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

Supporting document 1

Risk and Technical Assessment (at Approval)

Short Chain Fructo-oligosaccharides

Executive Summary

Short chain fructo-oligosaccharides (scFOS) can be produced by two discrete methods: enzymatic degradation of inulin or enzymatic condensation from sucrose. The Food Chemicals Codex has established specifications for scFOS that indicate analysis of the respective scFOS preparations should yield no less than 85.0% (w/w) scFOS with at least 30.0% trimer, 45.0% tetramer, and 5.0% pentamer and larger, with the remainder being glucose, fructose and sucrose on a dried basis. Inulin-derived substances (IDS) (including inulin-derived scFOS resulting from enzymatic degradation) are already a permitted addition to infant formula products, infant foods and formulated supplementary foods for young children (FSFYC) alone or in combination with galacto-oligosaccharides (GOS).

These oligosaccharide preparations are added to purposely better align the stool characteristics of formula-fed infants with the softer stools typically associated with breastfed infants. Despite having the same chemical specifications as inulin-derived scFOS ($scFOS_{inulin}$), sucrose-derived scFOS ($scFOS_{sucrose}$) is currently not permitted to be added to infant formula products, infant foods and FSFYC on the basis of its method of manufacture.

As part of an Application to amend the Australia New Zealand Foods Standards Code, to permit the use of $scFOS_{sucrose}$, this risk assessment was undertaken for the purpose of evaluating the technological suitability, safety and benefit of the proposed addition of $scFOS_{sucrose}$ to infant formula products, infant foods and FSFYC as an alternative to already permitted IDS. Additionally, an amendment to Standard 1.3.3 was sought for the enzyme β-fructofuranosidase produced by *Aspergillus niger* to be used as a food processing aid in the production of $scFOS_{sucrose}$.

The conclusions of this risk assessment are summarised as follows:

- $scFOS_{sucrose}$ is technologically suited to its proposed use and complies with international specifications.

- No public health and safety issues were identified with the proposed use of β-fructofuranosidase from *A. niger* as a processing aid in the production of $scFOS_{sucrose}$. An acceptable daily intake (ADI) “not specified” is considered appropriate.

- Results of laboratory animal studies confirmed that $scFOS_{sucrose}$ has no identifiable hazard at concentrations likely to be encountered under Good Manufacturing Practice.

- The digestion of $scFOS_{sucrose}$ was equivalent to IDS in an *in vitro* model of human colonic fermentation, producing comparable levels of short-chain fatty acids (SCFAs) and gas.

- No adverse effects on growth, hydration status, nutrient intake, frequency and nature of adverse events, gastrointestinal intolerance, stool consistency and frequency, or faecal flora, were observed in studies conducted in healthy infants or young children at amounts of $scFOS_{inulin}$, or $scFOS_{sucrose}$ up to 3.0 g/L for periods ranging from 1 week to approximately 3 months.

On the basis of the above considerations, it is concluded that $scFOS_{sucrose}$ produced by β-fructofuranosidase-catalysed condensation of sucrose is technologically justified and is as safe as IDS already permitted to be added to foods generally, and infant formula products, infant foods and FSFYC alone or in combination with IDS and/or GOS up to the currently permitted maximum amounts. Additionally, $scFOS_{inulin}$ and $scFOS_{sucrose}$ have the potential to soften infant stools and may reduce the incidence of constipation, both of which are considered beneficial effects.

Abbreviations

Time		**Weight**	
sec	Second	bw	Bodyweight
min	Minute	wt	Weight
d	Day	ng	Nanogram
wk	Week	µg	Microgram
mo	Month	mg	Milligram
yr	Year	kg	Kilogram
Length		**Dosing**	
nm	Nanometre	iv	Intravenous
µm	Micrometre	po	Oral
mm	Millimetre	mg/kg bw/day	mg/kg bodyweight/day
cm	Centimetre		
m	Metre		
Volume		**Concentration**	
µL	Microlitre	M	Molar
mL	Millilitre	ppb	Parts per billion
L	Litre	ppm	Parts per million
		w/v	Weight per volume
		v/v	Weight per weight
		cfu/g	Colony forming units per gram

Chemistry	
ALT	alanine aminotransferase
AST	aspartate aminotransferase
DP	Degree of polymerisation
GF_2	1-kestose
GF_3	nystose
GF_4	Fructosyl nystose
GOS	Galacto-oligosaccharides
HMO	Human milk oligosaccharides
IDS	Inulin-derived substances
SCFA	Short chain fatty acids
scFOS	Short chain fructo-oligosaccharides
$scFOS_{inulin}$	Short chain fructo-oligosaccharides derived from the enzymatic degradation of inulin
$scFOS_{sucrose}$	Short chain fructo-oligosaccharides derived from the enzymatic condensation of sucrose
U_h	Hydrolysis activity
U_t	Fructosyl transfer activity
Terminology	
ADI	Acceptable daily intake
AOAC	Association of Official Analytical Chemists
ATCC	American Type Culture Collection
EC	Enzyme commission (number)
FCC	Food Chemicals Codex
FSANZ	Food Standards Australia New Zealand
FSFYC	Formulated supplementary foods for young children
GMP	Good manufacturing practice
ICHSAG	Infant and child health scientific advisory group
NOAEL	No observed adverse effect level

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1. Introduction

On the 23rd September 2010, Food Standards Australia New Zealand (FSANZ) received an Application from GTC Nutrition d/b/a Corn Products International Inc (GTC Nutrition) seeking an amendment to Part 2.9 – Special Purpose Foods of the *Australia New Zealand Food Standards Code* (the Code) to permit the optional addition of short chain fructo-oligosaccharides synthesised from sucrose ($scFOS_{sucrose}$) to infant formula products (Standard 2.9.1), foods for infants (Standard 2.9.2) and formulated supplementary foods for young children (Standard 2.9.3 division 4) as an alternative to inulin-derived substances (IDS). The intention is to use $scFOS_{sucrose}$ as an optional alternative to IDS [i.e. either alone or in combination with IDS and/or galacto-oligosaccharides (GOS)] up to the same maximum amounts already permitted for infant formula products (3 g/L), foods for infants (0.8 g/100 g) and formulated supplementary foods for young children (FSFYC) (1.6 g/serve).

The Applicant also sought an amendment to Standard 1.3.3 – Processing Aids of the Code to permit the use of the enzyme, β-fructofuranosidase (also called invertase) from *Aspergillus niger* as a processing aid in the production of scFOS from sucrose. β-Fructofuranosidase (EC 3.2.1.26) from yeast (*Saccharomyces cerevisiae*) is already permitted as a food processing aid in Australia and New Zealand (Standard 1.3.3). The current Application therefore seeks to extend this permission by including a new source organism, *Aspergillus niger*.

1.1 Current permissions for IDS: implications for sucrose-derived scFOS

Inulin is a non-digestible plant fructan produced by members of the *Compositae* family, which includes chicory, and comprises mixtures of polymers of β (2→1)-linked fructose moieties, with or without a terminal glucose moiety. The internationally-recognised specification for inulin states that the degree of polymerisation (DP) of the mixture varies between 3 and 60 (Food Chemicals Codex 2012).

In addition to inulin, Food Chemicals Codex also defines scFOS as being indigestible carbohydrates synthesized from sucrose and fructose through an enzymatic process or from inulin by partial enzymatic hydrolysis. $scFOS_{sucrose}$ is described as a mixture of unbranched polysaccharides consisting of a sucrose molecule joined to additional fructose molecules via a β (2→1) linkage. An analysis of any scFOS preparation, irrespective of its mode of production, should reveal no less than 85.0% (w/w) scFOS with at least 30.0% trimer, 45.0% tetramer, and 5.0% pentamer and larger, with the remainder being glucose, fructose, and sucrose on a dried basis (Food Chemicals Codex 2012). The Applicant has confirmed that the $scFOS_{sucrose}$ proposed to be added to infant formula products, foods for infants and FSFYC has the following distribution; trimer (GF2) =36.2%, tetramer (GF3)=49.1%, pentamer (GF4)=10.7%). This fructo-oligosaccharide distribution conforms to that specified for a scFOS preparation.

Currently, Standard 1.1.1 of the Code defines IDS as: "*mixtures of polymers of fructose with predominantly β (2→1) fructosyl-fructose linkages, with or without a terminal glucose molecule and includes inulin, but does not include those polymers of fructose produced from sucrose by enzymatic action*". On this basis, $scFOS_{sucrose}$ (but not $scFOS_{inulin}$) is currently excluded from being added to infant formula products, foods for infants and FSFYC based solely on its mode of synthesis. This exclusion stems from FSANZ's previous evaluation of IDS and GOS in relation to Proposal P306 – Addition of Inulin/FOS and GOS to Food[1], when it was understood that there was no intention to add it to the food supply at that time; for this reason it was excluded from the scope of the evaluation on the grounds that there was

[1] http://www.foodstandards.gov.au/foodstandards/proposals/proposalp306addition3639.cfm

insufficient data to examine its effects. The current Application now proposes that $scFOS_{sucrose}$ be permitted to be added to certain special purpose foods and on this basis there is an opportunity to evaluate additional data on scFOS by considering an alternative manufacturing process for scFOS.

1.2 Risk assessment context

For the purpose of this risk assessment, the proposed addition of scFOS to infant formula, foods for infants and FSFYC in Australia and New Zealand will be considered in the context of the following:

- The Applicant is seeking permission to add $scFOS_{sucrose}$ to infant formula products, foods for infants and FSFYC as an alternative to the already-permitted IDS (including $scFOS_{inulin}$) – i.e. at the same concentrations.

- The Code already identifies $scFOS_{inulin}$ as a permitted substance. As the chemical specification for scFOS is the same irrespective of its mode of production, this risk assessment considers the use of scFOS in infant formula products, foods for infants and FSFYC.

- There is a history of safe use of infant formula products, foods for infants and FSFYC in Australia and New Zealand.

- Breastmilk contains a range of non-digestible oligosaccharides [so-called human milk oligosaccharides (HMOs)] at concentrations in excess of those currently added to infant formula and proposed to be added as part of the current Application. Data reviewed by FSANZ as part of Proposal P306 – Addition of Inulin/FOS and GOS to Food[2], indicated that the amounts of HMOs are maximal soon after birth (~25 g/L) and thereafter decline to ~15 g/L over the first three post-natal months. This contrasts with the maximum amounts of scFOS of 3 g/L proposed to be added to infant formula products as a substitute for already-permitted IDS.

- scFOS will be degraded like IDS and HMOs in the infant digestive tract. This degradation involves fermentation by intestinal microflora to produce short chain fatty acids (SCFAs) and gas. As the concentration of scFOS proposed to be added to infant formula products, foods for infants and FSFYC is equivalent to that already permitted for IDS, no change in digestion (i.e. net SCFA or gas production) is expected in infants or young children consuming infant formula, infant foods or FSFYC containing scFOS.

In May 2011, a new Ministerial Policy Guideline on the Regulation of Infant Formula Products[3] was notified to FSANZ. This Ministerial Policy Guideline requires a pre-market assessment by FSANZ of all substances proposed for use in infant formula products that do not have a history of safe use in these products. A pre-market assessment includes the requirement to not only assess safety but to substantiate the role of the substance in normal infant growth and development.

NOTE: Hereafter or unless otherwise specified, the term scFOS will be used to cover both $scFOS_{inulin}$ and $scFOS_{sucrose}$ because they have the same chemical specifications.

[2] http://www.foodstandards.gov.au/foodstandards/proposals/proposalp306addition3639.cfm
[3] http://www.foodstandards.gov.au/foodstandards/legislativeandgovernanceforumonfoodregulation/policyguidelines.cfm

1.3 Risk Assessment questions

For this Application, the risk assessment questions were developed in the context of the Section 18 Objectives of the *Food Standards Australia New Zealand Act 1991* and the Ministerial Policy Guideline on the Regulation of Infant Formula Products.

The following risk assessment questions are addressed in this report:

1. Does β-fructofuranosidase derived from *A. niger* achieve its technological function in the form and quantity used to produce $scFOS_{sucrose}$?

2. What are the technological properties of $scFOS_{sucrose}$ and how do these compare with IDS generated by enzymatic hydrolysis that are currently permitted in the Code?

3. Are there any public health and safety issues associated with the use of β-fructofuranosidase derived from *A. niger* as a processing aid?

In relation to the following population groups and products:

Population group	Products
Infants (0-12 months)	Infant formula
Older infants (6-12 months)	Follow-on formula; infant foods
Toddlers (1-3 years)	Toddler milk

4. What are the adverse physiological effects and health risks for the relevant population group consuming:
    a. Infant formula, follow-on formula, infant food or toddler milk containing $scFOS_{sucrose}$ up to current maximum limits?
    b. Infant formula, follow-on formula, infant food or toddler milk containing $scFOS_{sucrose}$ in combination with IDS and/or with GOS up to current maximum limits?
    c. How do the effects and risks for formula-fed infants in a) and b) compare with breastfed infants of comparable age?
    d. How do the effects and risks for formula-fed infants in a) and b) compare with infants fed infant formula or follow-on formula containing IDS generated by enzymatic hydrolysis?

5. What are the positive physiological effects and health benefits for infants of relevant age consuming?
    a. infant formula or follow-on formula containing $scFOS_{sucrose}$ up to the current maximum limits?
    b. infant formula or follow-on formula containing $scFOS_{sucrose}$ in combination with IDS and/or GOS up to the current maximum limits?

6. Would all conclusions in relation to healthy term infants be applicable to preterm infants and infants with gastrointestinal disease?

7. What are the health risks for the general population consuming $scFOS_{sucrose}$-containing foods?

2. Food Technology Assessment

2.1 Characterisation of the enzyme used to prepare $scFOS_{sucrose}$

2.1.1 Identity

Accepted name:	Invertase
Systematic name:	β-D-fructofuranoside fructohydrolase
IUMBM enzyme nomenclature:	EC 3.2.1.26
C.A.S. number:	9001-57-4
Other names:	β-fructofuranosidase, saccharase; glucosucrase; β-h-fructosidase; β-fructosidase; invertin; sucrase; maxinvert L 1000; fructosylinvertase; alkaline invertase; acid invertase

β-Fructofuranosidase can be either an endo or extraceullar enzyme depending upon its source. The invertase produced by *A. niger* (ATCC 20611), is an endocellular enzyme used specifically in the manufacture of scFOS from sucrose.

The source organism, originally classified as belonging to *Aspergillus niger*, was renamed *A. japonicus* in 1997 by the American Type Culture Collection (ATCC) based on morphological characteristics. For consistency with the Application and the Code, this report will refer to the source organism as *A. niger* (ATCC 20611).

The Applicant notes that methods to produce, purify and characterise the properties of this enzyme have been described in detail (Hidaka et al 1988; Hirayama et al 1989).

2.1.2 Enzymatic properties

β-Fructofuranosidase (EC 3.2.1.26) catalyses the hydrolysis of terminal non-reducing β-D-fructofuranoside residues in β-D-fructofuranosides. They also catalyse fructotransferase reactions.

The enzyme described in this Application acts as both an invertase on sucrose molecules and a fructosyltransferase between sucrose molecules and fructofuranosyl-sucrose molecules[4]. Specific reaction products are 1-kestose (GF_2), nystose (GF_3) and fructosyl-nystose (GF_4).

The properties of β-fructofuranosidase are described by Hirayama *et al* (1989). The purified enzyme has an estimated molecular weight of 340,000 by gel filtration, an optimum pH of 5.0-6.0, with almost no activity under pH 3.0 and above pH 10.0 and a temperature optimum of 50-60^{o}C, with above 81% of initial activity remaining at 50^{o}C.

Methodology to determine enzyme activity has been provided by the Applicant. Activity, measured in units, is based on the enzyme's ability to transfer fructose. One unit of enzyme preparation is defined as the amount required to produce one micromole (1 µmol) of GF_2 per hour from a 10% (w/v) sucrose solution at 40^{o}C.

The enzyme has an activity in the range 1.02×10^6 to 1.12×10^6 units/g.

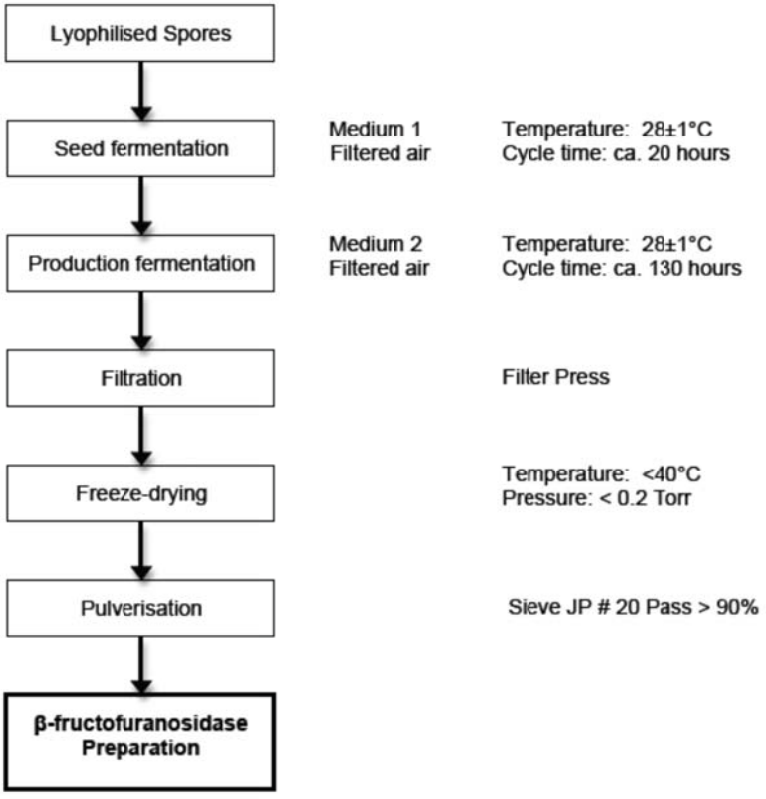
[4] Fructosfuranosyl-sucrose molecules are comprised of fructose chains with a terminal glucose molecule

2.1.3 Production of the enzyme

β-Fructofuranosidase is produced from a non-genetically modified *A. niger* deposited as ATCC 20611.

