavaud *et al*., 1998). These findings are consistent with what would be expected given the chemical structure of inulin and its similarities with other fermentable carbohydrates. That is, it is soluble in water, typically non-viscous and does not appear to bind bile acids (Schneeman, 1999).

Tolerance studies

Laboratory animals

A conventional 90-day oral (gavage) toxicity study conducted in young adult rats dosed with 2500 or 5000 mg/kg bw/day Vivinal ® GOS syrup (45% GOS) reported the No Observed Adverse Effect Level (NOAEL) to be 5000 mg/kg/bw/day (the highest dose tested) (Anthony JC. *et al*., 2006). This is equivalent to 2250 mg/kg bw/day of GOS.

Humans

A number of clinical studies have been conducted in human infants fed infant formula containing GOS and inulin-derived substances, in varying ratios and concentrations and for a range of durations (up to 6 months). The most commonly used GOS:inulin preparation is a 9:1 GOS:inulin preparation, added to infant formula at a level of 8 g/L. Concentrations of 4, 6 and 10 g/L have also been studied, as has GOS alone. The infants' ages vary between studies from preterm infants up to over 12 month-olds. Most of these studies have focused on the effect of GOS:inulin fortified formula on stool characteristics (frequency, consistency, bacterial content) and other gastrointestinal symptoms, compared to infants fed unfortified formula or breast milk. More detail on the effects of GOS and inulin-derived substances on stool frequency and consistency is given in Attachment 5 – Nutrition Assessment. The highest level studied is 10 g/L (9:1 GOS:inulin) in a 28-day study in preterm infants. In general, no adverse effects have been reported (see for example (Boehm *et al*., 2003; Moro *et al*., 2003; Schmelzle *et al*., 2003; Bakker-Zierikzee *et al*., 2005; Knol *et al*., 2005; Fanaro *et al*., 2005a; Ziegler *et al*., 2007; Alliet *et al*., 2007; Costalos *et al*., 2007; Bongers *et al*., 2007).

Other studies examined effects of GOS and oligofructose supplemented formula on the occurrence of atopic dermatitis, cholesterol, and HDL and LDL levels. No adverse effects were reported in these studies (Moro *et al*., 2006; Alliet *et al.,* 2007).

Some studies reported on gastrointestinal effects in infants given non-digestible oligosaccharides in infant formula products. Twenty-three infants less than 14-days received formula containing 4 g/L of the 9:1 ratio for 7 months. (Puccio *et al*., 2007). The formula also contained *Bifidobacterium longum* BL999. Occurrence of flatulence, spitting up/vomiting, comfort of the infant based on frequency of crying, restlessness and irritability and occurrence of parent diagnosed colic were recorded. More serious events (rhinitis, wheezing, cough, respiratory tract infection, diarrhoea, constipation, colic, fever, and rash) were also recorded based on inquires to the parents. Results indicated that compared with the control group, infants in the supplemented group were less likely to have flatulence. Data on frequency of crying, restlessness, colic, spitting, and vomiting showed no statistically significant differences between the two study groups. Occurrence of these events, serious or otherwise, was similar between groups.

In a 6-week study, 46 term infants were divided into three groups and fed formula supplemented with 2 g/L acidic oligosaccharides and 6 g/L maltodextrin, 2 g/L acidic oligosaccharides and 6 g/L of the 9:1 GOS:inulin preparation, or control formula containing 8 g/L maltodextrin. Possible side effects (crying, vomiting and regurgitation) were recorded and no differences were observed among the test groups (Fanaro *et al*., 2005b)

In other studies, similar results have been seen. Three and six month supplementation of formula-fed term infants with 2.4 g/L GOS alone had no influence on incidence of side effects (including crying, regurgitation, and vomiting) (Ben *et al*., 2004). In term infants given 0, 4 or 8 g/L of the 9:1 ratio for 28 days, supplementation had no influence on the incidence of side effects (crying, regurgitation, vomiting) or growth (Moro *et al*., 2002). Ninety-six infants younger than 4 months with colic given formula supplemented with 8 g/L of the 9:1 preparation for 14 days, had significantly decreased episodes of crying related to colic than those given a formula containing only 4 g/L GOS and inulin (Savino *et al*., 2006). Term infants with atopic dermatitis were given formula supplemented with 8 g/L 9:1 (GOS:inulin) for up to 6 months. Formula was well tolerated and there were significantly lower reports of regurgitation and crying in the group fed the GOS:inulin-supplemented formula relative to a group fed formula containing maltodextrin. There was no difference in the reported incidence of vomiting (Moro *et al*., 2006).

In a double-blind, randomised, controlled study of 120 days duration, 14-day old term infants were given either control formula (containing no oligosaccharides), or formula containing either 4 g/L GOS and polydextrose (50:50) or 8 g/L of polydextrose, GOS and lactulose (50:33:17) (Ziegler *et al*., 2007). Levels of GOS were therefore 0, 2 or 2.6 g/L. Parents rated infants' fussiness, fussiness relative to normal, gas, and gas relative to normal. Dropout rates were not statistically significantly different between groups; of 226 infants commencing the study, 164 completed it (24%, 22% and 36% dropout in each group). The most common reason for dropout (including the control group) was intolerance to the formula, including gas, fussiness and diarrhoea. Infants receiving the test formulas had more gastrointestinal effects (e.g. gas and diarrhoea) however given the presence of other prebiotics (polydextrose and lactulose) it is difficult to attribute this to the presence of GOS. The two test formulas had similar GOS levels (2 and 2.6 g/L), which are lower than those used in other studies where no adverse effects were observed.

There are a small number of published studies on the effects of formula supplemented with oligofructose alone in young infants. The longest study was in 297 healthy, term infants (<14 days old) given formula supplemented with 0, 1.5 or 3 g oligofructose (raflilose95)/L for 12 weeks (Bettler and Euler, 2006). Growth, clinical chemistry at baseline and 12 weeks (albumin, blood urea nitrogen, calcium, magnesium, phosphorus, creatinine, triglycerides, low-density lipoprotein, and cholesterol), and adverse events (*e.g.* abdominal pain, allergic reaction, constipation, diarrhoea, flatulence, irritability, loose stools, rash, spitting up and vomiting) were measured. Two-hundred and twelve infants completed the study (67%, 73% and 73% from the control, low- and high-dose groups respectively). Growth, tolerance of the formula and reported adverse events were similar between the groups. Mean values for growth and clinical chemistry results at 12 weeks were within the normal range, and it was concluded that both test formulas were safe for young infants. Fifty-five percent of infants had at least one event that was considered to be formula-related. The 3 g/L group had less formula-related events compared with the other groups. The 1.5 g/L group had slightly more events than the control group. None were considered serious.

In other studies on oligofructose alone, no adverse effects were reported in 36 preterm infants (mean gestational age 33.9 weeks, 0-14 days old) receiving supplemented formula (4 g/L) for 14 days relative to a control group of 20 similar infants receiving un-supplemented formula (Kapiki *et al*., 2007). More frequent stools were also observed in this study. Softer stools and increased stool frequency were also observed in a study in 14 infants with an average age of 12 weeks, given an average of 0.25g inulin /kg bw/day (average 1.5 g/day) for 3 weeks (Kim *et al*., 2007).

In a prospective, randomised, cross-over study in 58 infants (term, 2-6 weeks old) given formula containing either 1.5 g/L or 3 g/L oligofructose for one week out of five, an increased incidence of flatulence and regurgitation were reported during the week of oligofructose supplementation (Euler *et al*., 2005). Looser stools were also observed during the week of supplementation. These effects were less frequent in the 1.5 g/L dose group relative to the 3 g/L group: seven infants (21%) versus 10 (31%) with flatulence, four infants (12%) versus nine (28%) with regurgitation and five infants (15%) versus 10 (31%) with looser stools. No comparison of occurrence of flatulence or regurgitation was made with the control group of 14 human milk-fed infants, however stool frequency was increased and stool consistency was looser in the human milk-fed infants compared with either formula-fed groups.

FOS has not been studied in infant formula products to any extent.

In older infants fewer studies have been conducted. Oligofructose has been studied in older infants (4-24 months) in toddler formula or cereal, at levels from 0.67 g/day for 6 months (Duggan *et al*., 2003), 2 - 2.25 g /day for 3 weeks (Brunser *et al*., 2006; Waligora-Dupriet AJ *et al*., 2007), an average of 1.05 g/day with a maximum of 3 g/ day (Moore *et al*., 2003) and an average of 1.1 g/day or up to 0.8 g/kg body weight per day (duration not stated) (Saavedra and Tschernia, 2002) without any reported adverse effects. Brunser *et al* recorded observed gastrointestinal symptoms (flatulence, restlessness, cramping, pain, crying and vomiting), and the results indicated these were similar in the treated and control groups (Brunser *et al*., 2006). Scholtens et al reported feeding GOS/inulin (9:1 ratio) supplemented weaning products to 11 infants with a mean age of 4 months for 6 weeks. The mean intake was 4.05 g/day, no comment was made on the tolerance of the weaning foods (Scholtens *et al*., 2006).

Inulin-derived substances and GOS in the general food supply

Inulin is considered to be a dietary fibre in the general food supply. Dietary fructans including inulin occur naturally in plant foods such as wheat, bananas, Jerusalem artichokes, artichokes, onions and leeks. Average daily consumption of fructans has been estimated as 1-4 g in the United States and 3-11 g in Europe (Roberfroid and Delzenne, 1998). Other estimates of dietary exposure to inulin suggest adults may consume up to 10 g inulin per day from a range of foods containing naturally occurring or added inulin and inulin-derived substances.

Up to 20-30 g/day of inulin-derived substances and FOS can be tolerated by many adults (Briet *et al*., 1995), although increased flatulence was observed in adults taking 10 g/day 'fructo oligosaccharides' (DP not reported) for four weeks (Cummings *et al*., 2001a). However, out of 117 people starting the study in the fructo-oligosaccharide group, only three dropped out due to flatulence. A statistically significant increase in perceived well-being was also noted in the fructo-oligosaccharide group relative to a control group. A high level of consumption (e.g. over 30 g/day) may result in increased flatulence, and may cause more severe side effects such as stomach cramps and diarrhoea, however, these are common effects of over consumption of any dietary fibre. Gastrointestinal tolerance varies between individuals and is also influenced by diet (Coussement, 1999). In addition excessive consumption is likely to be self-limiting due to the unpleasant side effects (TGA, 1998).

There are two published reports of individual patients with an allergy to inulin (Gay-Crosier *et al*., 2000; Franck *et al*., 2005). Both individuals exhibited allergies to artichoke, and some processed foods containing inulin. However, the rarity of these cases indicates that from a public health and safety perspective inulin is an insignificant allergen. It is not recognised as a major allergen in Australia and New Zealand or elsewhere.

GOS is generally not present in foods, although it may be present at low levels in some processed dairy products, such as lactose reduced milk and yoghurt. Short term studies in adults have been done with GOS including 10 g GOS/day for 21 days, 15 g/day for 6 days and single intakes of 30 g, without reported adverse effects (cited in (Boehm *et al.,* 2005). However, intakes of GOS from the general food supply are likely to be negligible. The addition of GOS to the general food supply is not being considered as part of this proposal.

2. Discussion

Infants consuming breast milk tend to have a lower renal solute load[40] than those on conventional infant formula products. Similarly breast-fed infants tend to have softer stools than formula-fed infants. These effects are thought to be due to the presence of undigested or partially digested HMOs in the colon exerting an osmotic potential[41]. This osmotic effect is expected to be greatest in very young breast-fed infants without established colonic microflora as HMOs in milk will not fermented at all.

[40] The potential renal solute load of infant food is the sum of dietary nitrogen (expressed as mmol of urea divided by 28), sodium, potassium, chloride and phosphorus.

[41] Osmotic potential occurs when two solutions with differing solute concentrations are separated by a membrane that is only permeable to water. To achieve equilibrium, water is drawn from the side of the membrane that has a lower solute concentration to the side with the higher concentration in an effort to equalise the concentration on either side of the membrane.

Following microbial colonisation, around 60% of the HMOs are fermented, with only the remaining 40% having the potential to exert osmotic pressure and being excreted in the faeces.

Similar to HMOs in breast milk, inulin-derived substances and GOS are not digested to any great extent in the small intestine and reach the colon largely intact. In considering the safety of inulin-derived substances and GOS in infant formula products, it was important to consider whether the presence of undigested oligosaccharides in the colon could lead to excess water excretion in the faeces, causing diarrhoea and dehydration. A newborn infant's renal system is not fully developed and so has a limited capacity to concentrate urine. If excess water loss through faeces occurs then the renal concentrating ability may be exceeded leading to a negative water balance (dehydration). This issue was considered in two parts: for newborns without the ability to ferment oligosaccharides (no significant colonic microflora); and for young infants (< 12 months) with the ability to ferment oligosaccharides (colonic microflora).

In newborn formula-fed infants, prior to the establishment of colonic microflora, inulin-derived substances and GOS would not be fermented in the large intestine, and are excreted unchanged in the faeces. This is likely to contribute to a slightly increased osmotic potential relative to infants fed formula not containing 8 g/L GOS:inulin. However, in comparison to breast-fed infants, where the HMO concentration is up to 25 g/L, the levels of undigested oligosaccharides in the colon of infants fed GOS and inulin-supplemented infant formula products will be less than half those of breast-fed infants. Therefore, it is unlikely that there is any risk to these very young infants from the presence of inulin-derived substances and GOS in infant formula at the levels suggested.

