APPLICATION TO PERMIT THE OPTIONAL USE OF MILKFAT GLOBULE MEMBRANE ENRICHED WHEY PROTEIN CONCENTRATE IN INFANT FORMULA PRODUCTS

Applicant:

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Also associated with the Application:

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Statutory declaration

(Oaths and Declarations Act 1957)

I, [redacted] at Arla Foods Ingredients Group P/S, solemnly and sincerely declare that:

1. The information provided in this Application fully sets out the matters required; and
2. The information is true to the best of my knowledge and belief; and
3. No information has been withheld which might prejudice this application to the best of my knowledge and belief.

And I make this solemn declaration conscientiously believing the same to be true and by virtue of the Oaths and Declarations Act 1957. Declared at Århus this 21.06.2024

Signature

[redacted]

[redacted]

[redacted]

Declared before me

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Abbreviations

Abbreviation	Definition
ACC	Anterior cingulate cortex
AE	Adverse events
AFI	Arla Foods Ingredients P/S
ALA	Alpha-linolenic acid
Alk-SMase	Alkaline SMase
ANZ	Australia and New Zealand
AOM	Acute otitis media
ARA	Arachidonic acid
ASQ	Ages & Stages Questionnaire
AUD	Australian dollars
BAZ	Body mass index-for-age
BF	Breast-fed
BFR	Breastfed reference
BMI	Body mass index
Brown-ADD	Brown Attention-Deficit Disorder
BSC	Beta serum concentrate
BSSL	Bile salt-stimulated lipase
BSID	Bayley Scale of Infant Development
CANTAB	Cambridge Neuropsychological Test Automated Battery
CBCL	Child Behaviour Checklist
CCI	Confidential commercial information
CCP	Critical control point
CDI	Communicative Development Inventories
Cer	Ceramide
CF	Control formula
cGMP	Good Manufacturing Practices
CGMP	Casein glycomacropeptide
CHCL	Child Behaviour Checklist
ChP	Choline phosphate
CML	Complex milk lipid
CMPA	Cow's milk protein allergy
GD3	Disialoganglioside
GI	Gastrointestinal
GPC	Glycerophosphocholine
GPE	Glycerophosphoethanolamine
DAGs	Diglycerides

Abbreviation	Definition
DCCS	Dimensional change card sort
DOL	Days of life
DVFA	Danish Veterinary and Food Administration
ECCB	Exclusive capturable commercial benefit
EF	Experimental formula
EU	European Union
EV	Extracellular vesicles
FA	Fatty acid
FC	Functional connectivity
FFM	Fat free mass
FOF	Follow-on formula
FOS	Fructooligosaccharides
FSANZ	Food Standards Australia New Zealand
FSC	Food standards code
FSFYC	Formulated supplementary food for young children
GB	Guo Biao
GG	Gangliosides
GM3	Monosialoganglioside
HC	Health Canada
HCZ	Head circumference-for-age
HDL	High-density lipoprotein
IAP	Intestinal alkaline phosphatase
IF	Infant formula
IFG	Inferior frontal gyrus
IFO	Infant formula only
IFP	Infant formula products
IFSDU	Infant formula for special dietary use
IFPSDU	Infant formula products for special dietary use
IgG	Immunoglobulin G
IP	Intellectual property
ITT	Intent-to-treat
IOM	Institute of Medicine
LAZ	Length-for-age
LC-PUFAs	Long chain polyunsaturated fatty acids
LDL	Low-density lipoprotein
MAGs	Monoglycerides
MAIF	Marketing in Australia of Infant Formula
MH	Medial hypothalamus

Abbreviation	Definition
MF	Microfiltration
MFG	Milk fat globules
MFGM	Milkfat globule membrane
MFGM-L	Lipid-rich MFGM
MFGM-P	Protein-rich MFGM
MPI	Ministry for Primary Industries
MPLs	Milk polar lipids
MS	Mass spectrometric
MSE	Multiscale Sample Entropy
MWM	Morris Water Maze
N-CDase	Neutral ceramidase
NAM	National Academy of Medicine
Neu5Ac	N-acetylneuraminic acid
N-FA	N-fatty acyl
NIFI	New infant formula ingredients
NIFN	New Infant Formula Notice
NIP	Nutrition Information Panel
NIS	Nutrition Information Statement
NORT	Novel Object Recognition Test
NZ	New Zealand
OPN	Osteopontin
OPO	Oleic-palmitic-oleic
PC	Phosphatidyl choline
PE	Phosphatidyl ethanolamine
PEAL	Plain English Allergen Labelling
PGD	Prostaglandin
PI	Phosphatidyl inositol
PL	Phospholipids
PLB	Phospholipase B
PP	Per protocol
PRC	Peoples Republic of China
PS	Phosphatidyl serine
PUFA	Polyunsaturated fatty acid
Qb	Quantified Behaviour
RID	Radial immunodiffusion
RO	Reverse osmosis
RTF	Ready-to-feed
S29	Schedule 29

Abbreviation	Definition
SA	Sialic acid
SAE	Serious adverse events
SF	Standard formula
SL	Soy lecithin
SM	Sphingomyelin
SMase	Sphingomyelinase
SMPPi	Special medical purpose products for infants
Sph	Sphingosine
SPL	Sphingosine lyase
SPLs	Soy polar lipids
sPLA2 IB	Secretory pancreatic phospholipase A2 IB
SPK	Sphingosine kinase
TAG	Triacylglycerol
TRF	Teacher's Report Form
TTS	Toddler Temperament Scales
UF	Ultrafiltration
UK	United Kingdom
URTI	Upper respiratory tract infections
USA	United States of America
VF	Visual function
WAZ	Weight-for-age
WHO	World Health Organisation
WPC	Whey protein concentrate
XDH/XO	Xanthine oxidase
α-LA	Alpha-lactalbumin
β-LG	Beta-lactoglobulin

Executive Summary

Arla Foods Ingredients P/S (AFI) is a Danish food ingredient manufacturer supplying dairy-based ingredients, using standard whey processing techniques, for a wide variety of food applications including infant and medical nutrition formulations. Arla Foods Ingredients P/S has developed a dairy-derived whey protein concentrate (WPC) known under the tradename Lacprodan® MFGM-10. The unique feature of Lacprodan® MFGM-10, compared to typical WPC, is the enrichment of membrane components including phospholipids, glycolipids, membrane proteins, and sphingolipids. These stem from the three-layer milk fat globule membrane (MFGM) and single layer membrane of extracellular vesicles (EV) enriched in this product. MFGM is a component of all mammalian milks and is the primary delivery mechanism of fats in mammalian milk to offspring. For simplicity the MFGM and EV membrane materials are collectively referred to as MFGM as per the body of existing literature. All infants, breast-fed and formula-fed, therefore consume MFGM and its lipid and protein components, albeit at different levels. The components of MFGM are typically present at low levels in infant formula products that are milk-based or that contain milk-based ingredients. Vegetable oils, increasingly used over the last several decades as a fat source in infant formula products, typically lack the complex phospholipids present in MFGM, including sphingomyelin and gangliosides.

The addition of Lacprodan® MFGM-10 to infant formula products will ensure these products contain levels of phospholipids, sphingolipids, gangliosides and membrane proteins, that better align to the levels in human milk. The milk fat globule components represent only a fraction of the total whey protein concentrate, Lacprodan® MFGM-10. Due to complexity of assaying the total milk fat globule components present in Lacprodan® MFGM-10, sphingomyelin is used as the marker phospholipid to determine the level of addition of Lacprodan® MFGM-10. Clinical studies involving infants fed formula supplemented with Lacprodan® MFGM-10 demonstrate that Lacprodan® MFGM-10 is safe for consumption. These studies also show that infants consuming infant formula with added Lacprodan® MFGM-10 experience benefits in neurodevelopment and cognition endpoints compared to infants fed conventional infant formula products and approaching levels observed in breastfed infants.

The use of Lacprodan® MFGM-10 in infant formula products at 4 – 7 g/L is safe. This equates to a final proposed sphingomyelin (SM) range (1.8 – 7.5 mg/100 kJ) which also accounts for naturally occurring levels of SM in the dairy ingredients in infant formula products (IFP). Clinical studies on infants demonstrate the safety of the intended addition of Lacprodan® MFGM-10 to infant formula products at this level. The safe use of Lacprodan® MFGM-10 is also demonstrated by the history of safe consumption of infant formula products containing Lacprodan® MFGM-10 in over 20 countries, including countries in Europe, Asia and Central and South America. In the European Union, Lacprodan® MFGM-10 is not considered a novel food because it was supplied as a food ingredient prior to May 1997. In the Australia New Zealand context, Lacprodan® MFGM-10 is likely to be considered a nutritive substance when added to infant formula products and therefore requires permission in the Australia New Zealand Food Standards Code (the Code) before it can be added to these foods.

Arla Foods Ingredients P/S is requesting amendment to the Code to permit the addition of Lacprodan® MFGM-10 to infant formula and follow-on formula products in Australia and New Zealand at a level of 4 - 7 grams per litre (g/L). Arla Foods Ingredients P/S is requesting permission only for addition of

Lacprodan® MFGM-10 to infant formula products, including infant formula products for special dietary use, for infants up to 12 months of age. Permission is not sought, in this application, for the addition of Lacprodan® MFGM-10 to toddler milks or other foods that are formulated for young children.

Permission to add Lacprodan® MFGM-10 to infant formula products will provide the Australian and New Zealand market with infant formula products that more closely resemble the composition and the benefits provided by breastmilk. The optional addition of Lacprodan® MFGM-10 to infant formula products will increase the range of beneficial ingredients that can be used in IFP, providing additional benefits and increase consumer choice of products for formula-fed infants.

1 General Requirements (3.1.1)

1.0 Purpose of the application

This application seeks permission under the Australia New Zealand Food Standards Code (FSC) for the optional addition of milkfat globule membrane (MFGM) ingredient Lacprodan®MFGM-10, as a nutritive substance, to foods regulated within the FSC Part 2.9 Special purpose foods, specifically Standard 2.9.1 Infant formula products (IFP) (infant formula [IF, birth to 6 months], follow-on formula [FOF, 6 to 12 months] and infant formula for special dietary use [IFSDU, birth to 12 months inclusive]). Addition of Lacprodan® MFGM-10 will better align the composition infant formula with that of breast milk and support the developmental outcomes of infants.

Lacprodan® MFGM-10 is a whey protein concentrate sourced from bovine milk containing approximately two to four-fold higher enrichment of MFGM lipid and protein components compared to a standard whey protein concentrate widely used in infant formulas throughout the world. Arla Foods Ingredients Group P/S (AFI) manufactures the ingredient under the trade name Lacprodan® MFGM-10.

Permission to add nutritive substances to foods is regulated by Part 2.9 Special purpose foods, this application seeks to vary Standard 2.9.1 Infant formula products. Proposed options are laid out, as follows:

> **2.9.1-5 Use of substances as nutritive substances**
>
> *Use of nutritive substances*

The optional use of nutritive substances under 2.9.1- 5 (1) refers to the table of Schedule 29 (S29) section S29-5. Permission to add MFGM (as Lacprodan® MFGM-10) to infant formula products would be addressed by amending the table to S29-5 to include sphingomyelin as outlined in Table 1-1. Lacprodan® MFGM-10 is a complex ingredient with a number of components that are common to other dairy and non-dairy ingredients added to infant formula products. Sphingomyelin is suitable as an analytical marker of Lacprodan® MFGM-10 addition.

Table 1-1 Proposed amendment to the table to Schedule 29

Substance	***Permitted forms***	***Minimum amount per 100 kJ***	***Maximum amount per 100 kJ***
Sphingomyelin	Sphingomyelin	1.8 mg	7.5 mg

Conditions of use of the nutritive substance (Table 1-1), would comply with Standard 2.9.1 Infant formula products, part 5 Use of substances as nutitve substances.

The purpose of this application is consistent with policy guidelines as set out by the Food Ministers' Meeting (previously the Australia and New Zealand Food Ministerial Forum on Food Regulation). This includes the 'Policy Guideline for the Addition to Food of Substances other than Vitamins and Minerals' for the addition of MFGM to Special purpose foods. The addition of MFGM is aligned with the 'High Order' Policy Principles of the protection of public health and safety, informed consumer choice and the prevention of misleading or deceptive conduct. The permission to add MFGM to Infant formula products will promote consistency between domestic and international food standards and will help promote an efficient and internationally competitive food industry. The application is further aligned with the 'Specific Order Policy Principles – Any Other Purpose', where the purpose for adding

MFGM to Infant formula products is the provision of a safe bioactive substance that supports wellbeing associated with neural development and cognitive function in infants. Milkfat globule membrane components are naturally present in mammalian milks, including human, and as such has a safe history of consumption. The addition of MFGM from bovine milk to human food also has a history of safe use, having typically been added in the form and in quantities consistent for delivering the benefits subject of this application. The addition of MFGM to Infant formula products will not create a significant negative public health impact to the general population or sub-populations, nor will the presence of MFGM mislead consumers as to the nutritional quality of the food.

For products subject to Standard 2.9.1 Infant formula products, the Food Ministers' Meeting (Food Regulation Standing Committee – Regulation of Infant Formula Products) Policy Guideline provides guidance on expectations in the setting of new regulation for Infant formula products, in addition to the 'High Order' Policy Principles as above. This application is aligned with the Specific Policy Principles, in that:

- the addition of MFGM to Infant formula products does not negate the overarching recognition that breastfeeding is the normal and recommended way to feed an infant;
- the addition of MFGM to Infant formula products is consistent with national nutrition policies and guidelines of Australia and New Zealand (ANZ) that are relevant to infant feeding;
- the addition of MFGM to Infant formula products is based on a safe history of use, outside of ANZ, and takes into account the vulnerability of the infant population, recognising the importance of infant formula products in the diets of formula-fed infants;
- when used as the sole source of nutrition, infant formula containing MFGM supports the normal growth and development of healthy term infants similar to that of exclusively breastfed infants;
- Infant formula products containing MFGM are safe, suitable and meet the nutritional requirements to support the growth, development and dietary management of the infants for whom they are intended;
- used as the sole source of nutrition, infant formula containing MFGM supports the normal growth and development of term infants;
- Infant formula products, including infant formula, follow-on formula and infant formula for special dietary use, that contain MFGM are safe, suitable for the intended use;
- the addition of MFGM to infant formula products does not impact the essential composition of infant formula products prescribed in the FSC, formulas in accordance with Standard 2.9.1 that contain MFGM satisfy the nutritional requirements of infants;
- the addition of MFGM to infant formula products results in a composition that is more closely aligned with that of breast milk which contain significant levels of milkfat globule membrane components;
- the addition of MFGM to infant formula products available for sale in ANZ requires pre-market assessment. Whilst MFGM has been added to infant formula products manufactured in ANZ for a number of years, those products have been manufactured for export markets, and therefore the addition of MFGM at the proposed level does not have a known history of use in ANZ;

- the addition of MFGM to infant formula products has a substantiated beneficial role supporting the neural development and cognitive function of infants compared to formula-fed infants consuming formula not fortified with MFGM.

1.1 Justification for the application

The components of MFGM are naturally occurring in mammalian milks, including human milk and are associated with health benefits and supported by evidence supporting a key role in early life development. Milkfat globule membrane has been shown to support neural development and cognitive function in infants. Infant formula products typically have lower levels than human milk of the MFGM components. The proposed use of MFGM as a nutritive substance in infant formula products will produce infant formula products that more closely resemble the nutrient composition of human milk; a principle consistent with the Food Ministers' Meeting *Policy Guideline on the Regulation of Infant Formula Products*[1] and Codex's *Standard for Infant Formula and Formulas for Special Medical Purposes Intended for Infants*. The addition of MFGM enriched ingredients to IFP can help deliver the benefits detailed in Section 3.2.3 to formula-fed infants. The use of MFGM (as Lacprodan® MFGM-10) in IFP has been extensively studied and is widely used in international markets.

The Code does not currently explicitly permit the addition of MFGM to infant formula products. Therefore, this application seeks to clarify the permission to use and seeks to amend Schedule 29 of the Code to include MFGM (as Lacprodan® MFGM-10) as a permitted nutritive substance in infant formula products.

The optional addition of Lacprodan® MFGM-10 to infant formula products will extend the options of beneficial ingredients that may be used and hence provide additional benefits and increase consumer choice. Formula-fed infants in ANZ will have the opportunity to consume formula that delivers the benefits of MFGM, similar to formula-fed infants around the world.

Arla Food Ingredients P/S has not identified any disadvantages to permitting the addition of Lacprodan® MFGM-10 to infant formula products. Permission in the Code will be for optional addition. Lacprodan® MFGM-10 is sourced solely from bovine milk and is therefore required to be listed on product labels in accordance with allergen labelling requirements in ANZ (Standard 1.2.3 and Schedule 9). Allergen labelling requirements apply to existing standard milk-based infant formula products.

1.1.1 Regulatory impact

This application with provide regulatory clarification in ANZ with regards to the permitted use the MFGM enriched whey powder, specifically Lacprodan® MFGM-10, in infant formula products.

The applicant, AFI, is aware of the current Food Standards Australia New Zealand (FSANZ) reviewpertinent to this application; P1028 review of regulatory requirements for Infant Formula scheduled for Ministerial approval late-2024, and P1024 – revision of the Regulation of Nutritive Substances & Novel Foods (currently on hold pending FSANZ Act Review). There is no impact of those reviews affecting this application.

1.1.1.1 Cost and benefits

Manufacturers, suppliers and importers will be able to market and import Lacprodan® MFGM-10 and Lacprodan® MFGM-10 fortified products for sale in the Australian and New Zealand markets.

The facilitation of export trade of IFP manufactured by domestic manufacturers in Australia and New Zealand will be of direct benefit to them by increasing market opportunities.

[1] https://foodregulation.gov.au/internet/fr/publishing.nsf/Content/publication-Policy-Guideline-on-Infant-Formula-Products

Consumers in Australia and New Zealand will benefit from the availability of IFP with MFGM and the benefits associate with its consumption.

The incremental cost of the addition of Lacprodan® MFGM-10 is normally passed on as a nominal price premium for products to which it is added. Commonly products will contain more than one added optional ingredient. In the range of available IFP, historically the use of optional ingredients and associated price premiums has resulted in market segmentation into standard and premium product ranges, that have provided consumers with product and price range options. Such segmentation remains, however, there is a convergence of the segments as optional ingredients such as MFGM become more mainstream in international markets.

The cost of addition of Lacprodan® MFGM-10 to IFP products is dependent on ingredient cost and addition rate. Cost expectations are that the addition of MFGM in IFP that provide SM within the proposed range would add approximately Australian dolloars (AUD) 0.65 per 100 g of IFP powder. Products containing MFGM are expected to be at the premium end of the market and attract a higher price differential.

1.1.1.2 Impact on International trade

The permitted addition of Lacprodan® MFGM-10 to infant formula products (regulated under Standard 2.9.1) will better align products made in Australia and New Zealand with existing standards in other countries, facilitating international trade among jurisdictions in which Lacprodan® MFGM-10 is or is soon to be permitted.

Domestic manufacturers wishing to export Lacprodan® MFGM-10 fortified products to overseas markets will benefit from permission in the Code to add Lacprodan® MFGM-10 through alignment with international standards and no requirement to apply for exemptions under export regulations. There are significant market opportunity advantages with the ability to export locally compliant value-added products to various markets around the world, under for example Certificates of Free Trade.

1.2 Assessment procedure

Based on the criteria provided in the FSANZ "Application Handbook" (1 July 2019), AFI considers this application should be assessed by FSANZ under the general procedure. This application is for the variation of food regulatory measures required for the addition of a new nutritive substance to foods for vulnerable populations (infants) and the requirement for pre-market approval of the substance. Arla Foods Ingredients P/S notes that MFGM is a component of human milk and bovine milk; and no genetic modification has taken place, which may reduce complexity of assessment.

1.3 Confidential commercial information

Information is submitted separately under the terms of Confidential Commercial Information (CCI).

1.4 Other confidential information

Information is submitted separately under the terms of Information (CI).

1.5 Exclusive capturable commercial benefit

Arla Foods Ingredients P/S expects the application to confer an exclusive capturable commercial benefit (ECCB) to AFI once amendments to the Food Standards Code are made, and provided exclusivity is granted to AFI. Therefore, AFI commits to pay the fee to cover the assessment of the application. Justification for obtaining exclusivity and information to support an ECCB to AFI is outlined in Table 1-2.

Table 1-2 Justification for obtaining exclusivity and information to support an exclusive capturable commercial benefit to AFI

Why are you making this application? What are you hoping to get out its approval?	This application seeks permission for the optional addition of MFGM (as Lacprodan ® MFGM-10) to infant formula products in order to improve the compositional profile of IFP and deliver improved outcomes for formula-fed infants. The ability of add Lacprodan ® MFGM-10 to IFP manufactured in ANZ will enable AFI customers to align their products with international offerings, support product innovation and a range of differentiated product offerings, together with increasing consumer choice options. Arla Foods Ingredients P/S has made a significant investment globally over the last 2 decades in the development and manufacture of Lacprodan® MFGM-10 and its use in IFP supported by numerous scientific studies. There has been a significant investment by AFI in the preparation of this application, and the commitment to pay fees in full for the assessment.
How will you benefit from the approval of your application?	Approval of the application will enable infant formula products containing added MFGM to be sold in Australia and New Zealand. Arla Foods Ingredients P/S will benefit from supplying MFGM (as Lacprodan ® MFGM-10) to manufacturers in Australia and New Zealand, with increased demand for the ingredients in ANZ forecast on success of this application.
Who besides you, will benefit from the approval of your application? How and why will they benefit?	Approval of the application will ultimately benefit consumers. Manufacturers of infant formula products will benefit from being permitted to add MFGM to products they manufacture and market. The manufacturers will also benefit from technical support and knowledge shared with them by AFI.
If your application is approved, whose permission will be required before anyone can derive a benefit from that approval?	Arla Foods Ingredients P/S will enter into corporate partnerships with Australian and New Zealand infant formula manufacturers that wish to add Lacprodan® MFGM-10 ingredient, manufactured by AFI, to IFP products for consumers.
Who holds the intellectual property (IP) in the subject matter of your application?	Arla Foods Ingredients P/S holds proprietary information relating the manufacture of Lacprodan® MFGM-10. Currently AFI is not the only manufacturer of MFGM enriched milk-derived ingredients. However, Lacprodan® MFGM-10 is unique to

	AFI and much of the knowledge of the benefits of adding MFGM to IFP is based on research completed with Lacprodan® MFGM-10. The most recent AFI patent published relating to Lacprodan® MFGM is WO 2023/001783 A1 "Method of preparing a phospholipid-enriched, whey-derived composition having a low content of microorganisms, the composition as such and nutritional use of the composition". This patent covers both Australia and New Zealand. In addition WO 2023/001782 A2 "Method of preparing a whey-derived composition enriched in phospholipids and osteopontin, the composition as such, and nutritional use of the composition", is relevant in that it too encapsulates IP relevant to many of the properties of Lacrprodan® MFGM-10. Both Australia and New Zealand are covered in this patent.

AFI, Arla Foods Ingredients P/S; ANZ, Australia and New Zealand; IFP, infant formula products; IP, intellectural property

Arla Foods Ingredients P/S requests Exclusivity be granted for this application, enabling AFI to capture an Exclusive Capturable Benefit.

1.6 International and other national standards

1.6.1 International standards

The addition of MFGM to infant formula products is consistent with the intent and recommendations of relevant internationally recognised codes of practice and guidelines, and in particular with those by the Codex Alimentarius Commission (Codex). Specifically with regard to the use of MFGM in infant and follow-on formulas the safe history of use of MFGM and its components, together with extensive safety and clinical data addresses the recommendations for data requirements for changes to infant formula as recommended by the National Academy of Medicine (NAM) formerly called the US Institute of Medicine (IOM), Food and Nutrition Board guidelines that clarify the types and extent of safety testing necessary for new formula ingredients, particularly unconventional substances derived from novel sources or technologies (Institute of Medicine, 2004).

The Codex Alimentarius Commission (Codex)[2] provides a number of standards, codes of practice and guidelines relevant to special purpose foods, and for which the use of MFGM is consistent with their intent.

The Codex Standards for Special purpose foods for infants (Codex Standard for Infant Formula and Formulas for Special Medical Purposes Intended for Infants[3] (from birth through to 12 months)) allows for the addition of other ingredients which provide "substances ordinarily found in human milk and to ensure that the formulation is suitable as the sole source of nutrition for the infant or to provide other benefits that are similar to outcomes of populations of breastfed babies. The suitability for the

[2] http://www.fao.org/fao-who-codexalimentarius/en/

[3] https://www.fao.org/fao-who-codexalimentarius/shroxy/es/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXS%2B72-1981%252FCXS_072e.pdf

particular nutritional uses of infants and the safety of these substances shall be scientifically demonstrated. The formula shall contain sufficient amounts of these substances to achieve the intended effect, taking into account levels in human milk" (Codex Alimentarius, 2020).

The Codex Standard for Follow-up Formula[4] (for infants from 6 months and young children through 3 years)) is less definitive for optional ingredients however the addition of MFGM would be consistent with the requirements of the standard in that other nutrients may be added when required to ensure that the product is suitable to form part of a mixed feeding scheme intended for use from the 6th month on. That the usefulness of those ingredients is scientifically proven, and that when added, the food will contain significant amounts of the nutrients, based on the requirements of infants from the 6th month on and young children (Codex Alimentarius, 2017b).

The proposed addition of MFGM for Infant formula products under Standard 2.9.1 is aligned with the intent of the Codex infant and follow-up formula standards. Furthermore, the production and specifications set for MFGM are consistent with the Codex recommendations for raw materials and ingredients for use in infant and follow-up formula and formulae for special medical purposes for infants and young children (Codex Code of Hygienic Practice for Powdered Formulae for Infants and Young Children[5] (Codex Alimentarius, 2008).

The Codex Guidelines for Formulated Supplementary Foods for Older Infants and Young Children[6], allows for the use of other ingredient that may "improve the nutritional quality and/or acceptability of the Formulated Complementary Foods provided that they are readily available and have been proven to be suitable and safe for their intended purpose" (Codex Alimentarius, 2017a).

For infant formula products, the addition of MFGM and labeling of infant formula products fits consistently with the intent of infant formula marketing codes of practice; Marketing in Australia of Infant Formulas: Manufacturers and Importers Agreement 1992 (The MAIF Agreement, 1992); WHO International Code of Marketing of Breast-milk Substitutes (World Health Organization, 1981); The Infant Nutrition Council Code of Practice for the Marketing of Infant Formula in Aotearoa, New Zealand (Infant Nutrition Council, 2023).

1.6.2 Other national standards

Infant formula products containing Lacprodan® MFGM-10 are currently on the market in many countries, including (in alphabetical order) Argentina, Bulgaria, Brazil, Canada, Colombia, Czech Republic, Denmark, Ecuador, Finland, Hong Kong, India, Indonesia, Japan, Latvia, Lithuania, Malaysia, Mexico, Nigeria, Norway, Panama, Peoples Republic of China, Peru, Philippines, Poland,

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4 (https://www.fao.org/fao-who-codexalimentarius/sh-proxy/zh/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXS%2B156-1987%252FCXS_156e.pdf

5 https://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXC%2B66-2008%252FCXP_066e.pdf

6 https://www.fao.org/fao-who-codexalimentarius/sh-proxy/zh/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXG%2B8-1991%252FCXG_008e.pdf

Portugal, Russia, Singapore, South Korea, Spain, Sri Lanka, Sweden, Taiwan, Thailand, United States of America, and Vietnam. In many of these countries, the ingredient is listed simply as whey protein concentrate or whey protein concentrate (containing MFGM). In Mexico it is listed as milk solids.

1.6.2.1 European Union

In the European Union (EU) milk-derived ingredients enriched with MFGM components have been available and added to consumer products marketed prior to 15th of May 1997. This is the cut-off date for novel foods described in the Novel Food regulation, Regulation (EU) 2015/2283. Products and ingredients in the market prior to that date are exempt from the Regulation. Lacprodan® MFGM-10 is therefore not considered to be a novel food and for that reason the ingredient does not need a safety approval before marketing in the member states of the EU. All Infant formulas needs to be notified to the competent authorities of the member states. There has been no negative feedback from the competent authorities in the individual member states on infant formulas containing Lacprodan® MFGM-10. Lacprodan® MFGM-10 has been consumed in infant formula and follow-on formula in EU for the past 15 years.

1.6.2.2 United Kingdom

On exiting the European Union, the United Kingdom (UK) adopted the majority of the EU food laws, including approved novel foods, and those not deemed novel under the EU regulations. As Lacprodan® MFGM-10 falls into this category, it may be used in the UK for the manufacture of IFP and as an ingredient in IFP available for sale in the UK.

1.6.2.3 Peoples Republic of China

The addition of MFGM rich ingredients to IFP in China is permitted under the provisions for Optional Ingredients. In the absence of a national mandatory standard (Guo Biao (GB)), a Light Industry Standard (QB/T 5805-2023) for Milk (whey) protein powder with milk fat globule membranes was published for implementation on 1st February 2024 (Ministry of Industry and Information Technology of the PRC, 2023). This ensures clarity for the ongoing addition of MFGM-rich ingredients meeting the standard, to IFP for sale in China, prior to the long term development of GB standards.

A copy of the standard is provided with references.

1.6.2.4 Canada

Health Canada (HC) requires pre-market notification of New Infant Formula Ingredients (NIFI). A proposal[7] (28 November 2023) to modernise foods for special dietary use and infant foods clarifies the definition of a NIFI as “a substance:

a. that is intended for use as a food ingredient in infant formula, and
b. that is not added to meet the compositional requirements for infant formula, and
c. that is not a food additive or a novel food, and
d. for which a history of safe use in infant formula has not been demonstrated in Canada”.

To improve the efficiency of pre-market approval requirements, the proposal aims to place the responsibility of premarket authorisation of an NIFI with the NIFI manufacturer, rather that the infant formula manufacturer, and prior to submission of the IFP containing the NIFI, for approval. Prior to March 2024 NIFI approval was a part of the new infant formula pre-market notification process. [redacted]

[7] https://www.canada.ca/en/health-canada/programs/consultation-regulatory-modernization-foods-special-dietary-use-infant-foods/document.html

[redacted]

In response to on-going IFP supply issues HC issued a guidance document (update 25 March 2024): Transition strategy to prepare for the expiration of HC's interim policy to mitigate infant formula shortages[8]. The guidance is consistent with HC's modernisation proposal and allows for products currently in the Canadian market to remain once the interim policy on importation expires. As a part of the transition HC have published the list of NIFI cleared for use in IFP for sale in Canada (as of 1 March 2024).

Notably AFI Lacprodan® MFGM-10 is listed as a permitted bioactive (Figure 1-1).

Figure 1-1 NIFI cleared for use in IFP available for sale in Canada (as of 1 March 2024)

Bioactives - Oligosaccharides	Bioactives - Other (Nutrients)	Microbes
• NutraFlora®scFOS (Ingredion) • 2'-FL manufactured using genetically modified *E. coli* BL21 (DE3) strain #1540 or strain #1242 (Chr. Hansen, formerly Jennewein Biotechnologie GmbH) • 2'-Fucosyllactose (2'-FL) produced via fermentation using a genetically modified *E. coli* K12 MG1655 strain (DuPont Nutrition & Biosciences) • 2'-Fucosyllactose (2'-FL) produced via fermentation using a genetically modified *E. coli* K-12 (DH1) MDO MAP1001d strain [Glycom A/S (affiliated with DSM Nutritional Products Inc.)] • Vivinal®GOS Syrup (Friesland Campina Ingredients) • Dairy Crest GOS (Saputo Dairy UK) • Orafti® HP (Beneo GmbH), a long-chain inulin or long-chain fructo-oligosaccharide (lcFOS) in combination with Vivinal® GOS Syrup (Friesland Campina Ingredients) in a 1:9 ratio • VITAGOS™ (Vitalus Nutrition Inc.) • Vivinal®GOS Syrup LE (Friesland Campina Ingredients) • VITAGOST™ IF (Vitalus Nutrition Inc.)	• DHASCO-B (DSM, formerly Martek) • DHA from *Schizochytrium sp.* T18 (Mara Renewables) • DHA550 from Schizochytrium sp. FCC-3204 (Fermentalg) • Lacprodan milk fat globule membrane (MFGM)-10 (Arla Foods) • InFat (Frutarom, formerly Enzymotec)	• *Lactobacillus helveticus* R0052 (Lallemand): only for FUF (6 months and older) • *Bifidobacterium bifidum* R0071 (Lallemand): only for FUF (6 months and older) • *Lactobacillus reuteri* DSM17938 (BioGaia) • *Lactobacillus casei ssp. rhamnosus* GG/LGG (Chr. Hansen, originally by Valio Ltd.) • *Bifidobacterium lactis* Bb. 12

1.6.2.5 *United States of America*

In the United States of America (USA), MFGM-containing dairy ingredients are used in some products and may be labelled as whey protein concentrate. Label information may or may not contain a comment about being a source of MFGM. To date the MFGM containing ingredients are considered normal ingredients of IFP. Safety, physiological and technical requirements of the pre-market New Infant Formula Notice (NIFN) were supported by clinical trial studies.

1.6.2.6 *New Zealand*

Food manufactured in NZ for export outside of ANZ can be exempted from the compositional requirements of adopted joint food standards (including the FSC issued by FSANZ) and notices made

[8] https://www.canada.ca/en/health-canada/services/food-nutrition/public-involvement-partnerships/notice-stakeholders-transition-strategy-prepare-expiration-interim-policy-mitigate-infant-formula-shortages/document.html

under the Food Act 2014. The Ministry for Primary Industries (MPI) issues a Food Notice: Food for Export – Exemptions from Domestic Compositional Requirements (Ministry for Primary Industries, 2024) which lists exemptions for IFP and other food categories. The current Food Notice does not include exemptions for MFGM in any product category to any export market.

1.7 Checklists

Requirements	Comment and relevant sections covered	Page No
General requirements (3.1.1)		
A Form of application		
Application in English	Yes	
Executive Summary (separated from main application electronically)		17
Relevant sections of Part 3 clearly identified	Yes	(and as separate document)
Pages sequentially numbered	Yes	
Electronic copy (searchable)	Yes	
All references provided	Yes	
B Applicant details	Yes	2
C Purpose of the application	Section 1.0	19
D Justification for the application	Section 1.1	22
Regulatory impact information	Section 1.1.1	22
Cost and benefits	Section 1.1.1.1	22
Impact on international trade	Section 1.1.1.2	23
E Information to support the application	Section 2.1	33
Data requirements	Section 2.1.2	34
F Assessment procedure	Section 1.2	23
G Confidential commercial information (CCI)	Section 1.3	23
CCI material separated from other application material	Yes	
Formal request including reasons	Yes	
Non-confidential summary provided		
H Other confidential information	Section 0	23
Confidential material separated from other application material	Yes	
Formal request including reasons	Yes	
I Exclusive capturable commercial benefit	Section 1.5	24
Justification provided	Yes	
J International and other national standards	Section 1.6	25
International standards	Section 1.6.1	25
Other national standards	Section 1.6.2	26
K Statutory declaration	Yes	3
L Checklist provided with application	Section 1.7	30
3.1.1 Checklist	Yes	
All page number references from application included	Yes	
3.3.3 Checklist	Yes	
3.6.2 Checklist	Yes	

Requirements	Comment and relevant sections covered	Page No
3.6.3 Checklist	Yes	
Substances used for a nutritive purpose (3.3.3)	**Section 2**	**33**
A Information on the use of the nutritive substance	Section 2.1	33
A.1 Purpose of the use of the substance	Section 2.1.1	33
A.2 General data requirements for supporting evidence	Sections 2.1.2	34
B Technical information on the use of the nutritive substance	Section 2.2	40
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B.2 Chemical and physical properties	Section 2.2.3	58
B.3 Impurity profile	Section 2.2.4	62
B.4 Manufacturing process	Section 2.2.5	63
B.5 Specification for identity and purity	Section 2.2.6	67
B.6 Analytical method for detection	Section 2.2.8	82
B.7 Proposed food label	Section 2.2.9	83
C Information related to the safety of MFGM	Section 2.3	86
C.1. Toxicokinetics and metabolism, degradation products and major metabolites	Section 2.3.1	86
C.2 Animal or human studies	Section 2.3.1.1	86
C.3 International safety assessments	Section 2.3.4	94
D Information on the dietary intake of the nutritive substance	Section 2.4	95
D.1. List of food groups or foods likely to contain the nutritive substance	Section 2.4.1	95
D.2 Proposed maximum levels in food groups or foods	Section 2.4.2	95
D.3 Likely level of consumption	Section 2.4.3	96
D.4 Percentage of food group to use the nutritive substance	Section 2.4.4	97
D.5 Use in other countries (if available)	Section 2.4.5	97
D.6 Where consumption has changed, information on likely consumption	Section 2.4.6	99
E Information related to the nutritional impact of a vitamin or mineral	Not relevant to application	
E.1 Need to permit addition of vitamin or mineral	Not relevant to application	
E.2 Demonstrated potential to address deficit or health benefit	Not relevant to application	
F Information related to the nutritional impact of a nutritive substance other than vitamins and minerals	Section 2.52.5.1	99
F.1 Nutritional purpose (other than vitamins and minerals)	Section 2.5.1	99
G Information related to potential impact on consumer understanding and behaviour	Section 2.6	100
G.1 Consumer awareness and understanding	Section 2.6.1	100
G.2 Actual or potential behaviour of consumers	Section 2.6.2	102

Requirements	Comment and relevant sections covered	Page No
G.3 Demonstration of no adverse effects on any population groups	Section 2.6.3	103
Special purpose foods – Infant formula products (3.6.2)	**Section 3**	**104**
A Information related to the composition	Section 3.1	104
A.1 Purpose of compositional change	Section 3.1.1	104
A.2 Data for supporting evidence	Section 3.1.2	105
A.3 Specific information requirements	Section 3.2	105
Characterisation of proposed substance in breast milk	Section 3.2.1	105
Nutritional safety and tolerance	Section 3.2.2	105
Efficacy of proposed compositional change	Sections 3.2.3	146
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B Information related to the dietary intake or dietary exposure	Section 3.3	179
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B.2 Level of consumption	Section 3.3.2	181
B.3 Information relating to the substance	Section 3.3.3	182
C information related to the labelling requirements under Part 2.9 of the Code	Section 3.4	183
C.1 Safety or nutritional impact of labelling change	Section 3.4.1	183
C.2 Demonstrated consumer understanding of labelling change	Section 3.4.2	183
D Internationally recognised codes of practice and guidelines on labelling	Section 3.5	184

2 Substances used for a nutritive purpose (3.3.3)

2.1 Information on the use of the nutritive substance

2.1.1 Information on the purpose of the nutritive substance

The purpose of the use of Lacprodan® MFGM-10 in infant formula products is based on the weight of evidence of improved neurodevelopmental and cognitive outcome in formula-fed infants receiving Lacprodan® MFGM-10 fortified formula compared to standard formula not fortified with MFGM components. The addition of Lacprodan® MFGM-10 to IFP enriches these products with MFGM-derived lipid and protein components similar to those present in human milk. Breast-fed infants benefit from the MFGM associated components naturally present in human milk, whilst for infants reliant on IFP to support growth and development may not be able to experience the benefits of MFGM, unless it is added. This enables parents who formula-feed their infants the option to select a product that provides benefits more similar to those provided by the naturally occurring MFGM in human milk. The neurodevelopment and cognitive benefits associated with MFGM are discussed in Section 3.2.3.

Whilst Lacprodan® MFGM-10 is a MFGM enriched WPC, it is the MFGM fraction of the ingredient that is associated with the benefits of the ingredient when added to IFP. The whey component adds to the protein content and nutritional function of protein in formula, with the addition of whey into IFP a normal and common practice. The protein content of IFP is regulated by the Food Standards Code in Australia and New Zealand (Standard 2.9.1) and by other regulatory instruments around the world. It is the MFGM-lipid components of Lacprodan® MFGM-10 that are primarily associated with its physiological benefits and are also the key characterising components.

This application proposes the addition of Lacprodan® MFGM-10 to infant formula products (equivalent to 4 to 7 g/L as consumed). The presence of the added Lacprodan® MFGM-10 can be characterised by the quantification of the sphingomyelin content of the infant formula (Table 2-1). The proposed sphingomyelin content range allows for any intrinsic level from other dairy ingredients used in the IFP formulation, whilst necessitating the requirement for a MFGM ingredient to be added.

Components of the MFGM, together with those from extracellular vesicles (EV's), that are naturally present in human milk are known to deliver key physiological benefits for infants. Lacprodan® MFGM-10 contains many of these components and there is a significant level of support for the benefits and safety from both human and animal studies that has been established over a number of years.