The controlled fermentation process begins with sterilisation of the culture media and introduction in the batch of lyophilised spores. The process follows standard industry practices and uses appropriate substrates and nutrients. Fermentation is temperature controlled and lasts for approximately 150 hours. Once fermentation is complete, the preparation is concentrated by filtration and freeze drying of the liquid enzyme preparation. Residual amounts of soybean material, which is used as a fermentation nutrient, may remain in the final enzyme preparation. Residual activity of any incidental enzymes produced during normal fermentation processes are present only at relatively low levels and are considered insignificant in the final product.

The general manufacturing process is shown below.



2.1.4 Analysis and Specifications

It is stated in the Application that the enzyme preparation is produced using appropriate GMP controls and processes to ensure the finished product does not contain any impurities of a hazardous or toxic nature.

The Applicant has provided specifications for the commercial enzyme preparation (see Table 2.1). Certificates of Analysis provided in support of the Application demonstrate conformance to the stated specifications.

Table 2.1 *Specifications for the commercial enzyme preparation (as provided by the Applicant)*

	Specification
Enzyme activity	~ 1.0 x 10^6 units/g, not less than 8.0 x 10^5 units/g
Description	Pale brown powder
pH	6.0–7.0 (1 g/100mL)
Water loss	<7%
Arsenic	<1 mg/kg
Heavy Metals	≤10 mg/kg
Mesophylic total count	<50 000 cfu/g
Coliforms	<30 cfu/g
Salmonella	Absence in 25 g
Antibiotic activity	None
Mycotoxins and sterigmatocystin	Absence (<5 mg/kg)

Impurity and microbial specifications written for the enzyme meet international specifications relevant for enzymes prepared by the Joint FAO/WHO Expert Committee on Food Additives (JECFA 2006). The JECFA monographs are a primary reference source listed in Clause 2 of Standard 1.3.4 – Identity and Purity of the Code.

Based on the provided information, FSANZ agrees that β-fructofuranosidase produced from *A*. *niger* ATCC 20611 meets international specifications for enzyme preparations.

2.1.5 Technological function

B-fructofuranosidase is used specifically to produce scFOS from sucrose.

B-fructofuranosidases intrinsically have both hydrolysis (U_h) and fructosyl transfer (U_t) activity, with the ratio (U_t/U_h) varying depending on the specific source organism. Efficient production of fructooligosaccharides from sucrose requires B-fructofuranosidases with high productivity and a high U_t/U_h ratio. B-fructofuranosidase from *A. niger* ATCC 20611 has been demonstrated to have high enzyme productivity, strong transfructosylating ability compared with its hydrolysing activity and high regiospecificity for fructosyl transfer to the 1-OH group of terminal fructofuranosides when sucrose is used as substrate (Fernandez et al 2007; Hidaka et al 1988; Yanai et al 2001; Yun 1996).

2.2 Characterisation of $scFOS_{sucrose}$

2.2.1 Identity

C.A.S. number:	308066-66-2 [5]
Other names:	FOS, scFOS
Trade names:	Neosugar; NutraFlora; NutraFlora® P-95; Meioligo®; Actilight®

2.2.2 Properties of $scFOS_{sucrose}$

There is limited published data on the physiochemical properties of $scFOS_{sucrose}$. Compared to sucrose, the purified $scFOS_{sucrose}$ product is reported to have a similar taste profile but without any cooling effect, higher water retention, higher viscosity at similar concentration and higher thermal stability (Bornet 2001; Yun 1996).

$scFOS_{sucrose}$ are water soluble, non-reducing sugars, therefore they do not lead to Maillard

[5] This CAS number is listed as an agricultural product in 7CFR 205.606 (1-1-08).

reactions and are stable at pH values >3, refrigeration temperatures and temperatures up to 130°C (Bornet 2001). They are also very low in energy (~ 6 kJ/g; 1.5 kcal/g) and non-cariogenic (Maiorano et al 2008; Molis et al 1996).

The Applicant has supplied technical information for the commercial NutraFlora product which states the following physiochemical properties (see Table 2.3).

Table 2.3 *Physiochemical properties of Nutraflora (as provided by the Applicant)*

	Physiochemical property
Molecular weight	Variable, however typically 627 g/mol
Solubility	Highly (~ 80%) soluble in hot and cold water. Almost insoluble in most organic solvents
Water activity	0.1–0.2
pH (10% solution)	5.0–7.0
Calorific value	1.5 kcal/g

Fructose polymers, which include scFOS, may be added to foods for technological reasons (*i.e.* emulsifier, thickener, stabiliser and sweetener) or nutritional reasons (*i.e.* dietary fibre, prebiotic effects) intended to improve stool consistency.

There are no technical reasons restricting the addition of scFOS in combination with either IDS or GOS in infant formula products, infant foods and FSFYC.

2.2.2.1 Sweetness of $scFOS_{sucrose}$ compared with other saccharides

$scFOS_{sucrose}$ in solution has a similar sweetness [30-50% of sucrose (Niness 1999)] to IDS (30-65% of sucrose depending on DP, based on various product specifications) and GOS (30-35% of sucrose, e.g. Vivinal® GOS specification). Infant formulas typically contain 7.1% lactose [30-35% sweetness of sucrose (Parrish et al 1981)] and may contain a maximum of 0.3% added IDS or a maximum of 0.8% added GOS or total of IDS and GOS. Any additional level of sweetness contributed by scFOS would be minimal as it is proposed to be added at a maximum level of 0.3%. As scFOS would replace all or some of the IDS and as these substances provide similar levels of sweetness, there would be no difference in the final sweetness.

2.2.3 Production of $scFOS_{sucrose}$

Commercial production of the scFOS described in this Application is by the enzymatic action of β-fructofuranosidase on sucrose.

β-Fructofuranosidase (see section 2.1.2) cleaves sucrose into glucose (G) and fructose (F) and then links fructose via a β (2→1)- glycosidic linkage to a growing fructose chain to yield GF_2, GF_3 and GF_4 molecules. Each polymer chain produced by this enzymatic method will have one terminal glucose molecule per chain with the following configuration – kestose (glucose α(1→2) fructose β(2→1)- fructose); nystose (glucose α(1→2) fructose β(2→1)-fructose β(2→1)-fructose) etc.

Approximately 150 units/g of enzyme is added to a 50-60% sucrose solution (pH 5.5-6.0). Following the enzyme reaction (approximately 20 hours at 60°C), purification and concentration steps are performed. The only raw material used is sucrose and all processing aids used during manufacture are food grade. The commercial product may be in syrup or powder form. Each batch is analysed for specific performance and quality parameters including GF_2, GF_3, GF_4, ash, moisture, several heavy metals and microbiological contaminants. No enzyme or *A. niger* is present in the final scFOS product, as confirmed by analysis. A statement provided by the Applicant indicates no allergenic material is present in the final product.

The Applicant has provided specification data for a representative commercial powder product of $scFOS_{sucrose}$ (NutraFlora® P-95) (see Table 2.4).

For the powdered product, specifications state a minimum two year shelf-life from date of manufacture when sealed and stored under cool, dry conditions (25°C, 33% relative humidity). The syrup form of the product has a shorter shelf-life of approximately 3 months due to higher moisture content (~30%).

2.2.4 Analysis and Specifications

Specific methods for the detection and quantitative measurement of total scFOS have been developed (Ouarne et al 1999; United States Pharmacopeial 2008) which are similar to the AOAC method for fructan analysis (997.08). The assay is based on the determination of glucose and fructose released by enzymatic hydrolysis of scFOS by β-fructofuranosidase with modifications made for sucrose and free fructose and glucose in the sample.

A specification for scFOS was first published in the 6th Edition of the Food Chemicals Codex (FCC) (United States Pharmacopeial 2008) and updated in the 7th (2010) and 8th (2012) editions. The FCC is a primary reference source listed in Clause 2 of Standard 1.3.4 – Identity and Purity of the Code. Section 1.1 of this report describes the specification for scFOS. No distinctions are made between $scFOS_{sucrose}$ and $scFOS_{inulin}$ with regard to their properties, technological function, methods of analysis or impurity levels.

Based on the information provided by the Applicant, FSANZ concludes that $scFOS_{sucrose}$, as described in this Application (see Table 2.4), meets international specifications for scFOS.

Table 2.4 *Specifications for $scFOS_{sucrose}$ (as provided by the Applicant)*

	Specification
Form	Powder
Structure	α-Glu-(1→2)-[β-Fru(1→2)]$_{2-4}$ 100%
DP_{range}	3-5
DP_{av}	3.6
Purity	≥ 95% db
Kestose (GF_2)	36.2 (30–42)
Nystose (GF_3)	49.1 (45-57)
Fructosyl-nystose (GF_4)	10.7 (5-15)
Protein	<0.5% w/w
Ash	≤ 0.1 w/w
Moisture	≤ 5%
Heavy metals	
Arsenic (as As_2O_3)	≤ 0.1 ppm
Cadmium (Cd)	≤ 0.01 ppm
Lead (Pb)	≤ 0.05 ppm
Mercury (Hg)	≤ 0.01 ppm
Microbiological	
Enterobacteriaceae	≤ 3 cfu/g
Anaerobic thermophilic spores	≤ 100 cfu/g
Aerobic thermophilic spores	≤ 100 cfu/g
Anaerobic mesophilic spores	≤ 300 cfu/g
Aerobic mesophilic spores	≤ 300 cfu/g

DP = degree of polymerisation, db = dry basis, w/w = weight for weight, ppm = parts per million, cfu/g = colony forming units per gram

2.3 Conclusion of food technology assessment

The evidence presented in support of this Application provides adequate assurance that $scFOS_{sucrose}$ is technologically suited to its proposed use and complies with international specifications for scFOS.

3. Hazard Assessment

The scope of this hazard assessment was to:

- Assess the hazard of β-fructofuranosidase derived from *A. niger* when used as a processing aid in the production of scFOS.
- Evaluate the available toxicological data on scFOS to assist in determining the hazard at the levels currently permitted for IDS in special purpose foods.

3.1 Hazard assessment of β-fructofuranosidase from *A. niger*

The hazard of β-fructofuranosidase from *A. niger* has been assessed by considering the:

- production organism, including its history of safe use in food production processes
- history of safe use of β-fructofuranosidase in food production processes and any relevant published data on the hazard of the protein
- presence of the production organism and/or β-fructofuranosidase in the enzyme and scFOS preparations.

3.1.1 Hazard of A. niger

A. niger is a saprophytic fungus that is widely distributed in the environment. It is generally regarded as non-pathogenic to humans although it has been implicated as an opportunistic pathogen in immunocompromised individuals (US EPA 1997; Baker 2006). While *A. niger* is capable of producing mycotoxins (e.g. ochratoxin A) under certain environmental conditions, these are controlled in industrial settings (i.e. during fermentation) and therefore the organism is not hazardous (US EPA 1997; Schuster et al 2002; Nielsen et al 2009).

A. niger has a long history of safe use in the production of enzymes used as food processing aids. FSANZ has previously assessed the safety of a variety of enzymes derived from *A. niger*. Standard 1.3.3 of the Code lists the following enzymes derived from *A. niger* as food processing aids: α-amylase, α-arabinofuranosidase, asparaginase, carboxyl proteinase, catalase, cellulose, chymosin, endo-arabinase, α-galactosidase, β-galactosidase, β-glucanase, glucoamylase, glucose oxidase, α-glucosidase, β-glucosidase, hemicellulase endo-1,4-ylanase, hemicellulase multicomponent enzyme, inulinase, lipase (triacylglycerols), lipase (triacylglycerols, protein engineered variant, lysophospholipase, pectin lyase, pectin esterase, phospholipase A_2, 3-phytase, polygalacturonase or pectinase and transglucosidase.

Analysis of the β-fructofuranosidase enzyme preparation indicated that entire or fragments of mycelian filaments (but no soporiferous organs or spores) were evident in the final enzyme preparation (De Saint-Blanquet 1988). The β-fructofuranosidase enzyme preparation is screened for mycotoxins; certificates of analysis submitted with the Application indicated that there are no detectable (i.e. <5 ppb) aflatoxins, sterigmatocystin or ochratoxin A.

Based on the established history of safe use of various strains of *A. niger* to produce food grade enzyme preparations under highly controlled conditions, and the absence of detectable levels of the production organism in the final $scFOS_{sucrose}$ preparation, the extension of the current permission to include *A. niger* (ATCC 20611) as a new production organism for β-fructofuranosidase raises no public health and safety issues.

3.1.2 Hazard of β-fructofuranosidase

β-Fructofuranosidase hydrolyses terminal non-reducing beta-D-fructofuranoside residues in

beta-D-fructofuranosides. β-Fructofuranosidase is produced by a large number of organisms including bacteria, yeast, fungi and plants as a secreted and/or intracellular protein[6]. On this basis it is likely that humans are widely exposed to various forms of the protein in the diet. As previously mentioned, β-fructofuranosidase from *S. cerevisiae* is already permitted as a food processing aid in Australia and New Zealand.

The production of $scFOS_{sucrose}$ employs an enzyme inactivation step and a series of purification steps (including activated charcoal, filtration and chromatography) that ensures that no enzyme is present in the final scFOS preparation. Indeed the absence of β-fructofuranosidase has been demonstrated by the non-detection of protein (LOD = 0.5%). Therefore, there is limited potential for any dietary exposure to β-fructofuranosidase to occur.

3.2 Hazard assessment of scFOS

As IDS and GOS have previously been assessed as safe by FSANZ in Proposal P306, no further evaluation of these substances is included in this assessment.

The toxicological database for scFOS consists of both published and unpublished studies that were submitted by the Applicant. In addition, FSANZ conducted a search of the published scientific literature for any other relevant studies. The test material in the submitted toxicity studies was consistent with that intended for commercial use (i.e. compliant with the proposed specifications). FSANZ has independently evaluated the submitted toxicity studies on scFOS including studies on acute toxicity, repeat-dose toxicity, chronic toxicity/carcinogenicity and genotoxicity.

3.2.1 History of use of scFOS in infant formula

The Applicant provided information indicating that in the US, scFOS is permitted to be added to infant formula (2.5 g/L), follow-on formula (2.5 g/L) and toddler foods (1 g/serve). In some countries in Europe, scFOS is permitted to be added to infant formula and follow-on formula, while infant formula containing scFOS is marketed in Japan, Pakistan, China, Vietnam and Taiwan.

In Japan, scFOS has been added to infant formula products since 1987 (Yamamoto and Yonekubo 1993). A large nationwide survey reported soft and 'yellowish' stools in infants that consumed formula containing scFOS but no difference in health outcomes compared to breastfed infants (Yamamoto and Yonekubo 1993). The addition of scFOS to formula resulted in an increase in the frequency of soft stools but no effect in the incidence of watery stools.

3.2.2 Digestion of scFOS

Proposal P306 reviewed the digestion of HMOs, IDS (including oligofructose) and GOS. Like HMOs, oligosaccharides ingested in infant formula (e.g. IDS, GOS) are largely undigested in the small intestine, undergoing colonic fermentation by microorganisms to yield SCFAs.

In adults, scFOS are hydrolysed to a very limited extent by digestive enzymes in the small intestine and approximately 89% of ingested scFOS reach the colon intact (Molis et al 1996). Once in the colon, scFOS are completely fermented by colonic microorganisms and are metabolised to SCFAs (acetate, propionate and butyrate, which are either absorbed in the colon, or metabolised in the colon, liver or peripheral tissues), L-lactate, carbon dioxide, and hydrogen (Bornet et al 2002). scFOS is not excreted in stools, and small amounts are

[6] http://pir.georgetown.edu/cgi-bin/textsearch.pl

excreted in urine.

3.2.3 Acute toxicity

Takeda U (1981a) Acute toxicity study of Neosugar. Lab and Sponsor: Laboratory of Toxicology, Pharmacology and Toxicology Laboratories. Meiji Seika Kaisha Ltd, Yokohama, Japan. Unpublished.