In regard to young infants where the colonic microflora has become established, some of the undigested oligosaccharides will be fermented. In 1-month old, breast-fed infants only around 60% of ingested HMOs (16 g) are fermented; if a similar proportion of GOS and inulin-derived substances (at 8 g/L) are fermented in formula-fed infants, the remaining undigested oligosaccharides would be unlikely to pose a risk to infants. There is evidence which indicates that this is likely to be the case. Based on the presence of short chain fatty acids in formula-fed infants' stools, and *in vitro* studies indicating that colonic bacteria from formula-fed infants produce short chain fatty acids in the presence of GOS and inulin, it appears that a significant portion of GOS and inulin are fermented. It has also been shown that inulin is totally fermented in the large intestine of human adults; although there may be differences between young infants and adults in regard to fermentation capacity, where a substance is entirely fermented in adults, it is likely that some fermentation also occurs in infants. In addition, an *in vitro* study on the differences in fermentation capabilities between breast-fed and formula-fed infants suggests both groups have similar capability to ferment complex oligosaccharides into short chain fatty acids (Parrett and Edwards, 1997). Thus there is a high likelihood that GOS and inulin-derived substances would be fermented effectively in infants. Only around a quarter of the intake of added undigested oligosaccharides would need to be fermented to give a similar level in the colon as HMOs (given around 15 g/L HMOs of which 50-60% are fermented). The very high probability that such a level of fermentation occurs reduces the level of concern that water balance could be adversely affected by an increase in osmotic potential due to undigested inulin-derived substances and GOS in the colon. Furthermore, the level of proposed addition of non-digestible oligosaccharides to infant formula (8 g/L) is lower than the level of oligosaccharides in human milk (up to 25 g/L); even if no fermentation of inulin-derived substances occurred, the amount of oligosaccharides in the colon would be in the same range as breast-fed infants.

The contention that GOS and inulin are at least partially fermented in the colon, similarly to HMOs, and are therefore unlikely to affect water balance when used at levels no greater than those in breast milk, is supported by indirect evidence from a range of studies conducted in preterm, term and older infants. These studies indicate that the use of a 9:1 ratio of GOS to inulin in formula at a level of 8 g/L does not cause problems with water balance or other adverse effects.

In regard to the use of other ratios of oligosaccharides, or inulin-derived substances alone in infant formula, FSANZ has considered a concern expressed by EFSA relating to water balance. The available evidence that oligofructose and inulin are fermented by colonic microflora in formula fed infants as described above (presence of SCFA in stool, *in vitro* stool studies, and evidence from adult toleration studies), reduces the concern that water balance could be adversely affected by an increase in osmotic potential due to undigested inulin-derived substances in the colon. In addition, a 12-week study in term infants which indicated oligofructose at 3 g/L had no significant effect on growth, blood chemistry, or reported adverse events, supports the safety of inulin-derived substances in infant formula products. It has been shown that 8 g/L of GOS:long chain inulin is safe, levels as high as 25 g HMOs/L in breast milk is safe, and inulin-derived substances are likely to be fermented to a similar degree to GOS and HMOs, therefore, FSANZ concludes up to 8 g/L added inulin-derived substances will be safe for young infants. This conclusion applies equally to the use of GOS alone or any ratio of GOS:inulin-derived substances so long as the total level of oligosaccharides is no greater than 8 g/L.

The use of ratios of GOS to inulin other than 9:1 has not been reported in the published literature to any extent, therefore the safety of other ratios relies on a theoretical argument that the any effects will be similar to the 9:1 ratio. This seems reasonable provided the total concentration of oligosaccharides does not exceed 8 g/L.

Prebiotics such as GOS and inulin-derived substances are intended to selectively stimulate the growth and/or activity of beneficial bacteria (e.g. bifidobacteria and lactobacilli) in the colon. These bacteria do not generate gas as part of their metabolism; however other bacteria may also be stimulated, and therefore increased flatulence compared to standard formula-fed infants may occur.

In addition, evidence from studies in adults suggests that inulin-derived substances at a daily dose of 10 g cause gastrointestinal symptoms including flatulence and bloating in some individuals. The data from two studies where infants were fed formula containing oligofructose at 3 g/L do not provide clear evidence whether this would also occur in infants. There may be differences in colonic microflora between adults and formula-fed infants, and adults have a much more varied diet compared to infants; both these factors would impact on gas production. However, intestinal discomfort in very young infants is undesirable and appropriate studies (e.g. *in vitro* comparison of the volume of gas produced through fermentation of inulin-derived substances with the complex oligosaccharides found in human breast milk, or direct studies of gastrointestinal symptoms in infants with higher levels of intake) to demonstrate this would not occur are lacking. Therefore, it may be prudent to limit the addition of inulin-derived substances to levels which have been shown to be well tolerated in infants, i.e. 3 g/L in infant formula products.

Some gastrointestinal discomfort may initially be experienced by young infants changing from breast milk or conventional formula to oligosaccharide-supplemented formula.

The phenomenon of changed gastrointestinal effects is not uncommon for infants when their formula is changed. It is anticipated that this effect will be less evident in older infants (e.g. 6 months and over).

The effects of FOS have not been studied to any extent in infant formula and so it has not been considered it in this report.

Formulated Supplementary Foods for Young Children (FSFYC) and infant foods

Young children (1-3 years) have a more developed renal system that is able to more effectively concentrate urine relative to young infants. Therefore dehydration is much less of a concern in the older age group. For this group, FSFYC (toddler formula) and infant foods will not be the sole source of nutrition, so even if inulin-derived substances and GOS concentrations in these foods are higher than those in infant formula, exposure on a body weight basis is likely to be lower than for young infants exclusively consuming oligosaccharide supplemented formula. FSANZ considers the addition of GOS and inulin-derived substances to toddler formula and infant foods at levels similar to those added to infant formula is unlikely to pose a risk to children consuming these foods. Further information on exposure to inulin-derived substances and GOS by different age groups is detailed in Attachment 7 – Dietary Exposure Assessment.

Inulin in the general food supply

FSANZ considered the safety of inulin as part of Application A277 – Inulin and fructo-oligosaccharides as dietary fibre (FSANZ., 2001). Inulin, oligofructose and FOS are currently permitted as food ingredients and have been since prior to 1995, when Application A277 was first made. Since 2001, added inulin has been required to be labelled as dietary fibre, and has been added as an ingredient to a wide range of foods with the aim of increasing the fibre content. It is also used in some foods (e.g. margarine) as a bulking agent.

Although there are two reports of allergy to inulin, it is not a major allergen. Otherwise, no adverse effects have been reported due to the consumption of foods containing inulin, and FSANZ considers inulin-derived substances and FOS to have a history of safe use as food ingredients in the general food supply.

3. European regulatory assessments of GOS and inulin

The safety of GOS and inulin-derived substances in infant formula and in follow-on formula was considered by the Scientific Committee on Food (SCF) in 2001, initially in September (SCF, 2001b) and then again in December (SCF, 2001a). The data considered included six clinical studies in preterm and term infants given infant formula containing 4 or 8 g/L GOS and ‘high molecular weight oligofructose’ (at a 9:1 ratio) or ‘low molecular weight oligofructose’ alone (concentration not stated).

Stool frequency and consistency were found to differ significantly with the two concentrations of GOS and oligofructose. Increased stool frequency and greater watery or fluid stools were observed in the treated groups compared to infants fed non-supplemented infant formula.

Although it was noted that breast-fed infants generally have looser and more frequent stools than formula fed infants, human milk has a lower renal solute load than infant formula, and concern was expressed that increased faecal water losses might occur in infants fed formula containing oligosaccharides. This was of greater concern for infants already suffering from stress factors that may affect water balance.

It was also concluded that as by four to six months of age, infants have a more mature renal function and lower water turnover per unit body weight, that the adverse potential of added oligofructose and GOS would be very low in this group (SCF, 2001b).

Following this statement, further studies were submitted to the committee, which included two preliminary studies on growth and water balance. Growth data in both preterm and term infants fed different concentrations of GOS and oligofructose were considered. Other data submitted included individual observations of urine creatinine in preterm infants, observations of individual stool frequency, stool consistency, and indicators of protein metabolism in term infants, data on levels of minerals and potential renal solute load of different infant formulae, and preliminary information of urine osmolarity in term infants.

The SCF concluded that there was no evidence of adverse effects from the use of a formula with up to 8 g/L of a combination of 90% 'oligogalactosyl-lactose' (GOS) and 10% 'high molecular weight oligofructosyl-saccharose' (high molecular weight inulin). This conclusion was confirmed in 2003 in the SCF's Report on the Revision of Essential requirements of Infant Formulae and Follow-on Formulae (SCF, 2003).

The European Food Safety Authority (EFSA) Scientific Panel on Dietetic Products, Nutrition and Allergies considered the safety of oligofructose supplemented infant formula in 2004 (EFSA, 2004). Oligofructose at 0, 1.5 and 3.0 g/L in infant formula was given to healthy, term infants for 12 weeks (Bettler and Euler, 2006). Growth, serum markers of protein and mineral status and kidney function were all within the normal range. The committee stated that variable effects on the consistency and frequency of the infants' stools were observed, including increased adverse events (e.g. loose stools), in infants fed formula with added oligofructose. No measures were made to demonstrate satisfactory water balance, and the committee therefore could not exclude the possibility of increased risk of dehydration. A study on the effect of FOS on faecal microflora was also assessed at this time, and it was observed that oligofructose-supplemented formula (1.5 and 3 g/L) led to increased flatulence, regurgitation, irritability and looser stools (Euler *et al*., 2005). The committee concluded that there was no evidence of benefits to infants from the addition of oligofructose at the studied levels, and believed that there are reasons for safety concerns.

4. Conclusions

FSANZ has assessed the evidence on the potential of inulin-derived substances and GOS to cause adverse effects in infants and young children. In particular, the possibility of oligosaccharides increasing osmotic potential in the colon, which could lead to increased water loss and dehydration was considered.

The evidence indicates that inulin-derived substances and GOS, like naturally occurring HMOs, are not digested to any great extent in the small intestine. They reach the large intestine mostly intact and contribute to a small increase in osmotic potential in the colon.

However, this slight increase in osmotic potential for GOS and inulin-derived substances is not considered to be undesirable because breast-fed infants also have levels of undigested HMOs present in the colon.

A number of studies on the 9:1 GOS to long chain inulin preparation in infant formula products support the conclusion that 8 g/L of oligosaccharides will not pose a risk to young infants. This conclusion applies to GOS, inulin and oligofructose at any ratio to a total level of 8 g/L, based on data indicating that these oligosaccharides are fermented to a similar or greater extent than HMOs. The safety of this level (8 g/L) is further supported by the presence of higher levels of indigestible oligosaccharides (up to 25 g/L) in human milk.

However, given evidence in adults that increased intakes of inulin-derived substance may lead to gastrointestinal symptoms (e.g. flatulence and bloating), it may be prudent to limit the amount of these substances permitted in formula to those which have been shown to be tolerated by infants, ***i.e***. 3 g/L.

For young children, toddler formula and infant foods are not the sole source of nutrition, therefore similar or somewhat higher levels of GOS and oligofructose or inulin in these foods, leading to similar overall intakes of GOS and oligofructose or inulin as evaluated in the available studies, or as seen in young infants consuming oligosaccharide-supplemented infant formula products, is very unlikely to pose a risk to young children.

FSANZ considers inulin and FOS to have a history of safe use as food ingredients in the general food supply.

References

Alles, M.S., Hautvast, J.G., Nagengast, F.M., Hartemink, R., Van Laere, K.M. and Jansen, J.B. (1996) Fate of fructo-oligosaccharides in the human intestine. *Br.J Nutr* 76(2):211-221.

Alliet, P., Scholtens, P., Raes, M., Hensen, K., Jongen, H., Rummens, J.L., Boehm, G. and Vandenplas, Y. (2007) Effect of prebiotic galacto-oligosaccharide, long-chain fructo-oligosaccharide infant formula on serum cholesterol and triacylglycerol levels. *Nutrition* Article in press:

Anthony JC., Merriman, TN. and Heimbach JT. (2006) 90-day oral (gavage) study in rats with galactooligosaccharides syrup. *Food and Chemical Toxicology* 44:819-826.

Bakker-Zierikzee, A.M., Alles, M.S., Knol, J., Kok, F.J., Tolboom, J.J. and Bindels, J.G. (2005) Effects of infant formula containing a mixture of galacto- and fructo-oligosaccharides or viable Bifidobacterium animalis on the intestinal microflora during the first 4 months of life. *Br.J Nutr* 94(5):783-790.

Ben, XM., Zhou, XY. and Zhao, WH. (2004) Supplementation of milk formula with galacto-oligosaccharides improves intestinal microflora and fermentation in term infants. *Chin Med J* 117(6):927-931.

Bettler, J. and Euler, A.R. (2006) An evaluation of the growth of term infants fed formula supplemented with fructo-oligosaccharide. *International Journal of Probiotics and Prebiotics* 1(1):19-26.

Boehm, G., Fanaro, S., Jelinek, J., Stahl, B. and Marini, A. (2003) Prebiotic concept for infant nutrition. *Acta Paediatr.Suppl* 91(441):64-67.

Boehm, G., Jelinek, J., Stahl, B., van, L.K., Knol, J., Fanaro, S., Moro, G. and Vigi, V. (2004) Prebiotics in infant formulas. *J Clin Gastroenterol.* 38(6 Suppl):S76-S79.

Boehm, G., Stahl, B., Jelinek, J., Knol, J., Miniello, V. and Moro, G.E. (2005) Prebiotic carbohydrates in human milk and formulas. *Acta Paediatr.Suppl* 94(449):18-21.

Bongers, M.E., de, L.F., Reitsma, J.B., Groeneweg, M., Taminiau, J.A. and Benninga, M.A. (2007) The clinical effect of a new infant formula in term infants with constipation: a double-blind, randomized cross-over trial. *Nutr J* 6:8.