Table 2-1 Proposed amendment to the table to of S29-5 of Schedule 29

Substance	*Permitted forms*	*Minimum amount per 100 kJ*	*Maximum amount per 100 kJ*
Sphingomyelin	Sphingomyelin	1.8 mg	7.5 mg

2.1.2 General data requirements for supporting evidence

Literature searches have been completed to identify published studies supporting the safe use and tolerance of Lacprodan® MFGM-10 in IFP and studies that substantiate the neurodevelopmental and cognitive benefits of MFGM ingredients in IFP.

2.1.2.1 Human intervention studies related to neurodevelopment and cognition

A literature search was undertaken on the 11th of March 2024 to identify human intervention trials in infants with neurodevelopmental and/or cognitive outcome measures following intervention with MFGM. The search was completed using PubMed, Scopus and the Cochrane Library databases.

- *Search strategy*: ((MFGM) OR (Milk Fat Globule)) AND ((neurodevelopment) OR (cognitive) OR (behavio*)) AND ((infant) OR (child)) AND (trial)
- *Inclusion criteria*: generally healthy term infants (≤12 months); children (inclusion of assessment at ≥ 12 months of age); assessment of a physiological effect relevant to this application (neurodevelopment, cognition, cognitive outcomes); intervention (at ≤ 12 months) with formula, food or supplements containing MFGM, food or supplements containing specific MFGM components.
- *Exclusion criteria*: non-intervention studies (e.g. observational); older children and adults; studies without neurodevelopmental or cognitive outcome measures; review articles; study protocols; abstracts only (conference proceedings).

In addition to the search, references in relevant papers and reviews were searched for any further studies not identified in the database searches.

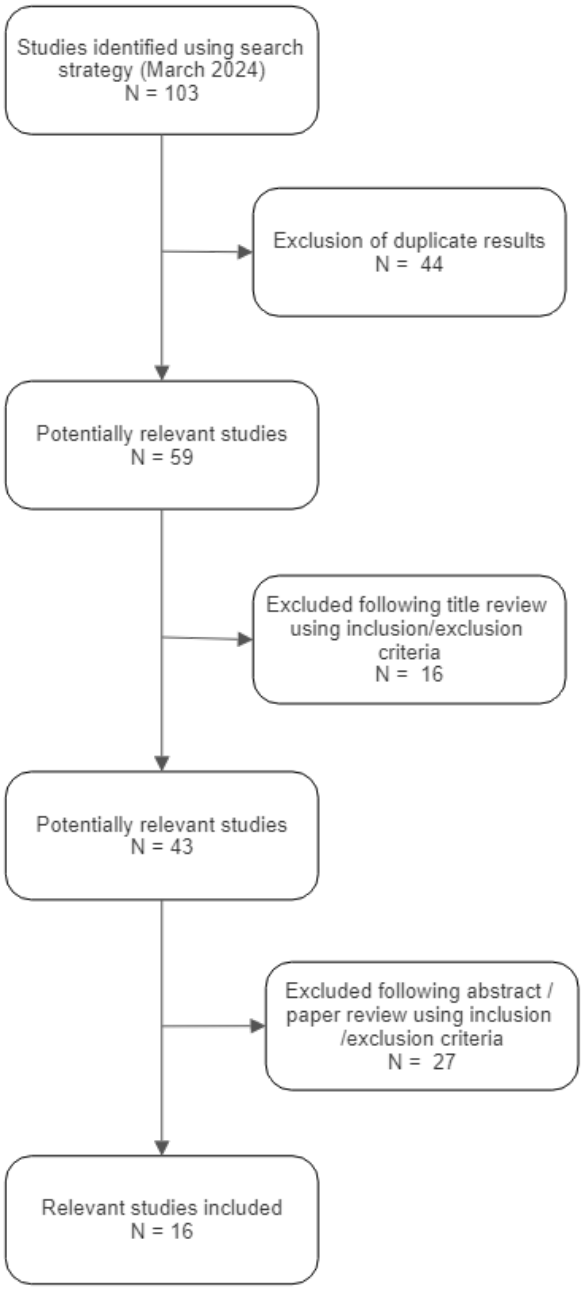
The search resulted in a total of 103 citations identified as meeting the criteria (Figure 2-1) with 44 excluded immediately based on duplication of results from the different databases. Using the inclusion/exclusion criteria. a further 16 results were excluded based on titles, and another 27 following abstract or paper review. A total of 16 publications were identified as relevant to supporting neurodevelopment and / cognitive development in infants, and full papers assessed.

Several studies identified in the search but excluded for various reasons, are included in parts of the application as they contribute to the evidence for MFGM or its components in neurodevelopment and / or cognition.

Excluded studies, with reason for exclusion, are listed in Appendix I.

A review of the evidence from clinical studies investigating the benefits of MFGM on infant neurodevelopment and cognition are discussed in Section 3.2.3.

Figure 2-1 Literature search for human intervention trials in infants to support neurodevelopment and cognition



2.1.2.2 Animal studies (preclinical) related to neurodevelopment and cognition

A literature search using the PubMed and Scopus databases was completed on the 14th of March 2024 using the following strategy.

The objective of this search was to identify preclinical studies in neonatal animal models that investigated neurodevelopmental, behavioural and / or cognitive outcomes following intervention with MFGM products.

- *Search strategy*: ((MFGM) OR (Milk Fat Globule)) AND ((neurodevelopment) OR (cognitive development) OR (behavio*) OR (brain development)) AND ((in vitro) OR (in vivo) OR (rat) OR (mouse) OR (pig) OR (piglet) OR (animal))

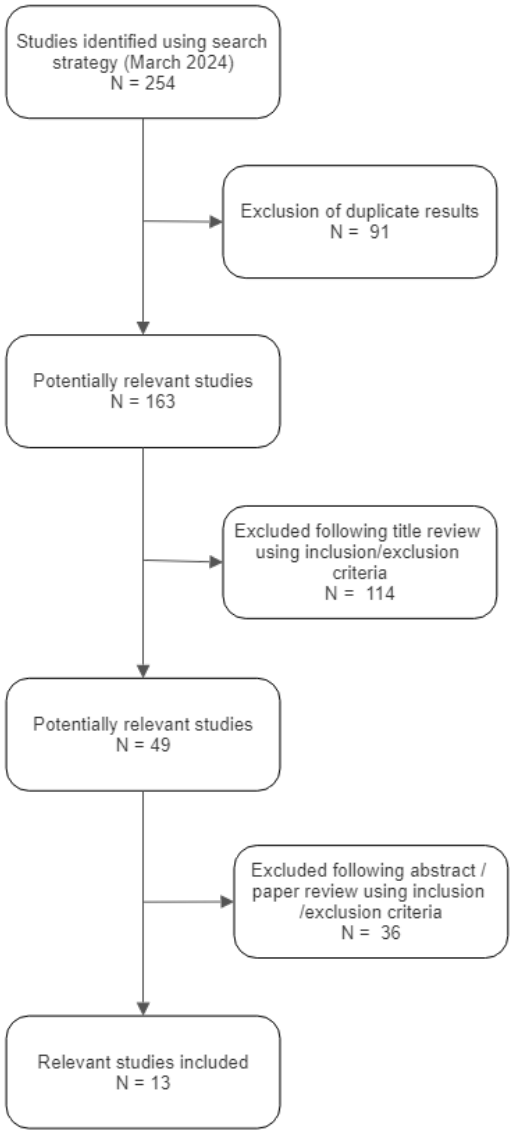
- *Inclusion criteria*: neonatal animal models (for rats and mice intervention initiated by postnatal day 10); assessment of a model relevant physiological effect relevant to this application (neurodevelopment, cognition, cognitive outcomes); oral (including gavage) administration of test material containing specific MFGM components.
- *Exclusion criteria:* pregnant/ lactating animals; juvenile/young adult animals; adult/aged animals; not neurodevelopment or cognitive outcomes measured; review articles; observational studies.

The search identified a total of 256 studies (118 in PubMed and 138 in Scopus), of which 91 were identified as duplicates and removed. One-hundred and sixty-five (165) records were reviewed for their fit to the inclusion criteria, with 15 studies meeting the criteria (Figure 2-2).

Excluded studies, with reason for exclusion, are listed in Appendix I.

A review of the evidence from preclinical studies investigating the potential benefits of MFGM relevant to infant neurodevelopment and cognition are discussed in Section 3.2.3.3.

Figure 2-2 Literature search for preclinical studies supporting neurodevelopment and cognition in neonatal models



2.1.2.3 Safety studies – clinical

A literature search was undertaken (6th April 2024) using PubMed, Scopus and the Cochrane Library databases to identify clinical trials in infants that address the safety and tolerance of Lacprodan® MFGM-10 and other MFGM-based ingredients.

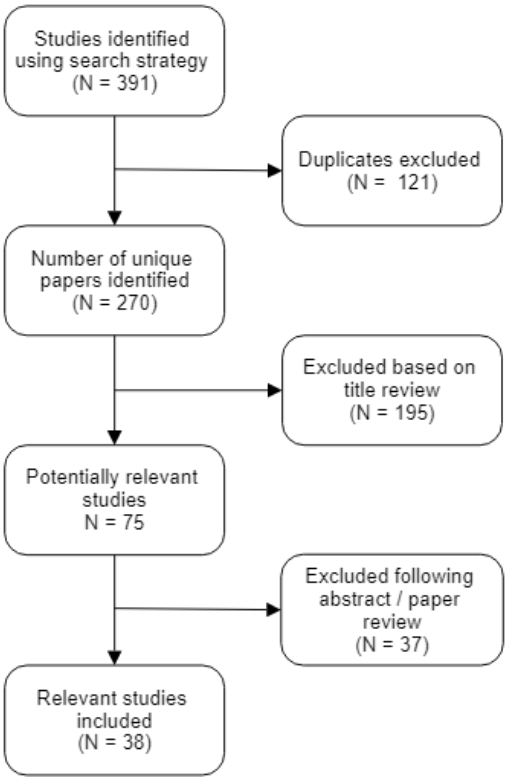
- *Search strategy*: ((mfgm) OR (MFGM) OR (sphingomyelin) OR (ganglioside) OR (milk fat globule\ )) AND (infant) AND (trial).
- *Inclusion criteria*: generally healthy term infants (≤12 months); children (inclusion of assessment at ≥ 12 months of age); reported measures of safety and tolerance.
- *Exclusion criteria*: non-intervention studies (e.g. observational); non-infant cohorts; studies without an MFGM-like ingredient; review articles; study protocols; abstracts only (conference proceedings).

In addition to the search, references in relevant papers and reviews were searched for any further studies not identified in the database searches.

The search resulted in a total of 391 citations identified as meeting the criteria (Figure 2-3) with 121 excluded immediately based on duplication of results from the different databases. Using the inclusion/exclusion criteria a further 195 results were excluded based on titles, and another 37 following abstract or paper review. A total of 38 publications, related to 13 independent clinical trials, were identified as containing data related to safety and tolerance outcomes, including growth, in infants and cohorts at follow-up over a number of years. Full papers were reviewed and grouped according to original trial as appropriate.

All search titles excluded are listed in Appendix I.

Figure 2-3 Literature search of safety and tolerance studies of Lacprodan® MFGM-10 and other MFGM-like ingredients in infants



2.1.2.4 Safety studies – preclinical

A literature search was conducted between the 10th - 24th March 2024 by Saxocon A/S to identify publicly available non-clinical (animal models) data to support the assessment of the safety and tolerance of MFGM, and to identify any potential to cause adverse events or effects, or toxicity following oral administration of MFGM.

The Web of Science core collection and Scopus databases were used for this literature search. These sources were chosen as they contain the most peer-reviewed scientific journals with a notable impact factor.

To define the gross scope of available literature regarding MFGM the relevant fields (i.e., title, abstract, and author keywords) were searched using the following search terms:

MFGM OR (milk fat globule)

After removing non-original articles (e.g., reviews and textbook content), articles lacking sufficient abstract information and duplicates, 4596 articles were identified.

To identify references containing data about MFGM's potential to cause the standard toxicity endpoints (i.e., genotoxicity or systemic toxicity) or any other adverse effect(s) the following search string was used to further refine the search:

AND (genotox* OR mutagen*) OR (tox* NOT toxin) OR (adverse OR safe*)

However this failed to identify any relevant articles. As Boolean search strings were not effective in this search to identify relevant studies, a cluster analysis was used, identifying clusters of terms and enabling screening based on the relevance of the cluster.

The literature search was then refined using exclusion terms associated with the unrelated clusters:

AND NOT (dairy AND process*) OR (cheese OR casein OR cream)

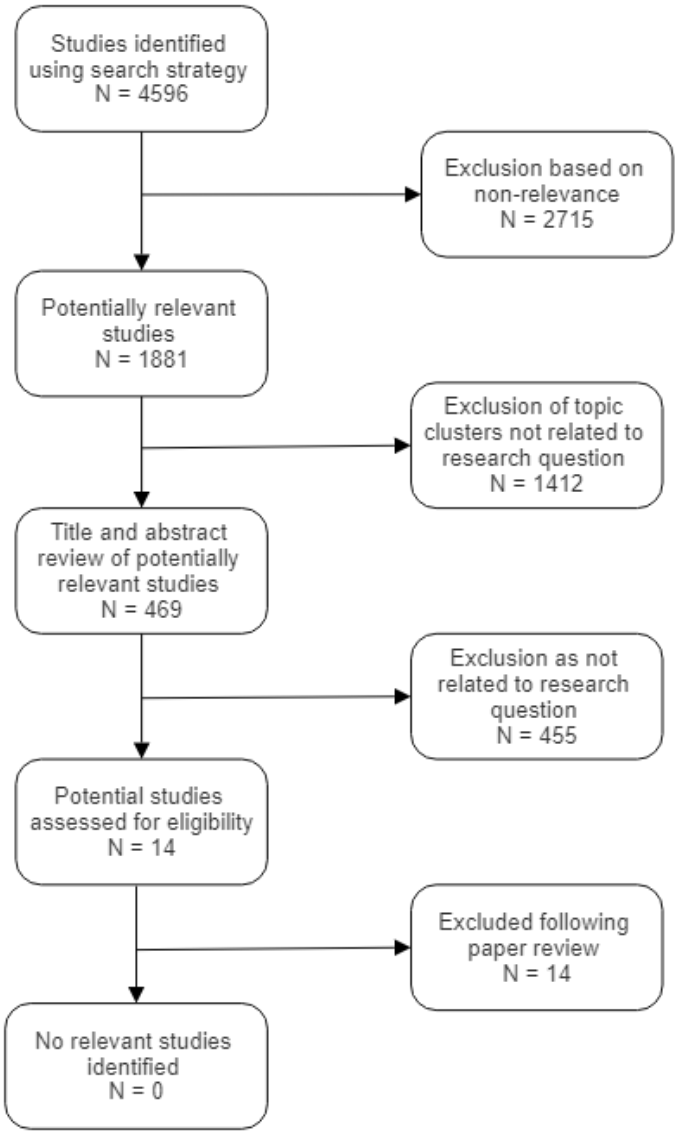
AND NOT e8 OR mfg-e8 OR mfge8 OR lactadherin

AND NOT human-breast OR (human AND breast) AND (cancer OR carc*)

The refined search identified 1881 papers written in English. Further cluster analyses were completed to refine the search based with 469 potentially relevant articles were identified. Titles and abstracts were screened for eligibility and 14 articles were identified for full review. None of the studies were found to specifically address safety aspects. Figure 2-4 provides an overview of their search results.

The full literature search report from Saxocon A/S is provided under separate cover under CI. This includes all search titles excluded in the Saxocon review.

Figure 2-4 Literature search for preclinical safety studies



2.2 Technical information on the use of Lacprodan® MFGM-10

The nutritive substance, Lacprodan® MFGM-10, is a complex whey dominant material derived from bovine milk. The manufacturing process (Section 2.2.5.1) results in a phospholipid enriched WPC. It is these phospholipid components that are the basis of this ingredient meeting the definition of a nutritive substance over and above the nutritional properties of standard WPC ingredients. Furthermore, the sphingomyelin component specifically allows for identification of added Lacprodan® MFGM-10 in infant formula products.

The lactating mammary gland packages and releases lipids by a unique mechanism to be secreted in milk. Lipid molecules are present in milk primarily as milk fat globules (MFG) and EVs (Sprenger, Ostenfeld, Bjørnshave, Rasmussen, & Ejsing, 2023). The origin of MFGM is therefore unique to the mammary gland and thus MFGM is only found in milk (Heid & Keenan, 2005). The physiological importance of MFGM to the mother-infant pair is supported by the fact that the genes associated with the synthesis of the milk fat and MFGM are among the most conserved lactation genes throughout evolution in mammals (German, 2011; Lemay et al., 2009). Extracellular vesicles are actively secreted into milk and are composed of a single membrane bilayer, which originates from shredding off the apical plasma membrane (rich in sphingolipids and cholesterol, and devoid of TAGs) as microvesicles or secretion of intracellularly formed exosomes (Blans et al., 2017; van Niel, D'Angelo, & Raposo, 2018). Both human and bovine milk contains EVs which can be absorbed by intestinal cells (Yung et al., 2024; Zempleni et al., 2017). The contribution and role of EVs is an emerging area of research contributing to the understanding of the phospholipid fractions present in milk and potential nutritional significance. [redacted]

Collectively the components of the MFGM and EVs are enriched in Lacprodan® MFGM-10. Until recently, research has focussed on the overall composition of the phospholipid enriched fraction of bovine milk, and this has collectively been referred to as MFGM. Accordingly, these contributing fractions are simply referred to as MFGM throughout this document.

Bovine milk contains about 3 to 5% fat, secreted as droplets or globules roughly 2 to 15 µm in diameter, surrounded by a membrane of polar lipids (phospholipids and sphingolipids) and proteins termed the MFGM (Lucey, Otter, & Horne, 2017). The core of the milk fat globule is composed primarily of triacylglycerols (TAGs), which represent 98% of total milk fat and provides approximately half of the infant's energy intake in addition to essential fatty acids required for growth and development (Innis, 2007).

In the cytoplasm of the mammary epithelial cells, droplets of triacylglycerol are surrounded by a coating consisting of a phospholipid/cholesterol monolayer with incorporated proteins. As the lipid droplets reach the apical cell membrane, an additional phospholipid bilayer encases the fat droplet before being extruded from the cell (Mather & Keenan, 1998). This tri-layer membrane is known as MFGM (Figure 2-5) and contains 60-70% of total milk phospholipids, as well as glycolipids, proteins, glycoproteins, cholesterol, and other lipids (Lee, Padhi, et al., 2018). The inner monolayer contains proteins and polar lipids derived from the endoplasmic reticulum. The outer double layer membrane

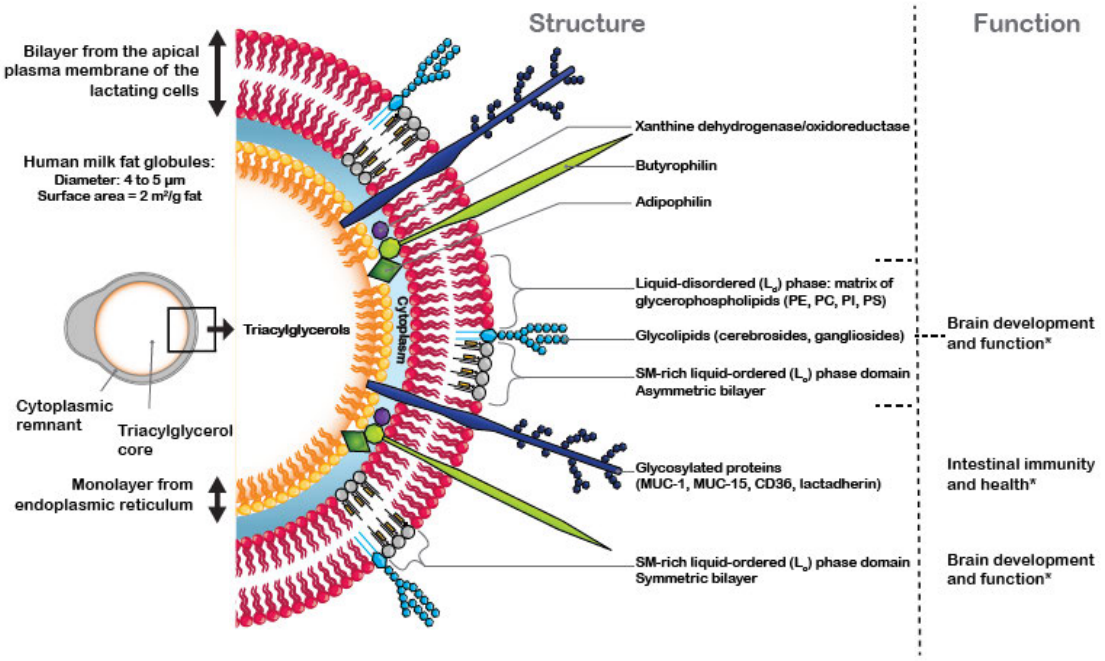
also contains polar lipids and proteins that come from the apical plasma membrane of the mammary epithelial cells which surround the fat globules as they are secreted (Guerin, Burgain, Gomand, Scher, & Gaiani, 2019; Heid & Keenan, 2005).

The MFGM consists of a variety of phospholipids, sphingolipids, membrane-specific proteins (mainly glycoproteins) triglycerides, cholesterol, enzymes and other minor components (Singh, 2006). A large number of reviews are regularly published detailing the composition and potential health functions of the constituent molecular species (Dewettinck et al., 2008; El-Loly, 2011; Guerin et al., 2019; Kosmerl, Rocha-Mendoza, Ortega-Anaya, Jiménez-Flores, & García-Cano, 2021; Lee, Padhi, et al., 2018; Mather, 2000; Singh, 2006; Smoczyński, Staniewski, & Kiełczewska, 2012; Caroline Thum, Roy, Everett, & McNabb, 2023; Yao, Ranadheera, Shen, Wei, & Cheong, 2023).

The mass of the MFGM is estimated to be 2 – 6% of the total milk fat globule, with the proteins and PL together accounting for up to 90% of the membrane dry weight (Singh, 2006). Typical ranges of bovine MFGM lipid and protein fractions based on data from a range of literature sources (Dewettinck et al., 2008; Bertram Y. Fong, Norris, & MacGibbon, 2007; Jiménez-Flores & Brisson, 2008; Sánchez-Juanes, Alonso, Zancada, & Hueso, 2009; Singh, 2006; Smoczyński et al., 2012) are summarised in Table 2-2.

Bovine MFGM proteins account for only 1 – 4% of the total bovine milk proteins (Cavaletto, Giuffrida, & Conti, 2008), but account for 25 – 70% of the MFGM depending on the source and method of extraction (Dewettinck et al., 2008; Singh, 2006), and other characteristics of the milk fat globule such as globule size (J. Lu, Argov-Argaman, et al., 2016; Caroline Thum et al., 2023). Similarly, reported fat content varies up to 80% of MFGM weight. Differences may be dependent on methodology and potential contamination of the triglycerides in the core of the MFG during isolation of the membrane for analysis (Singh, 2006).

Figure 2-5 Schematic of MFGM structure



Gallier et al. (2015) showed the incorporation of MFGM into standard infant formula resulted in fat globules that were more similar to that of breastmilk, with MFGM components forming a thin interface at the oil-droplet surface and concluded this may result in metabolic and digestive properties that are more similar to breast milk compared to standard IFP.

Table 2-2 Typical lipid and protein fractions of bovine MFGM

MFGM Component	% of MFGM	% MFGM Lipid	
Total MFGM lipid	50 - 80		
Triacylglycerols		56.0 - 62.0	
Diacylglycerols		2.1 - 9.0	
Monoacylglycerols		0.4	
Free fatty acids		0.6 - 6.0	
Sterols		0.2 - 2.0	
Phospholipids		26.0 - 46.0	**% of total PL**
Sphingomyelin			18.8 - 22.0
Phosphatidylcholine			27.4 - 36.0
Phosphatidylethanolamine			27.0 - 36.0
Phosphatidylinositol			11.0
Phosphatidylserine			4.0
Lysophosphatidylcholine			2.0
Total MFGM protein	25 - 70	**% MFGM Protein**	**Molecular**
BRCA1 and BRCA2			210
Mucin I (MUC1)			160 - 200
Xanthine oxidase (XO)		20	146 - 155
PAS III			94 - 100
CD36 or PAS IV		≤ 5	76 - 78
Butyrophilin (BTN)		20 - 43	66 - 67
Adipophilin (ADPH)			52
Lactadherin (PAS 6/7)			47 - 59
Proteose peptone (PP3)			18 - 34
Fatty acid binding protein (FABP)			13 - 15

adapted from Guerin et al. (2019),Mather (2000), Smoczyński et al. (2012), Singh (2006), Bertram Y. Fong et al. (2007)

2.2.1 MFGM components in human and bovine milks

2.2.1.1 Phospholipid composition of human milk

Phospholipids (PL) in human breastmilk are predominantly associated with the MFGM (Lopez & Menard, 2011), with SM accounting for approximately 37% of the total PL in breastmilk (Bitman, Wood, Mehta, Hamosh, & Hamosh, 1984), Table 2-3.

The mean concentrations of total PLs and individual PL (phosphatidylethanolamine (PE); phosphatidylinositol (PI); phosphatidylserine (PS); phosphtidylcholine (PC) and SM) components measured in mature human milk are listed in Table 2-3, and the % polar phospholipids are reported in Table 2-4. The mean concentrations of total PLs ranged from 11.38 to 61.35 mg/100 mL in mature human milk; particularly, mean SM concentrations ranged from 3.1 to 20.81 mg/100 mL. It is

important to recognise that these are ranges of mean PL and SM values, as opposed to a comprehensive total range of PL and SM levels. Differences in the analytical methods used may partly explain the wide range of the levels of total PLs and PL components reported in mature human milk, however it is well recognised that the PL levels of human milk vary greatly as a function of multiple factors such as time of sampling, full-breast expression, breast variation (Jensen 1995), diet, geography, metabolic stage, and gestational age at birth, in addition to different analytical methods (da Cunha, Macedo da Costa, & Ito, 2005; Robert G. Jensen, 1995; R. G. Jensen, 1999) and stage of lactation (Ma et al., 2017). Such variability was also noted by Cilla, Diego Quintaes, Barbera, and Alegria (2016) who completed a thorough review of reported PL contents of human breastmilk, evaluating and converting reported values to a common measurement basis and appraising the analytical methods used to determine the PL. Cilla et al. (2016) reported SM contents ranging from 31 to 153 mg/L in human breast milk, the range means being equivalent to 50 – 160 mg/L. W. Wei et al. (2019) monitored the temporal changes in PL content of breastmilk (colostrum through 3 months) from mothers who delivered prematurely and those with term delivery. The total PL content of the premature delivery cohort declined over the 3-month period (27.47 ± 7.38 to 20.18 ±4.85 µmol / 100mL, p <0.05), whereas the total PL in mothers delivering at term did not (25.83 ± 3.80 to 22.57 ± 1.13 µmol / 100mL, p >0.05). In both cohorts there was no significant change in the absolute level (µmol / 100mL) of SM across the study period. Interestingly however, the fact that the relative distribution of the individual phospholipids remains constant through the mature milk period (Table 2-4) indicates that some metabolic controls are maintained over the biosynthesis of these components. A comprehensive review by C. Thum et al. (2022), similarly identified the variability of PL content across lactation.

Table 2-3 Phospholipid content of human milk

Reference	Lactation period	Method	Total PL (mg/100mL)	PE (mg/100mL)	PI (mg/100mL)	PS (mg/100mL)	PC (mg/100mL)	SM (mg/100mL)
(Zeisel, Char, & Sheard, 1986)[a]	Mature	TLC					11.2±2.5	13.5±1.8
van Beusekom, Martini, Rutgers, Boersma, and Muskiet (1990)[b]	Transition - Mature	HPLC	26.7±5.13	1.94±0.41		2.19±0.48	4.26±2.88	
Holmes-McNary, Cheng, Mar, Fussell, and Zeisel (1996)[a]	Mature	HPLC/GC-MS					6.31±0.5	9.31±0.7
Holmes, Snodgrass,	Colostrum	^{1}H NMR					17.7±5.4	6.8±0.8

Reference	Lactation period	Method	Total PL (mg/100mL)	PE (mg/100mL)	PI (mg/100mL)	PS (mg/100mL)	PC (mg/100mL)	SM (mg/100mL)
and Iles (2000)[a]	Transition - Mature						37.0±5.4	7.5±0.8
Ilcol, Ozbek, Hamurtekin, and Ulus (2005)[a]	Colostrum Mature	HPLC-ELSD					11.2±1.4 8.0±0.8	9.7±1.0 7.1±0.7
Sala-Vila, Castellote, Rodriguez-Palmero, Campoy, and Lopez-Sabater (2005)	Colostrum Transition Mature	TLC	13.5±2.6 14.0±2.5 9.8±1.5					
Fischer et al. (2010)[a]	Mature	LC-MS					8.2±0.5	5.0±0.3
Blaas, Schuurmann, Bartke, Stahl, and Humpf (2011)	Not recorded	HILIC-HPLC-ESI-MS/MS						3.9 – 9.1
(Lopez & Menard, 2011)[c]	Mature	HPLC-ELSD	13.5±2.0	1.8±0.6	1.4±0.1	2.1±0.6	2.9±0.6	5.3±0.3
X. Q. Zou et al. (2012)[c] X. Zou et al. (2013)	Colostrum Transition Mature	HPLC-ELSD	16.8±1.5 22.3±1.3 19.2±1.4	1.6±0.1 2.9±0.5 2.9±0.4	1.4±0.1 1.5±0.1 1.6±0.1	2.1±0.1 3.1±0.2 3.2±0.2	4.8±0.7 5.7±0.5 4.1±0.4	6.9±1.0 22.3±1.3 19.2±1.4
C. Garcia et al. (2012)[d]	Not recorded	^{31}P NMR	25.03 [15.3;47.4]	4.15 [2.6;10.3]	1.1 [0.22;2.07]	2.21 [1.13;4.47]	6.03 [3.21;12.42]	7.83 [4.99;13.29]
Giuffrida et al. (2013)	Mature	HPLC-ELSD	23.8±3.4 (12.9-38.4)	6.8±1.9 (3.1-11.8)	1.1±0.3 (0.9-2.3)	1.4±0.3 (1.0-1.9)	6.0±1.3 (3.2-9.6)	8.5±1.7 (4.7-12.8)
Thakkar et al. (2013)	Mature	HPLC	22.68±7.27	7.07±2.70	1.29±0.52	0.80±0.33	5.28±1.80	8.28±2.48
Russo et al. (2013)	Not recorded	HPLC-ELSD	18.24±0.29	3.80±0.18	<LOQ	8.45±0.08	1.87±0.15	4.14±0.20

Reference	Lactation period	Method	Total PL (mg/100mL)	PE (mg/100mL)	PI (mg/100mL)	PS (mg/100mL)	PC (mg/100mL)	SM (mg/100mL)
Ma et al. (2017)	Colostrum	HPLC-MS/MS	33.09±15.13	8.57±2.42	1.53±1.07	11.91±5.70	6.9±5.01	3.83±2.34
	Transition		35.24±16.63	8.99±2.58	1.66±1.18	12.58±6.30	7.67±5.54	3.97±2.57
			26.64±5.70	8.13±2.94	1.12±0.33	9.81±1.84	5.0±1.29	2.33±0.57
	Mature		27.30±5.84	10.00±2.45	0.96±0.30	9.09±1.80	4.86±1.16	2.09±0.57
			17.00±8.00	4.60±2.26	0.74±0.47	1.74±1.00	2.61±1.72	6.54±3.76
			14.71±4.12	3.93±1.58	0.63±0.36	1.48±0.77	2.10±1.64	5.74±1.17
			21.00±10.01	7.36±4.02	0.67±0.42	1.83±1.04	2.69±1.64	7.85±3.94
			18.75±11.00	6.61±0.	0.59±0.	1.55±0.94	2.37±0.	7.04±3.68
			21.95±9.20	8.10±3.99	0.68±0.31	1.76±0.87	3.03±1.66	7.92±3.82
			20.47±8.17	7.84±3.28	0.65±0.25	1.62±0.75	2.76±1.36	7.15±2.82
Tavazzi, Fontannaz, Lee, and Giuffrida (2018)	Mature	HPLC-ELSD		7.49±3.00	1.07±0.72		5.08±2.03	8.47±2.47
		HPLC-MS/MS		2.85±1.56	1.82±0.72		5.39±2.77	9.28±3.25
(Ingvordsen Lindahl et al., 2019)	Colostrum	HPLC-MS	67.74±14.47	49.10±13.68			11.44±2.64	6.90±1.26
	Transition		48.65±18.11	37.86±14.00			6.56±3.26	4.23±1.88
	Mature		36.94±16.41	29.15±13.04			4.50±1.97	3.29±1.73
(Wu et al., 2019)	Colostrum	HPLC	35.07±10.84	4.61±2.11			20.32±6.61	10.14±3.39
	Transition		35.09±8.63	4.79±2.01			19.94±5.35	10.37±2.69
	Mature		28.10±7.86	3.63±1.53			15.41±5.09	9.07±2.52

Note: results are presented as mean ±SD, or range as reported in cited reference. LOQ: level of quantitation
[a] Conversion factors of 770 g mol^{-1} for PC and 751 g mol^{-1} for SM
[b] Weighted average of 2 populations (Dominica & Belize)
[c] Calculated based on an average human milk fat content of 3.8 g /100 mL (Cilla et al., 2016)
[d] Median values [1st; 99th percentile]

Table 2-4 Relative proportions of phospholipids in human milk

Reference	Lactation period	Method	PE (% total PL)	PI (% total PL)	PS (% total PL)	PC (% total PL)	SM (% total PL)
Harzer, Haug, Dieterich,	Colostrum	TLC	25.1±2.5	5.5±0.9	10.2±2.4	31.9±3.0	28.9±0.6
	Transition		28.0±0.1	5.2±0.1	83.9±0.8	28.2±1.1	30.4±0.7
	Mature		27.6±1.0	5.3±0.2	9.1±0.6	25.6±1.0	32.1±0.9

Reference	Lactation period	Method	PE (% total PL)	PI (% total PL)	PS (% total PL)	PC (% total PL)	SM (% total PL)
and Gentner (1983)							
Bitman et al. (1984)	Mature	TLC	19.7±0.2	6.0±0.6	8.6±0.2	27.8±1.5	37.8±1.5
Hundrieser and Clark (1988)	Not recorded	HPLC	23.8±.3	5.3±3.0	3.7±1.5	33.2±5.5	29.0±6.4
(Wang et al., 2000)	Transition	TLC	36.1±3.9	3.5±1.5	6.7±4.1	23.1±4.2	30.6±6.6
Sala-Vila et al. (2005)	Colostrum Transition Mature	TLC	5.9±0.6 8.6±1.2 12.8±1.2	6.0±0.6 5.2±0.5 5.9±0.5	7.9±1.1 8.2±1.0 10.4±1.3	38.4±3.1 37.7±4.9 31.3±4.8	40.5±3.6 39.2±3.6 41.0±3.4
Shoji et al. (2006)	Colostrum Transition Mature	TLC	14 ±0.7 15.0±1.4 13.5±0.7	7.3±1.1 7.3±0.4 7.0±1.4	5.3±1.1 5.3±0.4 6.0±0.1	26.8±7.8 25.5±0.7 24.9±3.3	36.0±0.1 37.5±2.1 39.2±4.5
(Benoit et al., 2010)	Mature	TLC	21.3±4.7	16.4±3.9 (PI + PS)		19.0±2.2	43.3±2.6
Lopez and Menard (2011)	Mature	HPLC-ELSD	13.2±1.7	10.1±1.4	15.4±3.1	21.1±2.2	40.2±4.7
X. Q. Zou et al. (2012) X. Zou et al. (2013)	Colostrum Transition Mature	HPLC-ELSD	9.3±1.1 13.2±0.9 15.0±1.5	8.3±1.0 6.8±0.4 8.2±1.2	12.7±1.6 13.9±1.6 16.7±1.8	28.5±1.6 25.7±2.2 21.3±2.3	41.2±2.0 40.4±3.3 38.8±3.9
C. Garcia et al. (2012)	Not recorded	^{31}P NMR	18.3 [12.4;25.6]	3.8 [1.1;5.2]	8.1 [5.4;15.2]	24.5 [19.8;30.2]	29.7 [25.7;33.8]
Wu et al. (2019)	Colostrum Transition Mature	HPLC	12.86±4.10 13.37±3.91 12.78±3.78			58.06±6.10 56.85±5.84 54.20±6.60	29.08±4.53 29.77±4.42 33.03±6.13

Note: results are presented as mean ±SD, or range as reported in cited reference
[a] Median values [1st; 99th percentile]

2.2.1.2 The phospholipid content of bovine milk

The phospholipid content of bovine milk is approximately 0.8% of the total lipid content, with the majority being membrane bound, and the remainder in the aqueous phase (MacGibbon & Taylor,

2006; Sprenger et al., 2023). Patton and Keenan (1971) identified the phospholipids present in skim milk and the MFGM are the same, and present in similar proportions.

Compared to the PL content of human breast milk, bovine milks contain the same major PL's however differ in the relative proportions of PL's with lower proportions (%) of SM, PC and PS, and higher relative contents of PE and PI (Table 2-5).

Like human milk, the fat globules in bovine milk also vary in size from ~2 µm to ~15 µm in diameter. Distribution of individual phospholipid components were looked at in two different sized bovine milk globule fractions (7.6-and 3.3-µmm average sizes) (J. Lu, Argov-Argaman, et al., 2016). The major phospholipids phosphatidyl choline (36-37%), sphingomyelin (23-26%) and phosphatidyl serine (8%) were not statistically different between the small and large bovine MFGM fractions. There was a statistically meaningful difference in phosphatidyl ethanolamine (19-27%) and phosphatidyl inositol levels (5-10%). The minor variations in phospholipid composition are attributed to fat globule size (J. Lu, Argov-Argaman, et al., 2016). Total phospholipid levels are comparable in human and bovine milk MFGM (Robert G Jensen, Ferris, Lammi-Keefe, & Henderson, 1990), although differences in specific phospholipid classes have been identified. For example, compared with bovine milk, human milk has higher levels of sphingomyelin, plasmalogens, and gangliosides (Cyrielle Garcia & Innis, 2013; C. Garcia et al., 2012).

Table 2-5 Relative proportion of phospholipids in bovine milk

Reference	Method	PE (% total PL)	PI (% total PL)	PS (% total PL)	PC (% total PL)	SM (% total PL)
Andreotti, Trivellone, and Motta (2006)	^{31}P NMR	23.5	12.0	3.6	24.0	24.2
Murgia, Mele, and Monduzzi (2003)	^{31}P NMR	25.8	14.0	1.5	26.8	26.8
MacKenzie, Vyssotski, and Nekrasov (2009)	^{31}P NMR TLC	26.1±0.1 22.1±0.3	7.5±0.1 7.0±0.2	11.7±0.2 10.4±0.3	26.5±0.3 28.1±0.3	20.8±0.5
C. Garcia et al. (2012)	^{31}P NMR	31.8±3.0	3.7±1.9	10.0±2.8	28.7±2.7	20.0±1.4
X. Zou et al. (2013)	HPLC-ELSD	30.23±2.69	9.89±0.87	7.32±0.99	25.20±1.88	27.36±1.07

2.2.1.3 Summary of MFGM phospholipids in human and bovine milk

Compared to the PL content of human breast milk, bovine milk contains the same major PL's however on average differ in the relative proportions of PL's with lower proportions (%) of SM and higher relative contents of PE (Cyrielle Garcia & Innis, 2013) (Table 2-6).

Table 2-6 Average distribution of major phospholipids in human and cow's milk

Analyte	% of total phospholipids	
	Human milk	Bovine milk
Sphingomyelin	31	22
Phosphatidyl ethanolamine	28	33
Phosphatidyl choline	24	29
Phosphatidyl serine	9	10
Phosphatidyl inositol	4.5	4
Total gangliosides (measured as total lipid bound sialic acid mg/L)		
Total gangliosides	9	4

from Cyrielle Garcia and Innis (2013)

2.2.1.4 The phospholipid content of infant formula products

The phospholipid content of infant formula products is a function of the product formulation and thus variation is expected (

Table 2-7,

Table 2-8). Sources of PL in IFP include the milk used as the base protein source of formulas, typically skim milk or WPC, added lecithin, typically soy lecithin, and more recently PL from dairy ingredients rich in MFGM. Many infant formula products have lecithin added, either as an additive to assist with emulsification or as a nutritional ingredient to increase the phospholipid content of formula. Soy lecithin consists of predominantly PC, with PE, PI and PS also present (Scholfield, 1981). Soy lecithin does not contain SM. W. Wei et al. (2019) presented the absolute values of PL of reconstituted and liquid IF available in the Chinese market. There was a significant amount of variation in both total PL content and relative levels of the individual PL's, noting that variation in PC may be as a function of lecithin addition, but importantly the only formula that can approximate the SM content of human breast milk are those formula that contain added MFGM ingredients.