Limited methodological and reporting details were given in the study report. $scFOS_{sucrose}$ (purity unspecified; ratio of the DP of 2, 3 and 4 of 31:55:14, respectively) was dissolved in water and administered (by gavage) to groups of six male and female JcL-IcR mice and Sprague Dawley (SD) rats at a dose of 3, 6 or 9 g/kg bw. Animals were sourced from Nippon Charles River Co (Japan). Mice were 4 weeks of age and rats were 6 weeks of age. Pre-treatment bodyweights were approximately 20/18 g for male/female mice and 169/120 g for male/female rats. The dose volume was 0.5 mL in mice and 2 mL in rats. Animals were observed for 7 days. There were no mortalities. Bodyweights were unremarkable and there were no gross pathological abnormalities. The LD_{50} in mice and rats was >9000 mg/kg bw.

3.2.4 Short-term repeat dose toxicity

Takeda U (1982b) Subchronic toxicological study of Neosugar. Lab and Sponsor: Laboratory of Toxicology, Pharmacology and Toxicology Laboratories. Meiji Seika Kaisha Ltd, Yokohama, Japan. Unpublished.

The level of reporting detail was limited. Two rat studies were undertaken with $scFOS_{sucrose}$ (ratio of the DP of 2, 3 and 4 of 31:55:14) respectively) and a mixture of 51% scFOS, 38% monosaccharides (presumably fructose and glucose) and 11% sucrose (purity, Batch No. and source unspecified). Dosing was by gavage (Study 1) or via the diet (Study 2). In both studies, male Wistar rats were used (6-8 weeks old; sourced from Nichi-ido Co, Japan).

In Study 1, groups of 18 male Wistar rats were dosed with 0, 1.5, 3 or 4.5 g/kg bw/day scFOS (or the scFOS mixture) for 6 weeks. Blood was collected at weeks 2, 4 and 6 and analysed for a limited number of clinical chemistry parameters. At sacrifice, the following organs were macroscopically examined: adrenals, pancreas, kidney, brain, heart, lung, spleen and testis. It was stated that no rats died during the study and there were no abnormal clinical signs. Graphically-presented data indicated that bodyweight gain was similar across all groups. There were a number of statistically significant differences ($p<0.01$) in haematology parameters between treated and control groups but in the absence of any numerical data it was not possible to evaluate the biological relevance of the differences. It was stated that distension of the caecum occurred 4/8 rats in the group dose with 4.5 g/kg bw/day scFOS. No further results were reported.

In Study 2, scFOS (or the scFOS mixture) was admixed in the diet at levels of 5 and 10% and fed *ad libitum* to Male Wistar rats for 6 weeks (~2500 and 5000 mg/kg bw per day, respectively). Separate groups of rats were dosed with sorbitol, glucose or sucrose at the same dietary concentrations. It was stated that no rats died during the study. Diarrhoea (of an unspecified severity and duration) was reported in an unspecified number of treated rats. At a dietary level of 10%, mean bodyweight was lower than the control group, without any difference in food consumption. Similar to Study 1, significant differences in haematology parameters were non-interpretable ($p<0.01$) because of the lack of numerical data. Also similar to Study 1, distension of the caecum was reported in treated rats (numbers affected unreported). Histopathology revealed dilatation of the renal distal tubules in 3/8 rats at a dietary concentration of 10% and in one rat in the 10% glucose group. The authors concluded that scFOS had the same toxicity profile as sucrose, glucose and sorbitol.

Tokunaga T, Oku T, Hosoya N (1988) Influence of chronic intake of new sweetener

fructooligosaccharide (Neosugar) on growth and gastrointestinal function of the rat. J. Nutr. Sci. Vitaminol **32**: 111-121.

Experimental
scFOS (purity and Batch No. unspecified; ratio of the DP of 2, 3 and 4 of 28:60:12, respectively; sourced from Mie Kariyo Co, Japan) was admixed in the diet and fed *ad libitum* to groups of six male Wistar rats (sourced from Nisseizai Co, Tokyo, Japan; 40-50 g bodyweight) at concentrations of 0, 10 or 20% for 6-8 weeks (~5000 and 10000 mg/kg bw per day, respectively). An additional group of rats was fed a diet containing 20% glucomannan (data not evaluated). All diets contained an equivalent concentration of total carbohydrate (67%), with the balance made up of corn starch.

One week prior to sacrifice, rats were housed individually in metabolism cages. Food intake was measured for 3 days. Faeces were collected for the analysis of neutral and acidic sterols, and volatile fatty acids. Over the remainder of the week, gastrointestinal tract (GIT) transit time was measured using carmine red as a marker. Rats were then fasted for 18 hours prior to sacrifice. The following organs were weighed: liver, kidney, small intestine, caecum and colon. Blood was also collected at sacrifice for the analysis of total cholesterol and triacylglycerol concentrations. Data were statistically analysed.

Findings
Mortalities and clinical signs were not reported. Graphically-presented data indicated that at a dietary concentration of 10%, mean bodyweight gain was lower than the control; this result was statistically significant ($p<0.01$). There was no treatment-related effect on food consumption. Mean faecal weight was significantly increased ($p<0.01$) in treated rats relative to the control group (0.58, 0.83, 1.15 g/day at 0, 10 and 20%, respectively). The increase in faecal weight was concomitant with a reduction in mean GIT transit time (27.7, 20.5 and 14.0 h at 0, 10 and 20%, respectively), which was statistically significant ($p<0.01$) at 10%.

There was a treatment-related increase in relative weight of the small intestine, caecum and colon relative to the control group, which was statistically significant ($p<0.05$ or 0.01) at 10 and/or 20% scFOS (Table 3.1). In the absence of histopathological analysis, it is unclear whether these findings are adverse but are likely to reflect the increased fermentation of scFOS in the gastro-intestinal tract. There was no treatment-related effect on any other organ weights.

Table 3.1 *Mean relative organ weights*

Parameter	Dietary concentration of scFOS (%)		
	0	10	20
Terminal bw	313.5±13.0	291.3±12.0	237.0±8.8**
Small intestine	1.85±0.05	1.94±0.15	2.29±0.16*
Caecum	0.17±0.01	0.37±0.04**	0.63±0.05**
Colon	0.29±0.01	0.35±0.02*	0.49±0.03**

*Results expressed as means ± 1 SD; *p<0.05; **p<0.01*

There was no treatment-related effect on total cholesterol. At dietary concentrations of 10 and 20% scFOS, mean triacylglycerol concentrations were significantly lower ($p<0.01$ and 0.05, respectively) than the control (209±21.8, 150±4.0 and 144.6±23.0 mg/100 mL serum at 0, 10 and 20%, respectively).

Graphically-presented data illustrated that the faecal excretion of total neutral sterols was significantly higher ($p<0.01$) than the control at 10 and 20% scFOS. Additionally, the faecal excretion of total bile salts was significantly elevated ($p<0.01$) at 20% scFOS.

There was an increase in the concentration of volatile fatty acids in faeces of rats that

ingested scFOS relative to the control group. Graphically-presented data illustrated that there was an increase in the excretion of acetic acid and propionic acid in faeces.

3.2.5 Chronic toxicity/carcinogenicity

Clevenger MA, Turnbill D, Inoue H, Enomoto M, Allen JA, Henderson LM, Jones E (1988) Toxicological evaluation of neosugar: genotoxicity, carcinogenicity and chronic toxicity. Journal of the American College of Toxicology **7**(5): 643-662.

Experimental
scFOS (>95% purity; ratio of the DP of 2, 3 and 4 of 37:51:12, respectively; sourced from Mie Kariyo Co, Japan; Batch No. unspecified) was administered to groups of 50 F344 rats/sex/group at dietary concentrations of 0, 800, 20,000 or 50,000 ppm *ad libitum* for 104 weeks. The highest concentration was based on the maximum amount of scFOS that could be incorporated into the diet. Rats were housed individually under standard conditions.

Rats were observed daily. Bodyweights were recorded weekly to week 26 then twice weekly thereafter. Food consumption was recorded weekly for each rat. Food conversion efficiency was calculated from bodyweight and food consumption data. All survivors were killed at the end of 104 weeks and blood collected for the analysis of standard haematology and clinical chemistry parameters (with the exception of clotting parameters and reticulocytes count). Selected organs from survivors were weighed (brain, adrenals, heart, spleen, lungs, testes, liver, ovaries and kidneys). All rats were necropsied and their tissues collected for histopathology. Results were statistically analysed.

Findings
Survival, bodyweight gain, food consumption and food conversion efficiency were unaffected by treatment. Doses of scFOS were calculated by the authors to be 341/419, 854/1045 and 2170/2644 mg/kg bw per day in males/females at 800, 20,000 or 50,000 ppm, respectively. There was no treatment-related effect on any haematology parameters. Statistically significant differences in a number of clinical chemistry parameters were determined in males between treated and control groups. On closer inspection these differences were concluded to be incidental in nature based on the lack of a dose-response relationship and the absence of any differences in females.

Organ weights were unremarkable. Selected non-neoplastic lesions are summarised in Table 3.2. There was an increase in the incidence of granulation of the lymph nodes in treated males relative to the control, which was significant ($p<0.05$) at every dietary concentration. However, given that the incidence of this lesion was within the historical control range (0-40%) in age- and sex-matched rats of this strain, and as no such difference was observed in females [a significantly lower ($p<0.05$) incidence in this lesion occurred at the highest dose], it is not considered treatment-related. In males, the incidence of dilated gastric glands was significantly higher ($p<0.05$) than the control at 20,000 and 50,000 ppm. Given the very high background incidence of this lesion (0-79%), it is not considered treatment-related but within the realms of normal biological variation. At 20,000 ppm females, adrenal hyperplasia was significantly higher ($p<0.05$) than the control, but in the absence of a dose-response relationship, the lack of a similar finding in males and as the incidence was within the historical control range (0-17%); it was not considered treatment-related.

Table 3.2 *Incidence (%) of non-neoplastic lesions in rats*

Lesion	Dietary concentration (ppm)			
	0	8000	20,000	50,000
Lymph node granulation				
Males	2	16*	24*	32*
Females	20	10	10	48
Dilated gastric glands				

Lesion	Dietary concentration (ppm)			
	0	8000	20,000	50,000
Males	4	14	20*	30*
Females	Not reported	Not reported	Not reported	Not reported
Adrenal hyperplasia				
Males	26	28	52	44
Females	0	2	14*	8

**p<0.05*

In males, the incidence of pituitary adenoma was significantly higher than the control at 20,000 and 50,000 ppm ($p<0.05$ and 0.01, respectively) (20, 26, 38 and 44% at 0, 8000, 20,000 and 50,000 ppm, respectively). In contrast, treated females showed a lower incidence of the same neoplasm relative to the control group (48, 38, 38 and 28% at 0, 8,000, 20,000 and 50,000 ppm, respectively). Based on this inconsistency between males and females, and the highly variable background incidence of pituitary adenoma in F344 rats (20-50%), these findings are considered to reflect normal biological variation and are not treatment-related.

Conclusions
The NOAEL was 50,000 ppm (equal to 2170/2644 mg/kg bw/day in males/females), the highest dietary level tested. There was no evidence that scFOS was carcinogenic.

3.2.6 Genotoxicity studies

Table 3.3 summarises the results of *in vitro* genotoxicity assays conducted on $scFOS_{sucrose}$. Positive and negative (vehicle) controls were tested in all studies and gave expected results. There was no evidence of genotoxicity.

Table 3.3 *Summary of genotoxicity studies on scFOS*

Test	Test system	Test article	Concentration	Result	Reference
Bacterial reverse mutation (Ames test)	*Salmonella typhimurium* strains TA98 TA100, TA1535 TA1537 TA1538 (±S9)	scFOS (Neosugar) Water vehicle	0, 50, 150, 500, 1500 and 5000 µg/plate	Negative	Clevenger et al (1988)
Bacterial reverse mutation	*Eschericia coli* WP2 uvrA (±S9)	scFOS (Neosugar) Water vehicle	0, 50, 150, 500, 1500 and 5000 µg/plate	Negative	Clevenger et al (1988)
Mammalian forward mutation	Mouse lymphoma L5178Y cells (±S9)	scFOS (Neosugar) Water vehicle	0, 2000, 3000, 4000 and 5000 µg/mL	Negative	Clevenger et al (1988)
Unscheduled DNA synthesis	HeLa cells	scFOS (Neosugar) Water vehicle	0, 25, 50, 100, 200, 400, 800, 1600, 3200, 6400, 12,800, 25,600 and 51,200 µg/mL	Negative	Clevenger et al (1988)

±S9 = study conducted in the presence and absence of an exogenous source of metabolic activation (S9 liver preparations from Aroclor 1254-induced rats).

3.2.7 Human studies

3.2.7.1 In vitro studies based on models of human digestion

Hernot DC, Boileau TW, Bauer LL, Middelbos IS, Murphy MR, Swanson KS, Fahey Jr GC (2009) *In vitro* fermentation profiles, gas production rates and microbiota modulation as affected by certain fructans, galactooligosaccharides, and polydextrose. J. Agri. Food Chem. **57**: 1354-1361.

Experimental

Using a model of large bowel fermentation (developed by Bourquin et al 1993), eleven different oligosaccharides (115 mg per 16 mL of medium), including scFOS (GTC Nutrition, Colorado, USA), were incubated with human faecal inoculum (pooled from 3 male volunteers) for 0, 4, 8 or 12 h (n=3). The production of gas (total, rate, time to maximum concentration, hydrogen and methane), change in pH, and the production of SCFAs (acetate, propionate, butyrate, total) and lactate, were analysed in samples collected at these times. Bifidobacteria, lactobacilli and *E. coli* were also quantified. Data for gas and SCFA production were fitted to a logistic model equation to determine the rate of production and time to maximum production. The interaction of substrate and time of fermentation for each parameter was statistically analysed.

Findings

Tables 3.4-3.8 summarise results for $scFOS_{sucrose}$, $scFOS_{inulin}$, inulin (derived from agave[7]) and GOS as these provide the most relevant comparisons for the current Application. Little difference was evident between $scFOS_{sucrose}$, $scFOS_{inulin}$, inulin and GOS with regard to gas production (Tables 3.4 and 3.5), changes in pH (Table 3.6) and the production of SCFAs (Table 3.7). The only difference between these compounds was that gas production was somewhat slower from the fermentation inulin (Tables 3.4 and 3.5). There was also no difference in the concentration of bacteria across the four treatments over time (Table 3.8) although the relatively short duration (≤12 hours) of the experiment limits the potential to discern any differences of biological significance.

Table 3.4 *Gas production from the fermentation of $scFOS_{sucrose}$, $scFOS_{inulin}$, inulin and GOS*

Test substance	Gas production (mL g DM)			Rate of gas production (mL/g DM h^{-1})	Time to reach max gas production (h)
	4 h	8 h	12 h		
$scFOS_{sucrose}$	120.2	134.2	147.6	73.2	3.2
$scFOS_{inulin}$	148.1	137.4	142.8	-[a]	-[a]
Inulin	52.2	143.3	148.0	38.7	4.6
GOS	110	114.2	126.2	79.3	3.1

Results expressed as means
DM = dry matter
[a] *Unable to be determined as fermentation did not follow a logistic model*

Table 3.5 *Production of hydrogen and methane from the fermentation of $scFOS_{sucrose}$, $scFOS_{inulin}$, inulin and GOS*

Test substance	H_2 production (mg/g DM)			CH_4 (mg/g DM)		
	4 h	8 h	12 h	4 h	8 h	12 h
$scFOS_{sucrose}$	3.9	3.4	7.8	0	1.6	2.2
$scFOS_{inulin}$	5.3	5.3	6.0	2.3	2.2	3.2
Inulin	0.9	6.3	7.2	1.1	1.2	3.0
GOS	3.9	4.1	5.0	0	2.4	2.5

Results expressed as means
DM = dry matter

[7] Agave are a genus of succulent plants.

Table 3.6 *pH changes from the fermentation of $scFOS_{sucrose}$, $scFOS_{inulin}$, inulin and GOS*

Test substance	pH change			
	0 h	4 h	8 h	12 h
$scFOS_{sucrose}$	6.52	-1.20	-1.41	-1.32
$scFOS_{inulin}$	6.5	-1.02	-1.03	-0.96
Inulin	6.48	-0.90	-1.26	-1.19
GOS	6.51	-0.84	-0.96	-0.96

Results expressed as means

Table 3.7 *SCFA production (mg/g DM) from the fermentation of $scFOS_{sucrose}$, $scFOS_{inulin}$, inulin and GOS*

SCFA	$scFOS_{sucrose}$	$scFOS_{inulin}$	Inulin[a]	GOS
Acetate				
4 h	201	246	173	234
8 h	242	254	226	282
12 h	265	278	229	278
Propionate				
4 h	62	61	43	62
8 h	74	68	52	72
12 h	77	71	52	74
Butyrate				
4 h	66	77	72	72
8 h	139	128	163	121
12 h	152	141	172	137
Total SCFA				
4 h	329	384	287	368
8 h	455	450	441	475
12 h	495	490	452	489
Lactate				
4 h	18	15	15	12
8 h	3	0	0	0
12 h	0	0	0	0

Results expressed as means
[a] *From the agave plant*

Table 3.8 *Effect of the fermentation of $scFOS_{sucrose}$, $scFOS_{inulin}$, inulin and GOS on bacterial numbers (log_{10} cfu/tube)*

SCFA	$scFOS_{sucrose}$	$scFOS_{inulin}$	Inulin	GOS
Bifidobacteria				
4 h	8.7	8.6	8.0	8.6
8 h	8.6	8.1	8.0	8.5
12 h	8.5	8.4	7.6	8.5
Lactobacilli				
4 h	9.5	9.5	9.5	9.4
8 h	9.5	9.4	9.7	9.3
12 h	9.2	9.2	9.3	9.1
E. coli				
4 h	8.6	8.7	8.7	8.7
8 h	8.6	8.5	8.6	8.7
12 h	8.4	8.3	8.3	8.4

Results expressed as means

Under the conditions of this study, there were no appreciable differences in the production of gas and SCFAs or any effect on bacterial populations over 12 hours from the fermentation of $scFOS_{sucrose}$, IDS and GOS. Despite differences in digestive tract microflora between infants and adults, these findings indicate that scFOS would behave no differently to already permitted fructans in infant formula.