Briet, F., Achour, L., Flourie, B., Beaugerie, L., Pellier, P., Franchisseur, C., Bornet, F. and Rambaud, J.C. (1995) Symptomatic response to varying levels of fructo-oligosaccharides consumed occasionally or regularly. *Eur.J Clin Nutr* 49(7):501-507.

Brunser, O., Gotteland, M., Cruchet, S., Figueroa, G., Garrido, D. and Steenhout, P. (2006) Effect of a milk formula with prebiotics on the intestinal microbiota of infants after an antibiotic treatment. *Pediatr.Res.* 59(3):451-456.

Castiglia-Delavaud, C., Verdier, E., Besle, J.M., Vernet, J., Boirie, Y., Beaufrere, B., De, B.R. and Vermorel, M. (1998) Net energy value of non-starch polysaccharide isolates (sugarbeet fibre and commercial inulin) and their impact on nutrient digestive utilization in healthy human subjects. *Br.J Nutr* 80(4):343-352.

Cherbut, C. (2002) Inulin and oligofructose in the dietary fibre concept. *Br.J Nutr* 87 Suppl 2:S159-S162.

Coppa, G., Pierani, P., Bruni, S., Carloni, C. and Gabrielli, O. (2001) Characterization of oligosaccharides in milk and feces of breast-fed infants by high-performance anion-exchange chromatography. *Adv Exp Med Biol.* (501):307-314.

Coppa, G.V., Gabrielli, O., Pierani, P., Catassi, C., Carlucci, A. and Giorgi, P.L. (1993) Changes in carbohydrate composition in human milk over 4 months of lactation. *Pediatrics* 91(3):637-641.

Coppa, G.V., Pierani, P., Zampini, L., Carloni, I., Carlucci, A. and Gabrielli, O. (1999) Oligosaccharides in human milk during different phases of lactation. *Acta Paediatr.Suppl* 88(430):89-94.

Coppa, GV., Pierani, P., Zampini, L., Gabrielli, O., Carlucci, A., Catassi, C. and Giorgi, PL. (1997) Lactose, oligosaccharide and monosaccharide content of milk from mothers delivering preterm newborns over the first month of lactation. *Minerva Pediatr.* 49(10): 471-475.

Costalos, C., Kapiki, A., Apostolou, M. and Papathoma, E. (2007) The effect of a prebiotic supplemented formula on growth and stool microbiology of term infants. *Early Hum.Dev.*

Coussement, P.A. (1999) Inulin and oligofructose: safe intakes and legal status. *J Nutr* 129(7 Suppl):1412S-1417S.

Cummings, J.H., Christie, S. and Cole, T.J. (2001a) A study of fructo oligosaccharides in the prevention of travellers' diarrhoea. *Aliment.Pharmacol.Ther.* 15(8):1139-1145.

Cummings, J.H., Macfarlane, G.T. and Englyst, H.N. (2001b) Prebiotic digestion and fermentation. *Am J Clin Nutr* 73(2 Suppl):415S-420S.

Duggan, C., Penny, M.E., Hibberd, P., Gil, A., Huapaya, A., Cooper, A., Coletta, F., Emenhiser, C. and Kleinman, R.E. (2003) Oligofructose-supplemented infant cereal: 2 randomized, blinded, community-based trials in Peruvian infants. *Am J Clin Nutr* 77(4):937-942.

EFSA. (2004) Opinion of the Scientific Panel on Dietetic Products, Nutrition and Allergies on a request from the Commission relating to the safety and suitability for particular nutritional use by infants of fructooligosaccharides in infant formula and follow-on formula (Request No EFSA-Q-2003-020). *The EFSA Journal* 31:1-11.

Engfer, M.B., Stahl, B., Finke, B., Sawatzki, G. and Daniel, H. (2000) Human milk oligosaccharides are resistant to enzymatic hydrolysis in the upper gastrointestinal tract. *Am J Clin Nutr* 71(6):1589-1596.

Euler, A.R., Mitchell, D.K., Kline, R. and Pickering, L.K. (2005) Prebiotic effect of fructo-oligosaccharide supplemented term infant formula at two concentrations compared with unsupplemented formula and human milk. *J Pediatr.Gastroenterol.Nutr* 40(2):157-164.

Fanaro, S., Boehm, G., Garssen, J., Knol, J., Mosca, F., Stahl, B. and Vigi, V. (2005a) Galacto-oligosaccharides and long-chain fructo-oligosaccharides as prebiotics in infant formulas: a review. *Acta Paediatr.Suppl* 94(449):22-26.

Fanaro, S., Jelinek, J., Stahl, B., Boehm, G., Kock, R. and Vigi, V. (2005b) Acidic oligosaccharides from pectin hydrolysate as new component for infant formulae: effect on intestinal flora, stool characteristics, and pH. *J Pediatr.Gastroenterol.Nutr* 41(2):186-190.

Flickinger, E.A., van, L.J. and Fahey, G.C., Jr. (2003) Nutritional responses to the presence of inulin and oligofructose in the diets of domesticated animals: a review. *Crit Rev Food Sci Nutr* 43(1):19-60.

Franck, P., Moneret-Vautrin, DA., Morisset, M., Kanny, G., Megret-Gabeaux, M. and Olivier, JL. (2005) Anaphylactic Reaction to Inulin:First Identification of Specific IgEs to an Inulin Protein Compound. *International Archives of Allergy and Immunology* 136:155-158.

FSANZ. (2001) *Full Assessment Report and Regulatory Impact Assessment A277 - Inulin and Fructo-oligosaccharides as Dietary Fibre*. http://www.foodstandards.gov.au/_srcfiles/A277_FAR.doc. Accessed on 6 September 7 A.D.

Gay-Crosier, F., Schreiber, G. and Hauser, C. (2000) Anaphylaxis from Inulin in Vegetables and Processed Food [Correspondence]. *The New England Journal of Medicine* 342(18):1372.

Gibson, G.R., McCartney, A.L. and Rastall, R.A. (2005) Prebiotics and resistance to gastrointestinal infections. *Br.J Nutr* 93 Suppl 1:S31-S34.

Gnoth, M.J., Kunz, C., Kinne-Saffran, E. and Rudloff, S. (2000) Human milk oligosaccharides are minimally digested in vitro. *J Nutr* 130(12):3014-3020.

Kapiki, A., Costalos, C., Oikonidou, C., Triantafyllidou, A., Loukatou, E. and Pertrohilou, V. (2007) The effect of a fructos-oligosaccharide supplemented formula on gut flora of preterm infants. *Early Hum.Dev.* 83:335-339.

Kim, S.H., Lee, d.H. and Meyer, D. (2007) Supplementation of baby formula with native inulin has a prebiotic effect in formula-fed babies. *Asia Pac.J Clin Nutr* 16(1):172-177.

Knol, J., Scholtens, P., Kafka, C., Steenbakkers, J., Grob, S., Helm, K., Klarczyk, M., Schopfer, H., Bockler, H. and Wells, J. (2005) Colon Microflora in Infants Fed Formula with Galacto- and Fructo-

Oligosaccharids: More Like Breast-Fed Infants. *Journal of Pediatric Gastroenterology and Nutrition* 40:36-42.

Molis, C., Flourie, B., Ouarne, F., Gailing, M.F., Lartigue, S., Guibert, A., Bornet, F. and Galmiche, J.P. (1996) Digestion, excretion, and energy value of fructooligosaccharides in healthy humans. *Am J Clin Nutr* 64(3):324-328.

Moore, N., Chao, C., Yang, L.P., Storm, H., Oliva-Hemker, M. and Saavedra, J.M. (2003) Effects of fructo-oligosaccharide-supplemented infant cereal: a double-blind, randomized trial. *Br.J Nutr* 90(3):581-587.

Moro, G., Boehm, G., Jelinek, J., Stahl, B. and Coppa, G. (2004) P1124 Digestibility of galacto-oligosaccharides and fructose-oligosaccharides in term infants. *Journal of Pediatric Gastroenterology and Nutrition* 39 Supplement 1: S484.

Moro, G., Arslanoglu, S., Stahl, B., Jelinek, J., Wahn, U. and Boehm, G. (2006) A mixture of prebiotic oligosaccharides reduces the incidence of atopic dermatitis during the first six months of age. *Arch.Dis Child* 91(10):814-819.

Moro, G., Minoli, I., Mosca, M., Fanaro, S., Jelinek, J., Stahl, B. and Boehm, G. (2002) Dosage-related bifidogenic effects of galacto- and fructooligosaccharides in formula-fed term infants. *J Pediatr.Gastroenterol.Nutr* 34(3):291-295.

Moro, G.E., Mosca, F., Miniello, V., Fanaro, S., Jelinek, J., Stahl, B. and Boehm, G. (2003) Effects of a new mixture of prebiotics on faecal flora and stools in term infants. *Acta Paediatr.Suppl* 91(441):77-79.

Moro, G.E., Stahl, B., Fanaro, S., Jelinek, J., Boehm, G. and Coppa, G.V. (2005) Dietary prebiotic oligosaccharides are detectable in the faeces of formula-fed infants. *Acta Paediatr.Suppl* 94(449):27-30.

Niness, K.R. (1999) Inulin and oligofructose: what are they? *J Nutr* 129(7 Suppl):1402S-1406S.

Ninonuevo, M.R., Park, Y., Yin, H., Zhang, J., Ward, R.E., Clowers, B.H., German, J.B., Freeman, S.L., Killeen, K., Grimm, R. and Lebrilla, C.B. (2006) A strategy for annotating the human milk glycome. *J Agric Food Chem.* 54(20):7471-7480.

Parrett, A.M. and Edwards, C.A. (1997) In vitro fermentation of carbohydrate by breast fed and formula fed infants. *Arch.Dis Child* 76(3):249-253.

Puccio, G., Cajozzo, C., Meli, F., Rochat, F., Grathwohl, D. and Steenhout, P. (2007) Clinical evaluation of a new starter formula for infants containing live Bifidobacterium longum BL999 and prebiotics. *Nutrition* 23:1-8.

Roberfroid, M.B. and Delzenne, N.M. (1998) Dietary fructans. *Annu.Rev Nutr* 18:117-143.

Rudloff, S., Pohlentz, G., Diekmann, L., Egge, H. and Kunz, C. (1996) Urinary excretion of lactose and oligosaccharides in preterm infants fed human milk or infant formula. *Acta Paediatr.* 85(5):598-603.

Saavedra, J.M. and Tschernia, A. (2002) Human studies with probiotics and prebiotics: clinical implications. *Br.J Nutr* 87 Suppl 2:S241-S246.

Savino, F., Palumeri, E., Castagno, E., Cresi, F., Dalmasso, P., Cavallo, F. and Oggero, R. (2006) Reduction of crying episodes owing to infantile colic: A randomized controlled study on the efficacy of a new infant formula. *Eur.J Clin Nutr* 60(11):1304-1310.

SCF (2001a) *Additional statement on the use of resistant short chain carbohydrates (oligofructosyl-saccharose and oligogalactosyl-lactose) in infant formula and in follow-on formula*. Report No. SCF/CS/NUT/IF/47 Final.

SCF (2001b) *Statement on the use of resistant short chain carbohydrates (oligofructose and oligogalactose) in infant formulae and in follow-on formula*. Report No. SCF/CS/NUT/IF/35 Final.

SCF (2003) *Report of the Scientific Committee on Food on the Revision of Essential Requirements of Infant Formula and Follow-on Formula*. Report No. SCF/CS/NUT/IF/65 Final.

Schmelzle, H., Wirth, S., Skopnik, H., Radke, M., Knol, J., Bockler, H.M., Bronstrup, A., Wells, J. and Fusch, C. (2003) Randomized double-blind study of the nutritional efficacy and bifidogenicity of a new infant formula containing partially hydrolyzed protein, a high beta-palmitic acid level, and nondigestible oligosaccharides. *J Pediatr.Gastroenterol.Nutr* 36(3):343-351.

Schneeman, B.O. (1999) Fiber, inulin and oligofructose: similarities and differences. *J Nutr* 129(7 Suppl):1424S-1427S.

Scholtens, P.A., Alles, M.S., Bindels, J.G., van der Linde, E.G., Tolboom, J.J. and Knol, J. (2006) Bifidogenic effects of solid weaning foods with added prebiotic oligosaccharides: a randomised controlled clinical trial. *J Pediatr.Gastroenterol.Nutr* 42(5):553-559.

Stahl, B., Bohm, G., Finke, B., Georgi, G., Jelinek, J. and Schmitt, JJ. (2005) Oligosaccharides mixture. AU 200120062 B2(782623): Australia.

TGA (1998) *Complementary Medicines Evaluation Committee Extracted Ratified Minutes, 7th Meeting*. http://www.tga.gov.au/docs/pdf/cmec/cmecmi07.pdf.

Waligora-Dupriet AJ, Campeotto, F., Nicolis, I., Bonet, A., Soulaines, P., Dupont, C. and Butel, MJ. (2007) Effect of oligofructose supplementation on gut microflora and well being in young children attending a day care centre. *International Journal of Food Microbiology* 113:108-113.

Ziegler, E., Vanderhoof, J.A., Petschow, B., Mitmesser, S.H., Stolz, S.I., Harris, C.L. and Berseth, C.L. (2007) Term infants fed formula supplemented with selected blends of prebiotics grow normally and have soft stools similar to those reported for breast-fed infants. *J Pediatr.Gastroenterol.Nutr* 44(3):359-364.

Attachment 7

Dietary Intake Assessment

Summary

A dietary intake assessment was deemed necessary for Proposal P306 in order to estimate the potential dietary intakes of inulin-derived substances and GOS in special purpose foods for infants and young children <3 years of age (i.e. infant and follow-on formula, infant foods and formulated supplementary foods for young children (FSFYC, for example, toddler formula).