Table 2-7 Phospholipid content of infant formula powders

Reference	Method	Formula type	Total PL (mg/100g)	PE (mg/100g)	PI (mg/100g)	PS (mg/100g)	PC (mg/100g)	SM (mg/100g)
Braun, Fluck, Cotting, Monard, and Giuffrida (2010)	HPLC-UV	Bovine IF Bovine GUM		38 ± 2 27 ± 2	35 ± 3		65 ± 1 77 ± 1	42 ±1 32 ± 2
B. Fong, Ma, and Norris (2013)	HPLC-MS/MS	Bovine		61 - 75	26 - 46	13 - 28	63 - 84	31 - 82
Tavazzi et al. (2018)	HPLC-ELSD	Bovine		19.4 – 44.9 16.5 – 82.6	13.5 - 79.6 17.1 – 90.3		32.2 – 99.3 40.0 – 109	18.0 – 23.8 18.0 - 23.8

Reference	Method	Formula type	Total PL (mg/100g)	PE (mg/100g)	PI (mg/100g)	PS (mg/100g)	PC (mg/100g)	SM (mg/100g)
	HPLC-MS/MS							
Zhu et al. (2019)	^{31}P NMR	Bovine		5.7 – 12.3				8.9 – 17.5

Publications reviewing the PL content of IFP have reported results on a powder basis (

Table 2-7) or on a reconstituted or ready-to-feed (RTF) basis (

Table 2-8). In a survey of 12 IFP Claumarchirant et al. (2016) identified 3 formula with added MFGM rich ingredients (

Table 2-8), reflected in the elevated SM contents and overall proportions of PL more aligned with that of human breast milk.

Table 2-8 Phospholipid content of reconstituted infant formula

Reference	Method	Formula type	Total PL (mg/100mL)	PE (mg/100mL)	PI (mg/100mL)	PS (mg/100mL)	PC (mg/100mL)	SM (mg/100mL)
(Zeisel et al., 1986)[a]	TLC	Bovine Soy					1.8 - 14.4 18.2 – 19.1	ND – 0.5 ND
Holmes-McNary et al. (1996)[a]	HPLC/GC-MS	Bovine Soy					2.7 – 7.7 10.0 – 16.6	0.8 – 4.3 0.8 - 2.9
Holmes et al. (2000)	^{1}H NMR	Bovine					3.1 – 9.2	2.3 – 7.5
Sala-Vila et al. (2005)	HPLC-ELSD	Bovine		2.2	1.4	0.6	6.2	1.0
Ilcol et al. (2005)	TLC	Bovine Soy					3.8 – 9.9 3.9	0.4 – 1.7 < 0.4
Claumarchirant et al. (2016)	HPLC-ELSD	Bovine 1 2 3 4 5[c] 6[c] 7 8 9[c] 10 11 12 13	 25.11±0.63 41.35±1.14 31.74±1.61 34.84±2.94 54.79±2.96 56.18±1.79 39.93±3.10 27.71±0.60 58.07±2.39 27.57±0.34 25.70±0.31 25.93±0.30	 6.24±0.32 15.09±0.63 10.75±0.64 10.17±0.96 20.74±1.09 24.17±1.13 13.36±1.45 6.30±0.19 26.23±0.84 6.56±0.14 6.77±0.13 6.49±0.07	 4.03±0.13 4.89±0.08 4.14±0.28 5.03±0.35 5.64±0.12 5.36±0.09 4.68±0.18 4.29±0.11 5.41±0.18 4.27±0.03 3.91±0.01 3.93±0.01	 4.06±0.12 4.90±0.09 3.79±0.23 4.68±0.24 5.94±0.21 5.66±0.04 4.79±0.17 5.20±0.25 4.67±0.13 4.70±0.04 4.09±0.01 4.17±0.01	 4.78±0.16 6.58±0.21 5.56±0.28 6.54±0.46 8.74±0.35 8.49±0.17 6.13±0.39 4.95±0.11 8.60±0.35 5.01±0.05 4.56±0.06 4.64±0.00	 6.01±0.10 9.90±0.46 7.50±0.35 8.43±0.94 13.73±1.30 12.50±0.85 8.96±0.94 7.51±0.11 12.63±0.84 7.01±0.16 6.37±0.20 6.70±0.22

Reference	Method	Formula type	Total PL (mg/100mL)	PE (mg/100mL)	PI (mg/100mL)	PS (mg/100mL)	PC (mg/100mL)	SM (mg/100mL)
			33.30±1.52	11.77±0.70	3.72±0.12	4.16±0.13	5.01±0.18	8.63±0.52
Deoni, Dean, Joelson, O'Regan, and Schneider (2018)	HPLC-ELSD	Bovine					8.5 5.8 6.0	2.81 6.2 2.81
Tavazzi et al. (2018)[b]	HPLC-ELSD HPLC-MS/MS	Bovine		2.8 – 15.1	2.8 - 14		6.7 – 18.2	2.9 – 4.0

[a] *Conversion factors of 770 g mol^{-1} for PC and 751 g mol^{-1} for SM*
[b] *Reported on reconstitution basis of 15g in 90 mL water*
[c] *IF supplemented with MFGM ingredient*

2.2.1.5 Ganglioside content of MFGM

Gangliosides (GGs) are complex lipids composed of a ceramide (Cer) and an oligosaccharide that contains one or more sialic acid (SA) residues such as N-acetylneuraminic acid (Neu5Ac). The structural diversity of gangliosides is due to variation in the oligosaccharide and ceramide parts, such as different sequences of monosaccharides as well as different lengths and saturation levels of the sphingoid base and N-fatty acyl (N-FA) substituents (Hewelt-Belka, Młynarczyk, Garwolińska, & Kot-Wasik, 2023).

In addition to phospholipids and sphingolipids, like human milk bovine MFGM also contains specific gangliosides; the major ganglioside in human milk is monosialoganglioside (GM3) whereas the main ganglioside in bovine milk is disialoganglioside (GD3). Although much of the focus on gangliosides has been on the carbohydrate and sialic acid, the sphingoid base fatty acids also show unexpected specificity and species-specific differences.

The distribution of the different GG structures differs in human and bovine milk. In humans, GD3 is predominant in colostrum, and GM3 is predominant in mature human milk (Laegreid et al., 1986, Pan and Izumi, 1999, 2000, Lee et al., 2013) (Table 2-9). On the other hand, GD3 is the predominant form of GGs in bovine milk (Laegreid et al., 1986, Pan and Izumi, 2000, Lee et al., 2013). GD3 amounted to 80% of total bovine milk GGs, compared to 25% of human milk GGs (Laegreid et al., 1986). In another study, GD3 represented 61.0% of cow's milk GGs compared to 31.8% of human milk GGs (Pan and Izumi, 2000). McJarrow et al. (2019) showed the variation in GG concentration in human milk across the time from parturition.

Table 2-9 Human milk ganglioside concentrations

Reference	Milk	n	GM_3 (mg/L ± sd)[a]	GD_3 (mg/L ± sd)[a]	Total GG (mg/L ± sd)
Giuffrida, Elmelegy, Thakkar, Marmet, and Destaillats (2014) China	Colostrum / Transition (0 – 11 days)	450	3.8 ± 0.4 (47)	4.3 ±0.9 (53)	8.1
Ma, MacGibbon, et al. (2015) Malaysia	Transition	12	8.3 ± 4.8 (44)	10.6 ± 4.3 (56)	18.9 ± 6.6
McJarrow et al. (2019) UAE	Transition (5-15 days)	41	9.5 ± 8.4 (45)	11.7 ± 9.5 (55)	21.2 ± 11.5
Ma, MacGibbon, et al. (2015) Malaysia	Mature (6 months)	42	21.4 ± 13 (85)	4.3 ± 5.5 (15)	25.3 ± 15.7
Ma, Liu, et al. (2015) China	Mature (6 months)	20	21.4 ± 9.5 (93)	1.5 ± 1.4 (7)	22.9 ± 9.9
McJarrow et al. (2019) UAE	Mature (6 months)	40	18.6 ± 9.7 (92)	1.6 ± 2.2 (8)	20.2 ± 9.8

[a] % of total GG is given in parenthesis from McJarrow et al. (2019)

Additional data of mean concentrations of total GGs found in mature human milk are listed in Table 2-10. Levels of GGs in human milk were found to range from 0.82 to 54.6 mg/L (note that this range is a range of means, and accounts, when applicable, for the individual mean values of the different lactation days). The weighted average of GGs was calculated at 9.99 mg/L, based on the combination of values reported in the different studies. As described previously, the analytical challenges for the measurement of GGs may explain the very wide variability of reported GG levels in human milk, as well as the difficulty in using GG as a key marker of MFGM. Due to this difficulty, a human milk range, defined as mean +/- 2SD, has not been calculated for this data.

The levels of GGs in bovine milk have been measured and compared to human milk in two of these studies (Table 2-11). Pan and Izumi (2000)) reported significantly lower levels of GGs in bovine milk compared to human milk (p <0.01), whether colostrum or later milk. Laegreid, Otnaess, and Fuglesang (1986) however reported comparable concentrations. Relatively limited understanding of the extent of similarity and difference of the GG profile in human and bovine milk remains. This is especially true given the wide variability of human milk GG levels.

Table 2-10 Mean concentrations of gangliosides in human milk.

Reference	Lactation day	Method of measurement	Total gangliosides (mg /L)
Takamizawa et al. (1986) *	40-390	TLC	26.1
Laegreid et al. (1986*)	60-300	HPTLC	11
Rueda et al. (1995)*	30-150	HPTLC	1.072
Pan and Izumi (1999)*	28-49	HPTLC	9.04
Pan and Izumi (2000)*	7-46	HPTLC	9.07
Uchiyama et al. (2011)*	30-60	HPTLC	41.2
Giuffrida et al. (2014)*	30-120	LC-MS	8.1

Table 2-11 Average ganglioside concentrations in human and bovine milk (mg/L)

Reference	Human		Bovine
	Colostrum	Later milk	
Laegreid et al. (1986)		11	11
Pan and Izumi (2000)	9.51	9.07	3.98

Bode, Beermann, Mank, Kohn, and Boehm (2004) showed both human and bovine milk gangliosides were selectively enriched with certain fatty acids compared whole milk lipids, and the fatty acid composition of milk gangliosides in the 2 species was significantly different. The amount of long-chain fatty acids (≥20 C atoms) was higher in bovine milk gangliosides (GM_3: 73.71 ± 3.39%; GD_3: 79.19 ± 2.79%) than in human milk gangliosides (GM3: 51.25 ± 0.65%; GD3: 34.04 ± 1.80%). Tricosanoic acid (23:0) dominated in bovine milk gangliosides (GM3: 24.05 ± 1.37%; GD3: 26.66 ± 1.24%), whereas it only played a minor role in human milk gangliosides (GM_3: 2.88 ± 0.10%; GD_3: 1.84 ± 0.29%) (Bode et al., 2004). More recently Hewelt-Belka et al. (2023) demonstrated the dynamic nature of GM3 composition across human lactation. The relative content of GM3 species containing very long N- -FA substituents with >22 carbon atoms decreased, while the content of GM3 species containing14:0, 18:0, 18:1, and 20:0 N-FA substituents increased in the later months of lactation (Hewelt-Belka et al., 2023).

There is limited data available on GG in IFP. Laegreid et al. (1986) showed the GG pattern of infant formula was identical to that of bovine milk but present at lower levels, 6 mg/L versus 11 mg/L respectively. Sanchez-Diaz, Ruano, Lorente, and Hueso (1997) estimated the GG intake of formula-fed infants to be approximately 20% that of breast-fed infants.

Infants receiving a GG enriched formula (9 mg/L as GD_3), through to 6 months of age, had significantly higher GG serum levels (p <0.002) compared to a control group fed standard formula (6 mg/L as GD_3), but did not differ from the breast-fed control group (Gurnida, Rowan, Idjradinata, Muchtadi, & Sekarwana, 2012). Compared to the standard formula, infants receiving the GG enriched formula had significantly increased scores for Hand and Eye coordination IQ (p < 0.006), Performance IQ (p < 0.001) and General IQ (p < 0.041). These cognitive development scores did not differ from the reference group. Gurnida et al. (2012) concluded IFP with increased GG content may have beneficial

effects on cognitive development in healthy infants aged 0–6 months, which may be related to increased serum ganglioside levels.

2.2.1.6 Cholesterol in MFGM

The lipid composition of human milk varies significantly from that of IFP and this difference includes cholesterol (Table 2-12). Human milk contains 90 to 150 mg/L compared to approximately 40 mg/L in milkfat-based IF and virtually none in vegetable oil-based formula (Delplanque, Gibson, Koletzko, Lapillonne, & Strandvik, 2015). The MFGM is the source of cholesterol in human milk. Cholesterol is necessary for synthesis of lipoproteins, bile acids, hormones and calciferols, therefore, essential to infant growth (Delplanque et al., 2015), neurological tissues and during the neuroplasticity period (Hussain et al., 2019; F. Lu, Ferriero, & Jiang, 2022). Brain cholesterol however is dependent on de novo synthesis by brain cells, as circulating cholesterol-containing lipoproteins cannot cross the blood-brain barrier (F. Lu et al., 2022).

C. Thum et al. (2022) showed there was significant variation in the cholesterol content of human milk across stage of lactation, putatively linked to changes in milk fat globule size (diameter ~ 3µm in colostrum versus ~ 5 µm in mature milk) and overall MFGM surface area. Z. Yang et al. (2022) suggested that milk cholesterol concentrations vary across ethnicities in China, in addition to stage of lactation.

The cholesterol content of infant formula was determined by Ramalho, Casal, and Oliveira (2011) with 2 samples of formula for infants up to 6 months of age having 12.48 ± 0.33 and 12.87 ± 0.27 mg/100mL and a formula for infants up to 1 year of age 9.59 ± 0.25 mg/100mL (mean $\pm$ standard deviation). No details as to the type of formula were recorded or if they contained milkfat or only vegetable oils.

Wong, Hachey, Insull, Opekun, and Klein (1993) found low levels of cholesterol in 3 commercial formulas available in the USA (3.6 ± 0.4; 2.2 ± 0.3; 1.0 ± 0.2 mg / 100mL (mean $\pm$ standard deviation).

Table 2-12 Cholesterol content of human milk

Reference	Analytical method	Colostrum (mg/100mL)	Transitional milk (mg/100mL)	Mature milk (mg/100mL)					
				1	2	3	4	5	6
Ramalho et al. (2011)	HPLC-DAD	29.2 ± 0.01		17.4 ± 0.5	12.0 ± 0.1		9.5 ± 0.1		
Boersma, Offringa, Muskiet, Chase, and Simmons (1991)	GC	36.0 ± 16.2	19.7 ± 0.7	19.0 ± 0.8					
Z. Yang et al. (2022)	HPLC	20	17.1	12.6					
Álvarez-Sala, Garcia-Llatas, Barberá, and Lagarda (2015)	E-S			11.3 ± 0.4					
Al-Tamer and Mahmood (2004)	E-S	23.8 ± 4.2							
Wong et al. (1993)	GC						14.2 ± 3.3		
Huisman et al. (1996)	GC-FID		16.6			12.8 ± 1.0			
Hamdan, Sanchez-Siles, Matencio, Garcia-Llatas, and Lagarda (2018)	GC E-S	20.7 ± 0.6 23.2 ± 1.1	14.8 ± 0.8 17.1 ± 0.8	12.8 ± 0.5 13.6 ± 0.5		10.9 ± 0.5 12.8 ± 0.2		10.1 ± 0.1 11.7 ± 0.1	
Kamelska, Pietrzak-Fiećko, and Bryl (2012)	ATR-FTIR	3.4 – 11.9	4.4 – 13.0						

Concentration is shown as mean ±standard deviation (mg/100mL)

NR, not recorded; E-S, enzymatic spectrophotometric; GC. Gas chromatography; AT-FIR, attenuated total reflectance-Fourier transform spectroscopy; HPLC-DAD, high performance liquid chromatography with diode array detector.

2.2.1.7 Proteins in MFGM

The protein composition of the MFGM varies as a function of stage of lactation, environmental factors, particularly immune assaults, and species. Bovine MFGM proteins account for approximately 1 – 4 % of total bovine milk protein (Cavaletto et al., 2008) and it is quite comparable to MFGM protein levels in human milk (Charlwood et al., 2002). MFGM proteins constitute a significant proportion (25 – 70%) of the MFGM (Guerin et al., 2019; Singh, 2006). There are over 40 MFGM proteins ranging from 15 to 240 kDa in size (Dewettinck et al., 2008; Mather, 2000).

A comprehensive review article summarised the nomenclature and identification of major proteins in milkfat globule membranes (Mather, 2000). MFGM proteins were mainly identified based on comparison of electrophoretic mobilities, staining of the gels, molecular cloning techniques, reaction with specific antibodies, and identification by N-terminal amino acid sequencing. Polyacrylamide gel electrophoresis (PAGE) had been used to elucidate the protein composition of the MFGM and major MFGM proteins were designated according to their relative mobility in sodium dodecyl sulphate (SDS)-PAGE and their ability to stain with Coomassie blue or the glycoprotein-specific periodic acid/Schiff (PAS) reagent. Bovine MFGM is resolved into 7 to 8 major bands of protein when separated by SDS-PAGE. Mucin 1, Xanthine dehydrogenase/oxidase, PAS III, PAS IV or CD (Cluster of Differentiation) 36, butyrophilin, lactadherin, and fatty acid binding protein (Mather, 2000). Figure 2-6 adapted from Singh (2006), shows major MFGM proteins separated by electrophoresis.

Proteomic approaches, including mass spectrometric (MS) analysis, provided rapid, unambiguous information on protein identity and further identification of minor proteins. Using a proteomic approach, human MFGM proteins have been identified (Cavaletto et al., 2008; Charlwood et al., 2002; Fortunato et al., 2003; Liao, Alvarado, Phinney, & Lönnerdal, 2011; Reinhardt & Lippolis, 2006). In a direct comparison between human and bovine milk, M. Yang et al. (2016) identified 411 MFGM specific proteins of which 232 were differentially expressed.

Functions of the 120 MFGM proteins identified by Reinhardt and Lippolis (2006) were associated with membrane/protein trafficking (23 %), cell signalling (23 %), unknown functions (21 %), fat transport/metabolism (11%), transport (9%), protein synthesis/folding (7%), immune proteins (4%) and milk proteins (2%).

In another study on bovine MFGM, 345 proteins are either uniquely presented or were significantly higher in abundance. These proteins were mainly involved in actin organization, vesicle mediate transport, carbohydrate catabolic process and response to bacterium (Lu et al. 2016b). In multiple species, 520 MFGM-enriched proteins in milk from the Holstein, buffalo, Jersey, yak, goat, camel, horse, and human were inferred through peptide identification and iTRAQ proteomic approach (Y. Yang et al., 2016; Y. Yang et al., 2015). In the MFGM-enriched protein compartment, a wide variety of proteins were identified from abundant fraction to minor fraction of milk protein, like several proteins such as alpha-S1-casein, alpha-S2-casein, kappa-casein, beta-casein, albumin, and alpha-lactalbumin.

Table 2-13 lists some of the major proteins identified in bovine MFGM. The main protein of the MFGM is the glycoprotein butyrophilin (about 40% of the total proteins of the MFGM), with xanthine oxidase, which comprises 12 to 13% of the total MFGM protein content, being the second most abundant. Other proteins are present in MFGM at concentrations of 5% or less. Almost 50% of the MFGM proteins have membrane/protein trafficking or cell signalling functions (Reinhardt and Lippolis 2006) and may play a role in synthesis and secretion of MFGM in milk.

Figure 2-6 Separation of Bovine MFGM Proteins by SDS-PAGE.

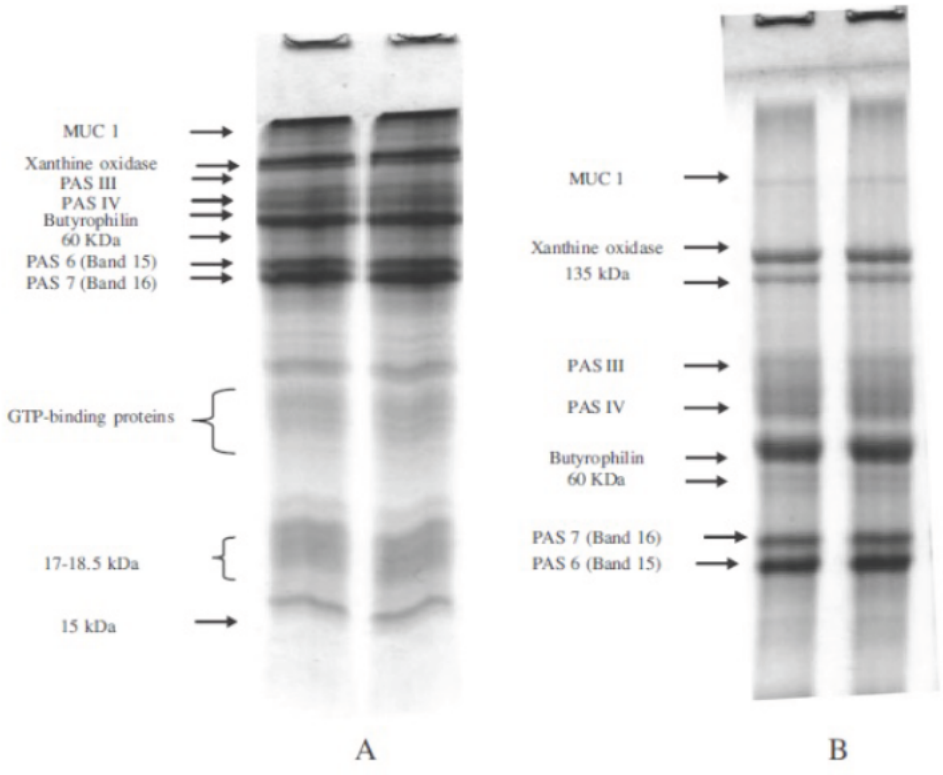


Table 2-13 Major proteins from bovine MFGM as detected by SDS-PAGE.

Protein	Type	Molecular weight (Da)	Reference
Mucin1	Glycoprotein	>160,000	Schroten et al. (1992)
Xanthine oxidase		150,000	Mather (2000), Vorbach, Scriven, and Capecchi (2002)
PAS III	Glycoprotein	~100,000	Mather (2000)
PAS IV or CD36	Glycoprotein	78,000	Greenwalt et al. (1992), Mather (2000),
Butyrophilin	Glycoprotein	66,000	Mather (2000)
Lactadherin	Glycoprotein	~50,000	Honan, Fahey, Fischer-Tlustos, Steele, and Greenwood (2020)
Fatty acid binding protein (FABP)		15,000	Spitsberg (2005)

The reversible phosphorylation of proteins is central to the regulation of most aspects of cell function biological processes (Cohen, 2002). M. Yang et al. (2020) used quantitative phosphoproteomics to investigate the MFGM phosphoproteins from human milk across the lactation cycle. A total of 323 phosphorylation sites on 203 phosphoproteins were identified in the human MFGM fractions, with 48 of the phosphoproteins differentially expressed between colostrum and mature milk. Osteopontin was the most heavily phosphorylated protein, with a total of 39 identified

phosphorylation sites; osteopontin included 38 and 36 phosphorylation sites in colostrum and mature MFGM, respectively (M. Yang et al., 2020). How these changes influence biological processes, or the effects of processing on their functionality is yet to be fully explored. Lee, Padhi, et al. (2018) undertook a detailed review of the properties and putative physiological functions of the major and minor proteins of the MFGM and their role in infant development.

2.2.2 Information to enable the identification of Lacprodan® MFGM-10

Lacprodan® MFGM-10 is a bovine milk derived WPC developed and marketed globally by Arla Foods Ingredients P/S. The unique feature of Lacprodan® MFGM-10 is the enrichment of bovine MFGM, particularly the phospholipid, sphingolipid, and membrane protein components, compared to typical WPC. As MFGM is a collection of components, it does not have a chemical name (according to Chemical Abstracts and the International Union for Pure and Applied Chemistry), a CAS registry number or a structural formula. In some jurisdictions Lacprodan® MFGM-10 has been considered as a WPC and does not require separate identification of its component parts.

The major protein components in Lacprodan® MFGM-10 are normal whey proteins, typical of regular whey-protein ingredients (e.g. WPC), they account for approximately 70% of the ingredient, and are not unique or novel to Lacprodan® MFGM-10. Thus, the routine quantification of MFGM proteins is challenging given their relatively low proportion of total protein in the product. Lacprodan® MFGM-10 is differentiated from regular whey protein products, in that the major lipid and protein components of the MFGM are enriched and present at two-to-four times the quantity present in typical WPCs (such as AFI's Lacprodan® WPC-80). The higher levels of membrane lipid components, such as phospholipids and sphingolipids, enables infant formula products containing MFGM to be compositionally closer to the phospholipid and sphingolipid content of human milk when compared to infant formula products containing typical WPCs.

For the purposes of identification, the major lipid components of the MFGM can provide measurable differences to typical WPCs that may be added to infant formula products. This can be complicated by the presence of phospholipid species from other ingredeints such as lecithin. However, as sphingomyelin and gangliosides are unique to milk these are suitable markers to determine the addition of MFGM components to IFP.

2.2.3 Information on the chemical and physical properties of Lacprodan® MFGM-10

2.2.3.1 Incorporation of Lacprodan® MFGM-10 into IFP food matrices

Lacprodan® MFGM-10 is a spray dried powder of homogenous composition. It is designed for addition into IFP during the wet mix phase of production of IFP powders. Lacprodan® MFGM-10 is essentially a whey powder, with a particle size distribution the same as that of standard WPC products. It is free flowing and easily added into the wet mix of IFP production where it is solubilised and distributed homogenously throughout the mix. Standard dairy processes such as homogenisation and turbulence are used in IFP wet mix production to ensure homogeneity of spray-dried powders. Lacprodan® MFGM10 may be produced according to specific customer requirements (Section 2.2.3.1) for dry blend applications. The physical and physicochemical properties of Lacprodan® MFGM-10 mean it blends homogenously with other dairy powders and readily disperses and dissolves during formula mixing. Accordingly, the particle size of Lacprodan® MFGM-10 is also not important in the context of considering any differences in nutritional status or toxicology when comparing to other WPCs and components of WPCs.

2.2.3.2 Chemical properties of Lacprodan® MFGM- 10

Comparison of the composition of Lacprodan® MFGM-10 to a standard WPC provides context of the key parameters that make it unique but also demonstrate compositional commonality.

2.2.3.2.1 Lacprodan® MFGM-10 lipids

The total phospholipid content in Lacprodan® MFGM-10 amounts to approximately 6.5 g/100 g of powder (as listed in the specification in Table 2-20). The two to four-fold higher level of fat content found in Lacprodan® MFGM-10 compared to Lacprodan WPC-80 results in enrichment of key phospholipids, sphingomyelin and gangliosides (Table 2-14). The composition of these complex lipids is constant across whey dominant streams. Partial supplementation of this complex lipid enriched whey protein concentrate (4 - 7 g/L) brings infant formula levels of sphingomyelin and gangliosides closer to average human milk levels.

Original data from 4 non-consecutive batches for Lacprodan® MFGM-10 and Lacprodan® 80 (WPC) fat and PL composition is submitted under Confidential Information.

Table 2-14 Typical compositional comparison of Lacprodan® MFGM-10 to WPC-80

Analyte	Lacprodan® MFGM-10 (n=5) (%)	Lacprodan® WPC-80* (n=4) (%)	Typical ratio
Total fat	18.6	5.5	3.4
Total PL	6.7	1.85	3.6
Phosphatidyl choline (PC)	1.86	0.50	3.8
Phosphatidyl inositol (PI)	0.38	0.136	2.7
Phosphatidyl serine (PS)	0.65	0.180	3.6
Phosphatidyl ethanolamine (PE)	2.0	0.46	4.3
Sphingomyelin (SM)	1.69	0.46	3.7

** Commercial fractions sold by Arla Foods ingredients P/S for infant formula applications*

Key phospho- and sphingolipids that are present in typical WPC-80 versus Lacprodan® MFGM-10 as a percentage of total fat are listed in Table 2-15. The phospholipid values reported for standard WPC-80 and Lacprodan® MFGM-10 are quite similar when reported as a percentage of total fat. Greater than 98% of total phospholipid contribution is from these five major PL components. The total and major individual phospholipid components listed in Table 2-20 are measured in every lot of Lacprodan® MFGM-10. Total gangliosides content is monitored periodically and measured at least 4 times a year.

Table 2-15 Typical phospholipid, sphingolipid and gangliosides levels in Lacprodan® MFGM-10 versus standard WPC-80 expressed as average of the batches in percentage of total fat

Analyte	Lacprodan® MFGM-10 (g/100 g fat) n=4	Lacprodan® WPC-80 (g/100g fat) n=5
Total phospholipids (PL)	35	32
Phosphatidyl choline (PC)	10.1	8.8
Phosphatidyl ethanolamine (PE)	9.1	8.1
Phosphatidyl inositol (PI)	2.0	2.4

Analyte	Lacprodan® MFGM-10 (g/100 g fat) n=4	Lacprodan® WPC-80 (g/100g fat) n=5
Phosphatidyl serine (PS)	3.5	3.2
Sphingomyelin (SM)	10.9	8.1
Gangliosides	1.43	1.25

2.2.3.2.2 Lacprodan® MFGM-10 proteins

The major protein and lipids components in Lacprodan® MFGM-10 are also present in typical whey-protein ingredients, albeit at lower levels, and are not unique or novel to Lacprodan® MFGM-10 (Figure 2-7).

Quantification of the levels of all MFGM-associated proteins in milk is relatively challenging. The relative abundance of major MFGM-associated proteins can be determined using semi-quantitative methods such as SDS-PAGE. As seen below, SDS-PAGE analysis of 7.5 µg of protein samples, from WPC-35, WPC-80, and Lacprodan® MFGM-10 ingredients, shows similar profiles between ingredients but higher levels of MFGM-associated proteins in the Lacprodan® MFGM-10. Lactadherin, butyrophilin, and xanthine oxidase (XDH/XO) are reported to be three of the most prominent MFGM-associated proteins (Demmelmair, Prell, Timby, & Lonnerdal, 2017). The presence of these proteins in other WPC ingredients (not deliberately enriched in MFGM) illustrates that MFGM-associated proteins are not novel to Lacprodan® MFGM-10.

Furthermore, the overall amino acid composition of Lacprodan® MFGM-10 is comparible with standard Lacprodan® WPC-80 (DI-8090) that is typically sold for infant formula applications (Table 2-16). As for all milk products, amino acid composition varies slightly due to natural conditions such as cow's feed, season, and lactation. Furthermore, there are minor variations in amino acid contents across the whey dominant streams.

There is similar distribution of essential amino acids between Lacprodan® MFGM-10 and Lacprodan® WPC-80 (used as protein source in infant formulas). These data and other calculations show that addition of a portion of Lacprodan® MFGM-10 (typical dose of 6 g/L) does not result in an increase in total protein or changes in protein quality in the product due to the similar amino acid profiles of WPC's.

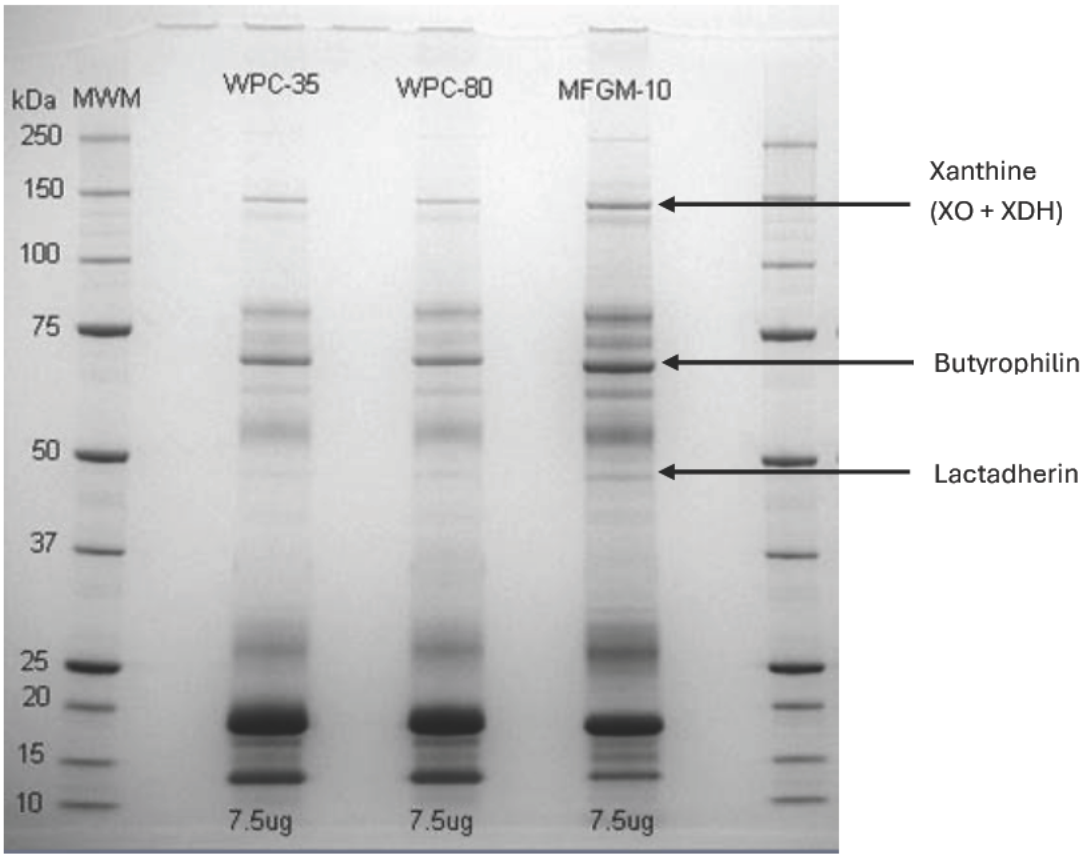
Table 2-16 Typcial amino acid composition of Lacprodan® MFGM-10 versus standard WPC-80

Amino acid	#Lacprodan® MFGM-10 (%)	#Standard WPC-80 – Lacprodan® DI-8090 (%)
Alanine	4.9	5.5
Arginine	3.7	2.7
Aspartic acid (asparagine)	10.3	11.3
Cysteine (cystine)	2.1	2.4
Glutamic acid (glutamine)	16.5	18.4
Glycine	2.2	2.0
Histidine*	2.4	1.9
Isoleucine*	5.6	6.6
Leucine*	10.8	11.4
Lysine*	9.1	9.9
Methionine*	2.1	2.3
Phenylalanine*	3.7	3.5
Proline	5.8	6.6
Serine	6.4	5.7
Threonine*	6.7	7.5
Tryptophan*	1.6	1.9
Tyrosine	3.8	3.2
Valine*	5.6	6.6

Amino acid	#Lacprodan® MFGM-10 (%)	#Standard WPC-80 – Lacprodan® DI-8090 (%)

#Commercial fractions sold by Arla Foods ingredients Inc. for infant formula applications
**Essential amino acids*

Figure 2-7 SDS-PAGE Protein Analysis of Lacprodan® MFGM-10 WPC compared to WPC



2.2.4 Information on the impurity profile

The production of Lacprodan® MFGM-10 as a MFGM enriched WPC is subject to regular monitoring for pesticides (Regulation (EC) No 396/2005) and contaminants (Commission Regulation (EU) 2023/915) under the EU regulatory framework, and as part of AFI's global monitoring program. Lacprodan® MFGM-10 is monitored for heavy metals, pesticide residues, and contaminants biannually by an external laboratory using validated analytical methods. The resulting analyses for pesticide residues and contaminants are within the regulatory limits established for these substances. Additionally, as Lacprodan® MFGM-10 is a powdered ingredient intended to be added to powdered infant and follow-on formula, the levels of arsenic, cadmium, lead, and mercury are regularly determined. Data from 4 representative non-consectuive batches manufactured in 2018/2019 are provided in Table 2-17. These show conformance with the established regulatory limits in Australia and New Zealand (Schedule 21 of the FSC) and other jurisdictions for powdered infant formula products.

Table 2-17 Heavy metal analyses for 4 representative batches of Lacprodan® MFGM-10

Analysis	Unit	Tested batches			
		MFGM-1 2018	MFGM-2 2018	MFGM-3 2018	MFGM-1 2019
Arsenic	mg/kg	<0.01	<0.01	<0.01	<0.01
Cadmium	mg/kg	<0.01	<0.01	<0.01	<0.01
Lead	mg/kg	No data	<0.003	<0.003	0.0045
Mercury	mg/kg	<0.005	<0.005	<0.005	<0.005

Microbiological safety and potential contamination are assessed by the routine analysis of every production batch of Lacprodan® MFGM-10. Microbiological analysis includes; total plate count following incubation at 30°C, aerobic thermophilic count, and levels of yeasts and moulds, *Bacillus cereus*, sulphur-reducing clostridia, coagulase-positive *staphylococci*, *Enterobacteriaceae*, and *Salmonella* in accordance with methods established by the International Organization for Standardization. Results from the analysis of 4 representative non-consecutive batches are presented in Table 2-18.

Table 2-18 Microbiological results of 4 representative batches of Lacprodan®MFGM-10

Parameter	Unit	Manufacturing batch numbers			
		MFGM-1 2018	MFGM-2 2018	MFGM-3 2018	MFGM-1 2019
Total plate count (30°C)	cfu/g	8700	<100	500	<100
Total plate count(55°C)	cfu/g	<100	<100	<100	200
Bacillus cereus	cfu/g	20	<10	<10	<10
Sulfite-reducing *Clostridia*	cfu/g	<10	<10	<10	<10
Enterobacteriaceae	cfu/g	<10	<10	<10	<10
Coagulase-positive staphylococci	cfu/g	Absent	Absent	Absent	Absent
Yeast and mold	cfu/g	<10	<10	<10	<10
Salmonella	cfu/g	Absent	Absent	Absent	Absent

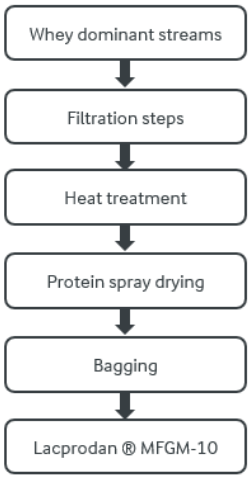
CFU = colony forming units.

Lacprodan® MFGM-10 is not intended for addition to IFP via a dry blend process but is for addition into wet blend processes that control the final microbiological quality of spray-dried IFP powders. However, where customers require Lacprodan® MFGM-10 for specific dry blend applications, it can be produced to a specification that has more stringent microbiological limits aligned with final IFP requirements.

2.2.5 Manufacturing process for Lacprodan® MFGM-10

Lacprodan® MFGM-10 is produced from bovine whey dominant streams produced using well-accepted dairy processing methodologies, which are used to separate an enriched fraction of MFGM components in the whey (Figure 2-8). The raw material feed for Lacprodan® MFGM-10 production is whey dominant streams conforming to the European Union Food Hygienic Guidelines and EU Regulation 853/2004.

Figure 2-8 Generic process flow diagram for Lacprodan® MFGM-10



The raw material (whey dominant streams) comes from authorised dairy product facilities that have implemented HACCP methods and comply with current Good Manufacturing Practices (cGMP). They are regulated in the European Union under Regulation (EC) No 852/2004 on hygiene of foodstuffs.

The production sites that manufacture Lacprodan® MFGM-10 have implemented quality control systems in accordance with cGMP and HACCP principles pursuant to Regulation (EU) No 852/2004, raw material analytical control, and physicochemical and microbiological final product controls.

Lacprodan® MFGM-10 is produced at Danmark Protein in Videbæk, Denmark. Due to capacity of the spray dryer at Danmark Protein, some Lacprodan® MFGM-10 liquid may be transported to the AFI factory Arinco, a plant 7 km from Danmark Protein, to be spray dried and bagged (Figure 2-9). Danmark Protein is certified under DS/EN ISO 50001: 2011, ISO 22000: 2005 / TS 22002-1: 2009 and FSSC 22000, and Arinco is certified under ISO 14001: 2015, ISO 22000: 2005 / TS 22002-1: 2009 and FSSC 22000.