3.2.7.2 In vivo studies

Human studies relevant to the current application have been evaluated in the subsequent chapter, *Physiological effects of scFOS in infants and young children*. Collectively these studies indicate that there were no adverse effects in infants and young children that consumed formula containing scFOS.

3.2.8 Supplementary data: studies conducted on scFOS$_{inulin}$

Studies on scFOS$_{inulin}$ submitted by the Applicant have been evaluated by FSANZ and included as supplementary data. The results of these studies are summarised in Table 3.9.

***Table 3.9:** Results of studies conducted on scFOS$_{inulin}$*

Study	Result	Reference
Digestibility study in humans	Dosing with 5 or 15 g/day scFOS for 5 weeks caused increased hydrogen gas production relative to the control, reaching statistical significance ($p<0.05$) at the highest dose. There was no effect on the incidence of GI complaints (bloating, pains or cramping, stool form or frequency), total concentration and proportion of SCFAs, faecal pH and faecal weight. Complete degradation of scFOS occurred in the large intestine.	Alles et al (1996)
Digestibility study in humans	~90% (range 60-109%) of an oral dose of scFOS (20.1 g/day) was not absorbed and was completely fermented by colonic flora. Small levels of FOS were detected in urine (0.05-0.30%).	Molis et al (1996)

3.3 Discussion

β-Fructofuranosidase is already permitted as a food processing aid in Australia and New Zealand. The proposed new source organism of this enzyme, *A. niger*, has a long history of safe use in the production of food-grade enzymes, with over 20 permissions for enzymes derived from this organism already included in the Code. Additionally, neither the source organism nor β-fructofuranosidase are detectable in the final scFOS$_{sucrose}$ preparation proposed to be added to infant formula products, infant foods and FSFYC. No public health and safety issues have been identified with the proposed use of β-fructofuranosidase from *A. niger* as a processing aid in the production of scFOS$_{sucrose}$. Based on these considerations, an ADI "not specified" is considered appropriate.

The substitution of already permitted IDS with scFOS$_{sucrose}$ does not increase the level of consumption of oligosaccharides in infant formula products, which is well below the concentration of HMOs in breast milk. In addition, as scFOS$_{sucrose}$ undergoes the same degradation process in infants to these permitted fructans, there is no increase in the potential total amount of SCFAs formed in the lower digestive tract and therefore no possible alteration in physiological effects. Indeed, this has been confirmed *in vitro* where gas and SCFA production resulting from the fermentation of scFOS by human microbiota was comparable to inulin and GOS (Hernot et al 2009). An *in vivo* human study on scFOS$_{inulin}$, indicated that SCFA production (including the proportion of individual SCFAs) was not increased following ingestion of up to 15 g/day scFOS$_{inulin}$ (Alles et al 1996).

Results of laboratory animal and human studies determined that scFOS irrespective of its mode of production is not hazardous.

- The acute oral toxicity of scFOS$_{sucrose}$ is very low (the LD_{50} in mice and rats was >9000 mg/kg bw) (Takeda 1981a).
- In the subchronic rat study by Tokunda et al (1988), no adverse effects occurred

following repeated dietary exposure to $scFOS_{sucrose}$ at dietary concentrations up to 20%. Increased excretion of SCFAs concomitant with an increased in caecal weights was not considered adverse but to have resulted from the fermentation of $scFOS_{sucrose}$ in the digestive tract.

- In a chronic rat study, the NOAEL was 50,000 ppm (equal to 2170/2644 mg/kg bw/day in males/females), the highest dietary level tested (Clevenger et al 1988). There was no evidence that $scFOS_{sucrose}$ was carcinogenic.
- $scFOS_{sucrose}$ was not genotoxic *in vitro* (Clevenger et al 1988).

3.4 Conclusions of hazard assessment

No public health and safety issues were identified with the proposed use of β-fructofuranosidase from *Aspergillus niger* as a processing aid in the production of scFOS. An ADI "not specified" is considered appropriate.

Results of laboratory animal studies at doses far in excess of those likely to be encountered by infants and young children confirmed that scFOS has no identifiable hazard at concentrations likely to be encountered under GMP.

The fermentations of $scFOS_{sucrose}$ were equivalent to IDS (including $scFOS_{inulin}$) in an *in vitro* model of human colonic fermentation, producing comparable levels of SCFAs and gas.

Overall, no toxicological issues were identified with regard to the addition of $scFOS_{sucrose}$ to infant formula at concentrations equivalent to current permissions for IDS, including $scFOS_{inulin}$.

4. Physiological effects of scFOS in infants and young children

4.1 Introduction

scFOS prepared from either sucrose or inulin has the same chemical purity and specifications. A range of preclinical toxicological studies or in vitro studies which measure fermentation gas release and fatty acid production yielded equivalent results (Section 3; Hernot, 2009). As the two production methods give rise to structurally and functionally similar scFOS, all the tolerance studies undertaken in infants were analysed together, irrespective of whether the scFOS was prepared using either sucrose or inulin

In addition to the assessment of potential adverse physiological effects of scFOS in infants and young children, the scope of this assessment also considers the potential beneficial physiological effect of scFOS in these population groups in light of the Ministerial Policy Guideline on the Regulation of Infant Formula Products (Ministerial Council 2011).

4.2 Evaluation

The physiological database for all scFOS consists of unpublished and published studies submitted by the Applicant in addition to published studies identified by FSANZ following a comprehensive search of the scientific literature. FSANZ undertook an initial electronic literature search on 24 January 2012, which was updated on the 10 October 2012, using PubMed (http://www.ncbi.nlm.nih.gov/pubmed/) and the search terms 'Fructo-oligosaccharide' OR 'Fructooligosaccharide'. For both searches, the following limits were used: Clinical trial, Meta-analysis, Randomized Controlled Trial, Humans, English, All infant (birth-23 months), and Preschool children (2-5 years). To be considered for detailed evaluation and inclusion in this physiological assessment studies were required to be published as a full report, described as randomised, blinded, and placebo-controlled, include only scFOS as the test material unique to the intervention group, and include healthy human participants that were ≤3 years old at recruitment.

Only two randomised controlled trials of the 20 studies identified from the literature searches met the inclusion criteria (Euler et al 2005; Bettler and Euler 2006). These studies are included in this assessment.

Additional searches were conducted in individual journals for randomised controlled trials using the keywords 'fructooligosaccharide' OR 'fructo-oligosaccharide'. Searches were conducted in:

- All American Society for Nutrition journals (American Journal of Clinical Nutrition, Journal of Nutrition, and Advances in Nutrition)
- All Nature.com publications (including European Journal of Clinical Nutrition and American Journal of Gastroenterology)
- Journal of Pediatric Gastroenterology and Nutrition
- Cochrane Library.

This search did not identify any additional studies that met the inclusion criteria.

Studies typically examined the effect of infant formula supplemented with scFOS up to a concentration of 3.0 g/L (i.e. equivalent to the already-permitted maximum concentration of IDS) on the following parameters:

- anthropometric parameters (bodyweight, length and occipital head circumference)
- formula intake and frequency of feeding
- adverse events

- gastrointestinal intolerance (spit-up/vomiting, flatulence, diarrhoea, burping, fussiness)
- stool consistency and frequency
- faecal flora.

In some studies, a limited number of clinical chemistry and urinalysis parameters were analysed, in addition to the analysis of scFOS in plasma and urine. Controls tested in each study included a non-supplemented formula group and/or a breastfed group. The duration of consumption ranged from one week to approximately 3 months. A summary table outlining the details of the studies can be found below, with a detailed evaluation of each study following.

Summary of studies

Test groups	Study duration	Result	Reference
Breast fed control (n=25 0 g/L scFOS (n=52) 3.0 g/L scFOS (n=50)	~112 days (term infants from birth)	No adverse effects. Stool Softening & improved lactobacillus status.	Pickering et al (1993)
0 g/L scFOS (n= 23) 1.5 g/L scFOS (n= 22) 3.0 g/L scFOS (n= 21)	29 days (term infants from birth)	No adverse effects.	Malacaman et al (1993)
0 g/L scFOS (n= 144) 3.4 g/L scFOS (n= 139)	16 weeks (10-24 month olds)	No adverse effects. Stool softening, reduced duration of diarrhoea, reduced middle ear infection & improved bifidobacterial counts.	O'Ryan et al (1996)
0.2-0.8 g/L scFOS (n=34) 0.2-0.8 g/L sucrose (n=21)	Up to 11 days (2-5 year olds)	No adverse effects. Stool softening.	Pollack et al (2001)
Breast fed control (n=14) 1.5 g/L scFOS (n=28) 3.0 g/L scFOS (n= 30)	1 week	No adverse effects. Stool softening.	Euler (2005)
Breast fed control (n=22) 0 g/L scFOS (n=24) 2.0 g/L scFOS (n=25) 3.0 g/L scFOS (n=26)	28 days (term infants from birth)	No adverse effects. Stool softening.	Merritt et al (2005)
0 g/L scFOS (n=98 1.5 g/L scFOS (n=98) 3.0 g/L scFOS (n=101)	12 weeks (term infants) from birth	No adverse effects. Stool softening and reduced constipation.	Bettler & Euler (2006)
Control formula (n=65) 2.5 g/L scFOS + sucrose (n=67 2.5 g/L scFOS + corn syrup (n=63)	Up to 35 days (term infants from birth)	No adverse effects.	Imeokparia & Lasekan (2009)

1. Pickering LK, Hofer J and Ziegler E (1993) The effect of an alternate carbohydrate on growth of healthy, term infants. Study No. CP-AE12a,b,c. Ross Products Division, Abbottt laboratories Pediatric Nutrition Research and Development Department. Unpublished.

Abbott Nutrition (2011d) Growth, tolerance and stool characteristics of term infants consuming short-chain fructooligosaccharides. Study No. AE04/AE12.

One hundred and two healthy term infants (1-8 days post-natal age) were randomised to receive cow milk-based infant formula containing iron (Similac®) or formula containing 3.0 g/L scFOS (source and Batch No. unspecified) to 112 days postnatal age. Analysis of scFOS-containing formula determined that it contained 1.3 and 1.2 g FOS as GF2 and GF3, respectively (~83% of total scFOS). A group of breastfed infants (n=25) served as a reference group. The study was blinded and conducted at three sites, with no additional nutritional supplementation given. Anthropometric measurements (weight, length and occipitofrontal head circumference) were recorded on post-natal day 8 (entry) and at visits on days 28, 56, 84 and 112. Formula intake, stool characteristics and the incidence of spit-up and vomiting were recorded by caregivers for 3 days prior to each visit. Median Rank Stool Consistency (MRSC) was scored as 1 = watery, 2 = loose, 3 = soft, 4 = formed and 5 = hard. Urine and faecal samples were collected at each visit. Stools were analysed for anaerobic microflora (bifidobacteria, lactobacillus, clostridia, *C. difficile* and bacteroides). Urine was analysed for GF2 and GF3, ketones, indicant/creatinin ratio and bilirubin. Blood was sampled at days 28 and 112 and analysed for scFOS, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and cholesterol.

Selected results are summarised in Table 4.1. There were no intergroup differences in growth, caloric intake and the percentage of feeding with spit-up or vomit. Breastfed infants had significantly ($p<0.05$) more feedings per day than both groups of formula-fed infants on days 28, 56 and 84. On day 28, stool frequency was significantly higher in breastfed infants than both groups of formula-fed infants but was comparable on days 56, 84 and 112. The stools of breastfed infants were significantly softer ($p<0.05$) than those of both groups of formula-fed infants on day 28, 56 and 84. The stools of infants consuming scFOS-supplemented formula were significantly softer ($p<0.05$) than those consuming unsupplemented formula on day 28 but were comparable at day 56 and 84. Plasma ALT and AST were comparable across all groups while cholesterol was significantly higher ($p<0.05$) in breastfed infants than those consuming formula. GF2 and GF3 were detected in 30-80% of urine samples and ranged in concentrations of 2-200 ppm. Analysis of anaerobic faecal flora indicated no significant difference in the concentration of bifidobacteria, bacteroides or clostridia between the scFOS-supplemented group and the formula control. However, in the scFOS group there was a significantly higher ($p<0.05$) proportion of infants with improved lactobacillus status compared to the formula control group.

Table 4.1: *Summary of findings in infants consuming 3.0 g/L scFOS-supplemented formula from birth to postnatal day 112*

Parameter	Breast fed	Formula	Formula + 3.0 g/L scFOS
N	25	52	50
Treatment failures	0	6	8
Protocol failures	2	12	6
Normal exit	23	34	36
Weight gain (g/day)	20.8±2.0	24.6±1.0	23.1±1.0
Head circumference gain[1] (mm/day)	0.3±0.0	0.4±0.0	0.3±0.0
Length gain (mm/day)	0.9±0.1	1.0±0.1	0.8±0.1
Feeding volume (mL/day)	Not reported	1040±49	974±45
Feeding frequency (No./day)	7.2±0.5	**6.2±0.4***	**6.0±0.3***
Spit up (%)			
Day 28	22	15	17
Day 56	27	23	9
Day 84	20	13	12
Day 112	24	25	15
Median No. of stools per day			
Day 28	3.7	**1.7***	**2.0#**
Day 56	2.0	1.0	1.0
Day 84	1.3	1.3	1.0
Day 112	0.8	1.0	1.0
MRSC[1]			
Day 28	2.0 (1.2-3.0)	**3.0*** (1.6-5.0)	**2.8#** (1.0-4.6)
Day 56	2.0 (1.0-3.0)	**2.6*** (1.1-3.8)	**2.5*** (1.0-3.3)
Day 84	2.0 (1.5-3.0)	**2.4*** (1.7-3.5)	**2.7*** (1.3-4.0)
Day 112	2.0 (1.6-3.0)	2.7 (1.0-3.7)	2.0 (1.0-3.0)
Cholesterol (mg/dL)[1]			
Day 28	139±5	**102±3***	**110±4***
Day 112	148±5	**117±3***	**119±4***
Degree of colonisation for lactobacillus frequency counts (%) [<low, detectable and >high][3]			
Day 8	94, 6 and 0	72, 24 and3	90, 7 and 3
Day 28	71, 18 and12	79, 14 and 7	72, 10 and 17
Day 56	53, 35 and 12	86, 10 and 3	**41, 38 and 21#**
Day 84	53, 29 and 18	79, 21 and 0	**48, 14 and 38#**
Day 112	41, 20 and 29	86, 10 and 3	**59, 24 and 17#**

Results are expressed as the mean ± SEM unless otherwise specified

*p<0.05 compared to breastfed infants

#p<0.05 compared to unsupplemented, formula-fed infants

[1]median rank (range)

[2]<low = $<10_4$, detectable = 10^4-10^9 and >high = $>10^{10}$

2. Malacaman EA, Choudhry I, Gheen D, Marks F, Forti W and Martens W (1993) The effect of an alternate carbohydrate on stool characteristics and tolerance in healthy, term infants. Study No. CP-AE04. Ross Laboratories, Abbottt laboratories Pediatric Nutrition Research and Development Department. Unpublished.

Abbott Nutrition (2011d) Growth, tolerance and stool characteristics of term infants consuming short-chain fructooligosaccharides. Study No. AE04/AE12.

Sixty-six healthy term infants (4-10 weeks post-natal age) were fed cow milk-based infant formula containing iron (Similac®) for two weeks and then randomised to receive whey-enriched formula supplemented with 0 (n=23), 1.5 (n=22) or 3.0 g/L (n=21) scFOS (Source and Batch No, unspecified) for 29 days. Analysis of scFOS-containing formula determined that the 1.5 g/L formula contained 0.65 and 0.60 g GF2 and GF3, respectively (~83% of total scFOS), while the 3.0 g/L formula contained 1.45 and 1.41 g GF2 and GF3, respectively (~92% of total scFOS). A group of breastfed infants (n=25) served as a reference group. Age at entry was significantly different ($p<0.05$) across the groups (39, 41 and 49 weeks). Bodyweight was recorded on days 1, 15 and 29. Formula intake, stool characteristics (MRSC and frequency were recorded daily) and the incidence of spit-up and vomit were recorded by parents. Stools and urine samples were collected on days 15 and 29 for the analysis of microflora, and scFOS and urine ketones, respectively. Blood was sampled on day 29 from 4 or 5 infants per group for the analysis of scFOS, ALT and AST.