FSANZ has received two Applications (from Heinz Wattie's Limited and Nutricia Australia), requesting amendments to the Code to allow addition of inulin-derived substances and GOS to infant formula products and in the case of Nutricia, to infant foods. The Applications and literature reviewed by FSANZ were used as the basis for assigning concentrations for the addition of inulin-derived substances and GOS in infant formula products, infant foods and FSFYC.

Dietary intakes of inulin-derived substances and GOS have been estimated in two ways:

- ***'Combined' Assessment*** – represents estimated intakes of inulin-derived substances and GOS collectively (i.e. summed) for each population group assessed in this proposal, i.e. based on the concentration of inulin-derived substances and GOS in special purpose foods for infants and young children as a total of 0.8 g/100 mL; and

- ***'Separate' Assessment*** – represents estimated intakes of inulin-derived substances and GOS separately for each of the population groups assessed in this proposal, based on the concentration at a maximum proposed level (inulin-derived substances and GOS at 0.8 g/100 mL in special purpose foods for young children).

Baseline dietary intakes of inulin-derived substances and GOS were estimated based on natural sources and added sources according to the current market uptake of these substances in processed food products. An additional intake estimate was then calculated to account for potential intakes from infant formula products, infant foods and FSFYC supplemented with inulin-derived substances and GOS.

As food consumption data were not available for children aged <2 years in Australia and <5 years in New Zealand, model diets were constructed for infants aged 3, 9 and 12 months for Australia and aged 3 months and 1-3 years for New Zealand for use in the intake assessments. FSANZ developed the model diets for Australian children based on the Australian National Nutrition Survey and New Zealand model diets were derived from the New Zealand total diet study. The model diets include the estimated intakes of infant formula (800 mL/day for a 3 month old), follow-on formula (545 mL/day for a 9 month old), toddler formula (between 285 and 425 mL/day for 1-3 year olds) and infant foods along with consumption of other foods where appropriate.

The estimates indicate that following the addition of inulin-derived substances and GOS to infant formula, 3 month old infants are likely to have the highest increase in mean intakes but the lowest total intake of these substances among the young children <3 years of age. Infants aged 3 months were assumed to be exclusively fed infant formula, thus their intake of inulin-derived substances and GOS at baseline is zero and the only contributor to intakes of inulin-derived substances and GOS for this age group is infant formula. As young children grow, they consume smaller amounts of formula, more of other foods, and greater amounts of food in total; hence their dietary intake of inulin-derived substances and GOS from added and the natural sources also increases. The estimated dietary intakes of inulin-derived substances and GOS increased for each age group assessed from 3 months to the 3 years age group.

The main source of inulin-derived substances and GOS in the diets of young children in Australia and New Zealand for the *'baseline'* for the combined assessment and GOS only assessment is from yoghurt. If the addition of inulin-derived substances and GOS to special purpose foods for infants and young children <3 years of age were to be approved, formula products[42] would be the main source of these substances for infants (≤1 year of age). The main source of inulin-derived substances and GOS intakes for New Zealand children (aged 1-3 years) were from yoghurts. Toddler formula was also a major contributor but was lower than yoghurts.

Intakes from naturally-occurring food sources and added sources in processed foods did not make a large contribution to the estimated intakes of inulin-derived substances and GOS for infants 9 months and 1 years of age.

1. Background

A dietary intake assessment was deemed necessary for Proposal P306 in order to estimate the potential dietary intakes of inulin-derived substances and GOS in special purpose foods for infants and young children <3 years of age (i.e. infant and follow-on formula, infant foods and FSFYC).

FSANZ had received two Applications (from Heinz Wattie's Limited and Nutricia Australia), requesting amendments to the Code to allow addition of inulin-derived substances and GOS to infant formula products and in the case of Nutricia, to infant foods also. These Applications were used as the basis for setting the concentration of added inulin-derived substances and GOS in infant formula products (Tables 1 and 2).

[42] Formula products include infant formula for 3 month old, follow-on formula for <1 year old.

Table 1: Proposed concentration of inulin-derived substances and GOS in infant formula products, as a *Ratio (FOS:GOS as a 1:9 ratio, maximum of 0.8 g/100 mL)*

Substance	Infant Formula Products and Infant Foods (g/100 mL)
	Ratio
Inulin	0.08
GOS	0.720

Table 2: Proposed concentration of inulin-derived substances and GOS in infant formula products, as *Minimum and Maximum (mg/100 kJ)*

Substance	Infant and follow-on formula (mg/100 kJ)	
	Minimum	Maximum
Inulin	30	110
GOS	145	290

2. Dietary modelling conducted to estimate inulin-derived substances and GOS intakes

2.1 What is dietary modelling?

Dietary modelling is a tool used to estimate dietary exposure/intakes to food chemicals, including nutrient intakes, from the diet as part of the FSANZ risk assessment process. To estimate dietary exposure to food chemicals, records of what foods people have eaten are needed along with reports of how much of the food chemical of interest is in each food. The accuracy of these dietary exposure estimates depends on the quality of the data used in the dietary models. Sometimes, all of the data needed are not available or their accuracy is uncertain so assumptions have to be made, either about the foods eaten or about chemical levels, based on previous knowledge and experience. The models are generally set up according to international conventions for food chemical dietary exposure estimates. However, each modelling process requires decisions to be made about how to set the model parameters and what assumptions to make. Different decisions may result in different answers. Therefore, FSANZ documents clearly all such decisions, model assumptions and data limitations to enable the results to be understood in the context of the data available and so that FSANZ risk managers can make informed decisions.

2.2 Population groups assessed

The primary target group was identified as young children (<3 years of age). Within this population group, dietary inulin-derived substances and GOS intakes were investigated for different age groups (Table 3).

Table 3: Population groups assessed for this Proposal

Australia: • Model diet for 3 month old infant; • Model diet for 9 month old infant; • Model diet for 1 yr old infant;
New Zealand: • Model diet for 3 month old infant; • Model diet for 1-3 years children.

2.3 Dietary survey data

DIAMOND contains dietary survey data for both Australia and New Zealand; the 1995 NNS from Australia that surveyed 13,858 people aged 2 years and above, and the 1997 New Zealand NNS that surveyed 4,636 people aged 15 years and above. Both of these surveys used a 24-hour food recall methodology.

As there were no data available from the 1995 Australian NNS for children aged < 2 years, theoretical diets were constructed to estimate dietary inulin-derived substances and GOS intakes for the target groups of children aged 3 months, 9 months and 12 months. Similarly, as there were no data available from the 1997 New Zealand NNS or 2002 New Zealand Children's NNS for children aged < 5 years, theoretical diets were used to estimate dietary inulin-derived substances and GOS intakes for the New Zealand children aged 3 months and 1-3 years. See Appendix 1 for further details on how the theoretical diets were constructed.

2.4 Dietary intake assessment approach

The dietary intakes of inulin-derived substances and GOS were estimated by combining usual patterns of food consumption, as derived from the theoretical diets, with current concentrations of inulin-derived substances and GOS in foods and the proposed levels of use of inulin-derived substances and GOS in infant formula, follow-on formula and Toddler formula. Concentrations were also assigned to infant foods where the data were available. The dietary modelling approach used for the intake assessment of inulin-derived substances and GOS for Australian and New Zealand population groups is as shown in Figure 1.

Dietary Intake = concentration x food consumption amount

Dietary intake assessments were estimated for both inulin-derived substances and GOS combined and also separately.

2.5 Inulin-derived substances and GOS concentration data

The proposed levels of inulin-derived substances and GOS added to special purpose foods for infants and young children (<3 years of age) that were used in the dietary intake assessment were derived from the aforementioned Applications. International analysis/literature (Appendix 2), industry use data and FSANZ analysis (Food Standards Australia New Zealand, 2002) provided current levels of inulin-derived substances and GOS in a range of other different foods consumed by the young children (<3 years of age).

Concentrations of inulin-derived substances and GOS, as described in Tables 1 and 2, were assigned to each of the food groups in the theoretical diets.

Concentration data from the literature were assigned to food groups as inulin-derived substances together (i.e. summed). For the purpose of dietary intake assessment, if levels of both inulin and FOS were available, the highest concentration available was used and if only levels of FOS were available for a food that FOS value was used. Where the concentration data were available as a range, an average of the lowest and highest values in the range was used. Some literature did not clearly distinguish between long chain and short chain oligosaccharides and in this case, also, the value cited was assigned to inulin-derived substances. Where the concentration data available did not distinguish between naturally occurring or added to foods, the value was included in the dietary intake assessments (for example, yoghurt).

FSANZ's assessment is based on the proposed maximum concentrations of inulin-derived substances and GOS in two ways:

- GOS and inulin-derived substance, with a maximum combined concentration of 0.8 g/100 mL; and
- proposed maximum concentrations of inulin-derived substances and GOS added separately.

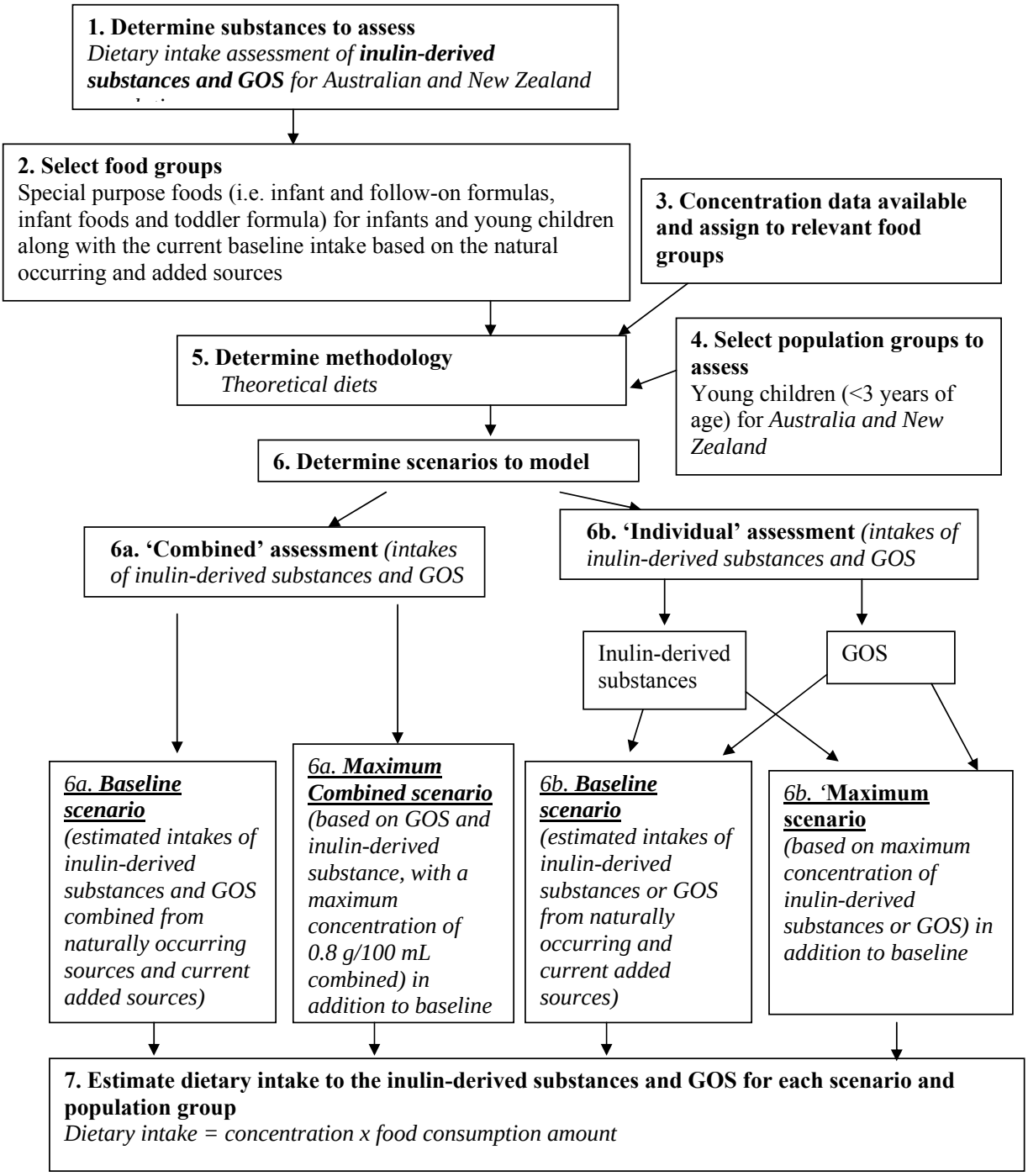


Figure 1: Dietary modelling approach used for the intake assessment of inulin-derived substances and GOS for the Australian and New Zealand population groups

The proposed maximum concentrations for infant formula products and foods for infants were also assigned to toddler formulas. The inulin-derived substances and GOS concentrations that were used in the dietary intake assessments are outlined in Table 4a for infant and follow-on formulas and Table 4b for infant foods and toddler formula.

Table 4: Inulin-derived substance and GOS concentrations used in the dietary intake assessments

a: Infant and follow-on formula concentrations

Substance	Infant and follow-on formula concentration (g / 100 mL)
	Maximum
Inulin-derived substance	0.80
GOS	0.80
Inulin-derived substance and GOS as combined	0.80

b: Infant foods and toddler formula concentrations

Substance	Infant foods and toddler formula concentration (g / 100 mL)
	Maximum
Inulin-derived substances	0.80
GOS	0.80
Inulin-derived substance and GOS as combined	0.80

2.6 Scenarios for dietary intake assessments

The scenarios that were investigated in the dietary intake assessments are outlined in Sections 2.6.1 and 2.6.2.