All processing equipment is suitable for food applications and in compliance with processing and production requirements set by the European Union legislation (Čapla, Zajác, Ševcová, Čurlej, & Fikselová, 2023) and Danish Veterinary and Food Administration (DVFA) (https://en.foedevarestyrelsen.dk). Arla Foods Ingredients P/S (AFI) is certified for the development, production, and sale of products based on whey protein and lactose by DVFA. Arla Foods Ingredients

P/S is a member of the Danish Agriculture and Food Council (https://agricultureandfood.dk/danish-agriculture/) and adheres to the practices set by the organisation.

2.2.5.1 General description of the manufacturing process for Lacprodan® MFGM-10

All process operations used in the manufacture of Lacprodan® MFGM-10 are standard dairy processing operations.

2.2.5.1.1 Feed material for Lacprodan® MFGM-10 manufacture

Lacprodan® MFGM-10 is produced from whey dominant streams, which are by-products from raw skim milk used to produce cheese or casein.

All raw milk is pasteurised (72°C for 15 seconds) prior to processing. This is a critical control point (CCP) at the dairy facilities, eliminating the risk of pathogens. The pasteurisers, all operate with divert valves, which are tested at the start of every production lot. The pasteurised milk is then processed to manufacture cheese or casein, yielding whey dominant streams that contain the components that are to be enriched in the Lacprodan® MFGM-10 production process. The whey dominant streams are clarified, separated, and undergoes additional pasteurisation (72°C for 15 seconds) or microfiltration (ceramic membrane <1.4 µm) prior to cooling. The whey dominant streams are transported under hygienic conditions at 5°C to Danmark Protein for further processing. (https://www.arlafoodsingredients.com/about/contact/locations/danmark-protein/)

Quality control parameters for the whey dominant streams include temperature, pH, and nitrate level as release parameters. Nitrate is controlled as an indicator of HNO3, a CIP cleaning residue. Gross composition is assessed through protein quantification, and selected microbial analyses are carried out.

In order to standardize the whey dominant streams for Lacprodan MFGM-10 production, the whey dominant streams pass through an ultrafiltration step to increase the concentration of the whey protein components, and reduce water and components such as lactose and minerals. If required, this step can alternatively be performed before the transportation to Danmark Protein

The whey dominant streams (after ultrafiltration) must comply with the below specification on the parameters: dry matter, protein and fat contents as well as pH (Table 2-19). If pH adjustment is required, NaOH, KOH or a combination of NaOH and KOH are used.

Table 2-19 Specification of whey dominant streams entering Lacprodan® MFGM-10 production

Parameter	Min (%)	Max (%)	Analytical method
Dry matter	8.4	10.3	ISO 6731:2010(E)/IDF 21:2010(E) mod. "Milk, cream and evaporated milk. Determination of the total solids content"
Protein as is	5.5	8.5	ISO 8968-3:2004(E)/IDF 20-3:2004: Determination of nitrogen content. Block digestion method (semi-micro rapid routine method)
Protein in dry matter	65	83	
Fat as is	0.31	0.78	ISO 7208/IDF 22: 2008 Skimmed Milk, whey and buttermilk. Determination of Fat Content. Röse-Gottlieb Gravimetric Method (Reference Method)
Fat in dry matter	3.7	7.6	
pH	5.6	6.8	IDF 115/ISO 5546 2nd ed 2010-06-01 (modified)

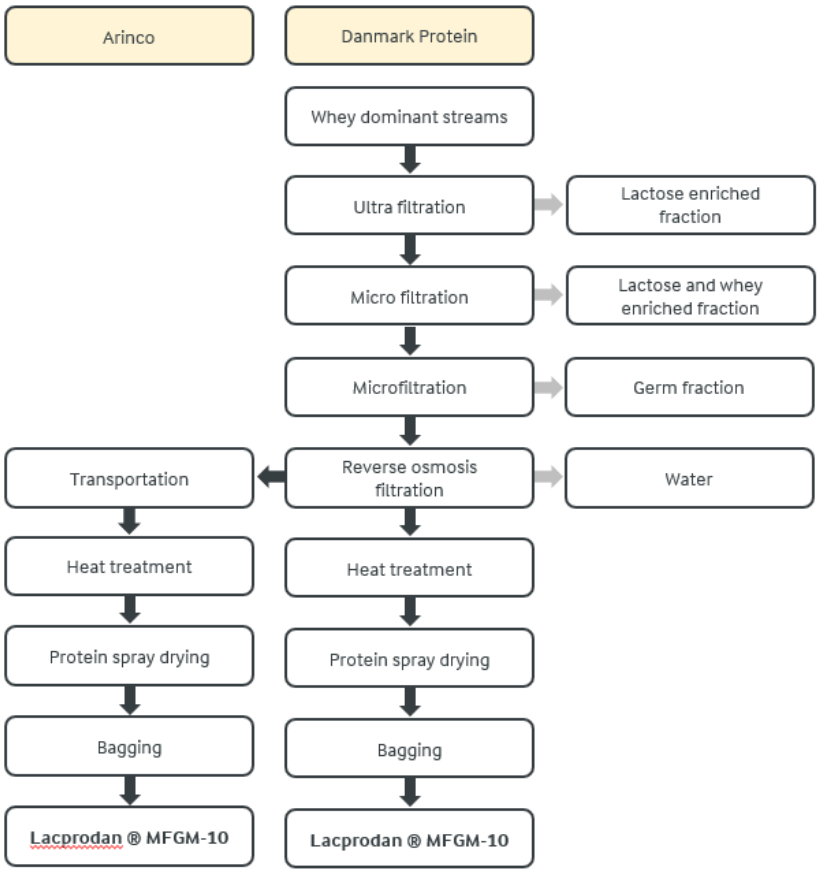
2.2.5.1.2 Lacprodan® MFGM-10 production

On receipt to the whey processing factory, the whey dominant streams are stored at 5°C prior to entering the Lacprodan® MFGM-10 manufacturing process. Maximum holding time is 72 hours prior to manufacturing.

A series of specific ultra- and micro-filtration process operations are completed that separates the whey dominant stream into 4 retentate and permeate streams (Figure 2-9):

i. Ultrafiltration (UF) retentate containing the MFGM components. This continues through the process;
ii. UF permeate – a high lactose enriched fraction containing minor amounts of components like non-protein nitrogen and minerals that is used for further processing;
iii. Microfiltration (MF) permeate – lactose and whey enriched fraction e.g. beta-lactoglobulin and alpha-lactalbumin that is used for further processing;
iv. MF retentate (germ fraction) containing most of the microorganisms from the raw material, which is discarded.

Figure 2-9 Detailed process flow diagram for Lacprodan® MFGM-10



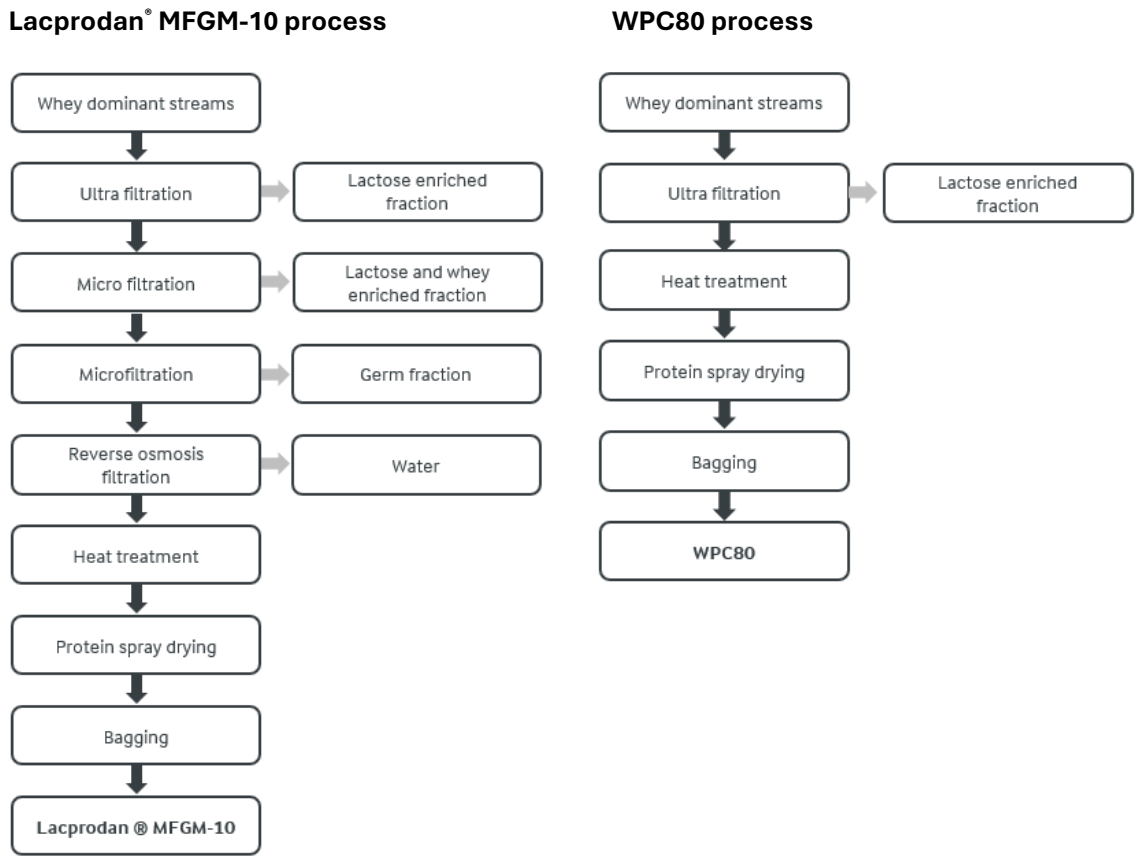
Following the selective separation by filtration, the MFGM enriched whey stream is concentrated using reverse osmosis (RO). Dependent on plant processing capacity, the concentrate stream may be sent to the nearby AFI Arinco plant where it is processed in the same way as at the main Danmark Protein plant. The product is transported liquid and is kept below 8 °C during transport.

At both manufacturing sites the product material is heat treated at 60-70 °C for approximately 20 s prior to being sprayed dried as part of the standard operational procedure. Following drying the powder passes through a sieve with a nominal mesh size of either 4 mm (Danmark Protein) or 2 mm (Arinco) and rotating magnet. The product is packed after drying, passing through a metal detector prior to packing into bags. There may be an intermediate bulk holding time of up to 2-3 weeks before packing into bags for customer supply.

Finished product analysis (compositional, functional, and physicochemical, and microbiological) is completed prior to release of the powder for customer supply.

For comparative purposes (Figure 2-10) shows the key differences between the manufacture of the MFGM enriched Lacprodan® MFGM-10 and an AFI WPC80.

Figure 2-10 Comparison of production methods for Lacprodan® MFGM-10 and WPC-80



2.2.5.2 Impact of processing on Lacprodan® MFGM-10

All processes used for the manufacture of Lacprodan® MFGM-10 are standard dairy processes and conditions and are not expected to impact the product any differently to other conventional dairy products.

The filtration steps used to manufacture AFI's Lacprodan® MFGM-10 concentrate lipids and MFGM components from whey to a level higher than that normally found in standard WPC. These filtration steps are the only difference between the manufacturing process of WPC and Lacprodan® MFGM-10 (Figure 2-10).

The filtration is not known to have any effects on secondary or tertiary protein structures that differ from commodity WPC manufacturing. The main causes for conformational changes and/or post-translational modifications in protein are heat treatments, enzymatic activity, shear, pressure, or UV. AFI has designed and optimized its production processes in order to avoid these processing influencers:

- Heat treatments: The raw material (whey dominant stream) is transported cold to our production plant, and it is kept cold until spray drying.
- Enzymatic activity: The whey dominant stream is pasteurized before processing at our plant, and bacterial growth is controlled during processing since everything is kept cold.
- Shear/pressure/UV: The production process for Lacprodan® MFGM-10 is not adding unusual shear or pressure, compared to standard processes for whey proteins. UV is not used in production.

2.2.5.3 Materials and processing aids

Raw materials used in the manufacture of Lacprodan® MFGM-10 include:

a. Whey dominant streams from the manufacture of cheese or casein in AFI production facilities (Section 2.2.5.1.1, Table 2-19).
b. If a pH adjustment is required of the incoming whey dominant stream, sodium hydroxide (NaOH), potassium hydroxide (KOH) or a combination of these are used.

No other materials or processing aids are used in the production of Lacprodan® MFGM-10.

2.2.6 Specification for identity and purity of Lacprodan® MFGM-10

The specification for the identity and purity of Lacprodan® MFGM-10 is shown in Table 2-20.

The full product specification is provided under Confidential Information.

Compositional results for 4 non-consecutive batches is provided under Confidential Information provided.

Table 2-20 Lacprodan® MFGM-10 specification

Analyte	Unit	Method	Specification
Total protein	%	ISO 8968-3 / IDF 20-3	69.0 - 76.0
Lactose	%	ISO 5765-2 / IDF 79-2	≤2.0
Fat	%	ISO 1736	16.0 - 22.0
Phospholipids	%	Phosphorus-31 NMR method	6.0 - 10.0
Sphingomyelin	%	Phosphorus-31 NMR method	1.3 - 2.3
Ash	%	NMKL 173	<3.0
Moisture	%	ISO 6731	<5.0
Minerals			
Sodium	%	AFI ICP analysis	<0.1
Magnesium	%	AFI ICP analysis	<0.1
Phosphorus	%	AFI ICP analysis	<0.50
Chloride	%	ISO 5943 / IDF 88	<0.10
Potassium	%	AFI ICP analysis	<0.30

Analyte	Unit	Method	Specification
Calcium	%	AFI ICP analysis	<0.40
Heavy Metals [1]			
Arsenic (As)	mg/kg	ICP-HRMS ISO 17294m:2016	≤0.2
Cadmium (Cd)	mg/kg	ICP-MS ISO 17294m:2016	≤0.1
Lead (Pb)	mg/kg	ICP-HRMS ISO 7294m:2016	≤0.05
Mercury (Hg)	mg/kg	ICP-MS ISO 17294m:2016	≤0.02
Microbiological			
Total plate count (30°C)	cfu/g	ISO 4833-1	≤10000
Total plate count(55°C)	cfu/g	ISO 4833-1: incubation at 55°C for 48hrs	≤1000
Bacillus cereus	cfu/g	ISO 7932	<50
Sulfite-reducing *Clostridia*	cfu/g	ISO 15213	<10
Enterobacteriaceae	cfu/g	ISO 21528-2	<10
Coagulase-positive staphylococci	cfu/g	ISO 6888-1	Absent/1 g
Yeast and mold	cfu/g	ISO 6611	<10
Salmonella	cfu/g	ISO 6579	Absent/250 g

[1] *The heavy metals As, Cd, Pb, and Hg are analyzed at least yearly as part of a monitoring program.*
ICP inductively coupled plasma; IDF International Dairy federation; NMKL Nordic Committee on Food Analysis; ISO International

Most of the parameters analysed for specification purposes use analytical methodologies or similar used globally for dairy products. The AFI ICP analysis used for mineral analyses is an internal method by Arla Foods Ingredients. We participate in proficiency testing using reference material offered by muva kempten GmbH

2.2.7 Stability of Lacprodan® MFGM-10

Lacprodan® MFGM-10 is a WPC that contains proteins typically found in other WPC with enriched levels of some MFGM-related proteins and lipids. Whey protein concentrates are common food ingredients with a long history of incorporation into a wide variety of food matrices, including infant formula products. Lacprodan® MFGM-10 has similar properties to other WPCs in relation to its incorporation and stability as a bulk ingredient and as an ingredient in food matrices. The stability of Lacprodan® MFGM-10 powder, across a variety of parameters is demonstrated in Section 2.2.7.1. The stability of Lacprodan® MFGM-10 in infant formula powders, is demonstrated in Section 2.2.7.1.

2.2.7.1 Stability of Lacprodan® MFGM-10 powder

Four batches of Lacprodan® MFGM-10 were stored at ambient conditions representative of climate zone 1 (21°C, 45% relative humidity (±5%)) to document the stability of the ingredient for 18 months as listed in the specification (Section 2.2.6). Lacprodan® MFGM-10 bags from commercial production were used; 15 kg powder packed in a paper bag with a polyethylene inner liner. This packaging material is light-proof and partially air-permeable.

The study report for commercially packed Lacprodan® MFGM-10 is provided in Appendix II.

2.2.7.1.1 Methodology

Stability was evaluated based on the following parameters: colour, water activity, water content, pH, protein content, fat content, ganglioside GD3, phospholipids, peroxide value, major whey proteins, immunoglobulin G (IgG), lactoferrin, microbiology and sensory properties (

Table 2-21). Samples were analysed at baseline and at 3, 6, 9, 12, 15 and 18 months. An intact bag was analysed at each time point.

Table 2-21 Methods for Stability Study

Analysis	Method	Laboratory
Colour (L*, a*, b*)[1]	Minolta color system	Internal
Water activity, a_w	Internal	Internal
Water content	ISO 6731	Internal
pH	ISO 5546	Internal
Protein content	ISO 8968-3	Internal
Fat content	ISO 1736	Internal
Gangliosides	GANGLIO-r – LC-MS/MS	External, NIZO
Phospholipid content	Phosphorus-31 NMR method	External, Spectral Service
Peroxide value	AOCS Cd 8b-90	External, Eurofins Steins
Major whey proteins	HPLC	Internal
IgG	Radial immunodiffusion (RID)	External, Eurofins Steins
Lactoferrin	ELISA method	External, Eurofins Steins
Microbiological analyses		Internal and external
Sensory properties	Descriptive evaluation	External, Aarhus University

[1] *Commission Internationale de l'Eclairage color space (L* indicates lightness, a* is the red/green coordinate, and b* is the yellow/blue coordinate)*

The characteristics that are considered to be prone to change during storage and are likely to affect the quality and use of Lacprodan® MFGM-10 are listed below (see Table 2-2) in the shelf-life specification, which has been created specifically for the shelf-life study.

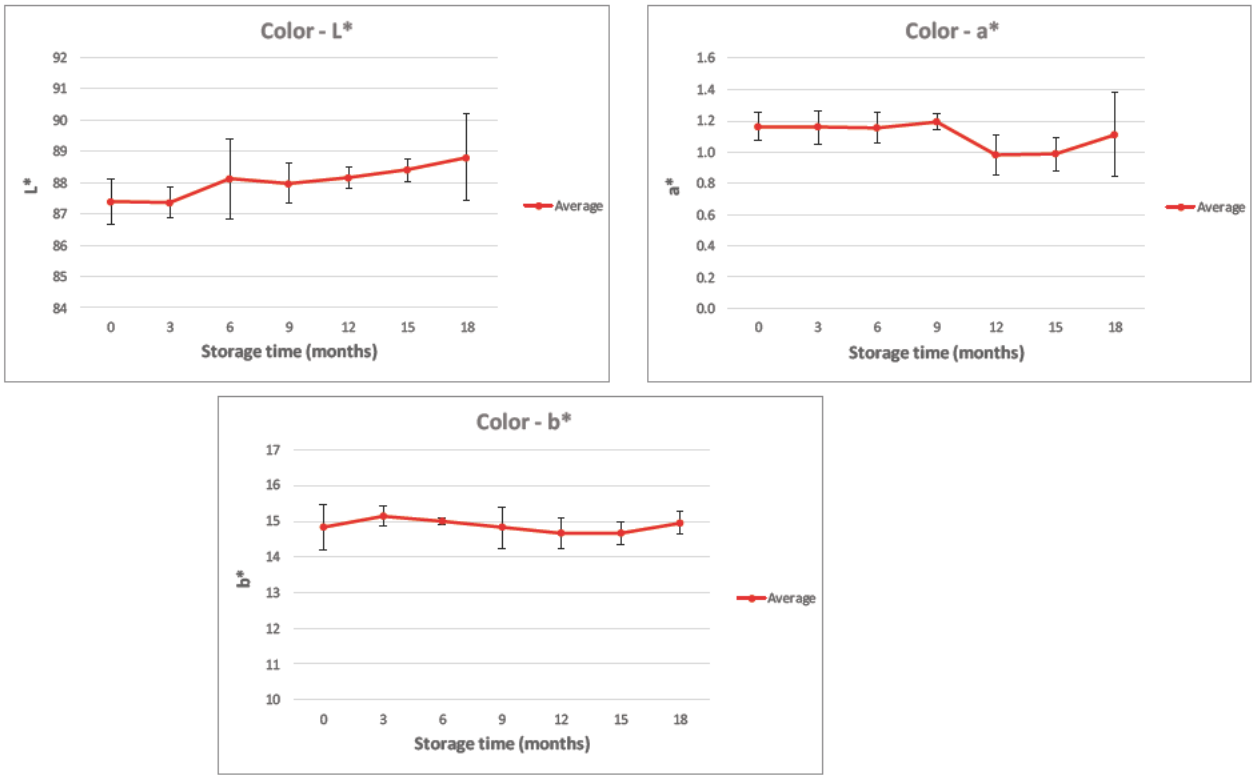
Table 2-22 Shelf-life specification for Lacprodan® MFGM-10

Shelf-life specification	Acceptance level
Colour change (ΔE*ab)	< 3
Water activity, a_w	≤ 0.5
Water content	≤ 6%
Total fat	≥ 16%
Protein content	≥ 73%
Phospholipids	≥ 6%
Gangliosides	N/A
Sensory analysis	No discrimination against fresh sample
Microbiology	Within release specification

2.2.7.1.2 Colour

Colour development in the 4 batches over 18 months is shown in Figure 2-11. The average L* value increased 1.4 unit from 0 to 18 months. No net change in the average a* and b* values were observed. In summary, the powder maintained its colour throughout 18 months of storage.

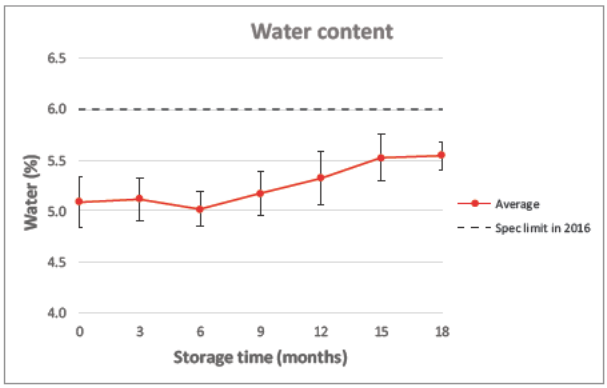
Figure 2-11 Colour changes during stability study



2.2.7.1.3 Water Content

An increase in water content was expected due to the hygroscopic nature of the powder that was stored in a partially air-permeable packaging material, and such an increase was observed (Figure 2-12)[9].

Figure 2-12 Changes in water content over stability study



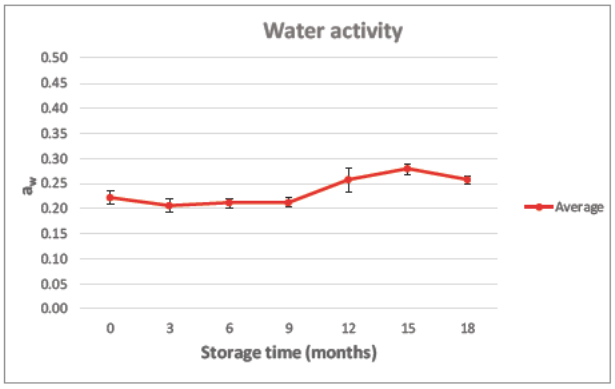
2.2.7.1.4 Water activity

There was no net change in the average a_w from 0 to 9 months (Figure 2-13). From 9 to 18 months, a small increase was observed. The water activity was still well below 0.5, sufficiently low to be

[9] At the time the stability study was performed, the specification for water content was ≤6%; it has since been reduced to ≤5%.

incompatible with microbial growth (Labuza 1971). The increase in a_w is attributed to a partially air-permeable barrier of the packaging material.

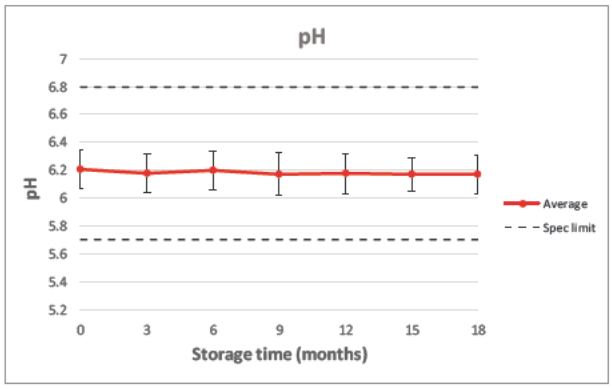
Figure 2-13 Changes in water activityt over stability study



2.2.7.1.5 pH

As shown in Figure 2-14, there was no change in pH over 18 months.

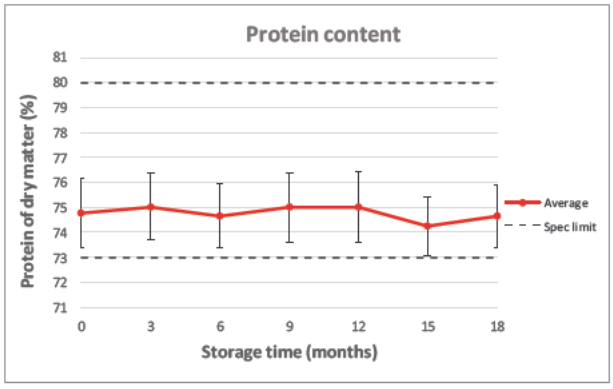
Figure 2-14 Changes in pH over stability study



2.2.7.1.6 Protein

There was no change in the protein content of the dry matter over 18 months (Figure 2-15).

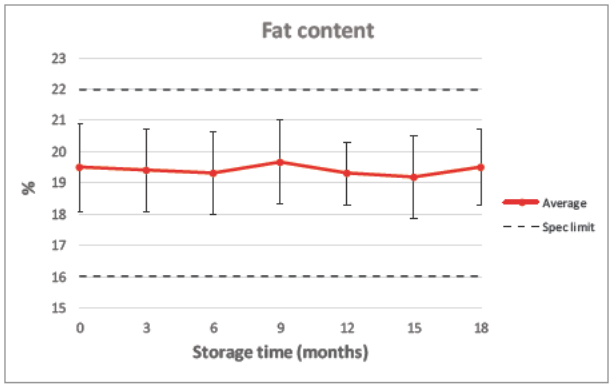
Figure 2-15 Changes in protein in dry matter over stability study



2.2.7.1.7 Fat

Total fat content did not change over 18 months (Figure 2-16**)**.

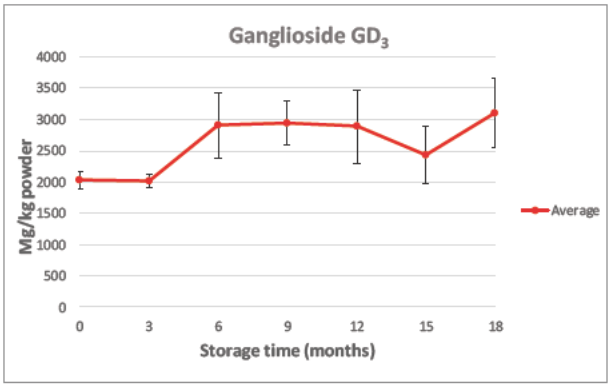
Figure 2-16 Changes in fat content over stability study



2.2.7.1.8 Ganglioside GD3

No net decrease in the average content of ganglioside GD3 was observed over 18 months (Figure 2-17).

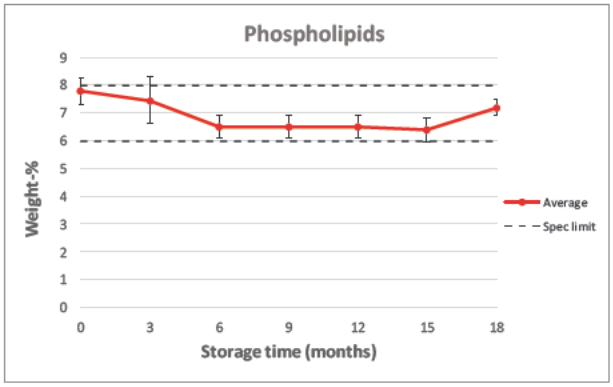
Figure 2-17 Changes in ganglioside GD3 over stability study



2.2.7.1.9 Phospholipids

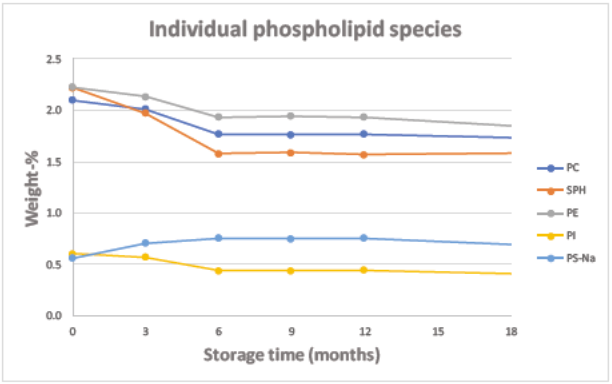
The average content of phospholipids remains above the minimum specification limit during 18 months of storage (Figure 2-18).

Figure 2-18 Changes in phospholipids over stability study



The content of individual species of phospholipids except phosphatidylserine decreased during the first 6 months of storage and were henceforth mostly stable, as shown in Figure 2-19. Data are from one representative batch.

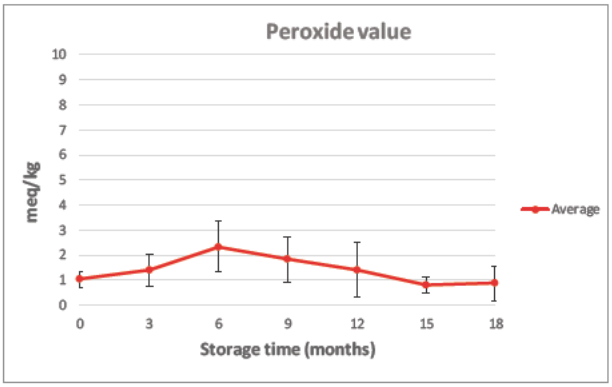
Figure 2-19 Changes in phospholipid species over stability study



2.2.7.1.10 Peroxide Value

Despite fluctuations of analysis results for peroxide value throughout the storage period, no significant net change in the average peroxide value was observed at 18 months of storage (Figure 2-20).

Figure 2-20 Changes in peroxide value over stability study



2.2.7.1.11 Major Whey Proteins

No significant change in measured native alpha-lactalbumin (α-LA), casein glycomacropeptide (CGMP) or beta-lactoglobulin (β-LG) was detected over 18 months (Figure 2-21, Figure 2-22 and Figure 2-23 respectively).

Figure 2-21 Changes in alpha-lactalbumin over stability study

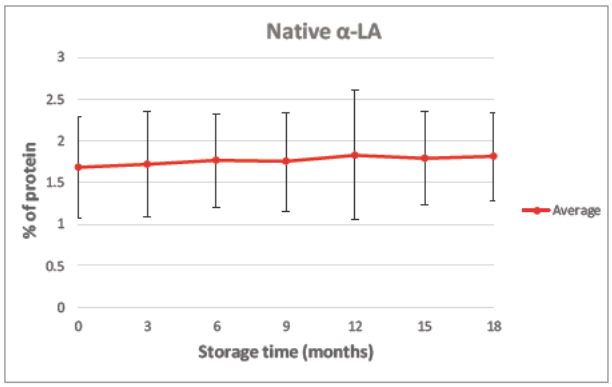


Figure 2-22 Changes in casein glycomacropeptide over stability study

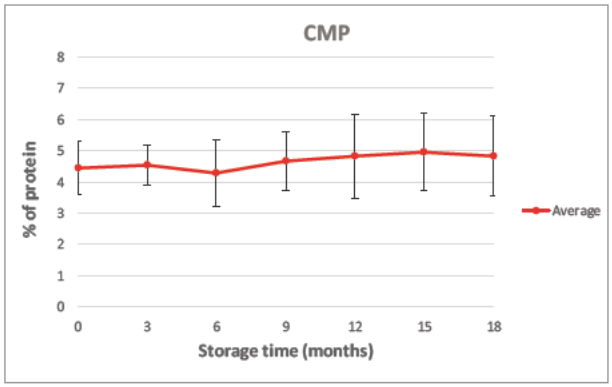
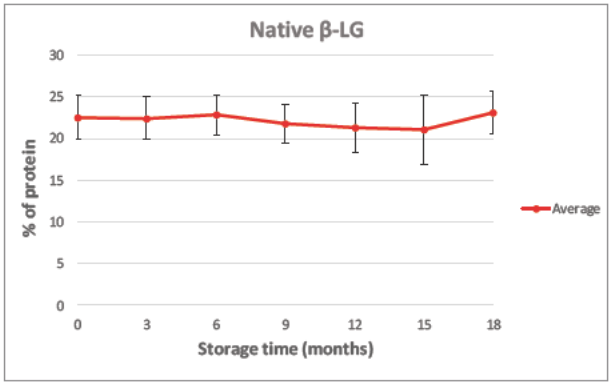


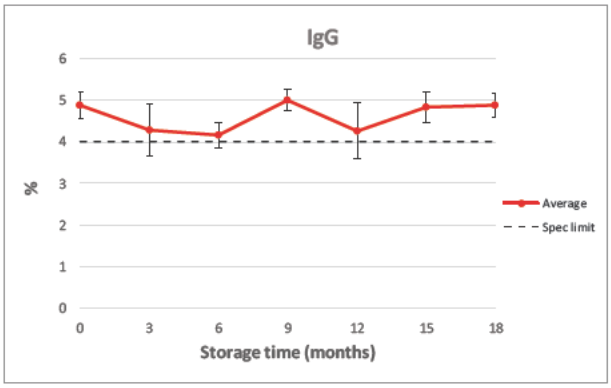
Figure 2-23 Changes in β-lactoglobulin over stability study



2.2.7.1.12 IgG

As shown in Figure 2-24, the IgG level showed some fluctuation, but overall did not change over the 18-month storage period.

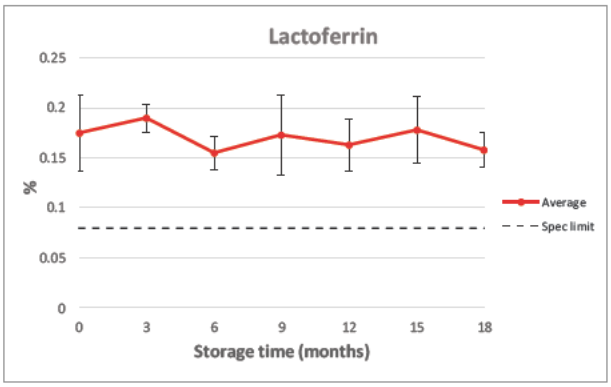
Figure 2-24 Changes in IgG over stability study



2.2.7.1.13 Lactoferrin

The lactoferrin content remained above the minimum specification limit during 18 months of storage and no significant decrease in the average content was observed (Figure 2-25).

Figure 2-25 Changes in lactoferrin over stability study



2.2.7.1.14 Microbiology

Microbiological compliance was assessed a end of the 18-month stability period (Table 2-23).

Table 2-23 Microbiological compliance over 18 month storage

Analysis	Acceptance Level	Compliant through 18 Months?
Total plate count	≤10,000 cfu/g	Yes
Enterobacteriaceae	≤10 cfu/g	Yes
Bacillus cereus	≤50 cfu/g	Yes
Staphylococcus aureus coagulase + a	Absent in 1 g	Yes
Yeast/mold	≤10 cfu/g	Yes
Salmonella spp.	Absent in 125 g	Yes
Cronobacter sakazakii	No standard	N/A (result: negative)

Analysis	Acceptance Level	Compliant through 18 Months?
Listeria monocytogenes	No standard	N/A (result: negative)
Sulfite reducing bacteria	No standard	N/A (result: <10 cfu)
Thermophilic count	No standard	N/A

2.2.7.1.15 Sensory Properties

Differences in sensory characteristics among samples were not significant, but some trends were evident to experienced tasters: an increase in cardboard, bitterness, stale flavour, teeth-coating, and drying-out mouthfeel were indicated with increasing age of samples. A trend towards a decrease in whey aroma and whey flavour was also noted. However, it was concluded that samples stored for up to 18 months cannot be distinguished from fresh samples based on taste, aroma, or mouthfeel.

2.2.7.1.16 Conclusion

All parameters assessed over the duration of the stability study remained within acceptance limits up to 18 months of storage. In conclusion, the stability data set documents a shelf life of 18 months for commercial packaged Lacprodan® MFGM-10 powder.

2.2.7.2 Stability of Lacprodan® MFGM-10 in IFP

A systematic series of stability studies of Lacprodan® MFGM-10 in infant formula powder was performed to assess the nutrient stability and sensory characteristics. This stability work was initiated in a variety of formulas targeted for infants at stage 1 (0-6 months) and stage 2 (6-12 months). Three independent stability trials were conducted to assess the overall stability of Lacprodan® MFGM-10 in finished powders.

2.2.7.2.1 Macronutrient stability in finished powders

[redacted]

The first study assessed macronutrient stability. In addition, key fatty acids and vitamins that are prone to lose stability over time in powders were also assessed. This trial was undertaken in formulas fortified with Lacprodan® MFGM-10 in addition to other nutrients. The fortification of Lacprodan® MFGM-10 was 5-6 g/L in infant formula products and approximately 2.5 g/L in follow-on products (Table 2-24). These formulas were packaged in conventional cans used for commercial sales.

Table 2-24 Products used in stability study 1

Formula Type	Formulation Number	Package
Infant formula	0844	Metal Can
Follow-on formula	0845	Metal Can

Samples of stage 1 and 2 products with added Lacprodan® MFGM-10 were tested for stability of macronutrients, key fatty acids, and vitamins at two different temperature conditions, 25ºC and 60% relative humidity (Climate Zone II) and 30ºC and 65% relative humidity (Zone IV), for at least 17 to 24

months (Table 2-25). The tested nutrients were chosen as those most prone to stability issues. For expediency, some stable nutrients were not measured at 24 months under the Zone II storage condition as Zone IV is the worst case. Analytical methods are shown in Table 2-26.

Table 2-25 Stability conditions

Storage Conditions	Temperature / Relative Humidity	Analysis Time Points (Months)
Climate Zone II	25ºC/ 60% RH	0, 17, 24
Climate Zone IV	30º C/ 65% RH	0, 17, 24

Table 2-26 Assay methodologies

Parameter	Instrument	Reference
Carbohydrates	Calculated by difference as 100 g minus the sum of grams moisture, protein, fat, and ash.	FSANZ - available CHO by difference (S11—3)
Fat	Mojonnier Model D Tester for Butterfat	AOAC 989.05
Loss on Drying	Vacuum Oven, Fisher Isotemp Model 281, or equivalent	AOAC 927.05
Protein (N * 6.25)	Erba Instruments Model 1500 ANA or equivalent Thermo Flash 2000 ANA	In-house method based on Dumas Method AOAC 968.06
C18:2n6 Linoleic Acid	Agilent Technologies 7890A Gas Chromatograph	AOAC 991.39
C18:3n3 Linolenic Acid	Agilent Technologies 7890A Gas Chromatograph	AOAC 991.39
C22:6n3 DHA	Agilent Technologies 7890A Gas Chromatograph	AOAC 991.39
Vitamin E	Agilent 1100 or equivalent Liquid Chromatograph	In-house method
Vitamin K	Hewlett Packard 1050 or equivalent Liquid Chromatograph + UV Wavelength Detector	In-house method
Vitamin C, Ascorbic Acid	Hewlett Packard 1100 or equivalent Liquid Chromatograph + UV Wavelength Detector	In-house method

Analytical results for each of the formulas are presented below (Table 2-27;Table 2-28).

Table 2-27 Stability of macronutrients, key fatty acids, and vitamins in infant formula fortified with Lacprodan® MFGM-10.

Parameter	Unit of Measure	Storage Conditions	Time (Months)		
			Zero	1	24
Carbohydrate	% w/w	Zone II	56.9	57.6	N/A
		Zone IV	56.9	57.4	57.4
Fat	% w/w	Zone II	26.7	26.5	N/A
		Zone IV	26.7	26.4	26.6
Loss on Drying	% w/w	Zone II	2.04	1.74	N/A
		Zone IV	2.04	1.89	1.79
Protein (N * 6.25)	%w/w	Zone II	11.33	11.32	N/A
		Zone IV	11.33	11.38	11.23
C18:2n6 Linoleic Acid	mg/100kcal	Zone II	877	851	N/A
		Zone IV	877	851	862
C18:3n3 Linolenic Acid	mg/100kcal	Zone II	75.8	74.2	N/A
		Zone IV	75.8	74.2	74.8
C22:6n3 DHA	mg/100kcal	Zone II	16.2	16.6	N/A
		Zone IV	16.2	16.5	16.2
Vitamin E	IU/100kcal	Zone II	3.35	3.49	3.31
		Zone IV	3.35	3.33	3.78
Vitamin K	mcg/100kcal	Zone II	10.98	10.05	9.87
		Zone IV	10.98	10.59	9.81

Parameter	Unit of Measure	Storage Conditions	Time (Months)		
			Zero	1	24
Vitamin C, Ascorbic Acid	mg/100kcal	Zone II	23.9	22.5	22.1
		Zone IV	23.9	22.5	21.9

M = months, Zone II = 25°C/60% relative humidity, Zone IV = 30°C/65% relative humidity

Table 2-28 Stability of macronutrients, key fatty acids, and vitamins in follow-on formula fortified with Lacprodan® MFGM-10.