Key finding are summarised in Table 4.2. There were no intergroup differences in formula intake (in the formula groups only) or the number of feeds per day, stool characteristics, the incidence of spit-up or vomit or the mean log counts of aerobic and anaerobic microflora. Average weight gain was comparable across the three groups. No scFOS was detectable in plasma. scFOS (GF2 and GF3) in urine was detected in 4/11 (~40-57 ppm) and 8/11 (~59-114 ppm) infants in the 1.5 and 3.0 g/L scFOS groups, respectively. There were no intergroup differences in ALT or AST. Infants consuming the scFOS-supplemented formula had significantly lower ($p<0.05$) clostridia colonisation (combined for both concentrations) compared to unsupplemented formula.

Table 4.2: *Summary of findings in infants that consumed 0, 1.5 or 3.0 g/L scFOS-supplemented formula for 29 days.*

Parameter	0 g/L scFOS	1.5 g/L scFOS	3.0 g/L scFOS
N	23	22	21
Age at entry (days)	39±2	41±3	49±3
Successful completer	20	17	16
Treatment failure - intolerance	1	2	3
Treatment failure - rejection	0	0	1
Protocol failures	0	3	0
TOTAL	21	22	20
Weight gain (g/day)	28.2	29.2	25.4
Feeding volume[1] (mL/day)	830±32	826±30	862±35
Feeding frequency (No./day)	5.9±0.2	6.6±0.4	6.1±0.3
Spit up (%) Week 2 Week 4	 8 13	 6 6	 17 25
Vomit Week 2 Week 4	 1 0	 0 0	 1 3
Median No. of stools/day Week 2 Week 4	 1.3 1.2	 1.2 2.7	 1.3 2.8
MRSC[1] Week 2 Week 4	 2.6 1.6	 2.7 1.6	 2.8 1.8
Degree of colonisation for clostridium (%) undetectable, detectable and beyond detectable]			
Week 2 Week 4	40, 60 and 0 20, 80 and 0	31, 63 and 6 41, 59 and 0	40, 60 and 0 60, 40 and 0

Results are expressed as the mean ± SEM unless otherwise specified

*p<0.05 compared to breastfed infants

#p<0.05 compared to unsupplemented, formula-fed infants

[1]median rank

3. Yamamoto Y and Yonekubo A (1993) A survey of physical growth, nutrition intake, fecal properties and morbidity of infants as related to feeding methods (IV). Journal of Child Health 52(4): 465-75

In a Japanese national survey (June-December 1989) involving 20,742 healthy infants (up to 4.5 months of age), the growth, nutritional intake and faecal properties of those consuming formula (including formula supplemented with fructo-oligosaccharides; details unspecified) was compared to those that were breastfed. Infants were classified into the following feeding groups: (1) breastfed (~36%); (2) breastfed and formula-fed (i.e. mixed); and (3) formula-fed infants (21%). The mixed feeding group was subdivided into those that were predominantly breastfed (23%) and those that were predominantly formula-fed (21%). Four different infant formulas were evaluated (A, B, C and D) but no details were provided on their composition; the exception was Formula A that contained an unspecified concentration of fructo-oligosaccharides. Bodyweight, nutrition intake (protein and caloric) was recorded at 0, 1, 2, 3 and 4 months. The frequency of bowel movements and faecal consistency was recorded presumably by caregivers. There were no significant differences in growth (i.e. bodyweight),

morbidity or health between breastfed and formula-fed infants. Infants that consumed formula supplemented with fructo-oligosaccharides had a higher proportion of softer stools (70%) than infants consuming unsupplemented formula (~64%). The authors concluded that the presence of fructo-oligosaccharides in the formula made faeces softer.

4. O'Ryan ML, Prado VJ and Soriano HP (1996) Effect of an alternate carbohydrate on incidence and severity of diarrhea. Study No. CP-AF97. Ross Products Division, Abbott laboratories Pediatric Nutrition Research and Development Department. Unpublished.

Abbott Nutrition (2011b) Effects of milk-based beverage with short chain fructooligosaccharides on tolerance and incidence of diarrhea in toddlers. Study No. AF97

Healthy children from 10 to 24 months of age consumed a scFOS-containing, milk-based beverage or a control milk-based beverage *ad libitum* for 16 weeks in addition to their normal diet. In phase I of the study, 73 children were randomised to receive a milk-based beverage containing 3.4 g/L scFOS and 72 to receive a control milk-based beverage. In phase II of the study, 66 and 72 children were randomised to receive a scFOS-containing, milk-based beverage or a control milk-based beverage, respectively. All children attended day care centres. Children were encouraged to drink at least 500 mL/day of the study beverage (2 x 250 mL at day care and *ad libitum* at home) and were not permitted to receive human milk. Tolerance was assessed by parental recordings of the following for three days prior to days 7, 28, 56, 84 and 112: occurrence of stomach cramps and vomiting associated with feeding; number, consistency (watery, loose/mushy, soft, formed or hard) and characteristics of stools; gas; and constipation. Weight and length were recorded at entry and on days 56 and 112. Stool samples were collected from all subjects at entry, day 112 and one of the four scheduled study visits for the analysis of faecal flora. Children were placed under active surveillance for the occurrence of diarrhoea; diarrhoea was defined as three watery or loose stools in 24 hours period as determined by a parent, guardian or day care centre worker. Children were evaluated on days 7, 28, 56, 84 and 112. A nurse attended the day care centre on a weekly basis to assess study compliance and identify episodes of diarrhoea and other illnesses.

Key findings are summarised in Table 4.3. Beverage intake, 3-day stool characteristics, weight, height and day care attendance were comparable between the scFOS-supplemented and control groups. On study day 7, MRSC of the scFOS group was significantly lower (p=0.03) than the control, which the authors attributed to a higher percentage of watery stools (10.6) compared to the control (5.74, p=0.05) and a significantly higher percentage of stools that were either watery or loose (27.11 *versus* 19.37 in the control, p=0.04). While episodes of diarrhoea and their aetiology were comparable between the two groups, the duration of diarrhoea in scFOS-treated infants was significantly shorter (p=0.036) than the control. With regard to other illnesses, the incidence of otitis media was significantly reduced (p=0.023) in the scFOS-treated group. The proportion of children with detectable Bifidobacteria counts (10^4-10^9) at entry was 53.1% in the scFOS group (34 subjects) and 46.9% in the control (30 subjects). At the end of the study, the proportion of children with greater than detectable bifidobacterial counts (>10^9) was significantly higher (p=0.028) in the scFOS-treated group than the control (56.5 *versus* 34.7%, respectively), which the authors suggested indicated an overall improvement. Statistical comparisons of lactobacilli counts found no differences between scFOS-treated and control children.

Table 4.3: *Effect of FOS on the occurrence of diarrhoea in toddlers*

Parameter	Control beverage	3.4 g/L FOS
Enrolled (phase 1 + 2)	144	139
Completed (phase 1 +2)	124	118
Treatment failures	6	3
Protocol failures	14	18
Median weight gain (entry to day 56) (g)	8.5	9.2
Median weight gain (day 56-112) (g)	5.5	6.1
MRSC		
Day 0	3.24	3.34
Day 28	3.17	3.25
Day 56	3.29	3.33
Day 84	3.37	3.40
Day 112	3.39	3.48
1 episode of diarrhoea	36	37
2 episodes of diarrhoea	7	3
3 episodes of diarrhoea	2	0
Mean duration of diarrhoea (days)	4.88	**3.91**[1]
Mean severity score for diarrhoea	5.55	5.05
Otitis media	33	**17**[2]
Bifidobacteria[3]		
Entry	46.9	53.1
Exit	34.7	**56.5**[4]

[1]p=0.036 compared to the control; [2]p=0.023 compared to the control; [3]% subjects with detectable (10^4-10^9) bifidobacteria counts; [4]p=0.028 compared to the control

5. Pollack PF, Chow J, Wof BW and Crane JC (2001) A randomized, double-blind, placebo controlled, parallel, multi-center acute serving size titration study of short-chain fructooligosaccharides versus sucrose in constipated children of ages 2 to 5 years. Study CP-BJ03. Ross Products Division, Abbottt laboratories Pediatric Nutrition Research and Development Department. Unpublished.

Abbott Nutrition (2011c) Fructooligosaccharides and laxation in children. Study No. BJ03

A study was undertaken to determine the dose of scFOS that would soften the stools of 50% of children with constipation. The authors defined constipation as “infrequent and/or difficult and painful evacuation of hard faeces”. Fifty-five children (2-5 years old) with a history of “simple” constipation without faecal impaction were randomised to receive either scFOS (NutraFlora®) (n=34; 29 completed) or sucrose (n=21; 17 completed) for a maximum of 11 days. Each treatment was ingested as a grape or cherry-flavoured syrup. Following a baseline period of three days, children then consumed 0.2 g/kg bw per day of scFOS or sucrose for two consecutive days. The dose was increased incrementally by 0.2 g/kg bw to a maximum of 0.8 g/kg bw. The doses were chosen based on adult data. Parents recorded bowel function (stool consistency and frequency) and gastrointestinal intolerance (burping, flatulence, fussiness and vomiting) on a daily basis. Stool consistency was scored based on the following scale, which was the inverse of that used to determine MRSC in other studies: 1 = hard, dry - pellets, small, hard mass; 2 = hard, formed - dry, stool remains firm and soft; 3 = soft, formed – moist, softer stool that retains shape; 4 = soft, unformed – stool pudding-like; 5 = watery – liquid that can be poured. The severity of intolerance was scored according to the following scale: 0 = absent; 1 = mild; 3 = moderate; 4 = severe.

There was a dose-related softening of stools in both groups although the increase in stool consistency scores from baseline was more pronounced in the scFOS group. The mean stool consistency of scFOS-treated children was statistically (p=0.004) and clinically different to sucrose-treated children at 0.6 g/kg bw (~1.3 *versus* 0.5, graphically-presented data). A difference in stool consistency was also noted at 8 g/kg bw (~1.2 *versus* 0.5, graphically-presented data) but the difference was not statistically significant. At 0.6 g/bw, the mean frequency of bowel movements was 1.33 per day in the scFOS group and 0.94 per day in the sucrose group compared to 0.74 per day during the baseline period. At 8 g/bw, the mean frequency of bowel movements in the scFOS group was 1.17 per day, while it was 1.06 per day in the sucrose group. Changes in the frequency of bowel movements from baseline were not significantly different between the scFOS- and sucrose-treated groups. There were no intergroup differences in intolerance signs.

6. Euler AR, Mitchell DK, Kline R and Pickering LK (2005) Prebiotic effect of fructo-oligosaccharide supplemented term infant formula at two concentrations compared with unsupplemented formula and human milk. Journal of Paediatric Gastroenterology 40: 157-164.

A 5-week prospective, randomised, crossover, single-site study was conducted in healthy term infants (2-6 weeks postnatal age) to determine whether the consumption of bovine milk-based formula supplemented with 1.5 or 3.0 g/L scFOS (Raftilose®P95) for one week would result in faecal flora similar to that of breastmilk-fed infants. Formula fed infants were randomised to one of four study groups, with all groups receiving control formula during weeks 1, 3 and 5. One of the two supplemented formulas containing 1.5 or 3.0 g/L scFOS was consumed during either week 2 or 4, with the control formula consumed during either week 4 or 2, respectively. A non-randomised breastmilk reference group was included in the study. Infants were visited each week and physically examined; bodyweight, length and occipitofrontal head circumference were recorded. Prior to each visit, caregivers used a 24-hour diary to record formula intake, stool frequency, size, consistency and colour. Stool consistency was assessed based on the following numerical classifications: 1 = hard, 2 = firm, 3 = soft, 4 = loose and 5 = watery (note: this is the inverse of the scale used in unpublished studies submitted by the Applicant). Caregivers were questioned about any adverse events at each visit. Stool samples were collected weekly for the analysis of bacteroides, lactobacilli, bifidobacteria, clostridia and enterococci; analysis for *Clostridium difficile* toxin was also undertaken.

Of the 87 infants enrolled in the study, 72 completed it (n = 14, 28 and 30 in the breastmilk, 1.5 and 3.0 g/L scFOS groups, respectively). All three groups grew normally throughout the 5-week study period. Statistically significant intergroup differences in bacterial counts were evident prior to supplementation with scFOS; these counts unaffected following one week of supplementation with scFOS. It was noted that mean bifidobacteria counts were significantly higher ($p<0.05$) in the 1.5 g/L scFOS group [9.1 log (base 10)] than the breastmilk group or 3.0 g/L scFOS groups [8.0 and 8.6 log (base 10)] but in the absence of a similar increase at the higher dose of scFOS, and as the increase from baseline was marginal [from 8.8 to 9.1 log (base 10)], this finding is unlikely to be treatment-related. Adverse events (which were summed across the whole 5 weeks of the study) and measures of tolerability and acceptability were not clearly reported in the paper; based on the information provided there was no apparent adverse effect of scFOS. The frequency of bowel movements and the stool consistency rating increased (i.e. it became softer) in infants that consumed formula supplemented with 3.0 g/L scFOS; the change in stool consistency was statistically significant ($p<0.01$) when compared to the 1.5 g/L scFOS group (Table 4.4). The results of this study indicated that the consumption of scFOS by health term infants was well-tolerated up to 1.5 and 3.0 g/L, and had a significant stool-softening effect at 3.0 g/L

Table 4.4: *Stool frequency and consistency in infants consuming scFOS-supplemented formula for 1 week*

Parameter	Breastmilk	1.5 g/L scFOS		3.0 g/L scFOS	
		-	+	-	+
Stool frequency (per day)	4.6*	2.1	1.5	1.5	2.0
Stool consistency rating	3.9*	2.6	2.7	2.7	3.2
Change in stool consistency	-	-	0.07	-	0.6#

*p<0.05 compared to the 1.5 and 3.0 g/L FOS groups; #p<0.01 compared to the 1.5 g/L FOS group

7. Merritt R, Williams T and Price P (2005) Effect of non-digestible carbohydrate on the fecal flora of term infants. Study No. AK16. Ross Products Division, Abbott Laboratories, Columbus, OH, USA. Unpublished.

Xia Q, Williams T, Hustead D, Price P, Morrison M and Yu Z (2012) Quantitative analysis of intestinal bacterial populations from term infants fed formula supplemented with fructo-oligosaccharides. J Pediatr Gastroenterol Nutr. 55(3):314-20.

In a randomised, controlled, multi-centre, single-blinded exploratory study, groups of 20 infants (0-5 days postnatal age) were enrolled to receive milk-based formula containing DHA and ARA (Similac® Advance®) or scFOS-supplemented formula (2.0 or 3.0 g/L; also containing DHA) for 28 days. A group of breastfed infants were also included. Three visits occurred during the study: enrolment, day 15 and day 28. Parents recorded formula intake, stool frequency and consistency, percent of feedings with spit-up/vomiting, signs of intolerance and medication use, on a daily basis. Mean rank stool consistency was determined using the following scale: 1 = watery, 2 = loose/mushy, 3 = soft, 4 = formed and 5 = hard. A stool sample was collected on day 28 and analysed for the total number of bacteria, bifodobacteria concentration relative to total faecal flora, Lactobacillus. *Clostridium difficile* and *Escherichia coli*.

Key findings are summarised in Table 4.5. Within the intention to treat (ITT) population, MRSC over the entire study was significantly lower in the 2.0 and 3.0 g/L scFOS groups (p=0.034 and 0.006, respectively) compared to the formula control group. The occurrence of watery stools was significantly higher in the 2.0 and 3.0 g/L scFOS groups over study days 1-14 (p=0.040 and 0.042, respectively) compared to the unsupplemented formula group, but was comparable to the breastfed reference group. The 3.0 g/L scFOS group also had a significantly higher (p=0.024) incidence of watery stools over study days 15-28. Over the entire study period, mean formula intake was significantly higher (p=0.022) in the 3.0 g/L scFOS group than the 2.0 g/L scFOS group. There were no intergroup differences in stool frequency or in the occurrence of adverse events. There were no significant intergroup differences in bifidobacteria, lactobacillus, *C. difficile* or *E. coli* concentrations relative to total faecal flora. Mean absolute counts of *C. difficile* were significantly higher than the breastfed group in the 2.0 and 3.0 g/L scFOS groups (p=0.005 and 0.012, respectively). In addition, mean counts of *E. coli* in the control formula were significantly higher ($p \leq 0.05$) than all other groups. Adverse events were comparable among the treatment groups.