2.6.1 'Combined' assessment (intakes of inulin-derived substances and GOS, collectively)

The *'combined' assessment* estimates intakes of inulin-derived substances and GOS combined for each population group assessed in this proposal. The two scenarios modelled for the *'combined' assessment* were the '*baseline scenario'* and the *'maximum combined scenario'*.

2.6.1.1 Baseline Scenario

'Baseline scenario' represents estimated intakes from naturally occurring inulin-derived substances and GOS in foods along with added sources based on the current market uptake for inulin-derived substances and GOS in processed foods. The concentration data used in dietary intake assessments are shown in Appendix 2.

2.6.1.2 Maximum combined scenario

The Applications are seeking a maximum concentration of 0.8 g/100 mL of total oligosaccharides. The '*maximum combined scenario'* takes into account estimated intakes of inulin-derived substances and GOS of 0.8 g/100 mL in infant and follow-on formulas, toddler formulas and foods for infants in addition to baseline intakes of inulin-derived substances and GOS from naturally-occurring sources in foods and added sources in other processed foods.

2.6.2 'Separate' assessment (intakes of inulin-derived substances and GOS individually)

The *'separate' assessment* represents estimated intakes of inulin-derived substances and GOS separately for each population group assessed in this proposal. The two scenarios modelled for each *'separate' assessment* were the '*baseline scenario'* and the *'maximum scenario'*.

2.6.2.1 Baseline Scenario

As per the *'baseline scenario'* in *'combined' assessment* (Section 2.6.1.1), but assessing inulin-derived substances and GOS separately.

2.6.2.2 Maximum Scenario

The Applications also provided proposed maximum concentrations of inulin-derived substances and GOS in infant formula for inulin-derived substances and GOS separately. The '*Maximum scenario'* takes into account the proposed maximum levels of use for inulin-derived substances and GOS in addition to baseline intakes of inulin-derived substances and GOS from naturally-occurring sources and added sources. In processed foods the proposed maximum levels of use for inulin-derived substances and GOS are as 0.8 g/100 mL.

2.7 How were the estimated dietary inulin-derived substances and GOS intakes calculated?

A detailed explanation of how the estimated dietary intakes were calculated can be found in Appendix 1.

3. Assumptions used in the dietary modelling

The aim of the dietary intake assessment was to make as realistic an estimate of dietary inulin-derived substances and GOS intakes as possible. However, where significant uncertainties in the data existed, conservative assumptions were generally used to ensure that the dietary intake assessment did not underestimate intake.

The assumptions made in the dietary intake assessment are listed below, broken down into several categories.

3.1 Consumer behaviour

- Consumption of foods as outlined in the theoretical diets represent current food consumption amounts for Australian and New Zealand young children (<3 years of age);
- consumers select products that, on average, contain inulin-derived substances and GOS at the concentrations specified;

- consumers do not alter their food consumption habits upon inulin-derived substances and GOS fortified products becoming more available on the market;
- infants aged 3 months are exclusively infant formula fed;
- infants aged 9 months consume follow-on formula and infant foods in addition to solid foods;
- all children aged 1-3 years consume Toddler formula and infant foods in addition to other solid and liquid foods;
- all milks consumed by children aged 1-3 years in the theoretical New Zealand diet is assumed to be toddler formula; and
- the substitution of Toddler formula for milk is on a "volume for volume" basis rather than on an energy basis.

3.2 Concentration Data

- It was assumed that the inulin-derived substances and GOS concentrations in foods were representative of Australian and New Zealand foods;
- where a food was not included in the intake assessment, it was assumed to contain a zero concentration of inulin-derived substances and GOS;
- the inulin-derived substances and GOS concentration of infant formula, follow-on formula, Toddler formula and infant foods is currently zero (i.e. at *'Baseline'*);
- the proposed inulin-derived substances and GOS concentration of follow-on formula was same as the concentration of infant formula;
- there was no contribution to inulin-derived substances and GOS intakes through the use of complementary medicines (Australia) or dietary supplements (New Zealand); and
- concentration data available were imputed to similar food groups for the dietary intake assessments (for example the concentration data from root vegetables (average value of burdock, murnong, yacon, salsify) were imputed to other root vegetables like carrot and potato).

3.3 General

- For the purpose of this assessment, it was assumed that 1 millilitre is equal to 1 gram for all liquid and semi-liquid foods (e.g. infant formula).

4. Limitations of the dietary modelling

Dietary modelling based on 1995 or 1997 NNS food consumption data provides the best estimate of actual consumption of a food and the resulting estimated dietary intake of a nutrient for the population. However, it should be noted that the NNS data do have limitations. These limitations relate to the age of the data and the changes in eating patterns that may have occurred since the data were collected. Generally, consumption of staple foods such as fruit, vegetables, meat, dairy products and cereal products, which make up the majority of most people's diet, is unlikely to have changed markedly since 1995/1997 (Cook, Rutishauser, and Seelig, 2001; Cook, Rutishauser, and Allsopp, 2001).

Over time, there may be changes to the ways in which manufacturers and retailers make and present foods for sale. Since the data were collected for the Australian and New Zealand NNSs, there have been significant changes to the Code to allow more innovation in the food industry.

As a consequence, a limitation of the dietary modelling is that some of the foods that are currently available in the food supply were either not available or were not as commonly available in 1995/1997 (e.g. toddler formula). Additionally, since the data were collected for the NNSs, there has been an increase in the range of products that are fortified with nutrients. FSANZ does update the food composition database through analytical programs and scans of the market place. However, with the market place continually changing it is difficult to account for all fortified products at a given point in time.

A limitation of estimating dietary intake over a period time using information from a recall method is that people may over- or under-report food consumption, particularly for certain types of foods. Over- and under-reporting of food consumption has not been accounted for in this dietary intake assessment.

Since the 1995 Australian NNS does not report on respondents aged below 2 years, the 1997 New Zealand NNS does not report on respondent aged below 15 years and the 2002 New Zealand National Children's Nutrition Survey (CNS) does not report on respondents aged below 5 years, theoretical diets were used to estimate dietary inulin-derived substances and GOS intakes for children in the target group of up to 3 years. FSANZ developed the model diets for Australian children based on an extrapolation from 2 years diet from Australian National Nutrition Survey and New Zealand model diets were derived from the New Zealand total diet study. Theoretical diets for Australian children aged 3 months, 9 months and 1 year and New Zealand children aged 1-3 years were used in this assessment. Mean food consumption amounts in the theoretical diets are used to represent food consumption patterns for an age group as a whole and may not be as accurate as the data derived for other population groups from the NNSs that use food consumption data of individuals.

Although some data on the use of complementary medicines (Australia) or dietary supplements (New Zealand) were collected in the NNSs, data were either not in a robust enough format to include in DIAMOND or have simply not been included in the DIAMOND program to date. Consequently, intakes of substances consumed via complementary medicines or dietary supplements could not be included directly in the theoretical diets.

While the results of national nutrition surveys can be used to describe the usual intake of groups of people, they cannot be used to describe the usual intake of an individual (Rutishauser, 2002). In addition, they cannot be used to predict how consumers will change their eating patterns as a result of an external influence such as the availability of a new type of food.

Concentration data available on natural sources and added sources based on the current market uptake of inulin-derived substances and GOS were limited. Also, the form or chain length of inulin-derived substances or GOS was often not indicated. How these limitations were dealt with was explained earlier.

Limited concentration data were available; therefore extrapolation of values from some foods to many other food groups was conducted. Concentration data and comments on data extrapolation used for the dietary intake assessments are as in Table A2.2.

5. Dietary intake assessment results

5.1 Estimated dietary intake of inulin-derived substances and GOS

Mean and 95th percentile dietary intakes of inulin-derived substances and GOS were estimated for the *'combined'* and '*separate' scenario assessments* based on maximum levels for inulin-derived substances and GOS. The results are shown in Figures 2 and 3 (full results in Tables 5 and 6). As young children grow, they consume smaller amounts of formula, more of other foods and greater amounts of food in total; hence their dietary intake of inulin-derived substances and GOS from supplemented and natural sources also increases. The estimated dietary intakes of inulin-derived substances and GOS increased for each age group assessed from 3 months to the 3 years age group.

This assessment has assumed infants aged 3 months were exclusively fed infant formula. Thus, the estimated dietary intakes of inulin-derived substances and GOS for this age group were exclusively from infant formula and, *'baseline'* inulin-derived substances and GOS intakes for infants aged 3 months were zero for both *'combined'* and *'separate'* assessments for Australia and New Zealand.

5.1.1 Combined assessment

Combined assessments represent estimated intakes of inulin-derived substances and GOS combined for each of the population groups assessed in this proposal, based on the concentrations at a maximum proposed level (0.8 gm/ 100 mL).

Infants 3 months for Australia and New Zealand:
The estimated mean and 95th percentile dietary intakes of inulin-derived substances and GOS for the *'maximum combined'* scenario were 6 g and 16 g per day respectively.

Infants 9 months for Australia:
The estimated mean and 95th percentile dietary intakes of inulin-derived substances and GOS were 5 g and 12 g per day respectively for the *'baseline'* scenario and 9 g and 23 g/day for the *'maximum combined'* scenario.

Infants 1 years of age for Australia:
The estimated mean and 95th percentile dietary intakes of inulin-derived substances and GOS were 7 g and 17 g per day respectively for the *'baseline'* scenario and 10 g and 26 g/day for the *'maximum combined'* scenario.

Children 1-3 years of age for New Zealand:
The estimated mean and 95th percentile dietary intakes of inulin-derived substances and GOS were 17 g and 42 g per day respectively for the *'baseline'* scenario and 19 g and 49 g/day for the *'maximum combined'* scenario.

5.1.2 'Separate' assessments

'Separate' assessments represents estimated intakes of inulin-derived substances and GOS separately for each of the population groups assessed in this proposal, based on the concentrations at different maximum proposed levels.

The intakes for the combined maximum scenarios will not be a sum of the two separate maximum scenarios and this is because of the special foods for young children. Adding the intakes from the two separate assessments would result in some "double counting" of inulin-derived substances and GOS from these foods.

5.1.2.1 Inulin-derived substances only Scenarios

Inulin-derived substances only scenarios represent estimated intakes of inulin-derived substances when added at 0.8 g/100 mL.

Infants 3 months for Australia and New Zealand:
The estimated mean and 95th percentile dietary intakes of inulin-derived substances for the *'maximum'* scenario were 2 g and 6 g per day, respectively.

Infants 9 months for Australia:
The estimated mean and 95th percentile dietary intakes of inulin-derived substances were 4 g and 9 g per day, respectively, for the *'baseline'* scenario and 5 g and 14 g/day for the *'maximum'* scenario.

Infants 1 years of age for Australia:
The estimated mean and 95th percentile dietary intakes of inulin-derived substances were 5 g and 14 g per day, respectively, for the *'baseline'* scenario and 7 g and 18 g/day for the *'maximum'* scenario.

Children 1-3 years of age for New Zealand:
The estimated mean and 95th percentile dietary intakes of inulin-derived substances were 12 g and 31 g per day, respectively, for the *'baseline'* scenario and 14 g and 34 g/day for the *'maximum'* scenario.

5.1.2.2 GOS only Scenarios

GOS only scenarios represent estimated intakes of GOS when added at 0.8 g/100 mL to special purpose foods for young children.

Infants 3 months for Australia and New Zealand:
The estimated mean and 95th percentile dietary intakes of GOS for the *'maximum'* scenario were 6 g and 16 g per day, respectively.

Infants 9 months for Australia:
The estimated mean and 95th percentile dietary intakes of GOS were 1 g and 3 g per day, respectively, for the *'baseline'* scenario and 5 g and 14 g/day for the *'maximum'* scenario.

Infants 1 years of age for Australia:
The estimated mean and 95th percentile dietary intakes of GOS were 1 g and 4 g per day, respectively, for the *'baseline'* scenario and 5 g and 12 g/day for the *'maximum'* scenario.

Children 1-3 years of age for New Zealand:
The estimated mean and 95th percentile dietary intakes of GOS were 5 g and 12 g per day, respectively, for the *'baseline'* scenario and 7 g and 18 g/day for the *'maximum'* scenario.

5.2 Major contributing foods to dietary intakes of inulin-derived substances and GOS

5.2.1 Combined assessment

The major contributors (≥ 5%) to intakes of inulin-derived substances and GOS for the 'combined' assessment for Australian and New Zealand young children (<3 years of age) are shown in Table 7.

With the exception of special purpose foods, the major contributors (≥ 5%) for all population groups assessed were similar between the *'baseline'* and *'maximum combined'* scenarios. For the *'maximum combined scenario'* the highest contribution was from infant formula products for the 9 month old and the 1 year old (47% and 33%, respectively) for Australia. The major contributors for *'baseline'* and *'maximum combined scenarios'* for New Zealand 1-3 year olds were from yoghurt (45% and 39%) and potatoes[43] (15% and 13%), respectively. Major contributors for *'maximum combined scenarios'* for New Zealand 1-3 year olds also includes toddler formula (11%).

5.2.2 'Separate' assessments

The major contributors (≥ 5%) to intakes of inulin-derived substances and GOS for the '*separate*' assessments for Australian and New Zealand young children (<3 years of age) are shown in Table 8.

The highest contributors for inulin-derived substances for the *'maximum scenarios'* among 9 month olds and 1 year olds in Australia were from infant formula products (30% and 23%), potatoes (19% and 20%) and yoghurt (11% and 12%), respectively. The highest contributors for *'baseline'* and the *'maximum'* scenarios among New Zealand 1-3 year olds were from yoghurt (29% and 27%) and potatoes (21% and 19%), respectively. Contributions from toddler formula were 9% for the *'maximum'* scenario for New Zealand 1-3 year olds.