Parameter	Unit of Measure	Storage Conditions	Time (Months)		
			Zero	17	24
Carbohydrate	%w/w	Zone II	60.5	60.6	N/A
		Zone IV	60.5	60.5	60.8
Fat	%w/w	Zone II	18.5	18.5	N/A
		Zone IV	18.5	18.6	18.6
Loss on Drying	%w/w	Zone II	1.72	1.65	N/A
		Zone IV	1.72	1.74	1.58
Protein (N * 6.25)	%w/w	Zone II	15.63	15.63	N/A
		Zone IV	15.63	15.66	15.51
C18:2n6 Linoleic Acid	mg/100cal	Zone II	662	648	N/A
		Zone IV	662	635	649
C18:3n3 Linolenic Acid	mg/100cal	Zone II	57.4	56.7	N/A
		Zone IV	57.4	55.6	56.0
C22:6n3 DHA	mg/100cal	Zone I	17.4	17.7	N/A
		Zone IV	17.4	17.2	16.8
Vitamin E	IU/100cal	Zone II	2.85	3.73	3.28
		Zone IV	2.85	3.22	3.03
Vitamin K	mcg/100cal	Zone II	10.25	9.76	10.68
		Zone IV	10.25	9.96	13.43
Vitamin C, Ascorbic Acid	mg/100cal	Zone II	24.9	24.4	22.4
		Zone IV	24.9	24.3	23.7

The stability data presented in this study shows that the macronutrients, fatty acids, and vitamins studied are stable through the label claim period of 24 months at climatic conditions where these products are intended to be distributed. This study assured that addition of Lacprodan® MFGM-10 had no impact on macronutrient, fatty acid, or vitamin stability in infant and follow-on formula matrices for at least 24 months.

2.2.7.2.2 Oxidative stability in finished powder

Lipid molecules are prone to oxidation, infant and follow-on formulas containing Lacprodan® MFGM-10 were assessed for oxidative stability. The infant formula products (infant formula and follow-on formula) were studied in 3 different storage conditions (Climate Zones II and IV and an accelerated condition at 40°C and 75% relative humidity) at multiple time points up to 24 months (Table 2-29). Infat formula and follow-on formula powders were packaged in two different commercial grade metal cans (one existing and one under development) (Table 2-30).

Table 2-29 Stability conditions

Storage Conditions	Temperature/ Relative Humidity	Analysis Time Points* (Months)
Zone II	25°C/60% RH	0, 2, 3, 4, 6, 8, 9, 12, 15, 17, 18, 21, 24
Zone IV	30° C/65% RH	0, 2, 3, 4, 6, 8, 9, 12, 15, 17, 18, 21, 24
Accelerated	40°C/75% RH	0, 2, 3, 4, 6, 8

**Results reported for highlighted time points, RH = relative humidity*

Table 2-30 Packaging used in stability trials

Formula Type	Package
Infant formula	Can 1
	Can 2
Follow-on formula	Can 1
	Can 2

To determine the oxidative status of infant formula containing Lacprodan® MFGM-10, free fat, propanol, hexanal, and free oxygen were measured in these products. Table 2-31 describes the assay methodologies used for each analyte.

Table 2-31 Assay methodologies

Analyte	Instrument	Reference
Free Fat	Mojonnier oven/Air Oven and Mojonnier Hotplate/Steambath	In-house method
Propanol	Static Headspace/Gas Chromato-graphy/Flame IonizationDetector (SHS/GC/FID)	In-house method
Hexanal	Static Headspace/Gas Chromato-graphy/Flame IonizationDetector (SHS/GC/FID)	In-house method
Oxygen	PBI-Dansensor Checkmate 9900	In-house method

Concentrations of the key analytes, free fat, propanol, hexanal, and oxygen, chosen to monitor oxidative stability for the three different formulas under the two climatic conditions and the accelerated condition are reported below in Table 2-32 through Table 2-35. These data reveal that little oxidation occurs over the time periods studied.

Table 2-32 Oxidative stability in infant formula, Can1

Analyte	Unit	Storage conditions	Time (Months)							
			Zero	2	3	4	6	8	17	24
Free Fat	% w/w	Zone II	0.62	N/A	0.69	0.35	0.71	0.73	0.75	0.82
		Zone IV	0.62	N/A	0.75	0.72	0.79	0.82	0.86	0.91
		Accelerated	0.62	0.90	0.99	1.27	1.36	1.80	N/A	N/A
Propanol	ppm	Zone II	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
		Zone IV	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
		Accelerated	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	N/A	N/A
Hexanal	ppm	Zone II	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
		Zone IV	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
		Accelerated	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	N/A	N/A
O2	%	Zone II	2.29	2.18	2.01	4.72	3.51	1.07	3.35	0.178
		Zone IV	2.29	2.17	1.75	4.65	3.43	1.09	3.24	0.124
		Accelerated	2.29	2.12	1.22	4.65	3.05	0.801	N/A	N/A

Table 2-33 Oxidative stability in infant formula, Can2

Analyte	Unit	Storage conditions	Time (Months)							
			Zero	2	3	4	6	8	17	24
Free Fat	%w/w	Zone II	0.52	N/A	0.43	0.58	0.53	0.57	0.6	0.54
		Zone IV	0.52	N/A	0.34	0.54	0.56	0.58	0.7	0.66
		Accelerated	0.52	0.68	0.49	0.78	0.94	1.32	N/A	N/A
Propanol	ppm	Zone II	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05

		Zone IV	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
		Accelerated	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	N/A	N/A
Hexanal	ppm	Zone II	<0.05	<0.05	<0.05	0.05	<0.05	<0.05	<0.05	<0.05
		Zone IV	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	0.05	<0.05
		Accelerated	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	N/A	N/A
O_2	%	Zone II	2.07	2.85	1.83	3.83	2.83	0.684	3.00	0.292
		Zone IV	2.07	4.76	1.27	3.40	2.50	1.39	2.54	0.169
		Accelerated	2.07	2.85	1.23	3.35	2.23	0.811	N/A	N/A

Table 2-34 Oxidative stability in follow-on formula, Can1

Analyte	Unit	Storage conditions	Time (Months)							
			Zero	2	3	4	6	8	17	24
Free Fat	% w/w	Zone II	0.28	N/A	0.33	0.32	0.34	0.30	0.32	0.25
		Zone IV	0.28	N/A	0.37	0.31	0.35	0.30	0.35	0.34
		Accelerated	0.28	0.38	0.41	0.38	0.40	0.41	N/A	N/A
Propanol	ppm	Zone II	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
		Zone IV	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
		Accelerated	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	N/A	N/A
Hexanal	ppm	Zone II	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
		Zone IV	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
		Accelerated	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	N/A	N/A
O_2	%	Zone II	2.16	1.99	1.51	4.10	2.78	0.943	3.14	0.220
		Zone IV	2.16	2.11	1.64	3.74	3.02	0.827	3.19	0.157
		Accelerated	2.16	2.57	1.11	4.21	2.86	0.785	N/A	N/A

Table 2-35 Oxidative stability in follow-on formula, Can2

Analyte	Unit	Storage conditions	Time (Months)							
			Zero	2	3	4	6	8	17	24
Free Fat	% w/w	Zone II	0.27	N/A	0.37	0.40	0.39	0.37	0.59	0.39
		Zone IV	0.27	N/A	0.29	0.39	0.35	0.36	0.61	0.38
		Accelerated	0.27	0.44	0.25	0.46	0.49	0.41	N/A	N/A
Propanol	ppm	Zone II	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
		Zone IV	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
		Accelerated	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	N/A	N/A
Hexanal	ppm	Zone II	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	0.05	<0.05
		Zone IV	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	0.05	<0.05
		Accelerated	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	N/A	N/A
O_2	%	Zone II	2.99	2.17	1.53	4.18	3.30	1.31	2.38	0.306
		Zone IV	2.99	8.82	1.72	3.43	2.75	1.49	2.73	0.213
		Accelerated	2.99	1.59	1.60	3.27	2.69	0.744	N/A	N/A

2.2.7.2.3 Stability of sphingomyelin in finished formula powders

Lacprodan® MFGM-10 contains higher phospholipid and sphingomyelin content compared to typical WPC (Section 2.2.3.2.1; Table 2-14). Addition of Lacprodan® MFGM-10 results in distinctly higher levels of sphingomyelin compared to non-fortified formulas and can be used as one of the markers to assure Lacprodan® MFGM-10 addition. Moreover, sphingomyelin levels can be quantitatively measured using established methods in finished infant formula.

To assess sphingomyelin stability in Lacprodan® MFGM-10-fortified products, two infant formulas with Lacprodan® MFGM-10 at 6 g/L and one toddler formula Lacprodan® MFGM-10 at 2.5 g/L were analysed at 0, 6, and 18 months. During the stability study period the formulas were stored at Zone II conditions (25°C and 60% relative humidity). Sphingomyelin analysis was carried out using quantitative 31P-NMR spectroscopy according to SAA-MET002-02.

Table 2-36 summarises the results of sphingomyelin content assayed at various time points. The different values in the table clearly reflect only analytic variability, not loss of sphingomyelin content.

Table 2-36 Sphingomyelin content (mg/g) in finished formula powders

Product	Months		
	0	6	18
Infant formula	1.10	1.05	1.10
Follow-on formula	1.05	1.10	1.20

2.2.7.2.4 Conclusion

In conclusion, in a variety of stability testing protocols of analytes (macronutrients, susceptible fatty acids, and vitamins; oxidation; and sphingomyelin content), at different packaging and storage conditions, infant and follow-on formulas containing Lacprodan® MFGM-10 showed acceptable stability of all parameters assessed.

2.2.8 Analytical method for detection

It is the presence of the elevated levels of MFGM phospholipids and sphingolipids that assigns Lacprodan® MFGM-10 its primary identity.

Several methods for the analysis of phospholipids are available. Arla Foods Ingredients P/S uses and recommends the use of $_{31}$P NMR (MacKenzie et al., 2009; Murgia et al., 2003). Phospholipid analysis is completed for Lacprodan® MFGM-10 by Spectral Services (Spectral Service, Köln, Germany). The $_{31}$P NMR method is recommended as the preferred method to measure total PLs including SM in milk-based matrices.

Spectral Service uses the soxhlet extraction procedure prior to $_{31}$P NMR analysis, which appears to have a better recovery for individual PL components, specifically SM (Figure 2-26).

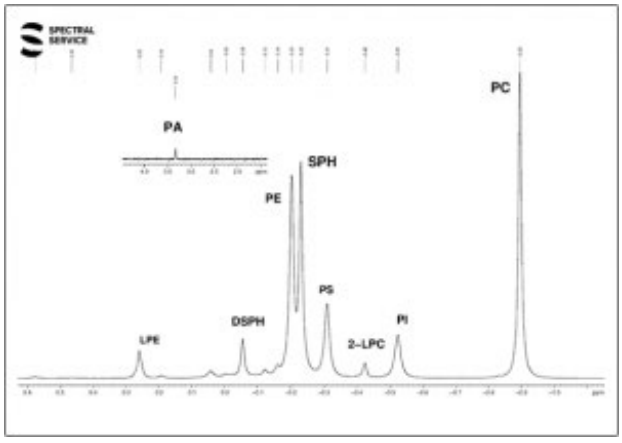
https://www.spectralservice.de/phospholipid-analysis-by-31p-nmr-spectroscopy/

In New Zealand, Callaghan Innovation routinely uses $_{31}$P NMR for the analysis of phospholipids in dairy products based on MacKenzie et al. (2009) on a contract laboratory basis.

It is noted the recently published Chinese Light Industry Standard (QB/T 5805-2023) uses HPLC-ELS for the identification and quantification of phospholipids including SM, and HPLC MS/MS for the quantification of gangliosides.

Quantification of PL by HPLC MS/MS has also been used.

Figure 2-26 Typical 31P-NMR spectrum of a milk sample, detail (phospholipids)(Spectral Services)



AOAC has established Standard Method Performance Requirements (SMPRs®) for the determination of phospholipids in infant and adult/paediatric nutritional formula (AOAC SMPR® 2021.017) (AOAC International, 2022). The scope of these requirements covers the quantitative determination of nutritionally relevant PL including PC, PE, PI, PS and SM. Any analytical method that meets the performance requirements is acceptable for the determination of PL in infant and adult nutritional products.

Given the complexity of Lacprodan®MFGM-10 composition, it is important that a marker protein or lipid is designated to assure the consistency of raw material from batch to batch and to assure incorporation into infant formula. For this nutritive substance, sphingomyelin is proposed as the identifying component for the following reasons:

i. Sphingomyelin is one of the major phospholipids present in MFGM fractions.
ii. Standardised analytical methods are available to assay SM in both the ingredient (Lacprodan® MFGM-10) and finished IFP matrices.
iii. Sphingomyelin is present in very low amounts in conventional protein fractions (WPC), not present in vegetable oils or lecithin and hence easily quantifiable in finished infant formula.
iv. Sphingomyelin levels of IFP made with whole milk are markedly less than the levels of SM found in IFP with added Lacprodan® MFGM-10.

2.2.9 Information on the proposed food label

2.2.9.1 Ingredient listing

A number of options are proposed as to how Lacprodan® MFGM-10 may be listed in the ingredients list of IFP:

- Lacprodan® MFGM-10 (**milk**)
- Whey protein phospholipid concentrate (**milk**)
- Phospholipid enriched whey protein concentrate (**milk**)
- Milk fat globule membrane enriched whey protein concentrate (**milk**)
- MFGM enriched whey protein concentrate (**milk**)
- Whey protein concentrate (containing milk fat globule membrane) (**milk**)
- Whey protein concentrate (containing MFGM) (**milk**)
- Whey protein concentrate (**milk**)* (* a source of MFGM)
- Whey protein concentrate (**milk**)* (* a source of milk fat globule membrane)

These options accurately reflect the nature of Lacprodan® MFGM-10.

2.2.9.2 Quantification in Nutrition Information Panel (NIP)

The identification of Lacprodan® MFGM-10 in the nutrition information panel (NIP) is proposed to be optional.

If included sphingomyelin is the MFGM component for quantification and should be included with other optional nutritive substances that are listed as any of the following nutrients:

- Sphingomyelin from MFGM
- Sphingomyelin from milk fat globule membrane
- MFGM sphingomyelin
- Milk fat globule membrane sphingomyelin
- Sphingomyelin* (* from MFGM)
- Sphingomyelin* (* from milk fat globular membrane)

This is in accordance with NIP guidelines in the FSC Schedule 29 29-10 (3). By way of example the proposed NIP format is provided in

Table 2-37. Sphingomyelin has been highlighted for convenience.

2.2.9.3 Plain English Allergen Labelling (PEAL)

Lacprodan® MFGM-10 is a dairy ingredient therefore is subject to the requirements of mandatory allergen labelling (Standard 1.2.3 Information requirements – warning statements, advisory statements and declarations).

Lacprodan® MFGM-10 must be labelled as the allergen **milk** in the ingredients list as shown above (Section 2.2.9.1)**.** Plain English allergen labelling (PEAL) requirements also require a separate summary statement.

Contains: milk

A sample Nutrition Information Statement (NIS) is shown inTable 2-37. This has been prepared based on proposed updates to the format of this information as outlined in P1028 – Infant formula (S29—10 Required format for a nutrition information statement in Attachment A – Draft variations to the Australia New Zealand Food Standards Code 2nd Call for Submissions – Proposal P1028). Sphingomyelin has been bolded for ease of identifying the line.

Table 2-37 Proposed NIS format

Nutrition Information	
	Average amount per 100
Energy	kJ
Protein	g
- Whey	g
- Casein	g
Fat	g
- Long chain polyunsaturated fatty acids	mg
- Docosahexaenoic acid	mg
- Eicosapentaenoic acid	mg
- Arachidonic acid	mg
Carbohydrate	g
- Lactose	g
- Galactose	g
Vitamins	
Vitamin A	µg
Vitamin B_6	µg
Vitamin B_{12}	µg
Vitamin C	mg
Vitamin D	µg
Vitamin E	µg
Vitamin K	µg
Biotin	µg
Niacin	mg
Folate	µg
Pantothenic acid	µg
Riboflavin	µg
Thiamin	µg
Minerals	
Calcium	mg
Copper	µg
Iodine	µg
Iron	mg
Magnesium	mg
Manganese	µg
Phosphorus	mg
Selenium	µg
Zinc	mg
Chloride	mg
Potassium	mg
Sodium	mg
Other nutrients	
Choline	mg
Inositol	mg
L-carnitine	mg
Additional	
Sphingomyelin (from MFGM)	mg

2.3 Data related to the safety of Lacprodan® MFGM-10

2.3.1 Information on the toxicokinetics and metabolism of Lacprodan® MFGM-10

Specific studies on the toxicokinetics of Lacprodan® MFGM-10 have not been undertaken. The ingredient is a complex mixture of components, of which the major groups of components (lipids and proteins) are discussed below.

The lipid fraction of MFGM contains polar lipids contains glycerophospholipids, sphingolipids (including sphingomyelin) and glycolipids (including gangliosides), cholesterol and triglycerides (Singh, 2006).

2.3.1.1 Absorption, distribution, metabolism and excretion of specific MFGM components

2.3.1.1.1 Phospholipids

Phospholipids comprise approximately 6% of the mass of MFGM; however, the majority of the phospholipid in milk is in the MFGM (Lee, Padhi, et al., 2018). Milk phospholipid digestion and absorption has been studied, with several some studies on the digestion and absorption of isolated phospholipid components.

Milk phospholipids are cleaved into monoglycerides, free fatty acids, and lyso-phospholipids by a variety of lipases, including lingual lipase, gastric lipase, pancreatic lipase, colipase, phospholipase A2, and BSSL (Å Nilsson & Duan, 2019). The main products of glycerophospholipid digestion in the luminal phase of digestion are the 1-acyle-phosphatidyl (lyso-phospholipid) compounds and these are the primary forms in which the glycerophospholipds are absorbed from the intestinal tract (Borgström, 1974).

The digestion and absorption of phosphatidylcholine has been extensively studied (Borgström, 1974; Å Nilsson & Duan, 2019; Parthasarathy, Subbaiah, & Ganguly, 1974). In the proximal small intestine, phosphatidyl choline is initially hydrolyzed to 1-lyso-phosphatidyl choline and free fatty acids by the pancreatic phospholipase A2 Ib. Lyso-phosphatidyl choline can be absorbed into mucosal cells or hydrolyzed further by jejunoileal brush border phospholipase B/lipase and mucosal-secreted phospholipase A2. Both pancreatic and mucosal phases of phosphatidylcholine digestion are mediated by the combined action of secretory pancreatic phospholipase A2 IB (sPLA2 IB) and secretory pancreatic phospholipase A2 IB (sPLA2 IB) (Åke Nilsson, Duan, & Ohlsson, 2021; Å Nilsson & Duan, 2019). Absorbed lyso-phosphatidyl choline is partitioned in the mucosal cells between degradation and re-acylation into chylomicron phosphatidyl choline (Å Nilsson & Duan, 2019). There is little or no absorption of intact phosphatidyl choline (Parthasarathy et al., 1974).

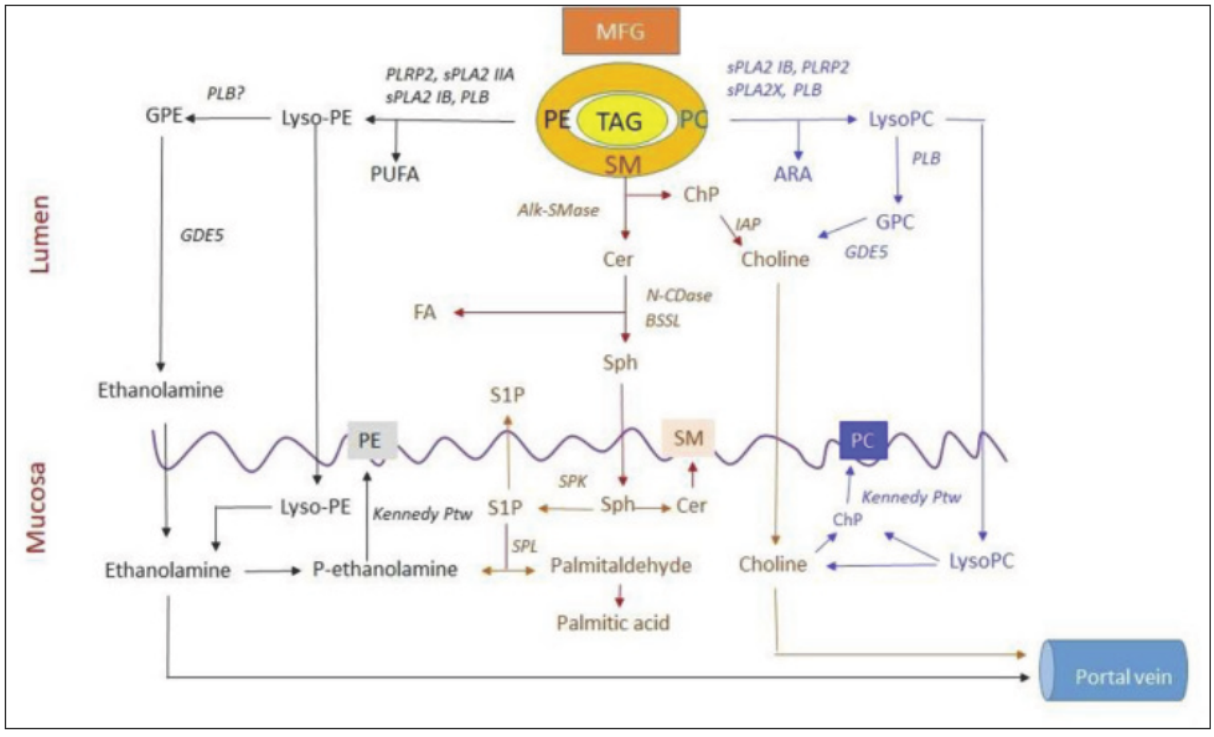
More recently Åke Nilsson et al. (2021) have reviewed the digestion and absorption of milk phospholipids in the neonate, demonstrating that the fate of the different phospholipids follow similar digestion and absorption processes to phosphatidyl choline, albeit producing different metabolites (Figure 2-27). The dialogue to Figure 2-27 of Åke Nilsson et al. (2021) provides an excellent overview of the digestion and absorption process:

"*Lipids in the milk are organized in the milk fat globule (MFG) with polar lipids in the membrane and triacylglycerol (TAG) in the core. The secretory pancreatic phospholipase A2 IB (sPLA2 IB), sPLA2, and sPLA2X hydrolyze phosphatidylcholine (PC) to lysoPC and may release arachidonic acid (ARA) for*

both phospholipid (PL) and prostaglandin (PGD) synthesis. Phospholipase B (PLB) can degrade both PC and lysoPC to glycerophosphocholine (GPC) and then by GDE5 to choline. Both lysoPC and choline are absorbed. Absorbed lysoPC may release choline that can, in turn, be phosphorylated to choline phosphate (ChP) in mucosal cells. Sphingomyelin (SM) is sequentially hydrolyzed by alkaline SMase (alk-SMase), neutral ceramidase (N-CDase), and bile salt-stimulated lipase (BSSL) to ceramide (Cer), sphingosine (Sph), and fatty acid (FA). The cleaved ChP is degraded to choline by intestinal alkaline phosphatase (IAP). Then, Sph, choline, and FA are absorbed. The Sph in mucosa may be phosphorylated by sphingosine kinase (SPK) to S1P, most of which is degraded by sphingosine lyase (SPL) to palmitaldehyde and further converted into palmitic acid. This reaction generates ethanolamine phosphate (ethanolamine-P). Both ethanolamine and choline are substrate for synthesis of phosphatidylethanolamine (PE) and PC, respectively, via the Kennedy pathway or transported via portal vein. PE is degraded by sPLA2 IB, sPLA2 IIA, PLRP2, and PLB to lysoPE for absorption. LysoPE can be degraded by PLB to glycerophosphoethanolamine (GPE) and then to ethanolamine likely by GDE5 for absorption and resynthesis of PE. Meanwhile, polyunsaturated fatty acid (PUFA) can be formed during PE hydrolysis."

There is little to no absorption or excretion of the intact phospholipids.

Figure 2-27 Digestion and absorption of the major polar lipids in milk



from Åke Nilsson et al. (2021)

Restoration of dietary phospholipid exposure in formula fed infants to levels of breastfed infants would be expected to follow similar digestion and metabolic pathways to the phospholipids in breast milk, producing comparable direct and indirect effects in the GI tract.

2.3.1.1.1.1 Effect of MFGM phospholipids on lipid digestion and absorption

Because MFGM acts as an emulsifier of milk fat (He et al. 2017), it may increase digestion of triacylglycerols (Luo et al., 2019). Berton et al. (2012) found that bovine MFGM improves the efficiency

of lipid digestion by human pancreatic lipase *in vitro*. Pancreatic lipase was more active against small milk fat globules than large ones, and the 25-fold increase in globule surface area brought about by homogenization resulted in a 2-fold increase in lipid digestion. The investigators attributed the enhancement of lipid digestion to the MFGM proteins (Berton et al., 2012).

Lecomte et al. (2015) compared the effects milk polar lipids (MPLs) to soy polar lipids (SPLs) on lipid digestion in an *in vitro* model and on lipid absorption in an *in* vivo murine model. They found an emulsion stabilised by MPLs enhanced lipid hydrolysis resulting in more rapid postprandial fat absorption in mice compared to an emulsion stabilised by SPLs. Mice fed with MPLs showed higher plasma TGs and NEFAs at 1 hour compared to mice fed with SPLs, indicating faster gastric emptying and intestinal absorption for MPL-fed mice (Lecomte et al., 2015). Similarly milk phospholipids enhanced lipid intestinal hydrolysis and promoted more rapid intestinal lipid absorption and sharper kinetics of lipemia than soy phospholipids (Mathiassen et al., 2015).

Michalski, Briard, Michel, Tasson, and Poulain (2005) compared the fat globule sizes of colostrum and breast milk through 4 days postpartum to that if infant formula finding a significant difference. The fat globule size of early breast milk being significantly larger than that of infant formula. Michalski et al. (2005) suggested the size and structure of fat globules in human milk may play a role in the digestion and absorption of lipids in neonates and that this is likely be a function of the MFGM.

Bach Korsholm Knudsen et al. (2021) investigated if emulsification of the lipid components of a diet in neonatal piglets differed between bovine milk derived emulsifiers containing various MFGM components and soy lecithin (SL), and any difference in lipid digestion occurred. Initially, lipid digestibility was determined *in vitro* in oil-in-water emulsions using four different milk-derived emulsifiers or SL and by measuring the degree of hydrolysis. Electron microscopy was used to assess the ultrastructural appearance of the emulsions. In the first of 3 *in vivo* trials, selected emulsions were added to a base diet and fed to preterm neonatal piglets. Initially, preterm pigs equipped with an ileostomy were fed experimental formulas for seven days and stoma output was collected quantitatively. Next, lipid absorption kinetics was studied in preterm pigs given pure emulsions. Finally, complete formulas with different emulsions were fed for four days, and the post-bolus plasma triglyceride level was determined. Milk-derived emulsifiers (containing protein and phospholipids from milk fat globule membranes and extracellular vesicles) showed increased effects on fat digestion compared to SL in an *in vitro* digestion model. Further, milk-derived emulsifiers significantly increased the digestion of triglyceride in the preterm piglet model compared with SL. Ultra-structural images indicated a more regular and smoother surface of fat droplets emulsified with milk-derived emulsifiers relative to SL. Relative to SL, milk-derived emulsifiers resulted in a different surface ultrastructure on the lipid droplets, and increased lipid digestion (Bach Korsholm Knudsen et al., 2021).

Using an *in vitro* model, T. Wei, Wu, Sun, Deng, and Li (2023) showed the inclusion of human milk phospholipids analogues in formula prevented hydrolysis of the structured fat triglyceride OPO (oleic-palmitic-oleic fatty acids on the glycerol backbone) during the *in vitro* gastric phase, resulting in the production of large amounts of diglycerides (DAGs) and monoglycerides (MAGs). *In vivo* experimental results showed that the phospholipid analogues may also then increase the gastric emptying rate of OPO and increase the hydrolysis and absorption of OPO at an early stage of intestinal digestion (T. Wei et al., 2023). The effect of this delay in fat hydrolysis may influence the maintenance of high serum lipid levels that in turm may be beneficial for sustainably providing energy for babies.

The role of the MFGM and its components on the digestion and absorption kinetics of lipids within the milk fat globule is complex, influencing not only the size and structure of milk fat globules, but also providing a number of components that participate in the digestive processes (Bourlieu & Michalski, 2015; Singh & Gallier, 2017).

2.3.1.1.1.2 Effect of MFGM phospholipids digestion products on microbiome and intestinal development

Feeding milk-based phospholipids (Lee, Zavaleta, Chen, Lonnerdal, & Slupsky, 2018; Nejrup, Licht, & Hellgren, 2017; Ortega-Anaya & Jiménez-Flores, 2019), enriched preparations of gangliosides (Rueda, Maldonado, Narbona, & Gil, 1998), or sphingomyelin (Norris, Jiang, Ryan, Porter, & Blesso, 2016) has been reported to normalize the gastrointestinal microbiome to resemble that of naturally fed animals, a finding which has been confirmed in clinical studies (He, Parenti, Grip, Lonnerdal, et al., 2019; Rueda, Sabatel, Maldonado, Molina-Font, & Gil, 1998). Microbiome effects of feeding MFGM preparations may be related to the glycoprotein moiety of MFGM (Guerin et al. 2019) or the persistence of sphingolipids throughout the GI tract (Larson, Falk, Hynsjö, Midtvedt, & Midtvedt, 1990). MFGM components, digested or incompletely digested may promote infant GI development directly and indirectly, thereby normalising intestinal development and function (R. C. Anderson, MacGibbon, Haggarty, Armstrong, & Roy, 2018; Bhinder et al., 2017; Huërou-Luron, Lemaire, & Blat, 2018; Norris, Milard, Michalski, & Blesso, 2019). He, Parenti, Grip, Lonnerdal, et al. (2019) provided further clinical evidence that the MFGM may have a role in the modulation of microbiota activity and function. In a study on to determine if MFGM supplementation in infant formula influence favourable changes in metabolism and gut microbiota to elicit benefits observed in prior studies Lee et al. (2020) found whilst MFGM supplementation did not induce significant compositional changes in the faecal microbiota it did suppress microbial diversity and altered microbiota-associated metabolites.

2.3.1.1.2 Sphingomyelin

Unlike the phospholipids, sphingomyelin (SM) is hydrolyzed by brush border alkaline sphingomyelinase (SMase) cleaving the phosphocholine head from SM to produce ceramide (Duan & Nilsson, 2000; Åke Nilsson et al., 2021). The ceramide is then hydrolysed by neutral ceramidase to sphingosine and free fatty acids, which are well absorbed (Nilsson and Duan 2018) (Figure 2-27). The key SMase responsible for digestion of SM in the gut is alkaline SMase (alk-SMase), which is attached to the surface of the intestinal brush border by a short intracellular domain with its active catalytic site exposed in the intestinal lumen (Cheng, Nilsson, Tömquist, & Duan, 2002; Liu, Nilsson, & Duan, 2000; Zhang et al., 2011).

In the intestinal tract, the total amount of glycerophospholipids is much higher than that of SM. These phospholipids may function as inhibitors of SM hydrolysis induced by alkaline SMase, delaying the digestion of SM until most of the phospholipids have been hydrolyzed by phospholipases and their products such as fatty acids, diacylglycerols, and lysophospholipids have been absorbed. Liu et al. (2000) suggested this may explain why hydrolysis of SM occurs mainly at the distal part of the jejunum and intact SM can be found in the colon and faeces even when fed in small amounts. Larson, Watsfeldt, Falk, Leffler, and Koprowski (1987) showed that faecal excretion of sphingolipids including sphingomyelin, may persist in neonates and young children up to 2 years of age. Later studies suggested a relationship between the persistence these compounds and the establishment of the microbiota. In particular, non-pathogenic species functionally specialised in degrading oligosaccharide chains.

There is little or no absorption of intact sphingomyelin (Duan & Nilsson, 2000).

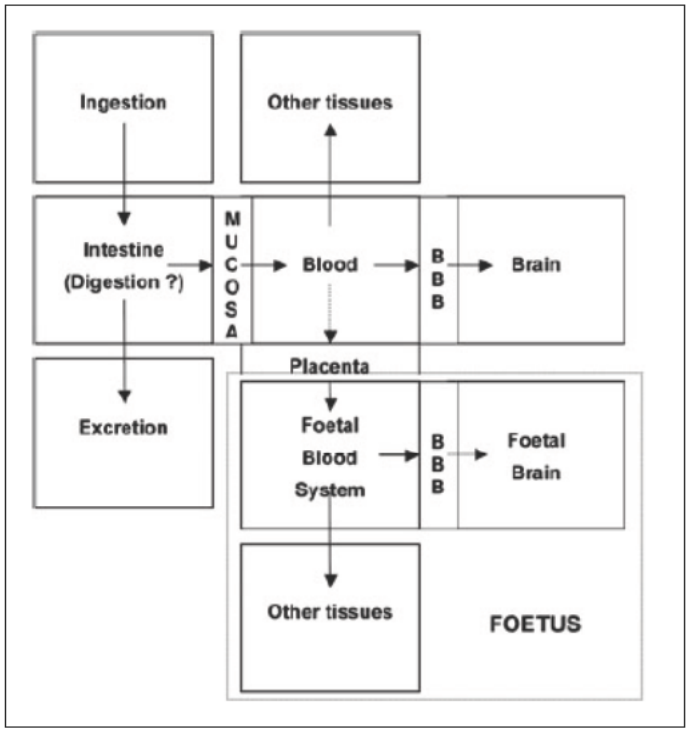
2.3.1.1.3 Gangliosides

The most abundant ganglioside in human milk is GM3 whereas GD3 is the predominant ganglioside in bovine milk (Laegreid et al., 1986).

Human milk gangliosides, GM3 and GD3, are not digested during the gastric phase reaching the intestinal region of the gastrointestinal tract intact and able to be absorbed in the small intestine for transport to different membrane sites in the body (McJarrow et al., 2009). The ability to gangliosides to be taken up by human intestinal cells and its metabolic fate determined by the site of uptake was shown by Schnabl, Larcelet, Thomson, and Clandinin (2009), using a human cell line. Saqr, Pearl, and Yates (1993) showed *in vitro* exogenous intact gangliosides could be taken up by a range of biological cells including blood cells enabling transport throughout the body to different tissues.

In rats fed a GD3 enriched diet for 2-weeks, Eek Joong Park, Miyoung Suh, et al. (2005) showed dietary gangliosides were absorbed in the small intestine and transported to various membrane sites. Changes in ganglioside levels were observed in the intestinal mucosa plasma and brain. Furthermore dietary gangliosides were shown to increase total retinal ganglioside and GD3 content during retinal development (Park, Suh, & Clandinin, 2005). Based on this work the research team concluded that the dietary availability of gangliosides may impact on the lipid composition of developing tissues and hence the may be biologically important (Eek Joong Park, Miyoung Suh, & M. Thomas Clandinin, 2005; Eek Joong Park, Miyoung Suh, et al., 2005). The study demonstrated that dietary gangliosides can be absorbed and distributed to tissues intact. McJarrow et al. (2009) summarised the uptake of dietary gangliosides as shown in Figure 2-28.

Figure 2-28 Uptake of dietary gangliosides by the body



from McJarrow, Schnell, Jumpsen, and Clandinin (2009)

Two metabolic fates are known for exogenous gangliosides (McJarrow et al., 2009):

i. In the Golgi body direct glycosylation resulting in higher homologous gangliosides or modified gangliosides may occur.
ii. In the lysozymes gangliosides may be catabolised to intermediates of both the saccharide and lipid components, and available for further distribution and metabolism.

From the Golgi body gangliosides enter the plasma membrane where they are assembled into microdomains consisting of cholesterol and sphingolipid-rich caveolae and rafts (McJarrow et al., 2009). E. J. Park et al. (2005) showed dietary gangliosides may be directly incorporated into the microdomains.

Larson et al. (1990) investigated the excretion of gangliosides in a small cohort of breast-fed and formula-fed infants. Whilst GM3 was detected only in the faeces of breast-fed infants, GD3 was present in the faeces of all infants through to 3 months, irrespective of feed type. There was a progressive reduction in faecal GD3 of breast-fed infants through 6 months, in contrast to children not receiving breast milk where no GD3 was detected at 6 months and beyond (Larson et al., 1990). This study provides that gangliosides are not completely digested and may be excreted intact. The relative amounts of dietary gangliosides that are excreted intact, to those that are absorbed or metabolised is unknown (McJarrow et al., 2009).

2.3.1.1.4 MFGM proteins

Kobylka and Carraway (1973) were among the first to study *in vitro* digestion of proteins in MFGM preparations. They showed comparable susceptibility to proteolysis of proteins in MFGM and proteins in fresh cream. The study used both trypsin and pronase to probe whether the membrane was intact with 'sidedness'; that is, whether membranes were intact with an inside that was distinct from an outside. There was no reduction of activity in any proteins in fresh cream compared to MFGM, which the investigators interpreted to mean that there is no barrier to proteolysis of MFGM proteins in the intact fat globule. Kobylka and Carraway (1973) contrasted these results to red-cell membranes, which remain intact through similar purification steps, limiting proteolysis of internal facing membrane proteins. While trypsin-mediated digestion appeared less active toward MFGM glycoproteins, pronase (which contains phospholipase A), showed activity against glycoproteins comparable to that against non-glycosylated proteins. These results indicate that there is no alteration in digestibility of MFGM proteins resulting from preparation of the MFGM material.

As information accumulated about the composition and structure of MFGM, methods also became more precise. Vanderghem et al. (2011)) used an enzymatic approach similar to that employed by Kobylka and Carraway (1973), extending the study to individual proteins of MFGM with two-dimensional gel electrophoresis and mass spectrometry (Vanderghem et al., 2011). These investigators determined that lactadherin, butyrophilin, and adipophilin were surface proteins readily digested (though a portion of lactadherin resisted digestion, possibly because of surface carbohydrate). Xanthine dehydrogenase/xanthine oxidase was distributed in two pools, some on the surface and some deeply embedded in the membrane (resistant to proteolytic attack), and the fatty-acid binding protein was also embedded. As these five proteins are among the six most abundant proteins in MFGM, they give a good first-order representation of the digestion of the approximately 191 proteins that have been detected in MFGM in bovine and human milk (Affolter, Grass, Vanrobaeys, Casado, & Kussmann, 2010; Cavaletto et al., 2008; B. Y. Fong & Norris, 2009; Liao et al., 2011; Reinhardt & Lippolis, 2006) and caprine milk (Juvarajah et al., 2018).

Aiqian Ye, Cui, and Singh (2010) and A. Ye, Cui, and Singh (2011) also used a proteolytic approach and two-dimensional electrophoresis to identify MFGM proteins but used pepsin to simulate gastric digestion (A. Ye et al., 2011) and pancreatic lipase to simulate upper intestinal tract digestion (Aiqian Ye et al., 2010). Xanthine oxidase was more readily lost to gastric digestion than butyrophilin or lactadherin, though comparable amounts of each were digested over the *in vitro* incubation period (A. Ye et al., 2011).

A further application of enzymatic study with two-dimensional gel electrophoresis for identification of individual MFGM proteins (Le et al., 2012) used a mix of enzymes (pepsin, trypsin, α-chymotrypsin, and pancreatin) to simulate human digestion. Mass spectrometry was used to identify resistant proteins. This study found that fatty acids were needed to protect PAS6/7 (lactadherin) from digestion, and that MUC-1 resisted initial digestion by pepsin and some proteins also resisted subsequent digestion by trypsin (Le et al., 2012).