***Table 4.5**: Key findings in infants consuming 2.0 or 3.0 g/L FOS-supplemented formula for 28 days*

Parameter	Breastfed	Control formula	2.0 g/L FOS	3.0 g/L FOS
N (ITT)	22	24	25	26
Adverse events	4	7	6	9
Formula/milk intake (ITT)				
Day 1-14	230.43±200.85	583.1±26.2	601.27±1.87	624.70±28.33
Day 15-28	722.28±0	749.51±27.75	727.45±37.31	816.84±37.01
% spit-up/vomit (ITT)				
Day 1-14	7.63±2.93	8.8±1.95	26.70±6.62	13.20±3.14
Day 15-28	10.62±4.43	10.04±1.75	15.74±5.71	11.09±3.45
MRSC (ITT)				
Days 1-14	2.18±0.124	2.67±0.142	2.37+0.130	2.42+0.154
Days 15-28	2.8±0.132	2.81±0.162	2.34+0.157	2.16+0.153
Daily stool number (ITT)				
Days 1-14	5.94±0.387	2.66±0.331	2.48±0.226	2.92±0.445
Days 15-28	5.27±0.442	1.88±0.305	1.77±0.308	2.32±0.338
% watery stools (ITT)				
Days 1-14	17.93±4.46	5.07±2.23	**17.17±5.73***	**17.64±4.69***
Days 15-28	9.94±4.99	12.06±5.59	21.80±7.13	**27.80±7.09***
Faecal flora (evaluable) (mean + SEM)				
% Bifidobacteria	19.78±5.40	17.45+4.42	14.71+4.71	20.29+4.32
% Lactobacillus	4.53±2.16	4.0±1.64	4.60±2.98	1.78±0.87
% *C. difficile*	0.03±0.02	0.03±0.021	0.45±0.30	0.09±0.041
% *E. coli*	0.39±0.017	1.38±0.46	0.36±0.15	0.37±0.13

*p<0.05 compared to the unsupplemented formula control; **p<0.01 compared to the unsupplemented formula control

8. Bettler J and Euler AR (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.

In a randomised, masked, multicentre trial, groups of healthy term infants (≤14 days of postnatal age and between the 10th and 90th centiles for weight and length) were fed bovine milk-based formula containing 0, 1.5 or 3.0 g/L scFOS (Raftilose®P95) for 12 weeks. A summary of the trial profile is presented in Table 4.6. Anthropometric measurements (weight, length and occipitofrontal head circumference) were recorded pre-treatment and at 4, 8 and 12 weeks. Adverse events (AE) were recorded during clinic visits at 4, 8 and 12 weeks and via telephone interviews at 2, 6 and 10 weeks; the severity and possible cause of any AE were evaluated by the investigator at each site. Blood was sampled pre-treatment and at 12 weeks for the analysis of clinical chemistry parameters (albumin, blood urea nitrogen, calcium, magnesium, phosphorus, creatinine, triglycerides, low-density lipoprotein and cholesterol.

Of the 297 infants enrolled in the study, 212 completed it. There were no intergroup differences in the occurrence of adverse events, reasons for discontinuation or length of study participation. At week 8, the mean length of the control group (58.2+2.6 cm) was significantly longer (p=0.046) than the 3.0 g/L scFOS group (57.5±2.1 cm). In the absence of any significant differences at week 4 or 12, or in weight or head circumference at weeks 4, 8 or 12, and as all anthropometric parameters were within the normal range of variation for age- and sex-matched infants, this difference was not considered treatment-related. In the

3.0 g/L scFOS group, the incidence of constipation (as assessed by the caregiver) was significantly lower (p=0.033) than the control. To exclude the possibility that the site with the highest enrolment may have affected the result, the authors repeated the analysis without data from this site and still found that the incidence of constipation was significantly lower (p=0.0099) than the control. While data on stool frequency and consistency were not reported, the lower constipation in the 3.0 g/L scFOS group is inferred to mean that stool frequency was higher and stools had a softer consistency than the control. Such a result would be consistent with the study of Euler et al (2005). There were no significant differences in the incidence of flatulence, diarrhoea, loose stools, dehydration or allergic reactions between the scFOS-treated and control groups. There was also no significant difference in formula tolerance and acceptance between the treated and control groups. The authors concluded that the addition of scFOS to bovine milk-based formula up to a concentration of 3.0 g/L "is safe and supports normal growth".

Table 4.6: Summary of trial profile

Parameter	Unsupplemented formula (control)	1.5 g/L FOS	3.0 g/L FOS
N	98	98	101
Completed	66	72	74
Adverse events	5	5	2
Failure to return	5	2	4
Protocol violation	2	4	0
Physician/family request Adverse event	20 13	12 9	21 15
Mean length of participation	40 days	48 days	41 days

9. Imeokparia M and Lasekan JB (2009) Comparative gastrointestinal tolerance of various infant formulas in health term infants. Study No. AK54. Abbottt Nutrition, Abbottt Laboratories, Research and Development and Scientific Affairs. Unpublished.

Abbott Nutrition (2011a) Comparative gastrointestinal tolerance of various infant formulas in healthy term infants. Study No. AK54.

A randomised, double-blind, multi-centre, parallel, tolerance feeding study was conducted in healthy, term-infants (0-8 days postnatal age) to assess the tolerance of a soy-based formula (Similac® Isomil® Advance®) supplemented with scFOS (2.5 g/L) until 35 days of age. One hundred and ninety-five infants were enrolled in the study, with 188 in the intention to treat (ITT) population. Three study groups were examined: (1) soy-based formula containing 20% carbohydrate as sucrose; (2) soy-based formula containing 20% sucrose and 2.5 g/L scFOS; and (3) soy-based formula containing 20% carbohydrate as corn syrup and 2.5 g/L scFOS. Formula intake and stool records were maintained daily by parents. AE were also recorded and assessed at each visit at enrolment, day 14 and day 35. MRSC was calculated using the following 5-point scale: 1 = watery, 2 = loose/mushy, 3 = soft, 4 = formed and 5 = hard. The number of stools passed per day and the % of feedings with spit-up or vomit (within one hour of feeding) was recorded. Anthropometric parameters were also recorded throughout the study.

Results are summarised in Table 4.7. There were no significant intergroup differences in growth, AEs, MRSC, stool frequency, feedings per day, formula intake per day or the % of feeding with spit-up or vomit. There were no significant differences in the mean specific gravity of urine between control and test groups of infants. It was noted that the specific gravity of urine collected from one infant receiving the scFOS-supplemented formula was slightly elevated (1.039 *versus* a cut-off value of 1.030) at day 14 but not day 35. This result seemed to be inconsistent with a slight increase in soy formula consumption recorded for the group on day 35. A detailed physical examination confirmed that the hydration status of the

infant appeared to be normal. The authors concluded that the study formulas were well–tolerated by infants.

Table 4.7: *Results of feeding infants a FOS-supplemented, soy-based formula for 5 weeks*

Parameter	Formula control (+20% sucrose)	2.5 g/L scFOS (+20% sucrose)	2.5 g/L scFOS (+20% corn syrup)
Total randomised	65	67	63
ITT	62	64	62
Evaluable group	62	62	62
% of feedings with spit-up (Evaluation group)			
Day 1-14	18.6±3.4	18.1±3.3	1.6±3.4
Day15-35	15.4±3.8	15.1±3.6	14.8±3.1
Day 1-35	17.2±3.4	16.0±3.2	17.6±3.2
Formula intake per day (mL)			
Day 1-14	555±17	559±20	570±20
Day15-35	673±22	739±27	726±35
Day 1-35	627±19	634±27	658±26
MRSC (Evaluation group)			
Day 1-14	2.5±0.1	2.6±0.1	2.5±0.1
Day15-35	2.6±0.1	2.7±0.1	2.5±0.1
Day 1-35	2.6±0.1	2.7±0.1	2.5±0.1
MRSC (ITT)			
Day 1-14	2.5±0.1	2.6±0.1	2.5±0.1
Day15-35	2.7±0.1	2.7±0.1	2.5±0.1
Day 1-35	2.6±0.1	2.6±0.1	2.5±0.1
Stool frequency (Evaluation group)			
Day 1-14	2.9±0.3	3.3±0.3	3.1±0.3
Day15-35	2.1±0.2	2.7±0.3	2.7±0.3
Day 1-35	2.4±0.2	3.1±0.3	2.8±0.3
Bodyweight gain (g) (Evaluation group)			
Day 1-14	36.4±3.2	29.3±2.9	29.0±2.2
Day15-35	35.2±2.1	36.8±1.9	37.8±1.8
Day 1-35	35.8±1.9	34.0±1.7	35.3±1.6
Length gain (cm)			
Day 1-14	0.16±0.02	0.19±0.03	0.16±0.02
Day15-35	0.13±0.01	0.13±0.01	0.13±0.01
Day 1-35	0.14±0.01	0.14±0.01	0.14±0.01
Urine specific gravity (mean)			
Day 1-14	1.0041	1.0038	1.0044
Day 15-35	1.0043	1.0034	1.0039
No. of subjects with at least 1 AE to day 35	29	33	28

Results expressed as the mean ± 1 SEM unless otherwise specified

4.3 Considerations by the Infant and Child Health Scientific Advisory Group (ICHSAG)

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

teleconference to discuss certain aspects of this physiological assessment. The consolidated discussion of key issues is at Appendix 1.

FSANZ sought advice from the ICHSAG on the results of unpublished studies by Pickering et al (1993), Malacaman et al (1993), Merritt et al (2005) and Imeokparia and Lasekan (2009), and published studies by Euler et al (2005) and Bettler and Euler (2006). The specific areas of focus included the effect of scFOS on infant hydration status, faecal flora, the sweetness of infant formula containing scFOS and the potential beneficial effect on infant growth and development, and stool consistency (including the effect on constipation). The ICHSAG concluded that scFOS:

- had no adverse effect on infant hydration status following the consumption of formula containing up to 3 g/L
- had no adverse effect on the microbiology of the infant digestive tract following the consumption of formula containing up to 3 g/L
- is unlikely to increase the sweetness of infant formula relative to oligosaccharides already permitted to be added to infant formula
- had no discernible effect on infant growth patterns and
- has the potential to soften stools and may reduce constipation

With regard to the relationship between stool consistency or frequency and infant well-being, the ICHSAG advised that there is a relationship between severe constipation and reduced infant well-being. However it would be difficult to identify such a relationship within what is the normal range of stool consistency and frequency in infants, including less severe constipation.

4.4 Discussion

This physiological assessment examined the potential of scFOS to cause adverse effects in infants, toddlers and young children when used as a replacement for already-permitted IDS in infant formula products, foods for infants and FSFYC. This assessment also considered the potential of scFOS to have a net beneficial effect on infants in view of Ministerial Policy Guideline on substances proposed to be added to infant formula products.

It is noted that most of the studies evaluated by FSANZ were conducted in infants (<6 months of age), which can be regarded as the most vulnerable group of the target populations relevant to this Application because infants aged up to 4-6 months acquire all of their nutrition from a single source. As such, evidence of safety in this vulnerable group can be taken as evidence of safety in older infants (10-24 months) and young children (2-5 years), which derive their nutrition from mixed sources.

4.4.1 Potential of scFOS to cause adverse physiological effects

Collectively the above findings provide a weight-of-evidence that the consumption of scFOS in infant formula in amounts up to 3.0 g/L is unlikely to cause adverse effects in healthy infants based on the absence of any adverse impact on anthropometric parameters (bodyweight, length and occipital head circumference), formula intake and frequency of feeding, the incidence of adverse events, gastrointestinal intolerance (spit-up/vomiting, flatulence, diarrhoea, burping, fussiness), stool consistency and frequency, or faecal flora (Pickering et al 1993, O'Ryan et al 1996; Euler et al 2005; Merrit et al 2005; Bettler and Euler

2006; Imeokparia and Lasekan 2009). In addition, the results of clinical intervention studies indicated that there was no adverse effect on infants' hydration status as assessed by the measurement of urine specific gravity (Imeokparia and Lasekan 2009), the occurrence of diarrhoea, expert clinical examination or formula intake (Pickering et al 1993, O'Ryan et al 1996; Euler et al 2005; Merrit et al 2005; Bettler and Euler 2006)

Only a small number of studies investigated the effects of scFOS-supplementation in young children (see studies by O'Ryan et al 1996 and Pollack et al 2001). However, in the absence of any evidence of adverse effects in healthy infants (which can be considered the most vulnerable population group) in conjunction with the absence of adverse effects in the studies in healthy young children, the addition of scFOS to foods for infants and FSFYC is unlikely to pose any safety issues.

4.4.2 Potential beneficial physiological effects of scFOS

The assessment of benefit as it relates to the addition of scFOS to infant formula products has focussed predominantly on the potential of scFOS to align the stool characteristics of formula-fed infants with their breastfed counterparts, which are typically softer. More general beneficial effects on growth and development are difficult to discern within the large range of normal infant growth and development. Indeed, this view was confirmed by the ICHSAG. Potential beneficial effects on faecal flora are evaluated in Section 5 "Microbiological Effects" where it is concluded that there are no apparent beneficial effects; the ICHSAG concurred with this conclusion.

One of the challenges in trying to elucidate a beneficial effect of a substance added to infant formula is the very large range of normal infant growth and development. A further challenge is that the studies underpinning the safety of scFOS were generally not designed to assess benefit within this large range of variability and as such the group sizes are somewhat modest. So individually, observations of stool softening are somewhat inconsistent across the studies but collectively there is a weight-of-evidence that scFOS has the potential to soften the stools of formula-fed infants in amounts of 3.0 g/L.

In the study of Pickering et al (1993), stool frequency was higher and stools were softer in infants consuming 3.0 g/L scFOS for 4 weeks compared to infants that consumed control formula; however, there was no difference in stool frequency or consistency over longer durations. O'Ryan et al (1996) observed that the duration of diarrhoea was significantly shorter in toddlers that consumed 3.4 g/L scFOS for 16 weeks. This study also found a significant reduction in middle ear infections in treated toddlers. The stools of infants that consumed scFOS-supplemented formula (2.0 or 3.0 g/L) for 28 days were softer than a formula control group (Merritt et al 2005). Additional evidence of stool softening comes from the study by Euler et al (2005), where an increased frequency of bowel movements and stool softening occurred in infants that consumed formula supplemented with 3.0 g/L scFOS compared to the group that consumed 1.5 g/L scFOS. In the largest study in the database, consisting of approximately 100 infants per treatment group, the incidence of constipation was significantly lower in infants that consumed formula supplemented with 3.0 g/L scFOS for 12 weeks (Bettler and Euler 2006)

On the basis of the available data, it is concluded the addition of scFOS to infant formula up to an amount of 3.0 g/L has the potential to soften stools and may reduce constipation, which are considered to be beneficial effects.

4.5 Conclusions of physiological assessment

The addition of scFOS to infant formula products, infant foods and FSFCY up to the currently permitted concentrations for IDS is unlikely to cause adverse physiological effects in the healthy target populations. The consumption of scFOS-supplemented formula supports normal growth in infants. It is further concluded that the addition of scFOS to infant formula up to an amount of 3.0 g/L has the potential to soften stools and may reduce constipation.

5. Microbiological effects

This assessment draws on animal and human studies to assess the effect of scFOS on the gut microflora of infants and young children. The human studies referred to in this section are evaluated in Chapter 4, with their microbiological findings summarised in Table 5.1.

The development of gut microflora in infants and young children has been described in Proposal P306. Accordingly, the dominance of *Bifidobacterium* and *Lactobacillus* species in the intestinal tract of breastfed infants and their associated effects generally accepted as beneficial to the host appear to be the result of a combination of factors present in breast milk. These factors include HMOs, lactoferrin, lactose, nucleotides and low concentration of proteins and phosphates.

Proposal P306 states that there is a significant knowledge gap about the complex interactions between the human host and the major anaerobes of the gastrointestinal tract such as *Bacteroides, Clostridium* and *Eubacterium.*

5.1 Formula-feeding and development of gut microorganisms in infants

Sherman et al (2009) discussed the potential roles and clinical utility of prebiotics in new-borns, infants and children and state that the addition of appropriate amounts of selected prebiotics in infant formula can enhance the growth of bifidobacteria or lactobacilli in the colonic microbiota, and thereby, may produce beneficial effects.

However, it is also noted that there is a potential concern that added substrate may promote the growth of either potential pathogens or opportunistic microorganisms (commensal luminous bacteria). These microorganisms may be capable of crossing the immature gut epithelial barrier and causing systemic disease as opportunistic pathogens.

Sherman et al (2009) state that the gut microflora of formula-fed infants are more diverse and similar to those observed in adults, with lower levels of bifidobacteria, but greater diversity and higher levels of other potentially pathogenic groups including *Bacteroides*, *Clostridium*, and Enterobacteriaceae. More recent studies, where reliable and accurate molecular methods were applied, are in general agreement with these trends. It is generally accepted that this description applies to formula-fed infants.

5.2 Breastfeeding and development of gut microflora in infants

Proposal P306 reviewed the development of gut microflora profiles in breastfed infants. In comparison to formula-fed infants, breastfed infants are reported to have lower rates of potentially pathogenic bacterial colonisation.

Jangi and Lamont (2010) investigated the relationships between *C. difficile* and the health of infants. *C. difficile* is recognised as a major cause of antibiotic-associated diarrhoea and colitis in adults, but is generally regarded as a harmless commensal in neonates and infants. Jangi and Lamont (2010) searched the PubMed database for studies that surveyed infants younger than 2 years of age with no overt evidence of gastrointestinal (GI) illness. They found that among healthy infants younger than one month of age, *C. difficile* was recovered from an average of 37% individuals. Between one and six months of age, colonisation decreased to an average rate of 30%, this trend continuing with recovery dropping to 14% between 6 and 12 months and 10% in > 1 year of age, in general irrespective of the feeding type. The asymptomatic carriage rate continues to fall after the first year of life, approximating the 0 to 3% carriage rate in adults thereafter. This pattern illustrates the ecological succession of microflora establishment within the gut. They noted that other *Clostridium* species isolated in the first week of life, such as *C. butyricum* and *C.*

sartagoformum are rarely found in adults. *C*. *difficile* carriage in some neonates and infants may be transient, whereas others may be colonised by different strains over time with stools positive for toxigenic and non-toxigenic strains intermittently. The infant gut appears to be resistant to the effects of *C. difficile* toxin A and B and clinical infection is rarely reported in infants.