The highest contributors for GOS for the *'maximum scenarios'* among the 9 month olds and 1 year olds in Australia were from infant formula products (80% and 69%) and yoghurt (12% and 19%), respectively. The highest contributors for GOS among New Zealand 1-3 year olds for the *'baseline'* scenario were yoghurt (84%) and cheese (7%) and for the *'maximum'* scenario were yoghurt (54%) and toddler formula (29%).

[43] The concentration data for potato has been imputed from other root vegetables.

Figure 2: Estimated mean dietary intake of inulin-derived substances and GOS for 'combined' and 'separate' assessments for Australia and New Zealand population groups

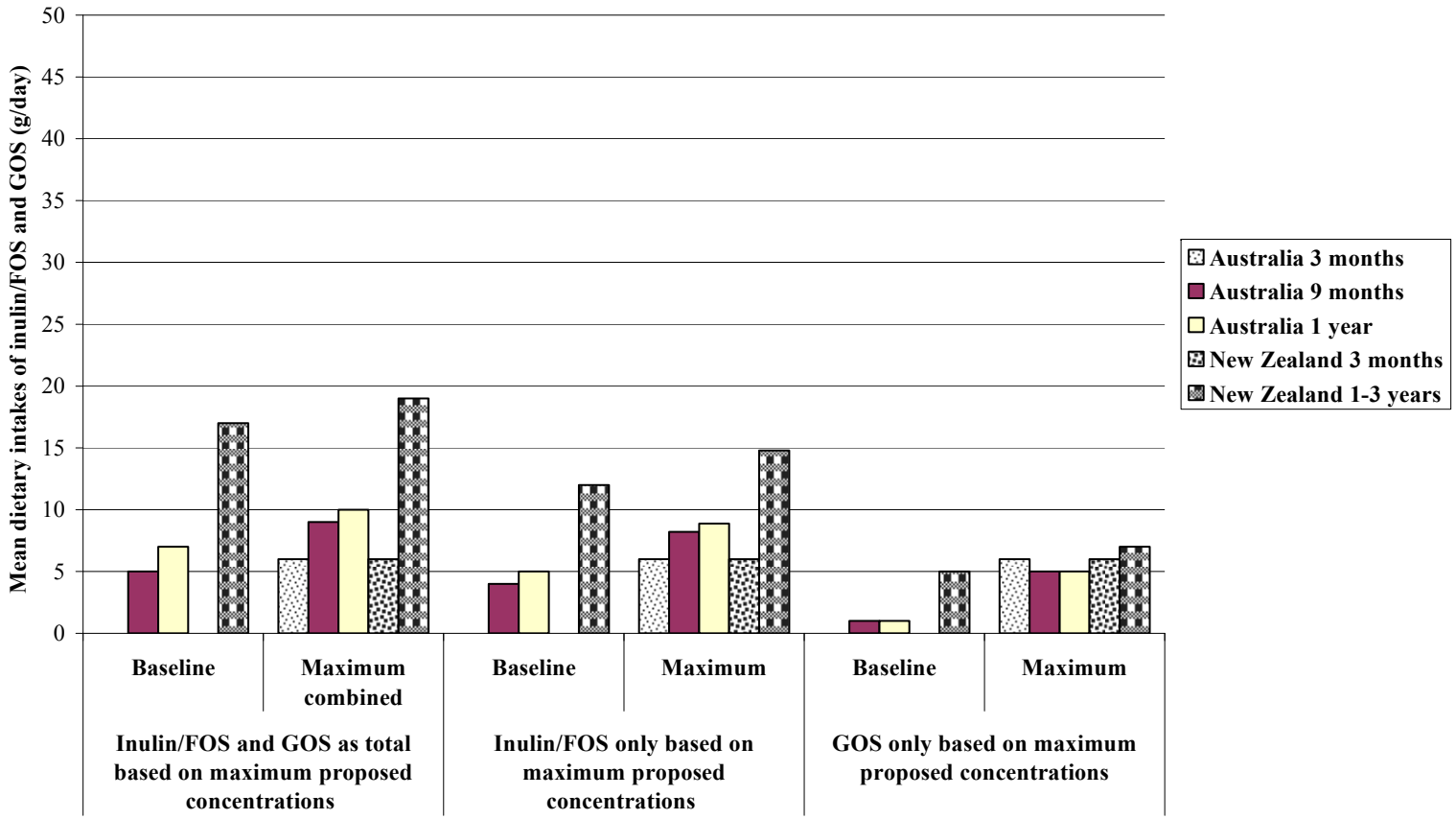


Figure 3: Estimated 95th percentile dietary intake of inulin-derived substances and GOS for 'combined' and 'separate' assessments for Australia and New Zealand population groups

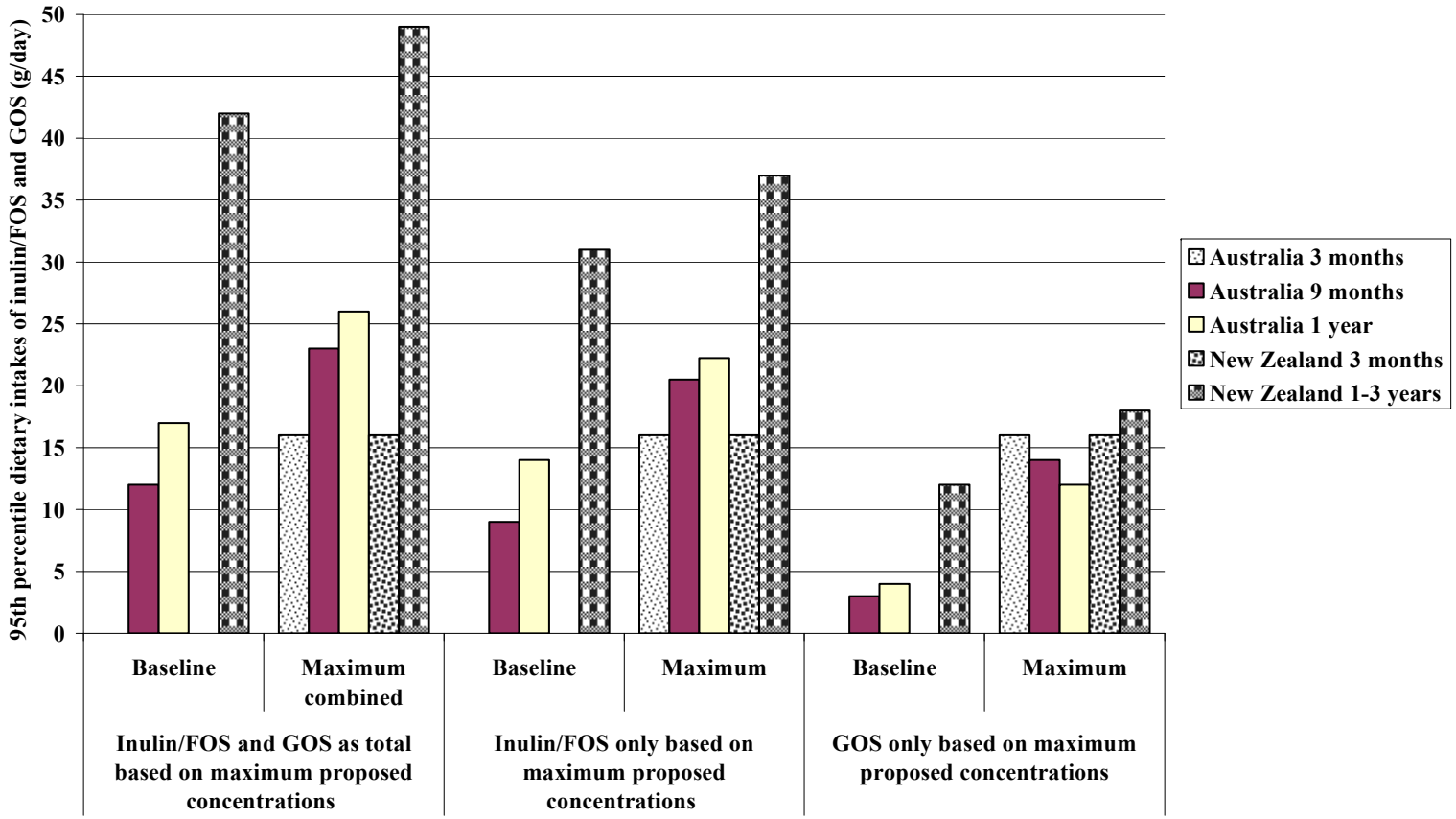


Table 5: Estimated mean dietary intake of inulin-derived substances and GOS for *'combined'* and *'separate'* assessments for Australia and New Zealand population groups

Population groups	Age	50th percentile body weight (kg)	Estimated energy requirement (kJ/kg bw/day)	Estimated intake of formula# (mL/day)	Combined assessment		Separate assessment			
					Inulin-derived substances and GOS as total based on maximum proposed concentrations		Inulin-derived substances only based on maximum proposed concentrations		GOS only based on maximum proposed concentrations	
					Mean (g/day)					
					Baseline	*Maximum combined*	*Baseline*	*Maximum*	*Baseline*	*Maximum*
Australia	3 months	6.4	343	800	0	6	0	6	0	6
	9 months	8.9	335	545	5	9	4	8	1	5
	1 year	9.6	345	425	7	10	5	9	1	5
New Zealand	3 months	6.4	343	800	0	6	0	6	0	6
	1-3 years	9.6	NA	280	17	19	12	15	5	7

Energy content of cow's milk based infant formula = 274 kJ/100 g
NA Not applicable

Table 6: Estimated 95th percentile dietary intake of inulin-derived substances and GOS for *'combined'* and *'separate'* assessments for Australia and New Zealand population groups

Population groups	Age	50th percentile body weight (kg)	Estimated energy requirement (kJ/kg bw/day)	Estimated intake of formula# (mL/day)	Combined assessment		Separate assessment			
					Inulin-derived substances and GOS as total based on maximum proposed concentrations		Inulin-derived substances only based on maximum proposed concentrations		GOS only based on maximum proposed concentrations	
					95th percentile (g/day)					
					Baseline	***Maximum combined***	***Baseline***	***Maximum***	***Baseline***	***Maximum***
Australia	3 months	6.4	343	800	0	16	0	16	0	16
	9 months	8.9	335	545	12	23	9	21	3	14
	1 year	9.6	345	425	17	26	14	22	4	12
New Zealand	3 months	6.4	343	800	0	16	0	16	0	16
	1-3 years	9.6	NA	280	42	49	31	37	12	18

Energy content of cow's milk based infant formula = 274 kJ/100 g
NA Not applicable

Table 7: Major contributors for Australia and New Zealand for the 'combined' assessments

1: Australia

Foods	% contribution					
	3 month old		9 month old		1 year old	
	Baseline	Maximum combined	Baseline	Maximum combined	Baseline	Maximum combined
Formula products*	NA	100	NA	47	NA	33
Yoghurt, fruit, full fat	NA	NA	26	13	25	17
Potatoes	NA	NA	21	11	21	14
Rice, white	NA	NA	9	<5	9	6
Bread, white	NA	NA	7	<5	7	<5
Ice cream, full fat, vanilla	NA	NA	6	<5	6	<5
Carrots	NA	NA	5	<5	5	<5

2: New Zealand

Foods	% contribution			
	3 month old		1-3 year old	
	Baseline	Maximum combined	Baseline	Maximum combined
Formula products*	NA	100	NA	11
Yoghurt	NA	NA	45	39
Potatoes	NA	NA	15	13

*Formula products include infant formula for 3 month old, follow-on formula for <1 year old and toddler formula for children 1-3 years old.
NA - Not applicable

Table 8: Major contributors for Australia and New Zealand for the *'separate'* assessments
a: Inulin-derived substances

1: Australia

Foods	% contribution					
	3 month old		9 month old		1 year old	
	Baseline	Maximum	Baseline	Maximum	Baseline	Maximum
Formula products*	NA	100	NA	53	NA	38
Potatoes	NA	NA	27	12	27	16
Yoghurt, fruit, full fat	NA	NA	16	7	16	10
Bread, white	NA	NA	10	<5	9	6
Ice cream, full fat, vanilla	NA	NA	8	<5	8	5
Carrots	NA	NA	7	<5	7	<5
Lettuce, raw	NA	NA	6	<5	6	<5
Rice, white	NA	NA	6	<5	6	<5

2: New Zealand

Foods	% contribution			
	3 month old		1-3 year old	
	Baseline	Maximum	Baseline	Maximum
Formula products*	NA	100	NA	14
Yoghurt	NA	NA	29	25
Potatoes	NA	NA	21	18
Bread, white	NA	NA	6	<5
Carrots	NA	NA	6	<5

*Formula products include infant formula for 3 month old, follow-on formula for <1 year old and toddler formula for children 1-3 years old.
NA - Not applicable

b: GOS

1: Australia

Foods	% contribution					
	3 month old		9 month old		I year old	
	Baseline	Maximum	Baseline	Maximum	Baseline	Maximum
Formula products*	NA	100	NA	80	NA	69
Yoghurt, fruit, full fat	NA	NA	63	12	63	19
Rice, white	NA	NA	21	<5	21	6

2: New Zealand

Foods	% contribution			
	3 month old		1-3 year old	
	Baseline	Maximum	Baseline	Maximum
Formula products*	NA	100	NA	29
Yoghurt	NA	NA	84	54
Cheese	NA	NA	7	<5

*Formula products include infant formula for 3 month old, follow-on formula for <1 year old and toddler formula for children 1-3 years old.
NA -Not applicable

REFERENCES

ACT Community Care. (2000) *From Milk to More...Introducing foods to your baby*. Canberra, Publishing Services.

Boehm, G. and Stahl, B. (2007) Oligosaccharides from Milk. *The Journal of Nutrition* 137: 847s-9s.