Chatterton et al. (2004) reported comparative *in vitro* digestion of human milk and infant formula using human neonatal gastric juice as the source of hydrolytic enzymes. The study was not directed specifically toward MFGM proteins, but the investigators reported that bovine lactadherin was stable to digestion at pH 4 and above, similar to human lactadherin. Some bovine proteins were more susceptible to digestion than their human milk counterparts. For example, human milk lactoferrin was resistant to the effects of digestion at pH 4 and above, whereas bovine lactoferrin was not hydrolyzed at pH 5.0 but was hydrolyzed below pH 5.0. Western blots using antibodies to human MUC-1 showed resistance of human milk MUC-1 to digestion even at pH 2, consistent with the *in vitro* digestion result using non-human enzymes. These results indicate similar vulnerability to digestion of MFGM proteins and other milk proteins and the similarity of digestion of human and bovine protein homologs.

Peterson et al. (1998) measured the immunoreactive levels of some major MFGM proteins in gastric aspirates after preterm infants were fed their mothers' milk. Significant amounts of mucin and lactadherin were found even 4 hours after feeding, while butyrophilin was rapidly degraded in most aspirates. Mucin and lactadherin survived at all gastric pH values, whereas butyrophilin was found only at pH > 4. MUC-1 has been detected in stools of breastfed infants (Hamosh et al., 1999), so incomplete gastric digestion of some MFGM proteins may represent the physiologic normal state. Dallas et al. (2014) studied production in the stomach of peptides from native proteins in human milk-fed infants. Two hours after feeding, there was an increase in the number of peptides produced from xanthine oxidase and lactadherin but not MUC-1, replicating the findings from *in vitro* studies. The lactadherin results are consistent with the results from Peterson et al. (1998) on preterm infants and the findings for butyrophilin and MUC-1 replicate findings from numerous *in vitro* studies of gastric and intestinal digestion of major MFGM proteins.

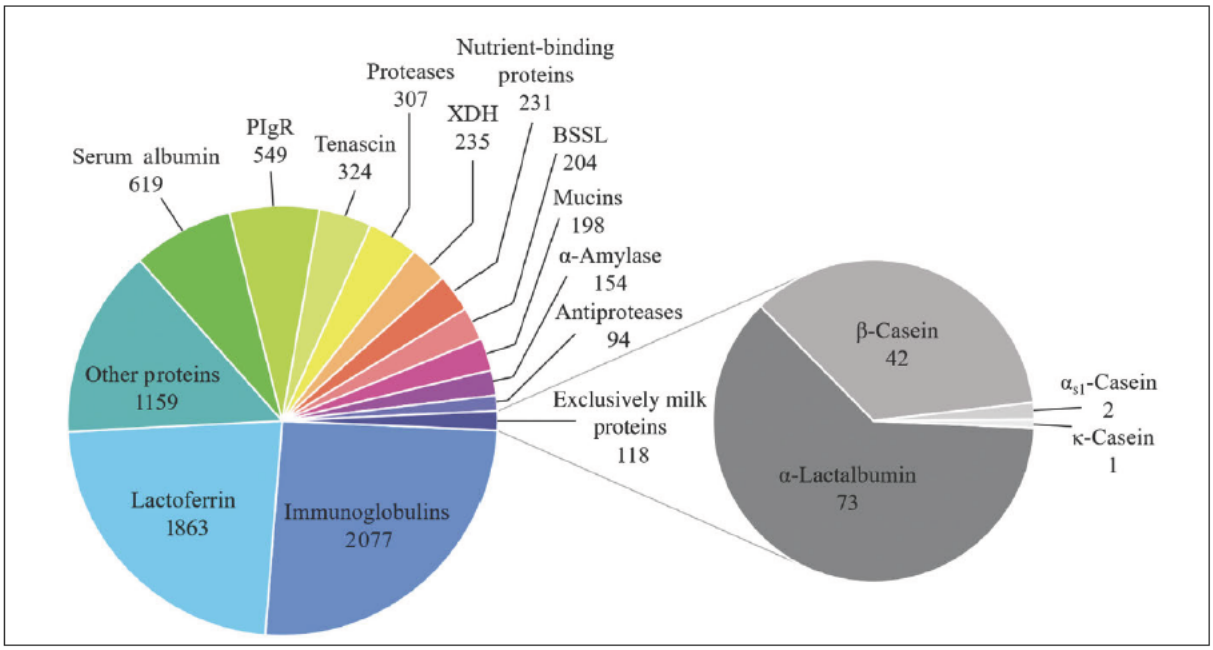
Few studies detail the excretion of protein metabolites in infants. Beverly et al. (2020) first reported the potential of milk peptides to survive digestion and to reach the stools of breastfed and formula-fed pre-term (< 34 weeks gestational age; n = 16) and term (> 34 weeks gestational age; n =10) infants. Stool samples were collected from preterm infants at 8/9 days of life (DOL) and/or 21/22 DOL, and from term infants at 8/9 DOL. In total 8132 peptides derived from 169 unique peptides previously identified in human milk were identified, however the majority of the peptides could potentially also be from endogenous sources (Beverly et al., 2020). There were 118 peptides derived from proteins exclusive to milk: 73 peptides from *α*-lactalbumin, 42 from *β*-casein, 2 from *αs*1-casein, and 1 from

κ-casein. Of the remaining peptides that are potentially derived from milk, lactoferrin was the single largest contributor with 1863 peptides Figure 2-29. Xanthine oxidase is a major MFGM protein and peptides derived from both xanthine oxidase and xanthine dehydrogenase are collectively identified within the peptides that may not be exclusive to milk proteins (Figure 2-29). The peptides identified were relatively well conserved across the infant age and type of feed. Supplemental data of Beverly et al. (2020) identified several peptides that may have been derived from the major MFGM proteins (m adherin (9); butyrophilin (18); FABP (7). This is consistent with the earlier *in vitro* work of Dallas et al. (2014), Hamosh et al. (1999) and Peterson et al. (1998).

Beverly et al. (2020) confirmed peptides from the digestion of milk proteins persist through to excretion in infant faeces and may retain some of their potential bioactive properties. This is also a likely fate for proteins associated with the MFGM.

From the *in vitro* and *in vivo* digestion studies, it appears that while there are some minor differences in the digestion of MFGM proteins from cows and human milk, there are marked similarities in the rank order and pH sensitivity to digestion of human and bovine homologs of MFGM proteins. Furthermore, that the digestion and metabolism of proteins in the infant results in a significant number of peptides excreted.

Figure 2-29 Milk peptides identified in infant stools



from Beverly et al. (2020)

Numbers underneath peptide names represent the unique peptides identified from all infant stool samples (n = 33). BSSL, bile salt-stimulated lipase; PIgR, polymeric Ig receptor; XDH, xanthine dehydrogenase/oxidase.

2.3.2 Information on the toxicity of MFGM

The essential nature of MFGM in breast and bovine milks, together with a long history of safe consumption of these components indicates a lack of toxicological concern. Safety studies identified by AFI in the scientific literature relate to the safety and tolerance of MFGM in formulations fed to

animals and human infants. Arla Foods Ingredients P/S is not aware of studies on MFGM that have investigated acute, short-term, and long-term toxicity and carcinogenicity, nor studies on reproductive toxicity, developmental toxicity, genotoxicity and immunotoxicity. Arla Foods Ingredients P/S does not consider these studies are required to establish the safety of MFGM in the context of incorporating the ingredient into infant formula products.

2.3.3 Potential allergenicity of MFGM

No new dairy proteins or components are introduced to infant formula products with the use of Lacprodan® MFGM-10.

Lacprodan MFGM-10 is manufactured from dairy streams (e.g. whey) that are already extensively and normally used for the manufacture of IFP. Additionally, the use of whole milk is not uncommon in the production of IFP. The manufacturing process for Lacprodan® MFGM-10 does not include any processing technologies or processing aids that change the chemical or physical properties of the protein components beyond that of standard dairy processing technologies. On this basis the potential allergenicity of MFGM is within the existing set of potential allergens from bovine milk to which infants consuming bovine-based or formula containing bovine milk derived ingredients will already be exposed to.

Like other dairy and dairy derived ingredients, the use of Lacprodan® MFGM-10 in IFP for specific dietary use in relation to cow's milk protein allergy (CMPA) is inappropriate.

2.3.4 Safety assessment reports prepared by international or national government agencies

Arla Foods Ingredients P/ S is not aware of any safety assessment reports by international or national government agencies.

In China the Chinese Society of Food Science and Technology produced a Scientific Consensus on Milk Fat Globule Membrane and Its Ingredients (Chinese Institute of Food Science and Technology, 2022) based on a literature review (1965 to January 2022). The review concluded that based on the extensive international use of MFGM ingredients in IFP globally, together with clinical trials reporting safety and tolerability there was no concern for the use of MFGM ingredients in IFP, and that MFGM ingredients are safe and well tolerated by infants.

2.4 Information on the dietary intake of the nutritive substance

2.4.1 Food and food groups proposed to contain Lacprodan® MFGM-10

This application is for the addition of Lacprodan® MFGM-10 to infant formula products (IFP only) as regulated under Part 2.9 Special purpose foods, of the Food Standards Code, specifically Standard 2.9.1 Infant formula products, including infant formula (birth to 6 months, or birth to 12 months), follow-on formula (6 to 12 months) and infant formula products for special dietary use (IFPSDU).

2.4.2 Proposed levels permitted in infant formula products

The proposed permissible addition rate of Lacprodan® MFGM-10 as 110 to 280 mg / 100 kJ of product as consumed. This equates to the addition of Lacprodan® MFGM-10 of between 4 to 7 g / L and allows for the regulatory limits for energy in IFP (minimum 2500 kJ/L for IF and FO; maximum 3150 kJ/L IF and 3550kJ/L FO). As the addition of Lacprodan® MFGM-10 as an ingredient cannot be quantified by analysis, sphingomyelin is proposed as the quantifying analyte by which to set the permitted levels (Table 2-38). Although there is a potentially a low baseline level of SM in non-enriched formula, the levels proposed in the specification can only be achieved with the addition of Lacprodan® MFGM-10, or potentially with other MFGM-based ingredients if they were permitted for addition. This application seeks only for permission to add Lacprodan® MFGM-10 manufactured by Arla Foods Ingredients P/S.

Table 2-38 Proposed specification for sphingomyelin levels in IFP (If, FOF & IFSMP)

Substance	***Permitted forms***	***Minimum amount per 100 kJ***	***Maximum amount per 100 kJ***
Sphingomyelin	Sphingomyelin	1.8 mg	7.5 mg

The proposed sphingomyelin levels are consistent with the range of SM levels found in breastmilk, and based on the addition rates used in clinical studies with Lacprodan® MFGM-10 used to enrich the MFGM content of formula (Table 2-39), with allowance for some natural contribution from other standard dairy ingredients used in the manufacture of IFP.

Table 2-39 Comparison of proposed levels of SM in human milk, intervention studies and made-up formula

Context	***Minimum amount***	***Maximum amount***
Proposed limits (mg/100kJ)	1.8	7.5
Typical concentration in human milk [a] (mg/L)	31	208
Estimated SM concentration of non MFGM enriched IFP (mg/L)[b]	60	99
Estimated addition of MFGM to IFP based on addition of 4-7 g/L Lacprodan® MFGM-10 (mg/L)	72	126

Context	*Minimum amount*	*Maximum amount*
Estimated total SM concentration of MFGM-10 enriched IFP (mg/L)	132	225
SM concentration in enriched formulas of intervention studies (mg/L)	84	141
Equivalent concentration range in made up formula based on proposed range (mg/L)[c]	49	204

[a] *Cilla et al. (2016)*
[b] *Claumarchirant et al. (2016)*
[c] *Based-on estimated energy intakes for infant formula products (Food Standards Australia New Zealand (FSANZ), 2016)*

2.4.3 Information on the likely levels of consumption of infant and follow-on formula

Typical consumption levels of infant formula products have been identified previously by FSANZ (Food Standards Australia New Zealand (FSANZ), 2016) as 0.8 L/day for infants from birth to ≤ 6 months and 0.6 L/day for infants 6 to ≤12 months, which is based on typical consumption of breastmilk of 0.8 L/day and 0.6 L/day, respectively (National Health and Medical Research Council, 2006). This assumes that an infant no longer receives breastmilk and is solely fed formula. In addition, infants 6 to ≤ 12 months would typically consume 200g/day of complementary food (Ministry of Health, 2012).

Consumption rates of infant and follow-on formula among New Zealand infants were estimated in the 2016 NZ Total Diet Survey are presented in Table 2-40 (Ministry for Primary Industries, 2018), with the levels used for modelling of infant (9-month old) food consumption patterns in Australia provided in the 24th Australian Total Diet Study (Food Standards Australia New Zealand (FSANZ), 2014).

The consumption patterns of infant formula products are unlikely to have changed significantly since the release of the latest data presented above, given that in infants IFP may either be the sole source of nutrition (highest consumption) or a supplement to breast-feeding in the first 6 months of life, and to complementary feeding after 6 months of life.

Table 2-40 Estimated intake of infant and follow-on formula in New Zealand and Australian infant

Food Group	*New Zealand infants[a] (g/d)*	*Australian 9-month-old infants[b] (g/day)*
Infant / follow-on formula	400	544[c]

[a] *Ministry for Primary Industries (2018)*
[b] *Food Standards Australia New Zealand (FSANZ) (2014)*
[c] *Non-soy*

The exposure of infants to the nutritive substance if added to infant formula products at the proposed levels is addressed in Section 3.3.

2.4.4 Percentage of food group to which Lacprodan® MFGM-10 is proposed or the percentage of the market likely to use the nutritive substance

The inclusion of Lacprodan® MFGM-10 in infant formula products in Australia and New Zealand is dependent on uptake and new product development initiatives of manufacturers and brand owners in the region. This may be aligned product development for export markets also.

Several manufacturers have expresses and interest in providing MFGM-enriched formula for Australasian infants.

Arla Foods Ingredients P/S proposes that up to 25% of available formula in Australia and New Zealand may be MFGM enriched using Lacprodan® MFGM-10 within the next 5 to 10 years. Recent formula consumption predictions[10]are that the current number of infants likely fed IFO (exclusively or with breast milk) by 6 months of age is 168,000 Australian infants and 45,000 New Zealand infants. These figures are estimated to increase approximately 10-fold over the next 10 years. Assuming 25% of available formula was to contain Lacprodan®MFGM-10 in 10 years' time that would equate to consumption by approximately 425,000 Australian infants and 125,000 New Zealand infants.

2.4.5 Information relating to the use of MFGM in other countries

The use of Lacprodan® MFGM-10 in IFP has been widely adopted in a large number of countries around the world (Section 1.6.2).

An independent market analysis (Innova Market Insights) was undertaken in April 2024 looking at product launches between 2019 to April 2024 that claim the inclusion of MFGM in the ingredients list (Appendix II).

In a global overview of product launches, infant formula (birth to 6 months) had the largest number of product launches observed with 176 launches, follow-on formula (6 – 12 months) had 147 and growing -up/toddler milks had 85 (Figure 2-30). The largest market for MFGM products was in the US, accounting for 46%, followed by Asia with 39% (

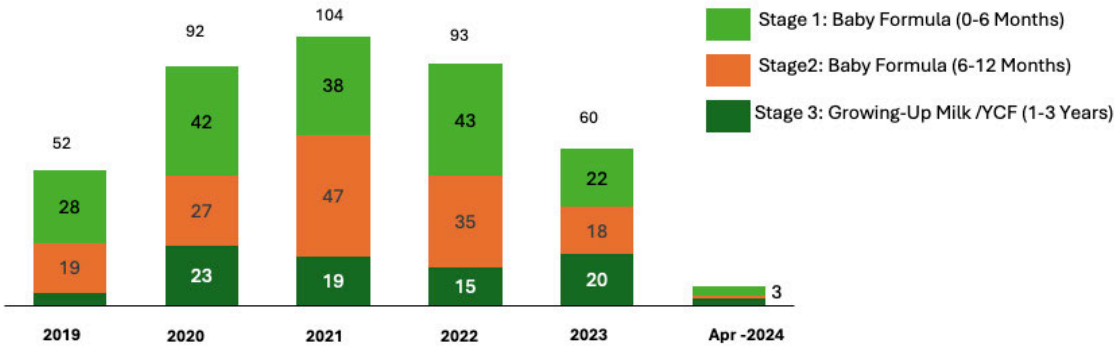
Figure 2-31).

Of note was a large number of products launched that made reference to the presence of MFGM in the product, however no specific MFGM enriched ingredients could be identified in the ingredient list.

[10] *FSANZ P1028 Infant formula: Appendix B – Detailed calculations of figures used in cost and benefit analysis*

https://www.foodstandards.gov.au/sites/default/files/food-standards-code/proposals/Documents/Supporting%20Document%204%20-%20Costs%20and%20benefits.pdf

Figure 2-30 Number of launches by product category containing MFGM in ingredients list (2019- Apr 2024)



The number of products with MFGM in the ingredients list launched identified in specific countries showed between 2019 to April 2024 the USA had the largest number of products launched (85) followed by Mexico (34) and South Korea (30) (Figure 2-32).

This data shows the commonality across countries and product categories where MFGM is used.

Figure 2-31 Proportion of products launched globally (2019 -2014) with MFGM in ingredients list

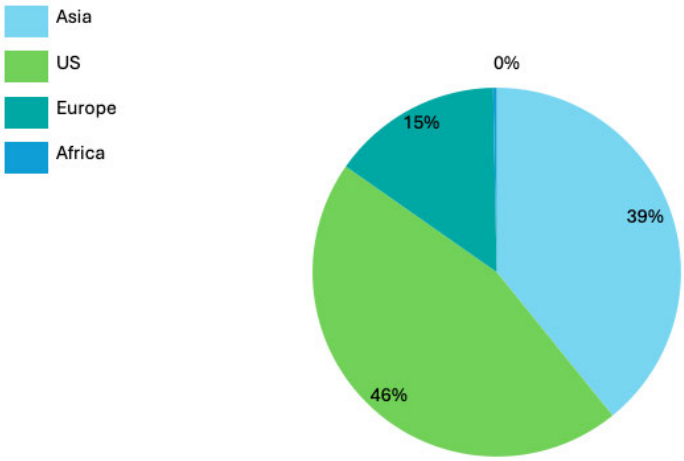
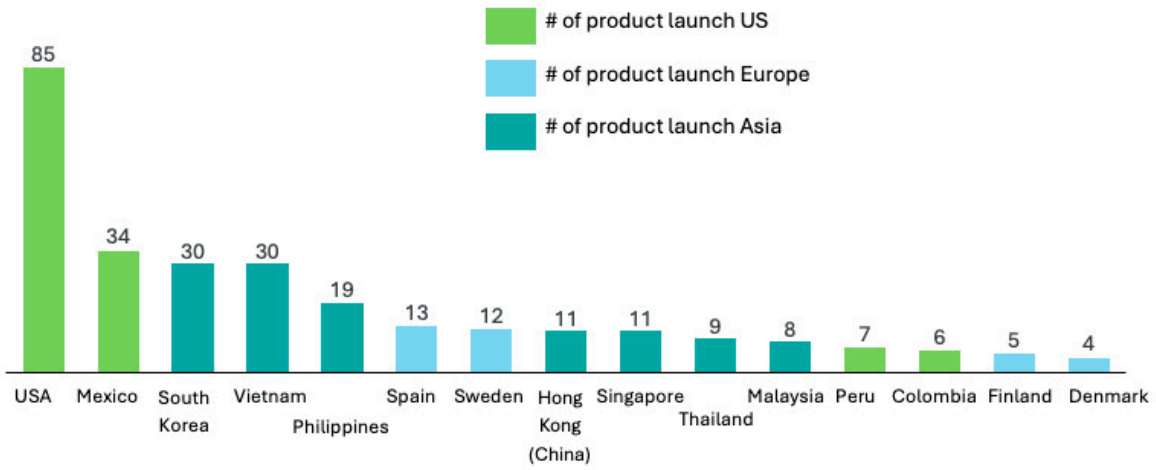


Figure 2-32 Products with MFGM in ingredients list launched 2019- Apr 2024 by country



2.4.6 Information on likely current food consumption for foods where consumption has changed in current years

There have been no reported or observed significant changes in intakes of infant formula products in Australia and New Zealand in recent years. According to the data presented in P1028[11], the Australian National Infant Feeding Survey in 2010-2011 found that in the day before the survey, approximately;

- 40% of infants aged 1 month old received non-human milk or infant formula products
- 55% of infants aged 6 months old received non-human milk or infant formula products

A similar pattern was discernible from New Zealand statistics. A 2007 report from the New Zealand Ministry of Health National Breastfeeding Advisory Committee found:

- 41% of infants were exclusively fed infant formula products at six months old
- 35% of infants were fed a combination of breast milk and infant formula at six months old.

2.5 Information related to the nutritional impact of Lacprodan® MFGM-10

2.5.1 Information related to the nutritional purpose of the use of Lacprodan MFGM-10

The nutritional purpose of the addition of Lacprodan ® MFGM-10 is discussed in detail in Section 3.1.1.

To summarise, the primary purpose of adding Lacprodan® MFGM-10 to infant formula products, based on the available evidence, to support the cognitive and neurodevelopment of infants in a more similar manner to that overserved for breast-fed infants. The addition of Lacprodan® MFGM-10 enables formula to be made that better approximates the phospholipid composition of breast milk

[11] *FSANZ P1028 Infant formula: Appendix B – Detailed calculations of figures used in cost and benefit analysis*

https://www.foodstandards.gov.au/sites/default/files/food-standards-code/proposals/Documents/Supporting%20Document%204%20-%20Costs%20and%20benefits.pdf

and helps ensure infants who cannot be breastfed do not miss out on the benefits of Lacprodan® MFGM-10.

2.6 Information related to the potential impact on consumer understanding and behaviour

2.6.1 Information to demonstrate the level of consumer awareness and understanding of MFGM in infant formula products

Whilst manufacturers and brand owners of infant formula products are aware of MFGM and its benefits as it has been used for some years in export products, this is a new ingredient for consumers in Australia and New Zealand. It has not previously been used as a dietary supplement and therefore expected awareness is anticipated to be currently low. Once permitted for use consumers (as in parents of infants consuming formula) are likely to seek information on the benefits of adding MFGM to IFP. In countries such as the USA, where MFGM enriched ingredients may be added to infant formula products, brand owner websites, together with product labelling (When asked about their attitudes towards purchase of products containing a specific ingredient 35 % of participants suggested they would prefer to purchase a product containing MFGM and 44% said it would not affect purchase choice. Of the remaining, 12 % were unsure and 10% said they would not purchase a product containing MFGM (Figure 2-35). The preference for MFGM was further analysed by responses by country surveyed (Figure 2-36), with over 30% of participants in India, China, Mexico, Indonesia, and the USA responding they would prefer a product with added MFGM.

Figure 2-33) often provide top level information providing consumers with a simple understanding of the ingredient benefits e.g. Enfamil NeuroPro™ Infant by Mead Johnson[12]. On pack and front-of-pack labelling of health and nutrition claims are not permitted in Australia and New Zealand, as they are in the USA and other countries, so it is likely consumers will rely more heavily on information available to support new products.

Consumer information is from markets where IFP with MFGM are already available has been collected in a series of surveys conducted on behalf of Arla Foods Ingredients P/S, first in 2018 and again in 2021; “The Mum Survey-2021” (Appendix II). The purpose of the survey was to gather consumer insights about the perception and purchase of IFP across 11 countries. In total 7600 college-educated women between the ages of 18 and 45 years who had children and / were pregnant at the time of the survey were interviewed for the survey.

Country and participant numbers were: United Kingdom, 507; France, 511, Indonesia, 508; Poland, 506; China, 2010; Russia, 514; South Korea, 500; Mexico, 504; Germany, 506; India, 523; and USA, 1011. Within the survey information on consumer knowledge and understanding of MFGM was sought, together with how it ranked amongst common and emerging ingredients in IFP, in relative terms of awareness. When asked about the most known ingredients used in IFP it was found that

[12] https://hcp.meadjohnson.com/s/product/a4R4J000000PpQdUAK/enfamil-neuropro-infant

Chinese women were the most knowledgeable of the different ingredients used compared to participants from other countries (Figure 2-34).

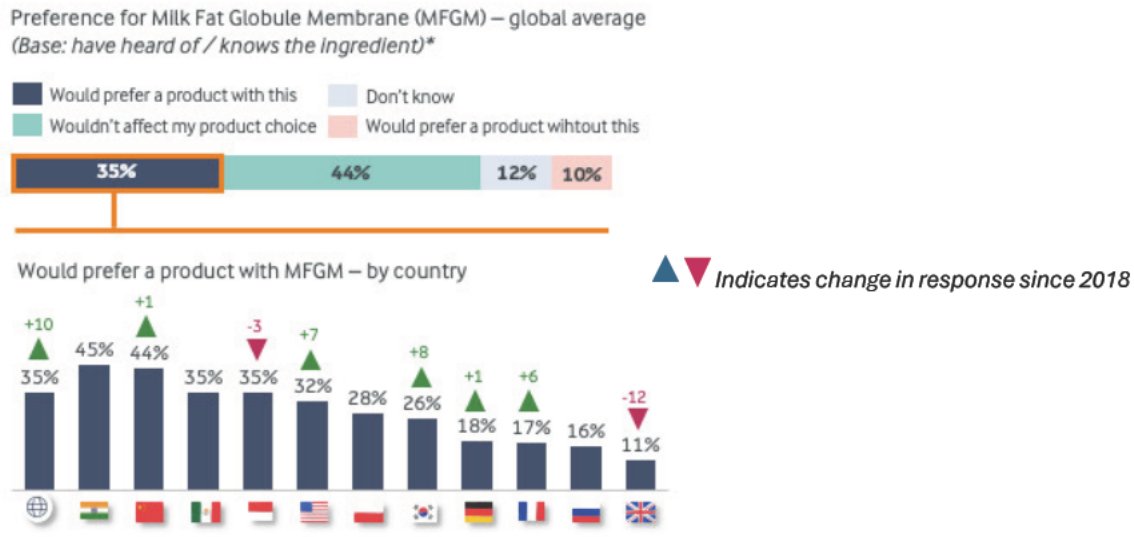
When asked about their attitudes towards purchase of products containing a specific ingredient 35 % of participants suggested they would prefer to purchase a product containing MFGM and 44% said it would not affect purchase choice. Of the remaining, 12 % were unsure and 10% said they would not purchase a product containing MFGM (Figure 2-35). The preference for MFGM was further analysed by responses by country surveyed (Figure 2-36), with over 30% of participants in India, China, Mexico, Indonesia, and the USA responding they would prefer a product with added MFGM.

Figure 2-33 Example of formula containing MFGM enriched ingredients in the USA

Figure 2-34 Percentage of participants with knowledge of various ingredients in IFP

Figure 2-35 influence of ingredient on purchase choice in 11 countries

Figure 2-36 Participant preference for MFGM by country



2.6.2 Information on the actual or potential behaviour of consumers in response to the proposed use of MFGM in infant formula products

Products that contain added Lacprodan® MFGM-10 will have the addition listed as an ingredient in the ingredient list (Section 2.2.9.1). The Nutrition Information Panel will include Sphingomyelin as the indication of the nutritive substance. Parents who have chosen to formula-feed and are aware of MFGM may choose a formula containing MFGM and thereby replace a similar formula not containing

MFGM. Product choice will be a substitution option. Arla Foods Ingredients P/S does not anticipate any nutritional concerns with this replacement. All infant formula products sold in Australia and New Zealand must meet strict regulatory standards.

Human breastmilk is the natural source of nutrition for infants and is rich in many bioactive components that provide unique benefits to infant growth and development. The addition of ingredients naturally found in breastmilk is seen as advantageous, however it is not equal or superior to breastmilk, and therefore will not be communicated as such. Research in Australian mothers found that the most frequently cited reasons for mothers to stop breastfeeding are perceived breastmilk insufficiency (not producing any/enough milk); resuming work; mastitis, nipple soreness and pain on feeding; and mothers felt it was the right time to stop (Magarey, Kavian, Scott, Markow, & Daniels, 2016). This research did not suggest that mothers stop breastfeeding because they believe formula is equivalent or superior to breastmilk. Arla Foods Ingredients P/S anticipates that parents who are already formula feeding their infants may wish to change to a formula containing Lacprodan® MFGM-10.

It is important to note that Standard 1.2.7-4 prohibits health and nutrition claims on infant formula products. Furthermore, nutritive substances can only be labelled as permitted by FSANZ e.g. in the ingredients lis and nutrition information. The inclusion of Lacprodan® MFGM-10 in IFP is only likely to be noted by those caregivers who pay attention to product composition when making a choice in Infant formula product selection, not a driver to initiate formula feeding.

Arla Foods Ingredients P/S does not anticipate that addition of Lacprodan® MFGM-10 will change overall consumption of Infant formula products, rather, it provides consumers with more choice.

2.6.3 Information to demonstrate the consumption of foods containing Lacprodan® MFGM-10 will not adversely affect any population groups.

There is no evidence to support the addition of Lacprodan® MFGM-10 will adversely affect infant populations consuming formula containing it. The composition of IFP is highly regulated and the consumption patterns for formula (serve size, volume and frequency) also well-defined based on the required intakes and feeding patterns of infants. The addition of Lacprodan® MFGM-10 to IFP will not change the gross composition or energy of IFP. Information on the safe use of IFP containing Lacprodan® MFGM-10 is detailed in Section 2.3.

3 Special purpose foods – Infant formula products (3.6.2)

3.1 Information related to the composition

3.1.1 Purpose of the compositional change

The purpose of the addition of Lacprodan® MFGM-10 to infant formula products is based on available clinical evidence that minor components in this ingredient support neurodevelopment and cognition in formula-fed infants that is more similar to that of breast-fed infants and leads to improved outcomes in infants compared to conventional infant formula. These benefits may persist through to adolescent years. Furthermore, the addition of Lacprodan® MFGM-10 to infant formula products results in the composition of infant formula products closer to the profile of human milk. This enables parents who are unable to breastfeed their infants to choose an infant formula product that provides benefits more similar to breast milk than standard formula options.

Human breast milk is the preferred sole source of nutrition for infants for the first 6 months of life and is recommended to be continued when solid foods are introduced from 6 months of age and beyond (National Health and Medical Research Council, 2013). However, when breast feeding is not possible, infant formula products should be used as an alternative to breast milk (National Health and Medical Research Council, 2013). Given the benefits that breastfeeding provides to infants it is important that when infant formula is required as a sole or partial source (along with breastfeeding) of nutrition that the composition of infant formula is as close as possible to human milk. This is reflected in Ministerial policy guidance on the regulation of infant formula products, which states *the composition of breastmilk should be used as a primary reference for determining the composition of infant formula and follow-on formula* (specific policy principle (h)); and in the Codex Standard for Infant Formula and Formulas for Special Medical Purposes Intended for Infants (CXS 72-1981).

Bovine milk components are routinely used in commercial infant formulas to more closely resemble the compositional and functional aspects of human milk. Ingredients enriched in individual protein fractions such as alpha-lactalbumin and lactoferrin have been added to mimic compositional and functional outcomes of their human-milk counterparts in infant formulas (Lönnerdal, 2011). The phospholipid content in bovine milk and human milk are quite similar in composition (J. Lu, Wang, et al., 2016) and bovine MFGM is a component that may be added to infant formulas to bring the compositional and functional outcomes of infant formulas closer to that of human milk. The MFGM is a milk component highly conserved across mammalian species, including bovine and human, and its components are not new, artificial, or foreign to the infant food supply. The addition of Lacprodan® MFGM-10 enables the formula composition to be closer to human milk, especially in phospholipids and sphingolipid content which are associated with neurodevelopment and cognition (Albi et al., 2022; Schneider et al., 2023).

Infant formulas containing dairy fats were widely used in the first part of the 20th century and are still used in some parts of the world, but their use has generally diminished (Delplanque et al., 2015). Most infant formulas sold across the globe typically use vegetable oils rather than dairy fats. While this approach permits formulas to achieve a fatty-acid composition similar to human milk, the

vegetable oil blend lacks some lipids, particularly minor bioactive lipids, found in human milk fat, including sphingomyelin and gangliosides. Moreover, in infant formulas based on vegetable fat, the fat globule is usually smaller than in breast milk, and the phospholipids are provided by emulsifying soy-derived lecithin, with a wide range of phospholipid species included (usually phosphatidyl choline or inositol) (Gallier et al., 2015). This is known to impact formula digestion (Zhao et al., 2024). Lecithin typically used to emulsify infant formulas is known to have different phospholipid compositions than commercial whey powder. Lecithin typically does not contain any sphingomyelin (Boyd, Drye, & Hansen, 1999).

The proposed use level of Lacprodan® MFGM-10 in infant and follow-on formula is intended to provide approximate levels of phospholipid and sphingolipids that are present in human milk, therefore more closely aligning the composition minor components of infant and follow-on formula products to that of human milk.

3.1.2 General data requirements for supporting evidence

See Section 2.1.2 for detail on the general data requirements for supporting evidence.

3.2 Specific information requirements for the nutritional safety, tolerance and efficacy of the proposed compositional change

3.2.1 Characterisation of proposed substance or the comparable substances in breast milk

The purpose of adding Lacprodan® MFGM-10 to infant formula products is to bring the composition of infant formula products closer to the profile of human milk, particularly in relation to complex lipid components, such as phospholipids and sphingomyelin, and minor proteins associated with the MFGM. Lacprodan® MFGM-10 is a MFGM enriched whey protein concentrate, and when added to infant formula is a significant contributor to the total whey protein content. However, it is the enriched MFGM components that provide improved compositional alignment with human milk.

The composition of human milk is dynamic across the lactation cycle, and this includes the composition of the components associated with the MFGM of human milk. The components of the MFGM are typically conserved across mammalian species (C. Garcia et al., 2012), and like human milk vary as a function of the lactational cycle.

The detailed characterisation of the MFGM-associated components in both human milk and Lacprodan® MFGM-10 is presented in Sections 2.2.1and 2.2.3 respectively.

3.2.2 Nutritional safety and tolerance of the proposed compositional change

3.2.2.1 Safety and tolerance of Lacprodan® MFGM-10 in infants

A large number of clinical trials in infants and young children have assessed the safety of ingredients added to formula that have retained MFGM material. The literature search (Section 2.1.2.3) identified 50 publications reporting measures of safety and tolerance of in infants. Of these, 38 publications related to 9 clinical trials where Lacprodan® MFGM-10 was added to infant formula (Table 3-1) , and the remaining 12 publications covering 6 clinical trials used other MFGM-based ingredients (Table 3-2).

In the first published study on the addition of MFGM added to infant food, marginally nourished Peruvian term infants (enrolled at 6-11 months old) received on a daily basis for 6 months (through 12-17 months of age) a complementary food containing Lacprodan® MFGM-10 as the protein source, with an average daily MFGM-10 intake of 5.9 g, or a control complementary food with skim milk as the protein source (Zavaleta et al., 2011). The two groups did not differ in growth measurements (weight/height, height/age, or weight/age ratios). Furthermore, anaemia and micronutrient status were not different between the two groups (Zavaleta et al., 2011). Further exploration of serum metabolome and immune markers was undertaken by Lee, Zavaleta, et al. (2018) using serum samples collected at the start and completion of the study for a subset (n = 100) of the cohort. This study found supplementation with MFGM tended to improve micronutrient status, energy metabolism, and growth reflected as increased levels of circulating amino acids and weight gain, particularly in female infants compared to those in the control group. No adverse events or negative effects associated with the consumption of the MFGM-enriched complementary food were reported. This study supports the safe use of MFGM- 10.

A follow-up of the original cohort 14 years of age has been undertaken evaluating nutrition status and health outcomes (Lazarte, Garcia, Lonnerdal, Slupsky, Murguia-Peniche, Heckmann, et al., 2021), body composition (Lazarte et al., 2023), cognitive development (Lazarte, Garcia, Lonnerdal, Slupsky, Murguia-Peniche, Heckmann, & Kvistgaard, 2021), and executive functions (Lazarte et al., 2022). Whilst some long term neurocognitive and metabolic benefits may be associated with the consumption of complementary food with added MFGM during infancy resulted in no differences in body composition at 14 years of age existed bewteen the trial groups. No adverse events or negative effects of the MFGM have been reported. Results to date support long-term safety of added dietary MFGM-10 during infancy.

In a Swedish study, Timby, Domellof, Hernell, Lonnerdal, and Domellof (2014) enrolled 160 healthy term infants less than 2 months of age, who were randomily assigned to receive either a Lacprodan® MFGM-10 supplementented, low energy, low protein experimental formula (EF; n = 80) or a standard formula (SF; n= 80) until 6 months of age. A breastfed reference group (BFR; n = 80) was also enrolled into the propsective, double blind, randomised controlled trial. The objective of the trial was to evaluate if the EF reduced differences in cognitive development and early growth between formula-fed and breastfed infants. Growth assessments were completed at baseline (<2 months) and 4, and 6 months, together with assessment of plasma amino acids, insuin and blood urea nitrogen. The EF group consumed significantly larger volumes of formula (p – 0.022) than the SF group, fully compensating for the lower energy density. Final growth measures were completed at 12 months (EF, n = 73; SF, n = 68; BFR, n = 72). There were no significant differences in linear growth, weight gain, body mass index (BMI), percentage body fat, or head circumference between the EF and SF groups. Significant differences in growth velocity (weight and length) were observed between the formula-fed BFR groups at 6 months, but not at 12 months. No adverse effects or tolerance issues were reported for this study. In addition to the main study publication (Timby, Domellof, et al., 2014) the cohort were also evaluated for cardiovascular risk markers (Timby, Lonnerdal, Hernell, & Domellof, 2014), incidence of infection through to 12-months and immune markers at 6-months (Timby, Hernell, et al., 2015), characterisation of the oral microbiota at 4 and 12 months (Timby et al., 2017). Across all of these studies there were no safety or tolerance conserns raised.

There were no significant differences observed between formula groups and EF and the BFR group across a wide range of cardiovascular and metabolic risk markers (serum lipids, adipokines, homocysteine, inflammatory biomarkers, and blood pressure) (Timby, Lonnerdal, et al., 2014).

Differences in serum cholesterol concentrations between the formula fed groups were apparent at 6 months, with the EF group having higher total levels than SF group, but approaching that of the BFR group. The EF group had a low-density lipoprotein (LDL) to high-density lipoprotein (HDL) ratio not significantly different from the SF group but lower than the BFR group. Timby, Lonnerdal, et al. (2014) suggested the outcome of this study raised the possibility of reducing the increased long-term risk of CVD in formula-fed infants. Furthermore, by 12 months there was a significantly ($p = 0.034$) lower incidence of acute otitis media (AOM) in the EF group compared to the SF group together with a reduced level of antipyretic use, but no difference from the BFR group. While some measures of stool consistency and frequency differed between formula-fed and BFR group, there were no differences in frequency of diarrhoea, abdominal pain, vomiting or laxative use between the 3 groups.

Timby et al. (2021) reported on growth (weight, height, head circumference, abdominal circumference), blood pressure and plasma cholesterol of the study cohort at 6 years of age. There were no differences in anthropometric measures or blood pressure at 6 years of age. The study team concluded the intervention of a low-energy, low-protein formula with MFGM supplementation was safe, because there were no severe adverse events in any of the study groups until 6.5 years of age, the EF group did not differ from the SF group in prevalence of chronic illness, medication, or allergy at 6 y of age, nor in hospitalisation or incidence of otitis between 1 and 6 years of age, and the EF group did not differ from the SF group in any of the anthropometric, biochemical, or neurodevelopmental outcomes at 6–6.5 years of age (Timby et al., 2021).

Billeaud et al. (2014) conducted a multicentre non-inferiority study in Italy and France. Healthy term infants aged 14 days or less were enrolled in the study and randomised to receive a standard formula (SF), a formula enriched with a lipid-rich MFGM fraction (MFGM-L) or a formula enriched with the protein rich Lacprodan® MFGM ingredient (MFGM-P). Infants consumed the allocated formulas from day 14 through to 4 months of age (day 112). The primary outcome of the study was mean weight gain per day from enrolment to 4 months (non-inferiority margin: -3.0 g/day). Secondary outcomes included length, head circumference tolerability, morbidity, and adverse events, together with exploratory outcome measures (plasma and red blood cell phospholipids, metabolic, and immune markers). Weight gain was non-inferior for both MFGM-L and MFGM-P groups compared to the SF, with weight z-scores and head circumference z-scores suggesting normal growth, with no significant differences between groups. There were no significant differences observed between the MFGM-enriched and standard formula, with overall tolerance high (94.2 – 100%). Of the morbidity indications observed over the duration of the trial (respiratory symptoms, fever, eczema, ear infection, colic, constipation, and diarrhoea) the only significant difference observed was for respiratory symptoms reported for the MFGM-P group at the day 56 visit. Compared with the control group, the MFGM-P group had a higher rate of mild respiratory symptoms (12.7% vs 2.1%), but a lower rate of moderate/severe symptoms (0.0% vs 8.4%). No between-group differences were observed in respiratory symptoms at the other clinic visits (Billeaud et al., 2014). Although pairwise group comparisons did not indicate any difference in the rates of eczema, a post hoc significance test across all formula groups to account for differences in group sizes, showed a significantly higher rate of eczema in the MFGM-P group compared to control ($p = 0.01$).