Clostridium spp. in general were recovered more frequently in formula-fed infants (49%-66%) compared to breastfed infants (6%-20%). The degree of specific colonization by *C*. *difficile* was found to be nearly 2-fold higher in formula-fed infants (30%) compared to breastfed infants (14%). When breastfed or formula-fed infants were prospectively studied, these differences in colonisation rates in early infancy seemed to disappear at 12 months, suggesting that breastfeeding may decrease colonisation by *C*. *difficile* in early infancy, with a "catch-up" phenomenon after weaning. Breastfeeding may have several beneficial effects, including enhancing the development of protective microorganisms that inhibit colonisation by *C*. *difficile.* High levels of maternal antibodies are also present in breast milk that inhibits colonisation of the gut by bacterial pathogens.

Lönnerdal (2010) states that specific strains of bacteria present in breast milk influence the early colonisation of the infant gut.

5.3 Approach to the microbiological risk assessment

For the purpose of this risk assessment, FSANZ considered lactobacilli and bifidobacteria as generally accepted beneficial microorganisms. Increases in potentially pathogenic microorganisms such as *C*. *difficile* and *E*. *coli* were considered undesirable effects. *Bacteroides* spp. were considered commensals.

Three unpublished studies on the effect of scFOS on the gut microflora of infants (as observed by studying faecal microflora) were provided by the Applicant and are summarised below. No additional studies were found in the scientific literature on the effects of scFOS on gut microflora in human infants, other than those submitted by the Applicant.

A summary of the microbiological studies is also provided in Table 5.1.

5.3.1 Effects of scFOS on potentially pathogenic bacteria

Infants consuming scFOS in infant formula products as a sole source of nutrition

Malacaman et al (1992) identified and enumerated *Bifidobacterium*, *Lactobacillus*, *Bacteroides*, *Clostridium*, *Peptostreptococcus*, *Escherichia*, enteric Gram-negative rods (GNR) other than *Escherichia*, *Pseudomonas*, *Enterococcus*, *Staphylococcus*, *Streptococcus* and *Candida* species in the stools of three groups of infants fed a) an unsupplemented control formula, n=20, b) scFOS at 1.5 g/L, n=17 and c) 3 g/L, n=16), using plate culture techniques. The infants were 4 to 10 weeks of age and the study was conducted over a period of two weeks.

The description of results in the abstract of the report contradicts the description given in the body of the report and no detailed microbiological data was provided. Therefore FSANZ has discarded this study from further consideration.

Subsequently, Pickering et al (1993) conducted a four month study with a single formula-fed group (scFOS supplemented at 3 g/L, n=36), in comparison with an unsupplemented control formula-fed group (n=34). A breastfed group of infants (n=23) was used as a reference group. Faecal microflora populations were obtained at the end of the baseline period (day 8) and at study days 28, 56, 84 and 112. An ANOVA on ranks of inputted logs revealed no

significant differences between formula-fed groups at any time points in faecal bifidobacteria, clostridia and *C. difficile*, however, data were not shown to support these findings.

When changes in anaerobic bacteria were evaluated from study days 8 to 112 in response to formula-feedings or human milk, there were no differences between Bifidobacteria, *Bacteroides* spp., clostridia and *C. difficile* counts between formula-fed groups (supplemented and unsupplemented) and the breastfed reference groups.

The decrease in the clostridia colonisation observed in the previous two week tolerance study carried out by Malacaman et al (1992) was not detected in this four month study, although again, no data to support these findings were provided.

It is noted at day 8, that 52% per cent of infants were found to have detectable bifidobacterial counts in the non-supplemented formula fed group. This was close to the percentage of infants with detectable bifidobacterial counts in the breastfed group (59%). However, only 36% the scFOS-fed group had comparative counts at day 8 of life. These results suggest that a pre-randomisation bias and/or confounding factors have been introduced into the experiment at the beginning of the trial. The approach to statistical analysis of the faecal microflora results is described in detail but no descriptive nor statistical microbiological data have been provided in this paper. Therefore the results cannot be assessed to determine if scFOS has produced any effects on gut microflora.

The above studies employed media based methods where selective plating and microscopic enumeration was carried out. The subsequent study carried out in 2005 employed molecular methods which may be more efficient in determining microbiological effects.

Abbott Laboratories (2005) assessed the abundance of *C. difficile* and *E. coli* as potentially pathogenic bacteria using species-specific real-time PCR assays in a randomised, controlled, multi-centre study. The study duration was 28 days. Four groups of infants were fed a control formula (CF), high (HFF) and low concentrations (LFF) of scFOS (3g/L and 2g/L respectively) and breast milk (HM) during the duration of the study. Out of a total of 101 infants enrolled in the study, 65 subjects provided stool samples for analysis. The number of samples analysed in this study for microbial abundance is assumed to be > 16 per treatment group (as explained in the statistical methodology) even though not specifically stated in the study.

Results were presented as absolute abundance (expressed as $\log_{10}$ *rrs* copies per g of wet samples) and relative abundance ($\log_{10}$ of *rrs* copies per million copies (cpmc) of total bacterial *rrs* genes) between the major bacterial groups and presented in the form of box-and whisker plots of absolute abundance of the major bacterial groups. The results stated that all but two stool samples had *E. coli* (one breastfed and one scFOS fed (3.0 g/L).

The relative and absolute abundance of *Bacteroides* spp. was lower in all three formula-fed groups in comparison to the breastfed group. The relative abundances of this group of bacteria are higher in both the scFOS fed groups in comparison to the control formula fed group. However, no significant differences were detected among the groups.

Both scFOS supplemented groups had higher mean counts of *C. difficile* (6.00 for the 1.5 g/L group and 5.68 for the 3.0g/L group) in comparison to breastfed (4.16) and control formula fed (4.96) groups. According to the data presented, it is noted that there were significant differences in absolute *C. difficile* counts between the human milk fed group and the 1.5 g/L fed group, the latter being significantly higher. This was consistent with observations of other studies comparing *C. difficile* counts in stools from breastfed and formula-fed infants. As previously discussed, while *C. difficile* is recognised as a major cause of antibiotic-associated diarrhoea and colitis in adults, the clinical significance of elevated *C. difficile* levels in infant

stools are unclear.

The relative abundance of *E. coli* was shown to be highly variable, with no significant differences detected among the groups. However, the mean values were demonstrated to be lower in both scFOS supplemented groups in comparison to the control formula-fed groups. This trend is repeated in the relative abundances. All formula-fed groups had higher absolute abundances of *E. coli* in comparison to the breastfed group.

Older infants and young children consuming scFOS in a formulated beverage

O'Ryan et al (1996) carried out a study on the efficacy of a milk-based beverage supplemented with scFOS on the incidence of diarrhoea in older infants and young children. Even though the faecal microorganisms assessed in this study included *C. difficile,* no results nor conclusions were reported on the changes of abundance of this pathogen in the stools of participants.

5.3.2 Effects of scFOS on bifidobacteria and lactobacilli

Infants consuming scFOS in infant formula products as a sole source of nutrition

Merritt et al (2005) conducted a quantitative analysis of intestinal bacterial populations in term infants using a stringent methodology. The total bacterial load was quantified using a real-time PCR assay, universal bacterial primers and a TaqMan® probe. The abundance of the *Bacteroides-Prevotella* group (as commensal bacteria), bifidobacteria and lactobacilli (as beneficial bacteria) were identified and determined using genus-specific real-time PCR assays. The quantitative comparisons indicated that stools of formula-fed infants tended to have higher faecal bacterial counts than breastfed infants. Results are presented as absolute abundance (expressed as $\log_{10}$ *rrs* copies per g of wet samples) and relative abundance ($\log_{10}$ of *rrs* copies per million copies (cpmc) of total bacterial *rrs* genes) between the major bacterial groups. They used two test formulae with low (1.5g/L) and high (3g/L) scFOS supplemented for comparison with an unsupplemented control group and a breast fed reference group.

The absolute abundance of bifidobacteria, lactobacilli and *Bacteroides-Prevotella* were reported as being not significantly different among the treatment groups. The relative and absolute abundances of *Bacteroides* spp. were lower in all formula fed groups in comparison to the breastfed group, but there were no significant differences detected.

On a relative basis, the group of infants fed 2 g/L of scFOS tended to have less bifidobacteria than the other three groups (3.97 in comparison to 4.36 $\log_{10}$ *rrs* copies in breastfed, 4.69 in the control fed group and 4.63 $\log_{10}$ *rrs* copies in the 3.0g/L fed group, although this result was not statistically significant.

The formula supplementation resulted in higher counts of bifidobacteria than the control group but also stimulated *Bacteroides* spp. The overall conclusions of this study state that supplementation of the control formula with scFOS at either level did not further increase the population of bifidobacteria or lactobacilli, nor decrease *E. coli* or *C. difficile.* FSANZ notes, however, the significant increase if *C. difficile* in the LFF group in comparison to the HM fed group.

The relative abundances of lactobacilli were found to be less than that of bifidobacteria, and varied among the treatment groups. No significant differences were detected in the faecal abundances of lactobacilli. Authors concluded that the milk-based infant formula used in this study supported both beneficial species in sizes comparable to the breastfed group. The

supplementation of the control formula with scFOS did not increase the relative abundance of beneficial bacteria.

The differences in intestinal microbiota levels in individual infants may have contributed to the inconsistent bifidogenic effect observed in the formula groups. It was noted that the addition of nucleotides to the milk-based formula may have influenced the results obtained. This conclusion suggests that effects were too small to observe a result. It is possible that the selected sample size (number of subjects) and exposure level (amount/frequency fed) precluded the detection of significant effects of the scFOS feeding and that high variability among individual test subjects obscured the detection of significant differences.

Older infants and young children consuming scFOS in a formulated beverage

Shibata et al (2009) carried out two studies on the clinical effects of feeding kestose on the treatment of atopic dermatitis (AD) in infants. The first study was an open pilot study (duration 12 weeks), where 12 children with AD participated in and completed the study (no mention of a control group is made). The mean age of the children was 21 months (ranging from 5 to 40 months). A significant increase in the bifidobacterial counts was found on week 12 in comparison with week 0 ($p=0.026$). Subjects whose basal counts were low (<6.0) showed the highest increase in response to treatment with kestose.

The second study was a randomised, double-blind, placebo-controlled trial which evaluated the relationship between the enhancement of bifidobacteria and the severity of AD. The study duration was 12 weeks. The study participants were under 3 years old, with AD. Initial stool bifidobacterial counts of all subjects were $< 10^{10}$ colony forming units (CFU)/g of faeces. The extent of AD was evaluated using a Severity Scoring of Atopic Dermatitis (SCORAD) and the bifidobacteria was enumerated using a real-time PCR method at weeks 0, 6 and 12. No significant correlation was found between the improvement of the SCORAD score and the count of bifidobacteria.

Shibata et al (2009) found no significant difference between the counts of bifidobacteria in the stools of infants given the placebo and the kestose-fed group ($p=0.23$). However, in infants with basal counts <9.0 $\log_{10}$, the kestose-fed group showed a significant enhancement of stool bifidobacterial counts on week 12 ($p=0.05$).

In the first trial, the significant differences may have been detected due to a higher proportion of study subjects (3 out of 12, or 25%) having low basal counts at the beginning of the study, whereas in the randomised controlled trial, just one out of the 15 subjects (7%) fed kestose had initial low basal counts.

The authors pointed out that there were differences in the species composition of the genus bifidobacteria in the intestinal microflora of allergic infants vs healthy infants and suggest that species specific increases may have occurred even though undetected in the present study. The PCR primers used in this study were designed for the detection and enumeration of the genus bifidobacteria rather than to differentiate species specific levels.

O'Ryan et al (1996) conducted a randomised, blinded, controlled study to assess the efficacy of a milk-based beverage supplemented with scFOS on the incidence of diarrhoea in toddlers. The 16 week study involved 283 healthy children. The beverages were fed *ad libitum* in addition to the usual diet as the child's sole source of milk-based beverage and encouraged to drink a minimum of 500 mL per day. Faecal samples were evaluated for lactobacilli and bifidobacteria, together with pathogenic microorganisms. The average intake of scFOS was 2.5 g (per child) at each assessment.

The results of the study showed that children who consumed the scFOS supplemented formula had greater detectable levels of faecal bacteria in comparison to the control group, but there was no effect on the faecal counts of *Lactobacillus* spp. The increases in bifidobacterial counts were significantly higher (p=0.028).

Additional Animal studies

FSANZ searched the PubMed database for recent literature on the effects of scFOS on gut microflora. Only one published study described its effect using an animal model. The effects of feeding scFOS and its modulating effects on faecal microflora were studied by Shen et al (2010). They used human flora-associated (HFA) piglets for this purpose, and analysed the faecal microflora at specified time points in a control group and a scFOS fed group (from day 1 to day 37). HFA piglets are considered suitable models for the study of human gut microorganisms as pigs share a higher similarity with humans in gastrointestinal anatomy and physiology, nutritional requirements, metabolism and omnivorous diet habit (Pang et al. 2007). They also have minimal individual variation, enabling the detection of significant effects. The authors employed qPCR and DGGE analysis of the 16S rRNA gene fragments for this purpose. It was found that *Bifidobacterium* genus was stimulated consistently, except during weaning. The effects of scFOS on non-bifidobacteria varied at different developmental stages of the animals.

5.4 Conclusion of microbiological effects assessment

Regarding the effect on potentially pathogenic bacteria, feeding infants scFOS supplemented formula does not result in significant changes to levels of *E. coli* in gut microflora compared to those fed an unsupplemented control. Higher absolute abundance and relative values of *C. difficile* have been observed in both scFOS-supplemented groups in comparison to the control formula fed group, however this was not statistically significant. This is consistent with the general increased prevalence of *C. difficile* in stools of formula-fed versus human milk-fed infants reported in the literature. The healthy infant gut appears to be resistant to the effects of *C. difficile* toxin A and B and clinical infection is rarely reported in infants.

FSANZ concludes that increased levels of *C. difficile* observed in healthy, full-term infants consuming an infant formula supplemented with scFOS up to 3.0 g/L do not pose an additional risk compared with infants consuming an unsupplemented infant formula from birth onwards.

The only comprehensive study reporting the effects of feeding scFOS on the gut microflora of infants was Merritt et al (2005). The results from the animal study carried out by Shen et al (2010) have also been considered in reaching conclusions by FSANZ.

In healthy, full term infants, scFOS supplementation of infant formula has not been shown to induce either bifidobacteria or lactobacilli, however a significant bifidogenic effect has been demonstrated in toddlers.

Table 5.1 *Summary of microbiological findings in the scFOS clinical studies*

Reference	Group	scFOS group compared with the control	scFOS group compared with the breastfed group
Infants			
Malacaman et al (1993)	1.5 g/L or 3.0 g/L	Decrease in Clostridia[b]	NA
Pickering et al (1993)	3.0 g/L	Increase in Lactobacillus No change in Clostridia, *E. coli*	No change
Merritt et al (2005)	2.0 g/L	No change in *Clostridium*, Bifido or Lactobacillus Increase in *C. difficile,* decrease in *E. coli*	No change in Bifido or Lactobacillus, increase in *Clostridium* and *E. coli* and *C. difficile*
	3.0 g/L	No change in *Clostridium*, Bifido or Lactobacillus Increase in *C. difficile, decrease in E. coli*	No change in Bifido or Lactobacillus, increase in *Clostridium* and *E. coli* and *C. difficile (statistically significant)*
Infants and young children			
O'Ryan et al (1996)	3.4 g/L	Increase in Bifidobacteria, no change in lactobacilli	NA
Shibata et al (2009)	1 g/day - infants 2 g/day – young children	Increase in Bifidobacterial counts in infants whose basal counts were less than 9.0 $\log_{10}$ cells per gram of faeces	NA

[a] *Not clear whether data were compared statistically between groups*
[b] *Not clear if the results were obtained for the combined groups or the 1.5 g/L fed group only*

6. Risk assessment conclusions

This risk assessment evaluated the technological suitability, safety and benefit of the proposed addition of $scFOS_{sucrose}$ to infant formula products, infant foods and FSFYC as an optional alternative for already permitted IDS, including $scFOS_{inulin}$.
Health risks to the target populations were assessed in the context of the following:

- The maximum concentration of $scFOS_{sucrose}$ proposed to be added to infant formula products, infant foods and FSFYC is equivalent to those amounts already permitted for IDS. As an optional alternative, there is no increase in the net concentration of oligosaccharides present in these foods.

- Chemically, scFOS is the same as IDS generated by enzymatic hydrolysis, which is already permitted to be added to these foods. The identity specification for scFOS, established by Food Chemicals Codex, is the same irrespective of whether it is synthesised from sucrose or enzymatically released from inulin.