Cook, T, Rutishauser, I. and Seelig, M. (2001) *Comparable data on food and nutrient intake and physical measurements from the 1983, 1985 and 1995 National Nutrition Surveys*. Canberra: Australian Food and Nutrition Monitoring Unit, Commonwealth Department of Health and Aged Care, Commonwealth of Australia.

Cook, T., Rutishauser, I, and Allsopp, R. (2001) *The Bridging Study: comparing results from the 1983, 1985 and 1995 Australian national nutrition surveys.* Canberra, Australian Food and Nutrition Monitoring Unit, Commonwealth Department of Health and Aged Care, Commonwealth of Australia.

Espinosa-Martos, Y. P and Ruperez. (2006) Soybean oligosaccharides. Potential as new ingredients in functional food. *Nutricion Hospitalaria* 21.1.

FAO. (2004) *Human Energy Requirements: Report of a Joint FAO/WHO/UNU Expert Consultation, Rome, 17-24 October 2001.* FAO Food and Nutrition Technical Report Series No 1. 2004. FAO, Rome. ftp://ftp.fao.org/docrep/fao/007/y5686e/y5686e00.pdf.

Food Standards Australia New Zealand. (2002) FSANZ Unpublished Data 2002.

Food Standards Australia New Zealand. (2007) *NUTTAB 2006*. Food Standards Australia New Zealand. 1-5-2007.

Hitchcock, N. E., *et al*. (1986). Nutrition and growth in infancy and early childhood: a longitudinal study from birth to five years. *Monographs in Paediatrics* 19: 1-92.

National Health and Medical Research Council. (2001) *Dietary Guidelines for Children and Adolescents In Australia Incorporating Infant Feeding Guidelines For Health Workers* (Draft).

New Zealand Food Safety Authority. (2006) *Bread and Toddler Milk Consumption - Omnibus Results* [September/October/November 2006] Draft Report.

Rutishauser, I. (2002) *Getting It Right:- How to Use the Data From the 1995 National Nutrition Survey*. Canberra: Commonwealth of Australia.

Spiegel, J. E, Rose, R, Karabell, P., Frankos, V. H, and Schmitt, D. F. (1994) Safety and Benefits of Fructoologosaccharides as Food Ingredients. *Food Technology* .

Van Loo, J., *et al*. (1995) On the Presence of Inulin and Oligofructose as Natural Ingredients in the Western Diet. *Critical Reviews in Food Science and Nutrition* 35(6): 525-52.

Vannoort, R. W. and Thomson, B. M. (2005) 2003/04 New Zealand Total Diet Survey. Wellington, New Zealand Food Safety Authority.

WHO. (1985) *Guidelines For The Study of Dietary Intakes of Chemical Contaminants.* 87. Geneva, World Health Organisation. WHO Offset Publication.

WHO. (2007) *The WHO Child Growth Standards*. http://www.who.int/childgrowth/standards/WFA_boys_0_5_percentiles.pdf.

Zuleta, A. and M Sambucetti. (2001) Inulin determination for food labelling. *J.Agric.Food Chem* 49(10): 4570-72.

Appendix 1

Theoretical diets used in the risk assessment

A1.1 How were the estimated dietary inulin-derived substances and GOS intakes calculated?

As there were no data available from the 1995 Australian NNS for children aged < 2 years, FSANZ developed theoretical diets for Australian children to estimate dietary inulin-derived substances and GOS intakes for the target groups of children aged 3 months, 9 months and 12 months.

Similarly, as there were no data available from the 1997 New Zealand NNS or 2002 New Zealand Children's NNS for children aged < 5 years, New Zealand theoretical diets from New Zealand total diet study were used to estimate dietary inulin-derived substances and GOS intake for the New Zealand children aged 3 months and 1-3 years.

Research conducted by UMR Research and the New Zealand Food Safety Authority reported that, for children aged 1-3 years who consume at least 200 ml of toddler formula per day, the average consumption was 460 ml per day (New Zealand Food Safety Authority, 2006). The theoretical diet for Australian children aged 1 year contained 423 g/day of Toddler formula; the theoretical diet for New Zealand children aged 1-3 years contained 267 g/day Toddler formula and approximately 15 g/day of infant formula/ follow-on formula. Therefore, the theoretical diets used in this assessment considered the complete replacement of milk with Toddler formula.

Since the theoretical diets were based on mean food consumption amounts only, individual records were not available to derive a distribution of food consumption amounts and hence a distribution of inulin-derived substances and GOS intakes. The 95th percentile dietary inulin-derived substances and GOS intakes were estimated using the internationally accepted formula (WHO, 1985) of:

95^{th} percentile intake = mean intake x 2.5

A1.1.1 Australian and New Zealand infants aged 3 months

The diet for the 3 month old was based solely on infant formula based on the assumption that milk could be the only food source for this age group. The recommended energy intake for a three-month-old boy (FAO, 2004) at the 50th percentile weight (WHO, 2007) was used as the basis for the theoretical diet. Boys' weights were used because boys tend to be heavier than girls at the same age and therefore have higher energy and food requirements. Dietary intakes of inulin-derived substances and GOS were calculated as follows:

1. Calculate the energy requirements for 3 month old infant:

   = Estimated energy requirement (kJ/kg bw/day) x body weight (kg)
   = 343 kJ/kg bw/day x 6.4 kg
   = 2195 kJ/day

2. Calculate the amount of infant formula required to meet energy requirements:

= Estimated energy requirement (kJ/day) ÷ energy content of infant formula (kJ/100g)

= $\frac{\text{2195 kJ/day}}{\text{274 kJ/100 g formula}}$

= 800 g infant formula per day

3. Calculate the estimated mean dietary intake of inulin-derived substances and GOS:

= Daily amount of infant formula x concentration of (inulin-derived substances and GOS) in formula

= 0.8 kg infant formula/day x concentrations (inulin-derived substances and GOS) per kg infant formula

A1.1.2 Australian infants aged 9 months

The theoretical diet for Australian children aged 9 months was based on information on recommended energy intakes, mean body weight and the proportion of milk and solid foods in the diet for a 9 month old child, and data from the 1995 NNS on foods consumed by a 2 year old child.

The recommended energy intake for a nine-month-old boy (FAO 2004) at the 50th percentile weight (WHO 2007) was used as the basis for the theoretical diet. The body weight of a 50th percentile 9 month old boy was 8.9 kg.

It was assumed that 50 per cent of energy intake was derived from follow-on formula and 50 per cent from solids (Hitchcock *et al*., 1986). The patterns of consumption of a two-year-old child from the 1995 NNS were scaled down and used to determine the solid portion of the 9 month old's diet. Certain foods such as nuts, tea, coffee and alcohol were removed from the diet since nuts can be a choking risk (National Health and Medical Research Council, 2001) and coffee and alcohol are unsuitable foods for infants (ACT Community Care, 2000). Consumption of breakfast cereals was assumed to be in the form of either infant cereal or single grain breakfast cereals, excluding bran-based cereals. All milk consumption was assumed to be in the form of follow-on formula.

A detailed description of the theoretical diet used for Australian children aged 9 month old can be found in Table A1.1

A1.1.3 Australian children aged 1 year

The theoretical diet for Australian children aged 1 year was based on information on recommended energy intakes, mean body weight and the proportion of milk and solid foods in the diet for a 1 year old child, and data from the 1995 NNS on foods consumed by a 2 year old child.

The recommended energy intake for a one year old boy (FAO 2004) at the 50th percentile weight (WHO 2007) was used as the basis for the theoretical diet. The body weight of a 50th percentile 1 year old boy was 9.6 kg.

It was assumed that 35 per cent of energy intake was derived from milk and 65 per cent from solids (Hitchcock *et al*., 1986). The patterns of consumption of a two-year-old child from the 1995 NNS were scaled down and used to determine the solid portion of the 1 year old's diet. Certain foods such as nuts (excluding peanut butter), coffee and alcohol were removed from the diet since nuts can be a choking risk (National Health and Medical Research Council, 2001) and coffee and alcohol are unsuitable foods for infants (ACT Community Care, 2000).

A detailed description of the theoretical diet used for Australian children aged 1 year can be found in Table A1.1

A1.1.4 New Zealand children aged 1-3 years

As there were no data available from the 1997 or 2002 New Zealand NNSs for children aged < 5 years, a theoretical diet was used to estimate dietary inulin-derived substances and GOS intakes for New Zealand children aged 1-3 years. The Simulated Diet for 1-3 year old toddlers that was used in the analysis of the 2003/04 New Zealand Total Diet Survey (NZ TDS) was used to estimate the mean dietary inulin-derived substances and GOS intake in this assessment (Vannoort and Thomson, 2005b). The Simulated Diet was a 14-day diet constructed to represent average consumers and was derived from regional studies, rather than national studies of food and nutrient consumption (Vannoort and Thomson, 2005a). Intakes of inulin-derived substances and GOS were divided by 14 to obtain daily intakes. In order to assume a 'worst-case' scenario, the body weight of a 1 year old child was used in the calculations of inulin-derived substances and GOS intakes in mg/kg bw/day.

A detailed description of the theoretical diet used for New Zealand children aged 1-3 years can be found in Table A1.2.

Table A1.1: Theoretical diet for Australian children aged 9 months and 1 year

Food/Food Group	**Food Consumption Amount (grams per day)**	
	9 months	1 year
Almonds	0.0	0.0
Apple, unpeeled	18.5	26.6
Avocado	0.2	0.4
Bacon	0.2	0.2
Baked beans, in tomato sauce, canned	3.1	4.4
Bananas	9.6	13.9
Beans, green	0.5	0.7
Beef steak, rib/ribeye/sirloin, grilled	1.1	1.6
Beer, 3.5% alcohol	0.0	0.0
Beetroot, canned	0.4	0.6
Biscuits, savoury	1.2	1.7
Biscuits, sweet, plain	2.7	3.9
Bread, multigrain	1.0	1.5
Bread, white	15.0	21.6
Bread, wholemeal	3.4	5.0

Food/Food Group	**Food Consumption Amount (grams per day)**	
	9 months	1 year
Breakfast cereal, mixed grain	0.0	4.3
Breakfast cereal, single grain	3.3	4.7
Broccoli, cooked	2.1	3.0
Butter, regular	0.4	0.5
Cabbage, cooked	0.3	0.5
Cake, chocolate, iced	2.7	3.9
Carrots, cooked	2.8	4.0
Celery, raw	0.5	0.7
Cheese, cheddar, full fat	2.2	3.1
Cheese, cottage	0.2	0.2
Cheese, processed, cheddar type	1.5	2.1
Chicken, breast, fillet	3.3	4.8
Chocolate, milk	2.1	3.0
Coconut, desiccated	0.5	0.7
Cream, pure (not thickened)	0.9	1.3
Cucumber, raw	1.0	1.5
Dairy blend (not reduced fat)	0.1	0.1
Eggs, boiled	2.3	3.3
Fish fillets	0.3	0.5
Fish, battered, takeaway	0.4	0.6
Fish, crumbed, oven bake	0.1	0.2
Grapes	2.6	3.7
Ham	1.8	2.5
Hamburger	0.0	0.0
Ice cream, full fat, vanilla	5.6	8.0
Infant cereal, mixed	3.0	0.0
Infant dessert, dairy based	1.8	1.3
Infant dessert, fruit based	2.0	1.1
Infant dinner, containing meat, chicken or fish	2.6	1.3
Infant formula	0.0	0.0
Juice, orange	113.7	163.8
Lamb chops, loin, grilled	0.6	0.9
Lettuce, raw	0.8	1.1
Liver, sheep	0.0	0.0
Mango	0.6	0.9
Margarine or margarine spread, polyunsaturated	1.4	2.1
Milk, full fat	0.0	0.0
Milk, modified, low fat	0.0	0.0
Mushrooms	0.4	0.6

Food/Food Group	Food Consumption Amount (grams per day)	
	9 months	1 year
Nori sheets	0.0	0.0
Oats, rolled	1.0	1.5
Oil, canola	0.3	0.5
Olives	0.0	0.0
Onions	1.7	2.5
Orange	8.5	12.2
Parsley, fresh	0.0	0.0
Pasta, white	7.0	10.1
Peach, canned in natural juice	3.4	4.9
Peach, fresh	2.8	4.0
Peanut butter	0.0	0.9
Peas, frozen, cooked	1.2	1.8
Pie, meat, individual size	3.2	4.6
Pineapple, fresh	1.1	1.6
Pizza, meat & vegetable containing	0.5	0.6
Pork chops, grilled	0.4	0.6
Potato crisps	2.3	3.3
Potatoes, cooked	12.3	17.8
Prawns, cooked	0.1	0.1
Pumpkin, cooked	1.7	2.5
Rice, white	8.8	12.6
Salmon, canned in brine	0.0	0.0
Salt, iodised	0.0	0.0
Salt, non-iodised	0.0	0.0
Sauce, tomato	0.9	1.3
Sausages, beef	2.4	3.5
Soft Drink	16.1	23.2
Soy Beverage, plain	0.0	0.0
Spinach, fresh, cooked	0.1	0.1
Strawberries	0.8	1.2
Sugar, white	5.9	8.4
Sultanas	1.6	2.3
Sweet corn, kernels, frozen	1.7	2.5
Tea	0.0	0.0
Tomatoes, raw	4.3	6.2
Tuna, canned in brine	0.3	0.5
Water, bottled still	0.0	0.0
Water, tap	0.0	0.0
Watermelon	2.2	3.2

Food/Food Group	Food Consumption Amount (grams per day)	
	9 months	1 year
Wine, white	0.0	0.0
Yoghurt, fruit, full fat	10.1	14.5