There are a number of factors that argue against the validity of these results. These factors include:

- The study was powered for growth, but not for eczema. To get accurate results in infants not at risk, a much larger study would be required.
- Consistent with this, eczema incidence was lower in this study compared to expected.
- The eczema finding was only found on post-hoc analysis of parental reports, daily records and

physician reported data, rather than by using accepted criteria (Schmitt et al., 2014).

- The authors state that "caution is therefore warranted in extrapolating this finding".
- While not reported in the original publication, the Timby study (above) also assessed skin reactions/rash and did not find an increased risk in the MFGM-10-supplemented group (Timby, Domellöf, Lönnerdal, & Hernell, 2015).

Timby, Domellöf, et al. (2015) provided commentary on the findings of Billeaud et al. (2014) stating that the finding of increased rates of eczema in the MFGM-P group should be interpreted with caution as both the small study observation numbers and lack of a systematic eczema scoring system reduce the certainty of results.

X. Li et al. (2019) enrolled 789 healthy term Chinese infants to participate in a prospective, randomised, double blind controlled study investigating the effects on infant growth and infection rates of 2 trial formula. Six hundred and seventy four (n = 674) infants completed the trial: standard formula enriched with MFGM (MFGM, n = 161) (containing Lacprodan® MFGM-10 at 3.8g / 100g powder), and SF with added probiotics (F19, n = 167) (Lactobacillus paracasei ssp. Paracasei F19), compared to the standard formula (SF, n = 167) and breast-feeding (BF, n = 179). Infants consumed trial formulas exclusively for the first 4 months, with all infants transitioning to the standard formula (SF) at the beginning of month 5 until month 6. Complementary foods were not used during the intervention period but could be introduced after children were 5 months of age. Growth was monitored at baseline (inclusion), months 1, 2, 3, 4, 5, 6, 9 and 12, with episodes of infection though to 12 months diagnosed and recorded by the study physician. Adverse events (AE) and serious adverse events (SAE) were recorded, with study physicians assessing for any potential relationship to the study formula. Both experimental formulas (MFGM and F19) were well tolerated, with a high level of compliance reported. Growth measures showed some minor differences between groups for some measures. There were no significant differences in weight z-scores between the 3 formula groups at any time point. The BF group tended to have higher mean weight compared to the formula groups, but after 4 months there was no significant difference between any of the groups. During the intervention, weight gain (g/day) did not differ among the formula-fed groups or between the formula-fed groups overall and the breastfed group. However, at 5–12 months, weight gain in the MFGM group was slightly (1.1 g/day) but significantly higher compared to the breastfed group ($p = 0.012$). Z-scores for body length did not differ significantly among the formula-fed groups at any time point, with gain in body length (cm/day) not differing among any of the groups during or after the intervention. Head circumference z-scores did not differ significantly among the formula-fed groups at any time between 1 and 12 months (X. Li et al., 2019). Although not statistically significant, the MFGM and F19 formula groups both had less episodes of fever and days with fever than the SF group, with no difference to the BF group. Compared to the BF group, infants receiving SF had significantly more episodes of fever ($p = 0.021$) and days with fever ($p = 0.036$). Similarly, the number of episodes of upper respiratory tract infections (URTI) did not differ between formula-fed groups, or between FB and all-formula fed. During the intervention phase there was no difference between the formula-fed groups related to skin infections, antibiotic use, vomiting or hospital visits. MFGM group did not differ from that of the BF group. There were no differences in the incidence or categories for AE between any of the groups both during and following the intervention period. A total of 21 SAE's were reported (SF, n = 4; MFGM, n = 7; F19, n = 6, BF, n = 4) with 16 being for LRTI. Twelve (12) of the SAE's were possibly linked to the formula, but not confirmed. X. Li et al. (2019) concluded that her MFGM formula was safe and well-tolerated, with few adverse events and outcomes measures that were more like those of BF infants, and that outcome measures for the MFGM-formula fed infants did not

differ to those of the BF group for any of the primary study parameters. In a further analysis of the study outcome measures on the serum metabolome and faecal microbiota(Lee et al., 2020), no negative effects of the MFGM formula were reported, and the authors reported consumption of the MFGM formula reduced some of the metabolic gaps observed between formula- and breast-fed infants.

Infant growth, formula tolerance, adverse events and health outcomes were key secondary objective of the multi-centre, randomised, double-blind controlled parallel group study by F. Li et al. (2019). Healthy term Chinese infants (n = 451) who had been formula-fed for at least 3 days, were enrolled and randomised to receive either a control formula (SF, n = 228) or the experimental formula (EF, n = 223) containing Lacprodan® MFGM-10 (5 g/L) and lactoferrin (0.6 g/L). A stage 1 formula was used exclusively to day 180, thereafter a stage 2 formula to day 365. A total of 292 infants completed study feeding through day 365 (SF, n = 148; EF, n = 144). Study visit evaluation days corresponded to 14 (-4 days; enrollment), 30 (± 3), 42 (± 3), 60 (± 3), 90 (± 3), 120 (+5), 180 (± 7), 275 (+10), 365 (+10), and 545 (± 7) days of age, and participants were eligible to continue in the study and complete neurodevelopmental testing at days 365 and 545 even if study formula consumption was discontinued after 180 days of age. Growth was evaluated as weight growth rate from 14 to 120 days of age, with other anthropometric measures recorded at each visit. No statistically significant differences by gender in growth rates based on weight (days 14 to 120), or for weight, length or head circumference (excluding some minor early length growth for girls to day 90); weight or weight for length z-scores through day 545 were detected. Overall formula tolerance (intake, fussiness, gas, stool frequency and consistency) did not differ between the formula groups. The overall incidence of AEs categorised by respiratory and gastrointestinal system (upper respiratory tract infections, cough, and diarrhoea), were significantly lower for the EF group than for the SF group, as were episodes of respiratory and diarrhea events. No group difference in the incidence of constipation and in the skin system (including eczema) were detected. No group difference was detected in the number of participants for whom at least one medically confirmed AE was reported (SF, 208, 91%; EF, 198, 89%; p = 0.43). No serious AEs were reported (F. Li et al., 2019). Follow-up studies (Chichlowski et al., 2021; Colombo et al., 2023) through 5.5 years of age reported no additional adverse events and no group differences in growth z-scores for weight for age, height for age or BMI for age. Together these results demonstrate safety, tolerance, typical growth and a significant reduction in the incidence of respiratory and gastrointestinal related through to 18 months of age in infants receiving the EF containing Lacprodan® MFGM-10 and lactoferrin compared to a standard formula.

Growth, tolerance and iron status of infants consuming a formula (EF) containing an MFGM-enriched fraction (Lacprodan® MFGM-10 (5 g/L)), modified protein, iron and arachidonic acid (ARA) concentrations compared to a control formula (CF) was evaluated in a multicentre, double-blind, randomised, controlled, parallel-group, prospective trial in the United States of America (USA) (Hedrick et al., 2021). Healthy term infants (EF, n = 182; SF, n = 191 who were exclusively formula-fed were enrolled and randomised at 10 – 14 days of age, consumed the allocated study formula exclusively through day 120. Participants who continued through day 365 were considered to complete the study even if study formula consumption discontinued or decreased to fewer than 2 feedings/day after day. Study visits corresponded to 14 (−4 days; enrolment), 30 (±3), 42 (±3), 60 (±3), 90 (±3), 120 (+5), 180 (±7), 275 (±7), and 365 (±7) days of age, with anthropometric measures (weight, length, head circumference) recorded at each visit. Tolerance (fussiness and gassiness) and stool characteristics (frequency and consistency) were recorded at each visit. Adverse events were coded

according to specific event and the body system involved. A similar dropout rate occurred in both groups with SF, n = 141and EF, n = 134, completing the study. No statistically significant group differences in the primary outcome, weight growth rate from day 14–120, were detected by gender. No statistically significant group differences were detected for weight, length, or head circumference growth rates by gender for any measured range. In addition, no statistically significant differences were observed for mean achieved weight, length, or head circumference at any measured time point up to day 365 with the exception of mean achieved weight in female infants at day 365 (SF, n = 60; 9892 ± 140, EF, n = 62; 9468 ± 138; p = 0.034). Through day 180 there were no statistically significant difference in daily formula intake volume, or duration of study formula intake (days) between he 2 formula groups. Parent reported tolerance (fussiness and gassiness) was similar between groups at all time points. With the exception of the 90-day visit, no significant differences in stool frequency or characteristics were observed. No statistically significant group differences were detected in the incidence of medically confirmed AEs by system: body as a whole; cardiovascular; endocrine; eyes, ear, nose and throat; gastrointestinal (GI); metabolic and nutrition; musculoskeletal; nervous system; skin; respiratory; and urogenital. Specifically within the skin system there was no difference in the incidence of medically-confirmed eczema between formula groups (SF n = 34, 18%; EF n = 32, 18%; p = 1.0). Serious adverse events were recorded for 30 participants (SF, n =17, 9%; EF: n = 13, 7%) all of which were unrelated to the study formula with the exception of one infant (INV-MFGM) considered intolerant to study formula (later diagnosed with oesophageal reflux) and one infant (INV-MFGM) diagnosed with cow milk protein allergy after study enrolment. This study provides further evidence that formula containing MFGM (Lacprodan® MFGM-10) are well tolerated, safe and support normal growth through to 1 year of age (Hedrick et al., 2021).

The effects of a formula containing 5 g/l of Lacprodan® MFGM-10 (EF) on growth, body composition and safety (Jaramillo-Ospina et al., 2022) neurophysiological outcomes (Algarin et al., 2022) and micronutrient, metabolic and inflammatory markers (Jaramillo-Ospina et al., 2023) through 2 years were assessed in infants in a single-centre, double-blind, randomized controlled, parallel group trial in Chile. Exclusively formula-fed healthy term infants (<120 days of age) were allocated to the EF group (n = 173) or SF group (n = 174), and infants exclusively breastfed (n = 235) to the BF reference group. Anthropometric (weight, length and head circumference) outcomes were recorded at baseline and clinic visits (180 (± 15), 365 (± 15), and 730 (± 15) days) and monthly phone calls to check on compliance through 365 days and AE though day 730 were completed. Growth at each time point and through day 730 was the primary out-come measured as longitudinal change in weight (kg), length (cm), HC (cm) and calculated body mass index (BMI; kg/m2). Growth z-scores: weight-for-age (WAZ), length-for-age (LAZ), body mass index-for-age (BAZ), and head circumference-for-age (HCZ) were estimated (Jaramillo-Ospina et al., 2022). Body composition (fat mass; fat-free mass, percentage body fat) was determined at baseline, 1 and 2 years of age. Adverse events and SAE were grouped by system, including infections, inflammatory, eczema (acute dermatitis, atopic dermatitis, contact dermatitis, and skin rash) and allergy symptoms, and gastrointestinal tract (including reflux, constipation, vomit, abdominal colic, diarrhoea and food allergy). Generally body composition did not differ significantly between formula groups, but small significant differences were detected between BF and formula-fed groups through day 730. Although FM at day 730 was higher in the EF than in the SF group and its trajectory from baseline to day 730 was higher in the EF than in the SF, %BF and FM trajectories from baseline to day 730 were similar between the two groups. Fat free mass (FFM) trajectories were higher for EF and SF than for HM from day 365 to 730 and baseline to 730 (p < 0.05). For EF versus HM, %BF was lower at day 180; however, this difference reversed from day 365. There were no differences between groups in the number of participants who had [3] 1 AE, or

difference in AE incidence rate. Respiratory and gastrointestinal tract events were the most frequently reported AE. There were no group differences in the incidence of SAE. In summary, this study demonstrated that an infant formula with added MFGM-10 was safe and well-tolerated when fed to healthy term infants through 12 months of age. Both randomised study formula groups were associated with higher growth z-score increases compared with the HM reference group between 6 and 24 months of age (Jaramillo-Ospina et al., 2022). This study further confirmed that infant formula containing MFGM supports typical growth and safety in infants.

Best et al. (2023) evaluated the effects of an MFGM enriched formula on rate of body weight gain in a multi-centre, randomised, double blind, controlled trial in late preterm (34 – 37 weeks gestation) weight appropriate for gestational age (AGA) infants. Comparator was a SF, and there was a breast-fed (BF) cohort as an observational reference group. Key compositional differences between the EF (22 kcal/30 mL) and SF (2 kcal/30mL) were that the EF contained Lacprodan® MFGM-10 (5 g/L), along with increased levels of inositol, vitamin D, butyrate and a higher whey:casein ratio. Infants received the study formula (EF or SF) through 120 days, after which they were provided unblinded SF until 365 days. Anthropometric measures (weight, length, head circumference) were completed at 40 weeks post menstrual age and 30, 60,90 120, 180, and 365 days. Body composition was assessed at enrolment and 120 days, dietary intake and tolerance (faecal characteristics and gassiness) at 30, 60,90 and 120 days. Atopic dermatitis was scored at each study visit. The sample size required to determine a change in weight between groups of 3 g / day was 100 per group; Recruitment challenges resulted in low enrolment rates (EF, n = 22; SF, n = 18, BF, n= 39) with numbers available for final analysis (EF, n = 17; SF, n = 18, BF, n= 36), and early termination of the trial, meaning results should be interpreted with caution (Best et al., 2023). There was no significant difference in rate of weight gain between the formula groups at 120 days, and no difference in weight, length and head circumference z-scores at 365 days. Compared with the BF group, infant weight and length z-scores were comparable; however, head circumference was smaller in infants randomized to the STF group compared to the BF group (p = 0.002). The EF but not SF groups differed from BF groups showing increased fat free mass, body mas and body volume at 120 days. There were no differences in atopic dermatitis scores or incidence of adverse events between any group at any time point. No SAE was related to the interventions. At 120 days there was a significant reduction in “infectious illness” in the EF group compared to SF (p = 0.02) and BF group (p = 0.01). Although results are potentially compromised by the small sample size this study provides further evidence of the safety and tolerance of Lacprodan® MFGM-10 in infant formula.

Table 3-1 Intervention studies assessing the safety and tolerance of Lacprodan®MFGM-10 in healthy infants (<12 months)

Reference	Objective (s)	Study design	Country	Study population, age at baseline and number	Study groups and intervention	Summary of findings relating to safety	Study limitations
Zavaleta et al. (2011)	To evaluate the efficacy of a milkfat globule membrane (MFGM)–enriched protein fraction in a complementary food, on diarrhoea, anaemia, and micronutrient status	Randomized, double-blind controlled study.	Peru	Healthy term infants aged 6-11 months with a birth weight ≥2500 g, primarily BF. The report includes 550 infants (with an even mix of males and females). MFGM n=277 control group n= 273 At the end of the study sample sizes were the following: MFGM n=253 Control group n=246 Reasons for dropout include: refused to continue, moved, dislike product, mothers work and final evaluation not completed.	Complementary food (40 g/d) divided into 2 servings Control Group: complementary food with skim milk (n=246) MFGM Group: complementary food enriched with MFGM protein fraction (Lacprodan®, Arla Foods Ingredients, Denmark; n=253) Duration: Daily, for 6 months	The primary outcome was diarrhoea morbidity. No difference was observed between the groups in the incidence of diarrhoea, but global prevalence of diarrhoea was significantly lower in the MFGM group (3.84%) compared with the control group (4.37%) (P<0.05). Furthermore, consumption of the MFGM protein fraction reduced episodes of bloody diarrhoea when adjusting for anaemia and potable water facilities as covariates. (odds ratio 0.54; 95% confidence interval 0.31–0.93, P=0.025). There were no differences between groups in anaemia, serum ferritin, zinc, or folate.	Due to the infants being recruited between 6 and 11 months of age, some were mostly over 1 year old during the study.
Lee, Zavaleta, et al. (2018)	To investigate the effects of MFGM in complementary food on the serum metabolome and immune markers of infants.			Serum samples (n= 50 MFGM group, n=50 control group) collected at baseline and end of intervention based on stratified random sampling plan.		MFGM supplementation had a beneficial impact on the micronutrient status and growth of infants in the MFGM group. Significant sex differences in growth parameters were observed (i.e., weight-for-age Z-score (WAZ) and/or final weight; males > females) within the control group, but was not significant within the MFGM group. Female infants in the control group showed reduced serum amino acid pool and less weight	

Reference	Objective (s)	Study design	Country	Study population, age at baseline and number	Study groups and intervention	Summary of findings relating to safety	Study limitations
						gain compared to male infants, but this was improved in female infants in the MFGM group.	
Lazarte, Garcia, Lonnerdal, Slupsky, Murguia-Peniche, Heckmann, and Kvistgaard (2021)	To evaluate the effects of early nutrition on cognitive outcomes at 14 years of age			Infants; 6-11 months n=499 in original study n=398 in this follow up study and 386 completed testing.		No safety related measures or adverse events reported.	
Lazarte, Garcia, Lonnerdal, Slupsky, Murguia-Peniche, Heckmann, et al. (2021)	To evaluate the effect of early nutrition on nutrition status and health outcomes at 14 years of age			Of the original study cohort (n=499), 398 adolescents (79.8%) were available for follow-up (n, % female; bMFGM: 199, 47%; Control: 192, 47.9%)		Anthropometrics, nutrition status (overweight, underweight, obesity) and biochemical measures (ferritin, zinc, insulin and haemoglobin) were similar between groups with the exception of higher prevalence of zinc deficiency in the Control group (P< 0.04). Cardiometabolic indicators were similar. There were no group differences in participants evaluated (bMFGM: 197; Control, 190) for total number of cardiometabolic risk factors. No safety related measures or adverse events reported.	
Lazarte et al. (2022)	To evaluate the effect of early nutrition on executive function at 14 years of age			Of the original study cohort (n=499), 398 adolescents (79.8%) were located for follow-up. A total of n = 386 participants completed the CANTAB assessments (MFGM, n=196; Control, n=190) at 14 years of age.		Main effects were primarily gender related with boys performing better than girls. However, a Group effect, $F(1, 244) = 4.47$, $p=0.036$) on the Strategic Working Memory Task, was observed with the MFGM group making significantly fewer errors than the Control group. Infants receiving MFGM showed significant advantages on a strategic working memory task at 14 years of age, even when important covariates were appropriately controlled.	

Reference	Objective (s)	Study design	Country	Study population, age at baseline and number	Study groups and intervention	Summary of findings relating to safety	Study limitations
						No safety related measures or adverse events reported.	
Lazarte et al. (2023)	To evaluate the effect of early nutrition on body composition at 14 years of age			A total of 365 participants completed body composition analysis at 14 years of age.		No differences in body composition at 14 years of age were detected	
Timby, Domellof, et al. (2014)	To test the hypothesis that feeding an infant formula with reduced energy and protein densities and supplemented with MFGM reduces differences in cognitive development and early growth between formula-fed and breastfed infants.	Prospective, double-blind, randomised controlled trial.	Sweden	160 healthy term infants, 2 months of age, randomly assigned to the experimental or control formula (n=80 per group) A breastfed reference (BFR) group (n = 80) N recruited = 80 in each group N in final analysis : Experimental formula N = 73 Standard formula N = 68 Breastfed N = 72. Dropouts were mostly "no cause given" or "moved from study site" (n=12). Most common causes of discontinued intervention were cows milk	Experimental formula: MFGM-supplemented, low-energy, low-protein experimental formula (EF) (6 g MFGM/L) Lacprodan® MFGM-10; Arla Foods Ingredients, Denmark Control formula: a standard formula (SF) Breastfed reference group The energy and protein contents of the EF and SF were 60 and 66 kcal/100 mL and 1.20 and 1.27 g/100 mL, respectively.	No significant differences in linear growth, weight gain, body mass index, percentage body fat, or head circumference were found between the EF and SF groups	The authors note that most parents started introducing complementary foods at 4-6 months and therefore the intervention was diluted. There is also the limitation that the intervention was both a low protein formula and MFGM supplementation.

Reference	Objective (s)	Study design	Country	Study population, age at baseline and number	Study groups and intervention	Summary of findings relating to safety	Study limitations
				allergy (n=3) and gastrointestinal symptoms (n=2)	Duration: from 2 months until 6 months of age		
Timby, Lonnerdal, et al. (2014)	To measure cardiovascular risk markers at 12 months of age			89% remained in the study at 12 months for this analysis. EF n=73 SF n=68 BFR n=72		During the intervention, the EF group had higher total serum cholesterol concentration than the SF group, reaching the level of the BF group. The EF group had an LDL:HDL ratio not significantly different from the SF group but lower than the BF group. These data indicate that raising the cholesterol intake between 2-6 months of age leads to higher total serum cholesterol levels not different from BF infants but without changing the LDL:HDL ratio.	
Timby, Hernell, et al. (2015)	Objective to measure incidence of otitis media, antipyretic use, serum IgG concentrations against pneumococcal.			At 12 months of age, the following submitted completed forms for assessment: EF n=57 SF n=58 BFR n=64		The cumulative incidence of acute otitis media was lower in the EF group than in the SF group (during the intervention) and did not differ from the BF reference group. There were no significant differences in the incidence or longitudinal prevalence of reported infection-related symptoms (fever, coughing, breathing difficulties, or skin rash) between the EF and SF groups, but the parents in the EF group reported significantly less antipyretics use during the intervention than the SF group. There were no differences in proportions of watery diarrhoea, loose, firm, or hard stools, abdominal pain, vomiting, or consumption of	

Reference	Objective (s)	Study design	Country	Study population, age at baseline and number	Study groups and intervention	Summary of findings relating to safety	Study limitations
						laxatives or probiotic drops between the EF and SF groups.	
Timby et al. (2017)	The objective was to characterize the oral microbiota in infants fed standard IF and MFGM-supplemented formula or breast milk at 12 months of age.			At 12 months of age, the following submitted completed forms for assessment: EF n=59 SF n=55 BFR n=52 Dropouts were due to either children starting the study before sampling was included (n=82), lost to follow up (n=9) or child did not cooperate (n=47)		No safety related measures or adverse events reported.	
Grip et al. (2018)	To investigate the lipidome in serum/plasma and erythrocyte membranes of infants fed EF compared to infants fed SF and a BF reference group			A subset of 90 infants were randomly selected (15 males and 15 females from each treatment group) from the infants described in Timby, Domellof, et al. (2014). For each treatment group 30 infants were tested at time point 4 and 12 months. However, for timepoint 6 months 213 infant samples (73 in the EF, 70 in the SF and 70 in the BFR groups) were tested out of 220 infants still in the study.		No safety related measures or adverse events reported.	

Reference	Objective (s)	Study design	Country	Study population, age at baseline and number	Study groups and intervention	Summary of findings relating to safety	Study limitations
He, Parenti, Grip, Domellof, et al. (2019)	To determine whether MFGM may impact metabolism as formula fed infants exhibit different metabolic profile than BF infants.			The same as above in Grip et al 2018		No safety related measures or adverse events reported.	
He, Parenti, Grip, Lonnerdal, et al. (2019)	To characterize the fecal microbiome and metabolome of infants fed a MFGM supplemented EF formula and compare this to infant fed standard formula and a BF reference group			The same as above in Grip et al 2018		No safety related measures or adverse events reported.	
Timby et al. (2021)	To evaluate neurodevelopment, growth, and plasma cholesterol status at 6 and 6.5 y of age in the same study population.			Of the original cohort: n=58 experimental formula, n=56 standard formula and n=64 breastfed.		There were no differences between the formula groups in weight, length, or head or abdominal circumferences, nor in plasma concentrations of homocysteine, lipids, insulin, or glucose. The MFGM group did not differ from the control in prevalence of chronic illnesses, medication or allergy at 6 years of age, nor in hospitalization or incidence of otitis between 1 and 6 years of age.	

Reference	Objective (s)	Study design	Country	Study population, age at baseline and number	Study groups and intervention	Summary of findings relating to safety	Study limitations
Billeaud et al. (2014)	The objective was to evaluate safety of formula with MFGM enriched fractions	Multicentre, randomized, parallel group, reference-controlled pilot study.	France and Italy	Healthy term infants aged ≤ 14 days with a birth weight of 2500-4500 g. This report includes 199 infants (57 in the control, 70 in the MFGM-L and 72 in the MFGM-P groups). There are fewer infants in the control group as there was a shortage of formula. at the end of the study there were 144 infants MFGM-L n=47 (23 withdrawn) MFGM-P n=52 (20 withdrawn) Control n=45 (12 withdrawn) Most dropouts were due to voluntary withdrawal or lost to follow up. After feedings started 1 dropped out due to gastroesophageal refulx, 1 due to vomiting, 2 due to colic, 1 due to constipation, 1 due to bronchitis and 2 due to major protocol deviation.	Control standard infant formula standard formula enriched with bovine-derived lipid-rich MFGM fraction (MFGM-L; Fonterra Cooperative Group) standard formula enriched with bovine-derived protein-rich MFGM fraction (MFGM-P; Lacprodan® MFGM-10, Arla Foods Ingredients, Denmark). Final levels of MFGM added was not reported. Infants received study formulas from 14±3 days until the age of 4 months. Hereafter they received standard formula until the age of 12 months. Dose duration: 0-12 months of age	Weight gain was non-inferior in the MFGM-L and MFGM-P groups compared with the control group. Among secondary and exploratory outcomes, few between-group differences were observed. Formula tolerance rates were high in all groups. Adverse event and morbidity rates were similar across groups except for a higher rate of eczema in the MFGM-P group. The authors commented that this difference was statistically significant only when the three study groups were compared in post hoc analysis performed to account for potential bias due to a lower number of infants in the control group compared with the MFGM group. Selected metabolic and immune markers were measured as exploratory outcome. No significant differences were found for any of these.	The authors note that limitations of this study include the small sample size and short duration of testing. As well as unequal allocation of the study groups.

Reference	Objective (s)	Study design	Country	Study population, age at baseline and number	Study groups and intervention	Summary of findings relating to safety	Study limitations
X. Li et al. (2019)	The objective was to evaluate effect on growth and infection rates of supplementing formula with probiotic *Lactobacillus paracasei* ssp. Paracasei F19 or WPC enriched in MFGM	Prospective, double-blind, randomized controlled intervention.	China	Healthy term infants aged 21±7 days with a birth weight of 2500-4500 g. n=800 infants. 200 infants were in each group of: Standard Formula (SF) MFGM formula *Lactobacillus paracasei* ssp. Paracasei F19 BF reference group. Infants completing the study included: 167 in the control, 161 in the MFGM group, 167 in the F19 group, 179 in the BF reference group. Number of drop-outs and the causes of drop-outs was similar between the groups	Infants received study formula exclusively from age 21±7 days until 4 months of age. From beginning of 5th month until 6 months all infants received standard formula (SF) with complementary foods permitted after 6 months. The infants were followed until the age of 12 months. Control standard infant formula standard formula enriched with 5 g/L bovine-derived MFGM fraction (Lacprodan® MFGM-10, Arla Foods Ingredients, Denmark) (3.88 g/100 g powder reconstituted in 129 g/L) standard formula supplemented with 1*108 cfu/L F19 BF reference group. Duration: 1-4 months of age	Both experimental formulas were well tolerated and resulted in high compliance. The formula-fed groups showed no difference from each other in weight, length, or head circumference z-scores at any time point. During the intervention, overall, the experimental formula groups did not have more episodes of diarrhoea, fever, or days with fever than the BF infants. However, compared to the BF infants, the SF group had more fever episodes and days with fever, but not diarrhoea. The F19-supplemented infants but not the other two formula groups had, compared with the BF group, more unscheduled hospitalizations and borderline more episodes of upper respiratory tract infections	The authors note the limitations of the study for investigating other outcomes include the number of sites, the absence of otitis media assessment, and the lack of cognitive development screening.
Lee et al. (2020)	The objective was to evaluate whether supplementation with MFGM in an IF would drive desirable changes in serum metabolism and gut microbiome.			From 150 samples that were randomly chosen for metabolomics analysis 124 qualified for further analysis. SF n=40 MFGM n=41 BFR n=42		No safety related measures or adverse events reported.	

Reference	Objective (s)	Study design	Country	Study population, age at baseline and number	Study groups and intervention	Summary of findings relating to safety	Study limitations
X. Li et al. (2021)	The objective was to compare the effects of supplementing bovine MFGM on cytokine, eczema and vaccination response			same as above Li et al 2019		The results showed that there were no differences in anti-diphtheria nor anti-poliovirus IgG concentrations between the SF and MFGM groups. Cytokine concentrations were comparable among the MFGM and BF groups. The study found no differences in the prevalence of doctor-diagnosed eczema at age 12 months among the groups. The results did not indicate increased eczema risk in infants fed MFGM.	
F. Li et al. (2019)	To evaluate neurodevelopment, growth, and health outcomes in infants receiving bovine milk fat globule membrane (MFGM) and lactoferrin in infant formula	Randomised, double-blind, controlled, multi-centre, parallel group.	China	Healthy term infants <14 days of age Enrolled: Control, n= 228; MFGM + Lf, n = 223 Day 365: Control, n= 148; MFGM + Lf, n = 144 Day 545: Control, n= 88; MFGM + Lf, n = 95	Control formula (stage 1 & 2) Test formula with MFGM (5g/L Lacprodan® MFGM-10, Arla Foods Ingredients, Denmark) + 0.6 g/L lactoferrin (Lf) (stage 1 & 2) Exclusive formula feeding to day 120 Stage 1 formula to day 180, stage 2 formula to day 365 Dose Duration: 0-12 months of age	No statistically significant differences in weight growth or other anthropometric measures by gender between groups. Formula intake and tolerance similar between groups. Significantly less adverse events related to respiratory illness and diarrhoea were found in the MFGM + Lf group. No differences in adverse events related to skin (rashes and eczema) or constipation were found between groups.	The authors note that a limitation is the lack of a breastfed reference group. This enriched formula also includes lactoferrin as well as MFGM. Finally there was a high dropout at 18 months due to lost to follow up.
Chichlowski et al. (2021)	The objective was to compare microbiota and metabolite profiles in a subset of study participants.			a subset of this cohort was tested for stool microbiota on day 120 MFGM n=27 control n=35 a subset was tested for metabolite profiles at day 120 MFGM n=26 control n=33		No safety related measures or adverse events reported.	

Reference	Objective (s)	Study design	Country	Study population, age at baseline and number	Study groups and intervention	Summary of findings relating to safety	Study limitations
Colombo et al. (2023)	To evaluate neurodevelopmental out-comes at 5.5 years of age in this same study population			116 infants enrolled and completed assessments (Control: 59, MFGM+LF: 57).		No group differences were detected for weight for age, height for age, or body mass index for age between groups	
Hedrick et al. (2021)	To evaluate growth, tolerance, and iron status in infants receiving added bovine MFGM and modified protein, iron, and arachidonic acid concentrations in infant formula at 1 year of age	Multicentre, double-blind, randomized, controlled, parallel-group, prospective trial	United States of America	Healthy term infants <14 days of age. 373 infants were enrolled control n=191 MFGM n=182 at the end of the study (day 365) there were 275 infants control n= 141 MFGM n=134 reasons for dropout were classed as "formula related" and "not formula related" . For the control group, 20 discontinued due to formula related issues and 29 for other issues. For the MFGM group, 17 discontinued due to formula related issues and 31 due to other issues.	Control formula was a standard infant formula (previously marketed as Enfamil ® Enriched formula included 5 g/L Lacprodan® MFGM-10, Arla Foods Ingredients, Denmark Dose duration: 0-12 months of age	No group differences in growth rate (g/day) or anthropomorphic measures (weight, length, head circumference) were detected. No group differences were detected in haemoglobin, haematocrit, or incidence of anaemia. No difference in parent reported mean formula intake. Parent reported gassiness and fussiness were not different between groups. No differences in stool consistency or frequency were found. No statistically significant group differences were detected in the incidence of medically confirmed AEs by system: body as a whole; cardiovascular; endocrine; eyes, ear, nose and throat; gastrointestinal (GI); metabolic and nutrition; musculoskeletal; nervous system; skin; respiratory; and urogenital. No difference in eczema was found. Within the eyes, ears, nose, and throat system, nasal/tear duct obstruction incidence was significantly different (control: 18, 9%; MFGM: 6, 3%; p = 0.019). Within the GI system, gas incidence was significantly lower for MFGM (9, 5%) versus control (24, 13%; p = 0.010). Within the “feeding problem” category, AE incidence was low but statistically significant (control: 0,	The authors note that study limitations include not having a breastfed reference group and that complimentary feeding would have started at 6 months, but participants must still have had at least 2 feedings a day of formula after 6 months old.

Reference	Objective (s)	Study design	Country	Study population, age at baseline and number	Study groups and intervention	Summary of findings relating to safety	Study limitations
						0%; MFGM: 7, 4%; p = 0.006); assorted AEs included feeding difficulty/intolerance, including that associated with beginning complementary foods (mild, 5) and newborn feeding problems (mild, 1; moderate, 1). No group differences were detected in the incidence of AEs associated with allergic manifestations or infection.	
Jaramillo-Ospina et al. (2022)	The aim of this study was to assess the effects of an experimental formula (EF) with added whey protein-lipid concentrate (5 g/L; source of bovine milk fat globule membrane [bMFGM]) on growth, body composition, and safety through 24 mo of age in term infants.	Double-blind randomized, controlled trial	Chile	Healthy infants < 120 days of age 582 infants were recruited: Standard formula: (n=174) Enriched formula: (n=173) Breastfed reference group: (n=235) At two years the sample size was as 478 infants (over 80% of the number recruited) Standard formula n=145 Enriched formula n=144 Breastfed n=187 Most dropouts were due to voluntary withdrawal or lost to follow up. Seven infants (3 SF and 4 EF) withdrew before 6 months sue to gastrointestinal symptoms.	Both study formulas had a prebiotic blend of polydextrose (PDX, Litesse Two Polydextrose; Danisco) and galactooligosaccharides (GOS; Vivinal GOS Galactooligosaccharide; Friesland Foods Domo; 1:1 ratio, 4 g/L) and the following (per 100 kcal): 17 mg docosahexaenoic acid (DHA), 25 mg arachidonic acid (ARA), 1.9 g protein, and 1.2 mg iron. Control formula: a standard infant formula Enriched formula with MFGM (5g/L Lacprodan® MFGM-10, Arla Foods Ingredients, Denmark) Duration: 0 to 12 months of age	At baseline, only weight-for-age was different between the formula groups (0.14 lower in EF versus SF group, P = 0.035). Weight-, length- and BMI- for- age trajectories were higher from baseline to days 365 and 730 in EF or SF compared with HM (all P < 0.05). No differences in changes in body composition were observed between the formula groups. For EF versus HM, %BF was lower at day 180; however, this difference reversed from day 365. Fat-free mass was higher in formula groups compared with HM at all time points. No group difference in adverse events (including, respiratory infections, GI infections, eczema, and others) were detected between groups.	The authors notes that limitations for this study include using different methods for estimating body composition at different time points which may make it hard to compare time points. As well, the effect of complementary feeding which could begin after day 180 on the body composition is an extra variable which they could not control. Finally fewer participants completed the body composition measurements than the other growth and

Reference	Objective (s)	Study design	Country	Study population, age at baseline and number	Study groups and intervention	Summary of findings relating to safety	Study limitations
Algarin et al. (2022)	To assess growth and tolerance in infants receiving an enriched infant formula.			A subset of children (n=122) underwent neurophysiological testing Standard n=42, Enriched n=35, Breastfed n=45		No safety related measures or adverse events reported.	adverse event measurements.
Jaramillo-Ospina et al. (2023)	To assess micronutrient (zinc, iron, ferritin, transferrin receptor), metabolic [glucose, insulin, Homeostatic Model Assessment of Insulin Resistance (HOMA-IR), insulin-like growth factor-1 (IGF-1), triglycerides (TGs), total cholesterol, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C)], and inflammatory (leptin, adiponectin, high sensitivity C-reactive protein) secondary outcomes through 24 mo of age in infants who received standard cow's milk-based infant formula (SF), similar			A subset of children were included: Standard n=80 Enriched n=80 Breastfed n=83		Only serum iron (þ22.1 µg/dL) and HDL-C (þ2.5 mg/dL) were significantly higher for EF compared with SF at D730. Micronutrient, metabolic, and inflammatory biomarkers were generally similar through 2 y in infants who received infant formula with or without added bovine MFGM. Over the 2-year study period, differences were observed between the formula and breastfed groups in terms of infant and maternal characteristics, growth parameters, and body composition. The formula-fed infants tended to have higher weight, length, and head circumference compared to the breastfed infants. However, there were no significant differences in body composition between the formula groups. Additionally, there were no significant differences observed between the formula and breastfed groups in terms of adverse events and serious adverse events.	

Reference	Objective (s)	Study design	Country	Study population, age at baseline and number	Study groups and intervention	Summary of findings relating to safety	Study limitations
	formula with added bovine MFGM (EF), or human milk (HM) through 1 y.						
Best et al. (2023)	To compare the effects of nutrient-enriched formula with standard term formula on rate of body weight gain of late preterm infants appropriately grown for gestational age.	Multicentre, randomized, double-blind, trial	Australia	Late preterm infants born week 34-36+6 40 infants were enrolled and received formula control: n=18 MFGM n=22 Breastfed reference n=39 At the end of the study (120 days) the following were still enrolled: control n=18 MFGM n=17 Breastfed n=36 4 infants withdrew from the intervention group and 4 from the breastfed reference group. 1 infant from the intervention group passed away. The study was terminated early due to low recruitment.	Control formula was a standard formula given to preterm infants. The enriched formula had 5 g/L Lacprodan® MFGM-10, Arla Foods Ingredients, Denmark. It is noted that the enriched formula had higher inositol, vitamin D, butyrate, and calories from increased protein per 100 kcal than the standard 2.8 g vs. 2.1 g All other nutrients, including calcium and phosphorous are consistent with recommended amounts for preterm infants Infants received the formula they were assigned until 120 days. After which they received standard formula until day 365 Dose duration: 0 - 120 days corrected age	There were no differences between randomized groups on weight, length, and head circumference z-scores at 365 days of age. When compared with the BFR group, infant weight and length z-scores were comparable, however, head circumference was smaller in infants randomised to the standard formula group compared to the BFR group. Adverse events were comparable across all groups. A lower incidence of "infectious illness" was observed in the MFGM group. Vitamin D status was higher in the MFGM group than both the other groups. No difference in butyrate were found between the groups.	This study was terminated early due to low recruitment and therefore has a very small sample size.

3.2.2.2 Safety and tolerance in infants of other sources of MFGM

The safety of ingredient sources of MFGM, other than Lacprodan® MFGM-10, used in IFP has also been assessed in several clinical studies involving infants and young children.

In a randomised, double-blind, controlled parallel group pilot study, healthy term infants (enrolled at 2 – 8 weeks of age) were fed an enriched formula (EF, n = 35) supplemented with MFGM-derived complex milk lipids (enriched with gangliosides (GG)), or a control unsupplemented formula (SF, n = 35) until 24 weeks of age (Gurnida et al., 2012). A breast-fed reference group (BF, n = 40) was included in the study. Anthropometric data (weight, length, head circumference) was collected at baseline and monthly to end of intervention period (6-months) and used to assess growth and nutritional status (z-scores for height-for-age, weight-for-age, and weight-for-height). There were no reports of allergy, diarrhoea, vomiting or colic, and no significant differences in reported illnesses between the two groups during the trial. Furthermore, no differences were observed for any of the growth parameters, suggesting that infant formula supplemented with polar milk lipids is well tolerated and is safe to be consumed by infants (Gurnida et al., 2012).

In Japan, preterm infants (n = 24, birth weight < 1500g) were recruited at birth into a randomised, double-blind, controlled parallel group trial to assess the effects of sphingomyelin (SM) on neurodevelopment (Tanaka et al., 2013). Infants were randomised to receive the SM enriched formula (EF, n = 12; SM 20% of all phospholipids in the formula) or the standard formula (S, n = 12; SM 13% of all phospholipids in the formula) in addition to breastmilk for the first 8 weeks of life. For the EF the added SM was derived from egg yolk lecithin. Anthropometric measure (weight, length, head circumference) were completed at follow-up evaluations (3, 6, 9, 12 ns 18 months corrected age). Tanaka et al. (2013) reported no significant difference in weight, height, or head circumference between the groups at 18-months of age, and that there were no side effects observed in the EF group from the SM enrichment during the trial period. This study supports the safe use of SM in infants.