- This is not an assessment of the safety of infant formula, infant formula products, foods for infants or supplementary formulated foods for young children, which have a history of safe use in Australia and New Zealand.

- The maximum amount of $scFOS_{sucrose}$ of 3 g/L proposed to be added as a substitute for already-permitted IDS is much lower than the total concentration of non-digestible oligosaccharides present in breast milk.

- $scFOS_{sucrose}$ is expected to undergo the same degradation as IDS and HMOs in the infant digestive tract.

Health benefits in infants were considered in the context of the Ministerial Policy Guideline on the *Regulation of Infant Formula Products*, which requires that a substance has a substantiated role in normal infant growth and development.

6.1 Responses to risk assessment questions

1. Does β-fructofuranosidase derived from *A. niger* achieve its technological function in the form and quantity used to produce $scFOS_{sucrose}$?

3. What are the technological properties of $scFOS_{sucrose}$ and how do these compare with IDS generated by enzymatic hydrolysis that are currently permitted in the Code?

Section of report	*Summary response/conclusion*
Section 2	Evidence submitted in support of this Application provides adequate assurance that: 1) β-Fructofuranosidase derived from *A. niger* achieves its technological function in the form and quantity used to produce $scFOS_{sucrose}$ 2) $scFOS_{sucrose}$ is technologically suited to its proposed use and complies with international specifications.

2. Are there any public health and safety issues associated with the use of β-fructofuranosidase derived from *A. niger* as a processing aid?

Section of report	*Summary response/conclusion*
Section 3	No public health and safety issues were identified with the proposed use of β-fructofuranosidase from *A. niger* as a processing aid in the production of $scFOS_{sucrose}$. An acceptable daily intake (ADI) 'not specified' is considered appropriate.

4. What are the adverse physiological effects and health risks for the relevant population group consuming:

a. Infant formula, follow-on formula, infant food or toddler milk containing scFOS up to current maximum limits?

b. Infant formula, follow-on formula, infant food or toddler milk containing scFOS or in combination with IDS and/ or GOS up to current maximum limits?

c. How do the effects and risks for formula-fed infants in a) and b) compare with breastfed infants of comparable age?

d. How do the effects and risks for formula-fed infants in a) and b) compare with infants fed infant formula or follow-on formula containing IDS?

Section of report	*Summary response/conclusion*
Section 3	Results of laboratory animal studies confirmed that scFOS has no identifiable hazard at concentrations likely to be encountered under Good Manufacturing Practice. The digestion of $scFOS_{sucrose}$ was equivalent to IDS in an *in vitro* model of human colonic fermentation, producing comparable levels of SCFAs and gas. Overall, no toxicological issues were identified with regard to the addition of scFOS to infant formula at concentrations equivalent to current permissions for IDS.
Section 4	The addition of scFOS to infant formula products, infant foods and FSFYC up to the currently permitted concentrations for IDS is unlikely to cause adverse physiological effects in the healthy target populations.
Section 5	The consumption of scFOS-supplemented formula does not result in adverse effects on gastrointestinal flora. It is concluded that scFOS does not pose an additional risk to healthy formula-fed infants.

5. What are the positive physiological effects and health benefits for infants of relevant age consuming scFOS?

a. infant formula or follow-on formula containing scFOS up to the current maximum limits?

b. infant formula or follow-on formula containing scFOS in combination with IDS and/or GOS up to the current maximum limits?

Section of report	*Summary response/conclusion*
Section 4	The addition of scFOS to infant formula up to a concentration of 3.0 g/L has the potential to soften stools and may reduce constipation, which are considered to be beneficial effects.
Section 5	No beneficial effect on gastrointestinal flora was evident in infants that consumed formula supplemented with up to 3.0 g/L scFOS. In toddlers, a significant bifidogenic effect was demonstrated at 3.0 g/L scFOS.

6. Would the conclusions in relation to healthy term infants be applicable to preterm infants and infants with gastrointestinal disease?

This risk assessment is based on data generated in healthy term-infants and young children and therefore the above conclusions are applicable to these groups. These conclusions may not be applicable to preterm infants or those with gastrointestinal disease, which lie outside of the boundaries of the general population. It is expected that these more vulnerable population groups would be under close medical supervision and as such the appropriateness of *any* formula or special purpose food (with or without scFOS or any other IDS) would be assessed by a paediatrician or clinical dietitian as appropriate.

7. What are the health risks for the general population consuming scFOS-containing foods?

The use of scFOS in the general food supply is considered as safe as IDS on the basis of the following considerations:

- scFOS occurs naturally in food consumed by the general population including artichoke, asparagus, chicory, onion and wheat (Van Loo et al 1995)
- scFOS has been assessed as safe when consumed by infants and young children up to the currently permitted levels for IDS
- scFOS will undergo the same degradative process in the digestive tract of the general population as in infants and young children.

6.2 Consolidated conclusion

On the basis of these responses, it is concluded that $scFOS_{sucrose}$.produced by β-fructofuranosidase catalysed condensation of sucrose is technologically justified and is as safe as IDS already permitted to be added to foods generally, and to infant formula products, infant foods and FSFYC alone or in combination with IDS and/or GOS up to the currently permitted maximum concentrations. Additionally, scFOS has the potential to soften infant stools and may reduce the incidence of constipation, both of which are considered beneficial effects.

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Appendix 1: Discussions of the Infant and Child Health Scientific Advisory Group (ICHSAG)

Food Standards Australia New Zealand (FSANZ)

KEY ISSUES

1. Brief summary of the project and FSANZ's preliminary risk assessment conclusions

2. Specific issues for discussion

   2.1 Water balance in very young infants

   2.2 Microbiological effects

   2.3 Sweetness of scFOS

   2.4 Potential beneficial effects

3. Summary of the outcome of discussions

KEY DISCUSSION POINTS

1. Brief summary of the project and FSANZ's preliminary risk assessment conclusions

1.1 FSANZ welcomed all to the meeting. As per the Terms of reference for this advisory group no members declared any conflict of interest with any of the agenda items.

1.2 FSANZ provided an overview of Application A1055, which seeks to permit the optional addition of scFOS to infant formula, infant foods and supplementary foods for young children as an alternative to inulin-derived substances (IDS). As part of the evaluation of this application, FSANZ is preparing a risk assessment report covering the potential of scFOS to cause adverse effects in the target populations. The assessment also considers the benefit (i.e. efficacy) of the addition of scFOS in light of Ministerial policy guidance on the addition of substances to infant formula.

1.3 ICHSAG Members noted the context in which the risk assessment was being undertaken by FSANZ including the existing permissions for IDS, the chemistry of scFOS in relation to IDS and how scFOS might reasonably be expected to behave in the infant digestive tract.

1.3.1 The application is for the addition of scFOS to infant and follow-on formula as a direct substitution for already-permitted IDS – i.e. at the same maximum concentration of 3 g/L.

1.3.2 The Australia New Zealand Food Standards Code (the Code) currently defines IDS as mixtures of polymers of fructose with predominantly β (2→1) fructosyl-fructose linkages, with or without a terminal glucose molecule and includes inulin, but does not include those polymers of fructose produced from sucrose by enzymatic action. So while the Code identifies inulin-derived scFOS as a subset of IDS, sucrose-derived scFOS is currently excluded on the basis of its method of production.

1.3.3 As the concentration of scFOS proposed to be added to infant formula is equivalent to that already permitted for IDS, no change in digestion is expected in infants consuming formula containing scFOS.

2. Specific issues for discussion

2.1 Assessment of hydration status in infants

2.1.1 FSANZ reported that the effect of oligosaccharides on the hydration status of young infants was previously considered under Proposal P306 - Addition of Inulin / FOS & GOS to Food. In relation to the current application, the issue is whether scFOS might increase the osmotic potential in the colon to the point where it is dehydrating rather than softening to stools to be more comparable with breast fed infants.

2.1.2 Four clinical intervention studies were described (involving the addition of between 1.5 and 3.0 g/L scFOS in infant formula) where the hydration status of infants consuming scFOS was assessed mainly via a physical assessment. Only one of the studies measured urine specific gravity as a surrogate for hydration status.

2.1.3 These studies found no evidence that scFOS had any effect on infant hydration status. In addition, scFOS is proposed to be added to infant formula as a replacement for already-permitted oligosaccharides at an equivalent concentration, and that scFOS is not expected to behave any differently to human milk oligosaccharides in the digestive tract (and indeed would be present at much lower levels). Based on a weight-of-evidence assessment, FSANZ had concluded that scFOS added to infant formula up to 3 g/L does not affect hydration status in healthy, full-term infants consuming supplemented infant formula.

2.1.4 FSANZ was advised that infant hydration status is difficult to assess objectively based solely on clinical examination. The measurement of urine specific gravity was more useful but only reflected hydration status in the previous 12-24 hours; it would not be expected to vary significantly. In the one study that measured urine specific gravity, it was noted that the level of variation was consistent across the treatment groups and on this basis did not seem to suggest any treatment-related effect on hydration status.

2.1.5 In a clinical setting, hydration status is more accurately assessed via recent changes in bodyweight gain. Members noted that in all four studies, bodyweight was consistent between scFOS-fed and control infants suggesting no effect on hydration status.

2.1.6 Members commented that Mean Rank Stool Consistency (MRSC) is a socially acceptable, qualitative measure of infant gastrointestinal health and advised FSANZ that the use of the descriptor “watery” was not equivalent to diarrhoea.

2.1.7 Members were uncertain as to whether observations in infants consuming soy-based formula containing scFOS could be extrapolated to other types of formulas (e.g. whey-based).

2.1.8 FSANZ was advised that in infants, there is no meaningful feedback mechanism controlling thirst and therefore modulating hydration status. This is because infant feeding is a passive activity based on how much fluid is offered by parents.

2.1.9 Based on the evidence provided, there was overall agreement with FSANZ's conclusion that there was no adverse effect on infant hydration status from the presence of scFOS in infant formula up to 3 g/L. However, one Member commented that the level of confidence in the data was questionable due to the small sizes of the studies.

2.2 Microbiological effects

2.2.1 Members noted that of the three clinical studies that assessed effects on gastrointestinal flora, only one was included in the microbiological assessment because of study limitations. In this study, there was a higher absolute and relative abundance of *Clostridium difficil*e observed at 2.0 and 3.0 g/L scFOS in comparison to the unsupplemented formula control group. This finding was not statistically significant. However, there was a significant difference in the abundance of *C. difficile* in the breastfed group compared with the group consuming 2.0 g/L scFOS (although not for 3.0 g/L scFOS). These findings are consistent with the general increased prevalence of *C. difficil*e in stools of formula-fed versus breastfed infants. FSANZ was advised that there are no clinical concerns with non-toxigenic strains of *C. difficile.*

2.2.2 FSANZ's preliminary conclusion is that increased levels of *C. difficile* in healthy, full-term infants consuming an infant formula supplemented with scFOS up to 3.0 g/L do not pose an additional risk compared with infants consuming an unsupplemented infant formula from birth onwards. Based on the information provided, ICHSAG Members concurred with this conclusion.

2.3 Sweetness of infant formula with added scFOS

2.3.1 FSANZ outlined the issue of the perceived potential of scFOS to sweeten infant formula leading to an increase in formula consumption and the possible development of a preference for sweet foods later in life.

2.3.2 scFOS has a similar sweetness profile to IDS and GOS, which are already permitted to be added to infant formula. As scFOS would replace all or some of the inulin-derived substances, and as these substances provide similar levels of sweetness, there is unlikely to be any difference in the final sweetness of infant formula containing scFOS up to 3 g/L.

2.3.3 Members noted that in the infant feeding studies, there were no consistent differences in formula intake between the scFOS treated groups and the control groups, which supports an expectation that there should be no preference to consume a lactose-based formula due to any increase in sweetness resulting from the addition of scFOS.

2.3.4 ICHSAG Members agreed with FSANZ's preliminary conclusion that scFOS added to infant formula up to 3 g/L is unlikely to increase the sweetness of formula compared with oligosaccharides already permitted in infant formula.

2.4 Potential beneficial effects of scFOS – infant growth and development

2.4.1 As scFOS is completely fermented in the colon, FSANZ described how its draft efficacy assessment focussed on gastrointestinal (functional) and microbiological effects. Longer term health outcomes, other than broad parameters of growth were not measured in the clinical trials, which had a maximum duration of 16 weeks.

2.4.2 FSANZ's preliminary conclusion is that scFOS added to infant formula up to 3 g/L does not have a beneficial effect on the gastrointestinal or microbiological outcomes that were measured in the four infant studies compared with a control formula. When compared with breastfed infants, stool consistency in the scFOS fed groups approached that of breastfed infants after 16 weeks and microbiological outcomes were similar. Higher weight gains in formula-fed compared with breastfed infants were as expected but there were no differences between the scFOS or control formula-fed groups.

2.4.3 Members advised that when comparing the growth of formula fed infants with the normal growth of breast-fed infants, the latter typically grows slower. Therefore, a substance (such as scFOS) could be judged to be efficacious if it were to slow the growth of formula-fed infants to be more like the growth of breast-fed infants.

2.4.4 ICHSAG Members commented that the assessment of efficacy presented some challenges in terms of the inherent variability in infant growth and development and what might be a suitable duration for monitoring growth in a clinical study. It was noted that unless there was a gross effect, the four infant feeding studies were too small to detect any small response. Ideally, they noted, there should be at least 30-35 infants/sex/group to have sufficient power to detect subtle changes.

2.4.5 It was noted that the assessment of bodyweight served two aspects of the risk assessment. On one hand it could be used to assess safety (i.e. growth and hydration status) but equally could be used to assess benefit (i.e. normal growth).

2.4.6 There was some discussion on the designs of the 4 infant feeding studies and whether they could reasonably be expected to detect a beneficial effect of scFOS. One view was that the studies should have been conducted for at least six months, a period during which infants are exclusively breast/formula fed. Another view was that studies should ideally run to one year.

2.4.7 FSANZ was informed that a paper had recently been presented at an international meeting, which suggested that the growth rate in the first four weeks after birth may be a useful predictor of later health status. While longer term observations (up to a year) might ideally be conducted, what happens in the first 4 weeks is a critical development window to focus on.

2.4.8 Members agreed with FSANZ's preliminary conclusion that the studies did not detect any beneficial effect on infant growth from scFOS.

3.5 Potential beneficial effects of scFOS – stool softening/constipation

3.5.1 FSANZ sought advice on the potential of scFOS to soften stools or reduce constipation and the extent to which these are beneficial effects, including the contribution to infant well-being.

3.5.2 Members noted that unpublished data submitted by the Applicant provided inconsistent evidence that scFOS up to 3.0 g/L increased stool frequency or had a stool-softening effect in formula-fed infants compared to an unsupplemented control group.

3.5.3 Two published studies identified by FSANZ were considered by the group. In the study of Euler et al (2005) (*J Pediatr Gastroenterol Nutr* 40: 157-164), the frequency of bowel movements increased and stools became softer in a group of thirty infants that consumed formula supplemented with 3.0 g/L scFOS for one week; the change in stool consistency was significantly different ($p<0.01$) to the change in stool consistency recorded at a level of 1.5 g/L FOS. In the study of Bettler and Euler (2006) (*Int J Probiotics Prebiotics* 1(1): 19-26), the incidence of constipation was significantly lower ($p=0.033$) in the 3.0 g/L scFOS group ($n=101$) relative to the unsupplemented control group after 12 weeks.

3.5.4 Some of the issues raised in relation to the study of Euler et al (2005) were the relatively short duration of treatment and the possibility that the effects were attributable to a change in infant formula *per se* rather than to scFOS. However, the apparent dose-related stool-softening indicated that the observations were attributable to scFOS.

3.5.5 In the second study by Bettler and Euler (2006), it was noted that there was no evidence that there had been any systematic analysis of adverse events, and no information how stool consistency/constipation had been assessed. Noted was the significantly lower incidence of constipation in the scFOS-supplemented group compared to the formula-fed control group. Some concern was expressed by one member that this difference was based on parent's assessment of constipation using an undefined method. However, FSANZ was advised that the assessment of constipation by caregivers was an appropriate means of identifying changes in stool consistency post-treatment. Therefore the significantly lower incidence of constipation in scFOS-consuming infants was concluded to indicate an effect consistent with stool softening.

3.5.6 ICHSAG concluded that the observations made in these studies provided a weight-of-evidence consistent with the hypothesis that scFOS has a potential stool-softening effect and may reduce constipation.

3.5.7 With regard to the relationship between stool consistency or frequency and infant well-being, the ICHSAG advised that there is a relationship between severe constipation and reduced infant well-being. However it would be difficult to identify such a relationship within what is the normal range of stool consistency and frequency in infants, including less severe constipation.

4. Summary of the outcome of discussions

Based on the available data, ICHSAG Members agreed with FSANZ's draft conclusions on the safety and efficacy of scFOS. These conclusions were that scFOS:

- had no adverse effect on infant hydration status following the consumption of formula containing up to 3 g/L

- had no adverse effect on the microbiology of the infant digestive tract following the consumption of formula containing up to 3 g/L

- is unlikely to increase the sweetness of infant formula relative to oligosaccharides already permitted to be added to infant formula
- had no discernible benefit on infant growth
- has the potential to soften stools and may reduce constipation.