Table A1.2: Theoretical diet for New Zealand children aged 1-3 years

Food	Food Consumption Amount	
	(grams per 14 days)	(grams per day)
Apple-based juice	380	27
Apples	350	25
Apricots, canned	60	4
Avocado	20	1
Bacon	30	2
Banana	490	35
Beans	15	1
Beans, baked	100	7
Beef, mince	120	9
Beef, rump	50	4
Beer	0	0
Beetroot	0	0
Biscuit, chocolate	115	8
Biscuit, cracker	60	4
Biscuit, plain sweet	165	12
Bran flake cereal, mixed	30	2
Bread, mixed grain	30	2
Bread, wheatmeal	115	8
Bread, white	425	30
Broccoli/Cauliflower	70	5
Butter	55	4
Cabbage	15	1
Caffeinated beverage	0	0
Cake	60	4
Capsicum	10	1
Carbonated drink	300	21
Carrot	115	8

Food	Food Consumption Amount	
	(grams per 14 days)	(grams per day)
Celery	15	1
Cheese	145	10
Chicken	60	4
Chicken nuggets	50	4
Chinese takeaway dish	0	0
Chocolate beverage	300	21
Chocolate, plain milk	20	1
Coffee beans, ground	0	0
Coffee instant	0	0
Confectionery	35	3
Corn, canned	30	2
Corned beef	35	3
Cornflakes	60	4
Courgette	10	1
Cream	20	1
Cucumber	15	1
Dairy dessert (child)	460	33
Egg	110	8
Fish fingers (child)	40	3
Fish in batter	45	3
Fish, canned	20	1
Fish, fresh	30	2
Flavoured snacks (child)	60	4
Fruit drink, powdered	830	59
Toddler formula	3,740	267
Grapes	20	1
Ham	70	5
Hamburger, plain	80	6
Honey	20	1
Ice-cream	150	11
Infant & follow-on formula	200	14
Infant weaning food, cereal based	0	0
Infant weaning food, custard/fruit dish	0	0
Infant weaning food, savoury dish	120	9

Food	Food Consumption Amount	
	(grams per 14 days)	(grams per day)
Jam	20	1
Kiwifruit	50	4
Kumara	30	2
Lamb/Mutton	40	3
Lambs liver	0	0
Lettuce	15	1
Margarine/Table Spread	35	3
Meat pie	90	6
Melon	30	2
Milk, flavoured	0	0
Milk, trim (0.5%)	0	0
Milk, whole	0	0
Muesli	15	1
Muffin/scone	70	5
Mushrooms	15	1
Mussels	0	0
Nectarines	30	2
Noodles, instant	160	11
Oats, rolled	120	9
Oil	35	3
Onion	15	1
Orange juice	280	20
Oranges	260	19
Oysters	0	0
Pasta, dried	150	11
Peaches, canned	50	4
Peanut butter	20	1
Peanuts	0	0
Pears	70	5
Peas	60	4
Pineapple	20	1
Pizza	70	5
Pork chop	20	1
Potato crisps	35	3

Food	Food Consumption Amount	
	(grams per 14 days)	(grams per day)
Potato, hot chips	210	15
Potatoes, peeled	240	17
Potatoes, with skin	60	4
Prunes	20	1
Pumpkin	80	6
Raisins/Sultanas	99	7
Rice, white	55	4
Salad dressing	0	0
Sausages, beef	150	11
Silverbeet	20	1
Snack bars	30	2
Soup	50	4
Soy, milk	100	7
Spaghetti in sauce (canned)	150	11
Strawberries	20	1
Sugar	25	2
Taro	0	0
Tea	0	0
Tomato	65	5
Tomato sauce	50	4
Tomatoes in juice	45	3
Water	3,500	250
Weet-bix	210	15
Wine, still red	0	0
Wine, still white	0	0
Yeast extract	25	2
Yoghurt	870	62

Appendix 2

Concentration data

Table A2.1: Concentration data obtained from the literature

Concentration Data	Concentration (g/100g)			Data Derivation
Food	**Inulin**	**FOS**	**GOS**	
Bar, Cereal And Milk Solids, Snack Or Breakfast Style	1			(Food Standards Australia New Zealand, 2007)
Artichoke, Jerusalem, Boiled	3.2			
Artichoke, Jerusalem, Raw, Peeled	3.0			
Capsicum	0.1			(Food Standards Australia New Zealand, 2002)
Raw Green Peas	0.5			
Onion, White	0.3			
Carrot, raw	0.3			
Banana				(Van Loo *et al*., 1995)
Raw	0.5	0.5		
Raw- Dried	1.4	1.4		
Asparagus				
Canned	0.2	0.2		
Raw	2.5	2.5		
Boiled	1.7	1.7		
Fried	3.4	3.4		
Chicory Root	41.6	22.9		
Dandelion Greens				
Raw	13.5	10.8		
Cooked	9.1	7.3		
Garlic				
Raw	12.5	5g		
Dried	28.2	11.3		
Globe Artichoke	4.4	0.4		
Jerusalem Artichoke	18	13.5		
Leeks				
Raw	6.5; 3	5.2		
Onions				
Raw	4.3	4.3		
Raw - Dried	18.3	18.3		
Cooked	3	3		
Baked	5	5		
Fried	5.8	5.8		
Wheat				
Bran - Raw	2.5	2.5		
Flour - Baked	2.4	2.4		
Flour - Boiled	0.4	0.4		
Barley				
Raw	0.8	0.8		
Cooked	0.2	0.2		
Rye				
Rye Flour - raw	0.75	0.75		
Rye Flour -baked	0.7	0.7		
Burdock - root	3.5-4			
Camas - bulb	12-20			
Murnong - root	8-13			
Yacon - root	3-19			

Concentration Data	Concentration (g/100g)			Data Derivation
Food	**Inulin**	**FOS**	**GOS**	
Salsify - root	4-11			
Tomato		0.15		(Spiegel *et al*., 1994)
Brown sugar		0.30		
Honey		0.75		
Soy Beans				(Espinosa-Martos and Ruperez, 2006)
Ripe Yellow Soybean Seeds			1.84-1.95	
Unripe Green Soybean Seeds			1.43-1.61	
Sweet Cookies	0.34			(Zuleta and Sambucetti, 2001)
Salted Cookies	0.55			
Skim Milk	1			
Ice-cream				
Lemon	5.09			
Vanilla	5.2			
Chocolate	4.91			
Cereal Bar	16.57			
Diet Cheese	1.61			
Breast Milk			7-12 g/L oligosaccharides	(Boehm and Stahl, 2007)
Bar	*			Confidential commercial information
Spreads Lite	*			
Spreads Olive Lite	*			
Yoghurt			*	Confidential commercial information
infant yoghurt			*	
infant dessert			*	
cheese			*	
smoothie			*	
spread			*	
Infant Formula	*		*	Based on proposed uptake
Infant food	*		*	

Table A2.2: Concentration data used for the dietary intake assessments and comments on data extrapolation

Concentration Data	Concentration (g/100g)			Comments
Food	**Inulin**	**FOS**	**GOS**	
Almonds				No values reported
Apple, Unpeeled				No values reported
Avocado	0.5	0.5		Value for banana = 0.5. Avocado in same category as banana (tropical fruit - inedible peel).
Bacon				No values reported
Baked beans, in tomato sauce, canned			1.52	Average of range (1.43-1.61) for unripe green soybean seed. Beans in same category as soy bean.
Bananas	0.5	0.5		Natural levels reported in raw banana.
Beans, Green			1.52	Average of range (1.43-1.61) for unripe green soybean seed. Common bean in same category as soy bean.
Beef Steak, Rib/Ribeye/Sirloin, Grilled				No values reported
Beer, 3.5% Alcohol				No values reported
Beetroot, Canned	8.19			Average of 4 values for burdock root, murnong root, yacon root and salsify root. Jerusalem artichoke and chicory also in same category but not used in averaged result due to very high levels in these foods in particular.
Biscuits, Savoury	0.55			Value derived from 'salted cookie'.
Biscuits, Sweet, Plain	0.34			Value derived from 'sweet cookie'
Bread, Multigrain	2.4	2.4		Value derived from 'wheat flour - baked'
Bread, White	2.4	2.4		Value derived from 'wheat flour - baked'
Bread, Wholemeal	2.4	2.4		Value derived from 'wheat flour - baked'
Breakfast Cereal, Mixed Grain	1			Value derived from 'Bar, Cereal And Milk Solids, Snack Or Breakfast Style'.
Breakfast Cereal, Single Grain	1			Value derived from 'Bar, Cereal And Milk Solids, Snack Or Breakfast Style'.
Broccoli, Cooked				No values reported
Butter, Regular	*			Confidential commercial information
Cabbage, Cooked				No values reported
Cake, Chocolate, Iced	2.4	2.4		Value derived from 'wheat flour - baked'

Concentration Data	Concentration (g/100g)			Comments
Food	**Inulin**	**FOS**	**GOS**	
Carrots, Cooked	8.19			Average of 4 values for burdock root, murnong root, yacon root and salsify root. Jerusalem artichoke and chicory also in same category but not used in averaged result due to very high levels in these foods in particular.
Celery, Raw	3.45			Average of raw asparagus and globe artichoke values (2.5g and 4.4g respectively). These foods are categorised in the same group as celery
Cheese, Cheddar, Full Fat	*			Confidential commercial information
Cheese, Cottage	*			Confidential commercial information
Cheese, Processed, Cheddar Type	*			Confidential commercial information
Chicken Breast, Fillet				No values reported
Chocolate, Milk				No values reported
Coconut, Desiccated				No values reported
Cream, Pure (Not Thickened)	*			Confidential commercial information
Cucumber Raw				No values reported
Dairy Blend (Not Fat Reduced)	*			Confidential commercial information
Eggs, Boiled				No values reported
Fish Fillets				No values reported
Fish, Battered, Takeaway				No values reported.
Fish, Crumbed, Oven Bake				No values reported.
Grapes				No values reported
Ham				No values reported
Hamburger				No values reported
Ice Cream, Full Fat, Vanilla	5.2			Value derived from 'Ice-cream'
Infant Cereal, Mixed		*		Confidential commercial information
Infant Dessert, Fruit Based	0.5	0.5		Assumes banana based
Infant Dinner, Containing Meat, Chicken Or Fish				No values reported
Infant Formula	*		*	Values imputed based on the information from the applications and maximum proposed levels for each scenarios
Juice, Orange				No values reported
Lamb Chops, Loin, Grilled				No values reported
Lettuce, Raw	27.55	16.85		Average taken from chicory root (41.6) and dandelion greens, raw (13.5) = 27.55 for Inulin. FOS = 16.85 (Average of 22.9 for chicory root and 10.8 for dandelion

Concentration Data	Concentration (g/100g)			Comments
Food	**Inulin**	**FOS**	**GOS**	
				greens).
Liver, Sheep				No values reported
Mango	0.5	0.5		Value for banana = 0.5. Mango in same category as banana (tropical fruit - inedible peel).
Margarine Or Margarine Spread, Polyunsaturated	*			Confidential commercial information
Milk, full fat	0.0	0.0		No values reported
Milk, modified, low fat	1			Value derived from skim milk
Mushrooms				No values reported
Nori Sheets				No values reported
Oats, Rolled	1.65	1.65		Value averaged from wheat (2.5) and barley (0.8) values for inulin and FOS.
Oil, Canola	*			Confidential commercial information
Olives				No values reported
Onions	4.3	4.3		
Orange				No values reported
Parsley, Fresh				No values reported
Pasta, White	0.4	0.4		Value derived from wheat, flour - boiled
Peach, Canned In Natural Juice				No values reported
Peach, Fresh				No values reported
Peanut Butter				No values reported
Peas, Frozen, Cooked			1.52	Value averaged from range of 1.43-1.61 for 'Unripe Green Soybean Seeds' = 1.52
Pie, Meat, Individual Size				No values reported
Pineapple, Fresh	0.5	0.5		Value for banana = 0.5. Pineapple in same category as banana (tropical fruit - inedible peel).
Pizza, Meat & Vegetable Containing				No values reported
Pork Chops, Grilled				No values reported
Potato Crisps	8.19			Average of 4 values for burdock root, murnong root, yacon root and salsify root. Jerusalem artichoke and chicory also in same category but not used in averaged result due to very high levels in these foods in particular.
Potatoes, Cooked	8.19			Average of 4 values for burdock root, murnong root, yacon root and salsify root. Jerusalem artichoke and chicory also

Concentration Data	Concentration (g/100g)			Comments
Food	**Inulin**	**FOS**	**GOS**	
				in same category but not used in averaged result due to very high levels in these foods in particular.
Prawns, Cooked				No values reported
Pumpkin, Cooked				No values reported
Rice, White		2.5	2.5	Value derived from wheat bran - raw as in same category
Salmon, Canned in Brine				No values reported
Salt, Iodised				No values reported
Salt, Non-Iodised				No values reported
Sauce, Tomato				No values reported
Sausages, Beef				No values reported
Soft Drink				No values reported
Soy Beverage, Plain			1.895	Value averaged from range of 1.84-1.95 for 'Ripe Yellow Soybean Seeds' = 1.895
Spinach, Fresh, Cooked	25.35	15.1		Average taken from chicory root (41.6) and dandelion greens, cooked (9.1) = 25.35 for Inulin. FOS = 15.1 (Average of 22.9 for chicory root and 7.3 for dandelion greens).
Strawberries				No values reported
Sugar, White				No values reported
Sultanas				No values reported
Sweet corn, Kernels, Frozen				No values reported
Tea				No values reported
Tomatoes, Raw				No values reported
Tuna, Canned In Brine				No values reported
Water, Bottled Still				No values reported
Water, Tap				No values reported
Watermelon				No values reported
Wine, White				No values reported
Yoghurt, Fruit, Full Fat	*		*	Confidential commercial information