Poppitt et al. (2014) evaluated the acceptability and efficacy of a ganglioside enriched formula (EF) against rotavirus infection in Indian infants aged 8 to 24 months. The prospective double-blind, randomised controlled trial compared a control formula (CF, whole milk powder) or the EF (whole milk powder + GG rich complex milk lipid) as supplements, with 225 infants randomised to each trial group. The intervention period was 12-weeks. Clinic visits were completed at baseline and 12-weeks, with study visits by fieldworkers twice weekly to dispense the supplement, collect faecal samples and collect health data. The safety evaluation based on incidence of adverse events (AE and SAE) for each group. All AE were recorded by fieldworkers during home visits, and defined as any symptom, disease, syndrome, intercurrent illness, and/or abnormal laboratory finding that emerged or worsened during the intervention, relative to baseline (Poppitt et al., 2014). During the trial, 405 AE were recorded of varying severity from mild to severe affecting 41% infants in the EF group and 46% infants in the SF group. Events were typically classified as mild and not related to the intervention. The proportion of AE was similar regardless of treatment, and no SAE occurred in the EF group (Poppitt et al., 2014), supporting the safe and potentially effective use of the EF supplement.

In a prospective, multi-centre, double-blind, randomised trial in China, healthy term infants aged <14 days, were assigned randomly to be fed a MFGM enriched formula (EF, n = 108) or a standard formula (SF, n = 104) for 6 months and then switched to stage 2 EF and SF formula until 12 months. A reference group (n=206) contained healthy breastfed infants (BF)(B. Jiang et al., 2022; Xia et al., 2021). The

MFGM material was a GG rich complex milk lipid. Anthropometric measures (weight, length, head circumference) to determine growth were assessed at baseline, 24 ± 5 days, 4,6, 8, and 12 months, with data converted to weight-for-age, length-for-age and HC-for-age z-scores. Tolerance was evaluated based on parental records at each visit with digestive tolerance was based on the volume of formula intake and any other dietary intakes, stool characteristics, including frequency of predominant stool colour (brown, yellow, green, red or black) and consistency for each stool, diarrhoea and mucus, frequency of spitting up or vomiting, crying after 15 min feeding, night crying and sleep behaviour and periods of restlessness (B. Jiang et al., 2022). All gender-based growth parameters were within normal ranges across all groups, with no significant difference in weight-for-age (p = 0.60 and 0.57), length- for-age (p = 0.90 and 0.98), HC-for-age (p = 0.30 and 0.82), and BMI-for-age (p = 0.53 and 0.34) z-scores in male and female infants respectively. At 12 months of age the were no significant differences in anthropometric outcomes between any of the study groups. There was no significant difference in the incidence of gastrointestinal event (constipation, diarrhoea) between groups over the 12-month study (p = 0.55). Differences in stool colour, particularly between the EF and SF groups were no longer apparent by 8 months. Between enrolment to 6 months skin rash [MF, n=4 (4%); SF, n=0 (0%); BFR, n=12 (6%); p=0.002] and upper respiratory infection [EF, n = 7 (6.5%); SF, n = 1 (1%); BF, n = 15 (7%); p=0.003] were the most frequent adverse events. The incidence of regurgitation and vomiting at 42 days, 4 or 6 months did not vary (p>0.05) between groups (B. Jiang et al., 2022; Xia et al., 2021). This study provides additional evidence of the normal growth and tolerance of formula containing MFGM ingredients.

The efficacy of a formula containing higher levels of DHA, ARA, iron, folic acid, vitamin B12, and an alpha-lactalbumin-enriched whey protein concentrate with higher levels of sphingomyelin and phospholipids than the control product on developmental myelination, cognition and behaviour to 6 months (Schneider et al., 2022) and through 2-years (Schneider et al., 2023) was undertaken in a cohort of healthy term American infants (EF, n = 39; SF, n = 42; BF, n = 108). The multi-centre prospective, longitudinal, double-blind, randomised controlled clinical included an intervention period of 12 months, with infants assessed at the study visits; 6 weeks and 3, 6, 9, 12, 18 and 24 months (EF , n = 32; SF, n = 35; BF, n = 108). Growth was assessed using anthropometric measures (weight, length, and head circumference) with data converted to weight-for-age, length-for-age and HC-for-age z-scores. Body composition was assessed using air displacement plethysmography at baseline, 6 weeks, 3, 6, and 9 months (if the infant met weight and length criteria for the measurement unit). Safety was assessed based on parent reported adverse events. At 3- and 6-months body weight and length were similar across all groups. The rate of constipation as an AE was (EF, n = 6/32; SF, n = 2/34; BF, n = 3/108), and 1 AE in each formula group was considered related to the intervention product , but no specific details provided (Schneider et al., 2022). At 24-months no significant differences in growth were found between the EF and SF groups. A tendency toward higher weight-for-age Z-scores was observed at 12, 18, and 24 months of age in the formula-fed groups compared to the breastfed reference group (Schneider et al., 2023). Changes in body composition were generally similar across all groups with fat mass increasing to 6 months with a concomitant reduction in fat free mass. This study supports the safe use and tolerance of MFGM in infant formula.

A prospective, multi-centre, double-blind, randomised, controlled equivalence study (Netherlands, France, Belgium and Singapore) was designed to evaluate the safety and tolerance of a concept infant formula with large, milk phospholipid-coated lipid droplets containing vegetable and dairy fats in healthy term infants (Breij et al., 2019). Healthy term infants, with normal growth measures for age

and gender, and ≤ 35 days postnatal, who were either fully formula- fed or fully- breastfed were enrolled. The primary outcome was daily weight gain (g/d) from enrolment until 17 weeks of age. Secondary outcomes included length, head circumference, formula intake, tolerance parameters (gastrointestinal symptoms including cramps, diaper rash, regurgitation and vomiting, stool consistency), plasma parameters, and AE's. Data was collected at visits (baseline, 5, 8, 13, and 17 weeks of age) and through diaries and planned investigator calls between visits.

Detailed outcomes of the Singapore (Chinese, Malay or Indian ethnicity) cohort (Shek et al., 2021) were reported by Teoh et al. (2022), with the inclusion of a second control formula. The 3 study formulas were isocaloric: the EF concept formula, a control formula also containing a probiotic mix (SF+p), and a second control formula without the probiotic mix (SF-p).Due to recruitment ceasing prematurely the number of infants fully formula fed by 28 days was only 117 – compared to the required sample size for equivalence analysis (n = 249). The number of infants enrolled as intent-to-treat (ITT) was 453 in total (EF, n = 152; SF+p, n = 146; SF-p, n = 155; BF, n = 67). Growth outcomes were assessed on a per protocol (PP) basis, equivalence of weight gain not demonstrated between any of the formula groups, was shown between the EF and mean of the 2 control formula when analysed to 17-weeks of age, but not the control without prebiotics alone. Compared to the BF group, equivalence in daily weight gain was demonstrated for the EF and CF+p formula groups, but not the CF-p group. Mean weight, length and head circumference were not statistically significantly different for any of the intervention group pairwise comparisons at any visit until 17 weeks of age. No significant differences in daily stool frequency were observed between the formula groups until 17 weeks of age, apart from a slightly lower daily stool frequency in the FP-p group. Compared to formula-fed infants, the breastfed reference group consistently showed a higher daily stool frequency from 1 to 4 months. he percentages of infants with absent, mild, moderate, or severe regurgitation were not statistically significantly different between intervention groups, and regurgitation typically declined over time. No statistically significant differences in the distribution of vomiting categories were observed. There was no statistically significant difference in the incidence of SAE and AE between the study groups. None of the adverse events that were documented during the study were considered related to the study product by the investigators. Based on the outcomes described above, there was no safety concern related to the occurrence of any (S)AE during the study (Teoh et al., 2022).

Long-term follow-ups of the study by Breij et al. (2019) has been reported by Abrahamse-Berkeveld et al. (2024) who investigated the impact of the EF on longitudinal anthropometric measurements, specifically BMI and BMI-for-age z-score, and Lidewij Schipper et al. (2023), who evaluated the effects of the EF on cognitive performance at 3, 4 and 5 years of age and compared it to the erythrocyte fatty acid composition of the study groups at 17 weeks of age. Anthropometric data was collected at 1, 3, 4, and 5 years of age, along with blood pressure at the 5-year visit to assess the impact of the EF on long-term BMI trajectories and blood pressure (Abrahamse-Berkeveld et al., 2024). At the 5-year follow up 149 of the original 116 enrolled, from 3 of the 4 original study countries (EF, n = 49; CF, n = 51; BF, n = 49). Throughout the study, mean BMI values and BMI-for-age z-scores observed in the EF group were much closer to the BF group. In contrast, from 12 months of age onwards, the CF group had consistently higher mean absolute BMI and BMI-for-age z-scores compared to the breastfed group. Consistently lower BMI and BMI-for-age z-score were observed in the EF group compared to the CF group. Through 5-years of age, in pairwise comparisons, the weight-for-age and head circumference-for-age z-scores were not statistically significantly different at any time point between

any of the study groups. No apparent differences in waist circumference at 3, 4, and 5 y of age or in skinfolds from 3 months to 5 years of age or their derived parameters, were observed between any of the study groups and there were no significant differences in childhood overweight or obesity observed between the study groups through to 5 years. Although the study cannot attribute the findings to any particular aspect of the EF, including MFGM material, it does suggest the presence of large, milk phospholipid-coated lipid droplets enriched with dairy lipids in IMF may have a lasting beneficial impact on BMI trajectories and childhood blood pressure at 5 y of age and further narrow the gap in functional health outcomes of formula-fed infants to those of breastfed infants (Abrahamse-Berkeveld et al., 2024). Mean values for body weight, length, head circumference, and BMI remained within the adequate growth ranges of the WHO standards, indicating that the EF was well-tolerated and did not result in any adverse effects on growth parameters.

Ambrożej, Dumycz, Dziechciarz, and Ruszczyński (2021) conducted a systematic review with meta-analysis to evaluate the safety and benefits of MFGM supplementation in infants. Growth parameters at 4 months of age were chosen as the primary outcome. The meta-analyses (Figure 3-1) included 4 studies (n = 814) comparing MFGM-supplemented formula to standard formula, and 2 studies (n = 549) comparing MFGM-supplemented formula to breast feeding. The review specifically identifies outcomes related to Lacprodan® MFGM-10 compared to other MFGM sources.

At 4 months of age there were no differences in mean weight, length and head circumference (Figure 3-1 (a), (c), (e)) observed between infants receiving a standard formula or those receiving the MFGM-enriched formula (Ambrożej et al., 2021). Compared to breastfed infants, those fed MFGM-supplemented formula had slightly lower mean body weight and head circumference (Figure 3-1 (b), (f)) however, body length (Figure 3-1 (d)) did not differ between groups.

Ambrożej et al. (2021) considered MFGM treatment-emergent adverse events reported to be respiratory tract and gastrointestinal infections, skin diseases, and formula intolerance, observing none of the studies raised safety or tolerance concerns regarding MFGM-supplemented formula and concluding a good safety profile for MFGM.

Ruiz et al. (2017) first reported on a prospective, randomised, double-blind intervention study in healthy term Spanish infants. A total of 170 infants between <2 months of age were randomised to receive either a standard infant formula (SF, n=85) or a formula containing long chain polyunsaturated fatty acids (LC-PUFAs), milk fat globule membrane [redacted] (contributing 10% wt/wt of total protein), symbiotics and gangliosides (collectively referred to as Nutriexpert® factor) (EF, n=85). The control group of infants who had been exclusively breastfed (BF) was recruited. The formula-fed groups received infant formula (SF or EF) though to 6 months, then follow-on formula (SF or EF) until 18 months of age. No difference in growth (Nieto-Ruiz et al., 2019) or linear growth velocity (Ruiz et al., 2017) was observed between the 3 groups at 18 months. Head circumference was measured at birth, 6, 18 months and 2.5 years. There were no differences between the two study groups in HC measurements at any of the timepoints, however at 2.5 years in a follow-up subset of the original formula fed cohorts (n = 75) male children fed the EF (n = 27) had a larger HC (p = 0.019), higher percentile HC/age (p = 0.006) and Z-Score HC/age (p =0.011), compared to those fed SF (n=13). No differences were found in girls (Campoy & Ruiz, 2016). Faecal microbiota and reported illness frequency were also assessed at 1, 6, 18 months and 2.5 years (Campoy, Cerdo, et al., 2018). Infants in the EF group had fewer respiratory tract infections (p = 0.033) and total infections (p = 0.018) compared to SF and BF infants. At 12 months of life, BF infants showed a higher rate of conjunctivitis

(p = 0.042) and unclassifiable febrile episodes (p = 0.005) than infants fed FEF. At 18 months of life, infants fed EF showed a lower rate of conjunctivitis (p =0.013) than BF infants. No growth or morbidity data was reported in studies at 2.5-year (Nieto-Ruiz, Diéguez, Sepúlveda-Valbuena, Herrmann, et al., 2020) and 4-year follow-ups (Campoy, Nieto-Ruiz, Sepulveda-Valbuena, et al., 2018; Cerdó et al., 2022; Nieto-Ruiz, Diéguez, Sepúlveda-Valbuena, Catena, et al., 2020). At the 6-year follow-up, Nieto-Ruiz et al. (2022) there were no differences in anthropometric measures (including BMI, HC, waist circumference) of children in the 3 study groups. Diéguez et al. (2022) also reported no differences between the BMI and HC of children in the 3 groups but found while all mean blood glucose levels were within a normal range, BF children had lower mean glucose levels than children in the SF group (p = 0.027). There was no significant difference in blood glucose levels between BF and EF group children. Diéguez et al. (2023) explored the 6-year follow-up data further and found no differences between groups in body fat mass, or rates of obesity/thinness. Blood glucose data obtained from continuous monitoring devices was used to evaluate Multiscale Sample Entropy (MSE) which provides information about the regularity, fluctuation, and complexity of glucose levels over time. Lower MSE values indicate higher regularity, while higher values indicate greater irregularity and complexity. At 6 years, the SF group had lower MSE than BF children, but there was no difference in MSE between EF and BF children (Diéguez et al., 2023). This study of Spanish infants, with follow-up through to 6 years, has not raised any safety or tolerance concerns regarding the addition of MFGM [redacted] to infant and follow-on formula (fed up to 18 months).

Table 3-2 Intervention studies assessing the safety and tolerance of other MFGM and MFGM-like ingredients in infants (<12 months)

Reference	Objective (s)	Study design	Country	Study population, age at baseline and number	Study groups and intervention	Summary of findings relating to safety	Study limitations
Gurnida et al. (2012)	To assess the impact of infant formula supplemented with gangliosides from complex milk lipid on cognitive functions of normal healthy infants.	Double-blind, randomized, controlled parallel group clinical pilot study.	Indonesia	Healthy term infants aged 2-8 weeks with a birth weight of ≥ 2.5 kg. This report includes 110 infants with 35 infants in each of the two study formula groups and with 40 infants in a BF reference group. At 6 months of age, 91 infants remained in the study Control: n=30 Enriched n=29 Breastfed reference group n=31 A total of 19 babies dropped out of the trial. These included five babies in the control group: one infant was withdrawn due to consuming complementary feeding, two infants were withdrawn due to consuming different infant formula, and two infants were withdrawn due to consuming different infant formula and complementary feeding; six babies in the treatment group were withdrawn due to consuming different infant formula and/or complementary feeding; eight babies in the reference group: due to	Control standard infant formula; Experimental standard formula with added complex milk lipid (Fonterra Cooperative Group) to increase the ganglioside GD3 content by ~2-3 mg/100 g. Dose duration: 2-6 months of age	No differences in measures of growth were found between formula groups. The number of infants with reported minor illness such as fever and cough did not differ significantly between the control and the treatment groups throughout the trial (data not shown) and there were no instances of diarrhoea, allergy, vomiting or colic in either of the groups	The authors note that the small sample size is a limitation in this study

Reference	Objective (s)	Study design	Country	Study population, age at baseline and number	Study groups and intervention	Summary of findings relating to safety	Study limitations
				complementary feeding or receiving infant formula.			
Tanaka et al. (2013)	The objective was to examine the effects of nutritional factors, especially sphingomyelin on the mental, motor and behavioural development of premature infants	Double-blind, randomized, controlled parallel group clinical pilot study.	Japan	Infants were recruited at birth after admission to the NICU until 18 months of age. 24 very low birth weight (less than 1500 grams) preterm babies were recruited. Control: n=12 Enriched: n=12	Breast milk was given priority and shortage was covered by one of the two formulas. Sphingomyelin fortified milk (SM 20% of all PL in milk). Added Phospholipids originated from a milk source to reach a higher dose of sphingomyelin. Control milk (13% of all PL in milk). Added phospholipids originated from egg yolk lecithin. Dose duration is unclear from the text. Intervention should have been at least 8 weeks.	Results for head circumference, height and body weight at 18 months of age were not significantly different between the trial group and the control group. All subjects in the trial group had no side effects from SM fortified milk (e.g., reduction in number of platelets) during the trial period	The authors note the small sample size as a limitation
Poppitt et al. (2014)	The objective was to assess acceptability and efficacy of a high-ganglioside complex milk lipid (CML) for prevention of rotavirus.	Prospective double-blind randomized controlled trial.	India	Healthy term infants aged 8-24 months. This report includes 450 infants (284 males; 166 females) Control n=225 intervention n=225 11 discontinued the intervention, and 19 discontinued the control.	Control supplement contained 5 g whole-milk powder; CML supplement contained 2 g complex milk lipid (Fonterra Cooperative Group) + 3 g whole-milk powder. Both supplements were provided in individual sealed sachets. Dose duration: 12 weeks in a time between 8-24 months of age	During the trial similar numbers of infants reported adverse events with the majority of events classified as mild and not related to the intervention. The seasonal prevalence of rotavirus was therefore not high enough for demonstrating any difference between groups.	The authors note that the prevalence of rotavirus and diarrhea was unseasonably low at baseline. Throughout the trial there were only 110 cases of diarrhea, of which 10 were

Reference	Objective (s)	Study design	Country	Study population, age at baseline and number	Study groups and intervention	Summary of findings relating to safety	Study limitations
							because of rotavirus, making it hard to draw any conclusions.
Xia et al. (2021)	To evaluate neurodevelopment and growth of healthy term infants fed formula supplemented with MFGM	Prospective, multi-centre, double-blind, randomised.	China	Healthy term infant >14 days of age. 418 infants were recruited Standard infant formula n=104 Enriched infant formula n=108 Breastfed reference group n= 206 After 12 months, 61 infants had dropped out. The group numbers were: Standard infant formula n=83 Enriched infant formula n=92 Breastfed reference group n= 182 There was no significant difference in dropout rate among the formula-fed groups. The most common reason for discontinuation was related to formula intolerance, as evidenced by constipation (EF, n=3; SF, n=5), vomiting (SF, n=1), and allergic reaction (SF, n=1). The most common reason for withdrawing from the breastfed reference group was the perception of insufficient milk production. Other reasons were loss of contact, voluntary withdrawal, and inability to follow protocols.	Formulas were made with the same macro and micronutrient composition. A stage 1 formula was used for 0-6 months and a follow-on formula given from 6-12 months. Control formula (stage 1 & 2) Test formula with MFGM (Fonterra, NZ) with a minimum ganglioside concentration of 17.9mg/100g (first formula) and 16.9 mg/100g (follow-on formula) Duration: 0 to 12 months of age.	No differences were found in measures of growth between formula groups. No differences in daily intake of formula milk volume, energy, protein fat or carbohydrates were found between formula groups throughout the study. There was no indication of adverse events due to formula group.	The authors note that the study did not consider the effect of complementary feeding on these parameters.

Reference	Objective (s)	Study design	Country	Study population, age at baseline and number	Study groups and intervention	Summary of findings relating to safety	Study limitations
B. Jiang et al. (2022)	To evaluate the safety and tolerability of MFGM supplementation in formula for infants 0 to 12 months.			As above in Xia et al 2021		No differences in the frequency of stools were found between the formula fed groups. Colour of stool varied slightly by group (e.g. breastfed had more golden colour stools) as well as frequency of loose stools. No differences in the rate of vomiting or milk spit up were found between groups. The standard formula had a higher frequency of night crying than the enriched or breastfed groups. At the 6 months assessment the enriched formula group had more adverse events than the other two groups. There was no statistical difference in the frequency of all adverse events among the three groups on the 42-day, 4-monthand 12-month visits. There was a lower incidence of diarrhea in the enriched group at the 8 month visit. From 4 to 12 months, the increase in body weight, recumbent length, head circumference and BMI of infants between the 2 formula-fed groups or among the 3 groups were not significantly different. BMI was highest in the breastfed group, followed by the enriched group and then the standard group at 42 days.	
Schneider et al. (2022)	The study aims to investigate the efficacy of a blend of docosahexaenoic acid (DHA), arachidonic acid (ARA), iron, vitamin B12, folic acid, and sphingomyelin (SM) from a uniquely processed whey	Prospective, longitudinal, two-centre, double-blind, randomized, controlled, parallel group design	United States of America	Healthy infants 2-5 weeks of age 189 infants enrolled control n=42 Enriched n=39 Breastfed reference group n=108 At the end of the 6 months the following infants remained control n=34 enriched n=32 breastfed reference group n=108	Intervention products were bovine milk-based infant formulas manufactured by Wyeth Nutrition. The alpha-lactalbumin enriched whey protein concentrate for the control product was almost devoid of phospholipids and SM, while the alpha-lactalbumin enriched whey protein concentrate used in the investigational product contained higher levels of SM and	Safety findings were largely similar across groups. Body weight and length values were between 10th and 90th percentiles for most infants at 3 and 6months. Regarding adverse events, 2/34 participants in the control group, 6/32 in the investigational group, and 3/108 in the breastfeeding group were reported to have had constipation. One AE in each group was considered related to the respective study product. No serious AE was reported.	The authors note that the COVID-19 pandemic limited recruitment resulting in a smaller sample size and potentially confounding environmental factors.

Reference	Objective (s)	Study design	Country	Study population, age at baseline and number	Study groups and intervention	Summary of findings relating to safety	Study limitations
	protein concentrate enriched in alpha-lactalbumin and phospholipids compared with a control formulation on myelination, cognitive, and behavioral development in the first 6 months of life.			One child in each formula group dropped out due to adverse events. Others were lost to follow-up or without explanation. There were 29 dropouts before the last scan in the breastfed reference group but all data points were used.	phospholipids due to the unique manufacturing process of ingredients. The investigational product contained higher levels of DHA, ARA, iron (fortified through ferrous sulfate heptahydrate), folic acid, and vitamin B12 (fortified through cyanocobalamine) than the control product. Dose duration: 1-12 months of age		
Schneider et al. (2023)	To assess the impact of a myelination blend on myelination during the first two years of life.			Some infants from Scheider et al 2022 were lost to follow up. The group numbers were as follows: Control n=35 Enriched n=32 Breastfed n=108		Growth measures (weight-for-age, height-for-age, and head-circumference-for-age Z-scores) were not found to be different between groups A tendency toward higher weight-for-age Z-scores was observed at 12, 18, and 24 months of age in the formula-fed groups compared to the breastfed reference group.	
Breij et al. (2019)	To evaluate whether a concept IF with large, milk phospholipid-coated lipid droplets is equivalent to standard IF with regard to growth adequacy and safety in healthy, term infants.	Randomized, double-blind, controlled, prospective, multi-country trial	Netherlands, Belgium, France and Singapore	Healthy term infants with a postnatal age of ≤ 35 days. EF, n = 115 CF, n = 108 BF, n = 88	The formulas were similar in energy content, total lipid content and n-3 and -6 PUFA composition Standard formula with vegetable oil. Lipid droplets were small (~0.5 um) The sole difference between the two control formulas was the absence (Control w/o prebiotics) or presence (Control) of 0.8 g/100 mL of the specific scGOS/lcFOS (9:1) mixture. Enriched (concept) formula: the vegetable oil was partially replaced	Measures were completed at 17 weeks of age. No apparent differences in mean daily formula intake (ml/day) or intake per kg body (mL/kg/day) were observed across intervention groups Equivalence of daily weight gain was demonstrated between the Concept and Control group after additional correction for ethnicity and birthweight No clinically relevant group differences were observed in secondary growth outcomes, tolerance outcomes or number, severity or relatedness of adverse events. EF supported adequate growth and is well tolerated and safe for use in infants.	The authors noted the multiple differences in lipid composition and structure of the EF mean the study outcomes cannot be attributed to just 1 factor. The study was conducted at 17 sites – investigator training may have increased

Reference	Objective (s)	Study design	Country	Study population, age at baseline and number	Study groups and intervention	Summary of findings relating to safety	Study limitations
Lidewij Schipper et al. (2023)	To evaluate the effects of an infant formula mimicking human milk lipid composition and milk fat globule structure on childhood cognitive performance.			This trial includes those that completed cognitive testing at 5 years of age. CF, n = 47 EF, n = 41 BF, n = 49	by dairy lipids (48%), and milk PL derived from bovine MFGM were added. Lipid droplets in this formula were large (mode diameter of ~3–5 um) Dose duration: 0 to 17 weeks of age.	No safety related measures or adverse events reported.	variation. The inclusion of Asian and Caucasian ethnicities may have impacted variation in growth trajectories.
Abrahamse-Berkeveld et al. (2024)	To evaluate in a follow-up study of a randomized, controlled trial whether a Concept IMF with large, milk phospholipid-coated lipid droplets enriched with dairy lipids (EF) beneficially impacts long-term body mass index (BMI in kg/m2) trajectories and blood pressure at school age.			This trial includes those that completed the follow up testing at 5 years of age CF, n= 51 EF, n= 49 BF, n= 49		Throughout the study period until 5 y of age, the weight-for-age, and head circumference-for-age z-scores were not statistically significantly different at any time point between any of the study groups Compared to Control, Concept group children had consistently lower mean BMI values during follow up. Mean values were close to the breastfed group. At 5 y of age, the Concept group had a lower mean diastolic and arterial blood pressure compared with the Control group	
Shek et al. (2021) Teoh et al. (2022)	To evaluate whether a concept IF with large, milk phospholipid-coated lipid droplets is equivalent to standard IF with regard to growth adequacy and safety in healthy, term Asian infants	Randomized, double-blind, controlled, prospective, multicentre trial	Singapore	Healthy term infant <35 days of age 453 infants were enrolled and received the formula Concept / PL enriched formula n=152 Control formula n=146 control formula without prebiotics n=155 Breastfed reference group n=67	The formulas were similar in energy content, total lipid content and n-3 and -6 PUFA composition Standard formula with vegetable oil. Lipid droplets were small (~0.5 um) The sole difference between the two control formulas was the absence (Control w/o prebiotics) or presence (Control) of 0.8 g/100 mL of the specific scGOS/lcFOS (9:1) mixture.	Measures were completed at 17 weeks of age. No apparent differences in mean daily formula intake (ml/day) or intake per kg body (mL/kg/day) were observed across intervention groups Equivalence of daily weight gain was demonstrated between the Concept and Control group after additional correction for ethnicity and birthweight No clinically relevant group differences were observed in secondary growth outcomes,	Study recruitment was stopped prematurely, with lower than expected full formula feeding resulting in a small sample size. Including Malay, Indian and Chinese infants may have introduced

Reference	Objective (s)	Study design	Country	Study population, age at baseline and number	Study groups and intervention	Summary of findings relating to safety	Study limitations
				In this study only those infants that were fully formula fed by 28 days of age were included therefore final numbers were Concept/enriched formula n=35 Control formula n=29 control formula without prebiotics n=28 Breastfed reference n=66	Enriched (concept) formula: the vegetable oil was partially replaced by dairy lipids (48%), and milk PL derived from bovine MFGM were added. Lipid droplets in this formula were large (mode diameter of ~3–5 um) Dose duration: 0 to 17 weeks of age	tolerance outcomes or number, severity or relatedness of adverse events.	confounding variables in growth trajectories. Feeding practises in these etnicities may be different as indicated by a higher number of Chinese infants in the breastfed group.
Ruiz et al. (2017)		Prospective, randomized double-blind, nutritional intervention study.	Spain	Healthy term infants >2 months of age Standard formula (n=85) Enriched infant formula (EF, n=85) Breastfed reference group n= 50 Exclusively breastfed for at least 2 months, were included between 0–6 months of age	Infants received infant formula from randomization (0-2 months) until 6 months, after which they received a corresponding follow-on formula from 6-18 months of age Standard infant formula: (Stage 1 and follow on) Enriched formula included MFGM (10% of total protein content (wt:wt), MFGM-10), synbiotics, LC-PUFAs, gangliosides, sialic acid and nucleotides Duration: from 2 to 18 month of age	No differences in linear growth velocity were found between the three study groups.	This clinical trial involes an enriched formula with multiple added ingredients which make it difficult to attribute any effects to MFGM alone. Many follow up studies have quite a low sample size due to high dropout rates (over 35% by 18 months). By providing formula until 18 months, there would have also been complementary feeding that may have impacted the results.
Campoy, Nieto-Ruiz, Arias, et al. (2018) Nieto-Ruiz, Diéguez, Sepúlveda-Valbuena, Catena, et al. (2020)	To analyse the long-term effects of a new infant formula enriched with bioactive compounds on healthy children's language development at four years old.			Healthy term infants >2 months of age Standard formula (n=85) Enriched infant formula (EF, n=85) Breastfed reference group n= 50 Exclusively breastfed for at least 2 months, were included between 0–6 months of age Up to 18 months of life, a total of 40 infants were excluded in the SF and EF groups as follows: 24		No safety related measures or adverse events reported.	

Reference	Objective (s)	Study design	Country	Study population, age at baseline and number	Study groups and intervention	Summary of findings relating to safety	Study limitations
				were excluded in the SF group (1 infant due to perinatal hypoxia, 1 infant had growth deficiency, 15 infants did not take the infant formula, 2 had colic of the infant, 3 were excluded due to lactose intolerance, 1 infant due to digestive surgical intervention, and 1 infant suffered hydrocephalus); 16 infants were excluded in the EF group (2 infants presented growth deficiency, 2 infants lactose intolerance, 11 infants did not take the infant formula, and 1 was excluded due to epileptic seizure). Furthermore, one infant of the BF group was excluded because he/she was not breastfed This follow-up study involves a subset of those children. Standard formula n =46, Enriched formula n = 43 Breastfed n = 33.			
(Campoy & Ruiz, 2016)	To evaluate the influence of early nutrition on head circumference			Head circumference (HC measured at birth, 6, 12, 18 months and 2.5 years. Follow up at 2.5 years in 75 formula-fed children.		There were no differences between the two study groups in HC measurements at any of the timepoint performed. However, male children only fed EF (n=27) showed a larger HC (p=0.019), higher percentile HC/age (p=0.006) and Z-Score HC/age (p=0.011), compared to those fed SF (n=13).	

Reference	Objective (s)	Study design	Country	Study population, age at baseline and number	Study groups and intervention	Summary of findings relating to safety	Study limitations
Campoy, Cerdo, et al. (2018)	To compare the effect of a standard infant formula (F1) with a new one designed by Ordesa Laboratories SL and supplemented with LC-PUFAs, Milk Fat Globule Membrane (MFGM) components and synbiotics (NutriexpertR factor) (F2) on the gut microbiota composition of infants during the first 18 months					Infants receiving enriched formula showed fewer respiratory tract infections (p=0.033) and total infections (p=0.018) compared to standard formula and BF infants. At 12 months of life, BF infants showed a higher rate of conjunctivitis (p=0.042) and unclassifiable febrile episodes (p=0.005) than infants fed enriched formula. At 18 months of life, infants fed enriched formula showed a lower rate of conjunctivitis (p=0.013) than BF infants. They observed that the gut microbiota of enriched formula-fed infants was more similar to that of breast-fed than standard formula-fed ones.	
Nieto-Ruiz et al. (2019)	To analyze the influence of a new enriched-infant formula with bioactive compounds on growth, neurodevelopment, and visual function (VF) in healthy infants during their first 18 months of life.			At 18 months the sample size was the following: Standard n= 48 Enriched n= 56 Breastfed n=37		There were no differences in measures of growth between the three groups.	

Reference	Objective (s)	Study design	Country	Study population, age at baseline and number	Study groups and intervention	Summary of findings relating to safety	Study limitations
Nieto-Ruiz, Diéguez, Sepúlveda-Valbuena, Herrmann, et al. (2020)	To analyse the effects of a bioactive nutrients-enriched-infant formula on children's behaviour up to 2.5 years, compared to a standard infant formula or breastfeeding			This follow-up study involves a subset of those children. Standard formula n =29, Enriched formula n = 41 Breastfed n = 33.		No safety related measures or adverse events reported..	
Nieto-Ruiz et al. (2021)	To analyse the long-term effects of an infant formula supplemented with bioactive nutrients on brain structure and neurocognitive function in healthy children aged 6 years.			This follow-up study involves a subset of those children. Standard formula n =30, Enriched formula n = 25 breastfed n = 33.		No safety related measures or adverse events reported.	
Cerdó et al. 2022	To compare the dynamics of gut microbiota maturation and explored its association with neurodevelopment at 12 months and 4 years of age in infants fed standard or enriched infant formulas.			This follow-up study involves a subset of those children. Standard formula n =48 Enriched formula n = 56 Breastfed n = 37.		No safety related measures or adverse events reported.	

Reference	Objective (s)	Study design	Country	Study population, age at baseline and number	Study groups and intervention	Summary of findings relating to safety	Study limitations
Diéguez et al. (2022)	To analyse potential long-term differences depending on the diet with an experimental infant formula (EF), compared to a standard infant formula (SF) or breastfeeding (BF) during the first 18 months of life on children's hypothalamic functional connectivity (FC) assessed at 6 years old			This follow-up study involves a subset of those children. Standard formula n =22 Enriched formula n = 20 Breastfed n = 20.		No difference in BMI or head circumference was found between groups. While all mean glucose levels were in a normal range, BF children showed lower mean glucose levels compared to the SF-fed group (p = 0.027), and there were no differences between children fed with BF and EF.	
Nieto-Ruiz et al. (2022)	To analyse the long-term effects of an experimental infant formula (EF) on neurocognitive function and brain structure in healthy children aged 6 years compared to those fed with a standard infant formula or breastfed.			This follow-up study involves a subset of those children. Standard formula n =37 Enriched formula n = 39 Breastfed n = 32.		At 6 years old, children from the three study groups did not differ in their anthropometric characteristics, including BMI and head and waist circumferences.	

Reference	Objective (s)	Study design	Country	Study population, age at baseline and number	Study groups and intervention	Summary of findings relating to safety	Study limitations
Diéguez et al. (2023)	To analyse the long-term effects of early nutrition on glycaemic variability in healthy children.			This follow-up study involves a subset of those children. Standard formula n =32 Enriched formula n = 32 Breastfed n = 28		At 6 years old, BF children had lower mean glucose levels and higher multiscale sample entropy (MSE) compared to those fed with SF. No differences in MSE were found between EF and BF groups.	

3.2.2.3 Preclinical safety and tolerance of Lacprodan® MFGM-10

There have been many studies using MFGM in neonatal animals. Specific examples related to cognitive health effects are detailed in Table 3-4 (Section 3.2.3.3). In both rats (L. R. Brink, Gueniot, & Lonnerdal, 2019; Collins et al., 2022; Jiang, Du, Brink, & Lönnerdal, 2022; Moukarzel et al., 2018) and pigs (Berding et al., 2016; Fil et al., 2019; Zhang et al., 2023), no issues have been found for body weight gain and no studies have noted any problems associated with toxicity or illness. However, there have been no preclinical studies specifically focused on safety and tolerance identified.

3.2.2.4 Safety and tolerance – conclusion

In conclusion, the extensive use of the Lacprodan® MFGM-10 as a source of MFGM in a growing number of clinical trials and follow-up studies, has enabled the safe use to be established and evaluated on-going.

Lacprodan® MFGM-10 is used worldwide as a component of infant formulas, as are similar products from other manufacturers, with no evidence that demonstrates, or suggests reasonable grounds to suspect any safety or tolerance issues to infants from its use. Furthermore, Lacprodan® MFGM-10 has been studied in 9 prospective, randomized, double-blind, placebo-controlled or parallel-arm studies (Best et al., 2023; Billeaud et al., 2014; Hedrick et al., 2021; Jaramillo-Ospina et al., 2022; F. Li et al., 2019; X. Li et al., 2019; Ruiz et al., 2017; Timby, Domellof, et al., 2014; Zavaleta et al., 2011) which have included 2890 formula-fed infants, 1314 of whom received Lacprodan® MFGM-10 at levels of 5-6 g/L for up to one year. The studies were completed in different infant populations – Chinese, Swedish, French/Italian, Spanish, American, Chilean, Australian, and Peruvian, representing a range of ethnicities.

Across other studies with formula supplemented MFGM-like ingredients safety and tolerance of the formula are consistently reported.

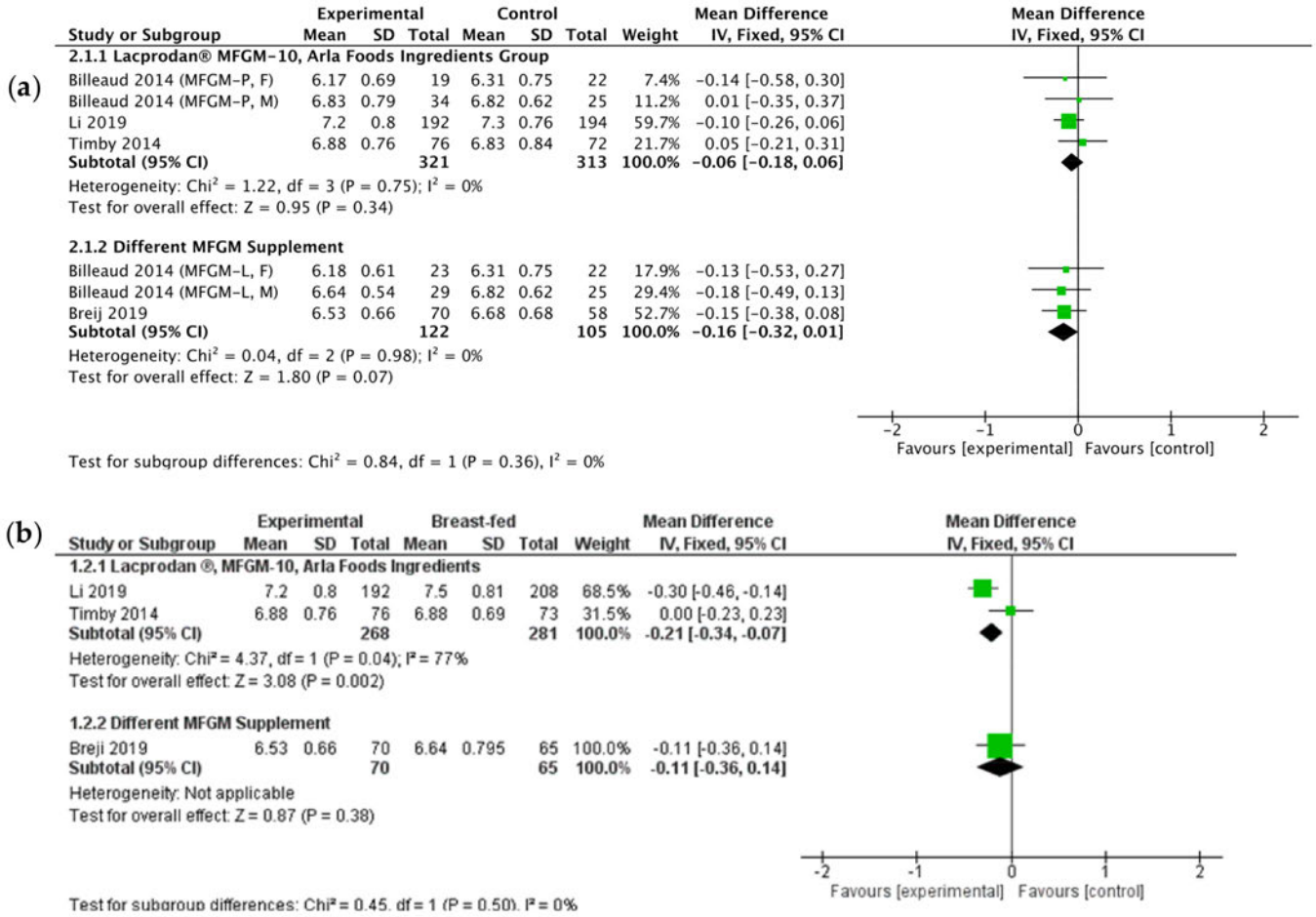
In conclusion, the growth, tolerance, AE, and morbidity outcomes reported across all relevant studies provide direct evidence for safety and tolerance of formulas containing Lacprodan® MFGM-10 and similar MFGM products.

3.2.2.5 After-marketing surveillance

Arla Foods Ingredients P/S as the ingredient supplier is unable to directly gather after-marketing data related to the safety and tolerance of Lacprodan® MFGM-10 as that information is held by its customers and brand-owners of the IPF. In the time that Lacprodan® MFGM-10 has been used in IFP, well over a decade, no concerns regarding the safety or tolerance of the ingredient has been related back to AFI by its customers.

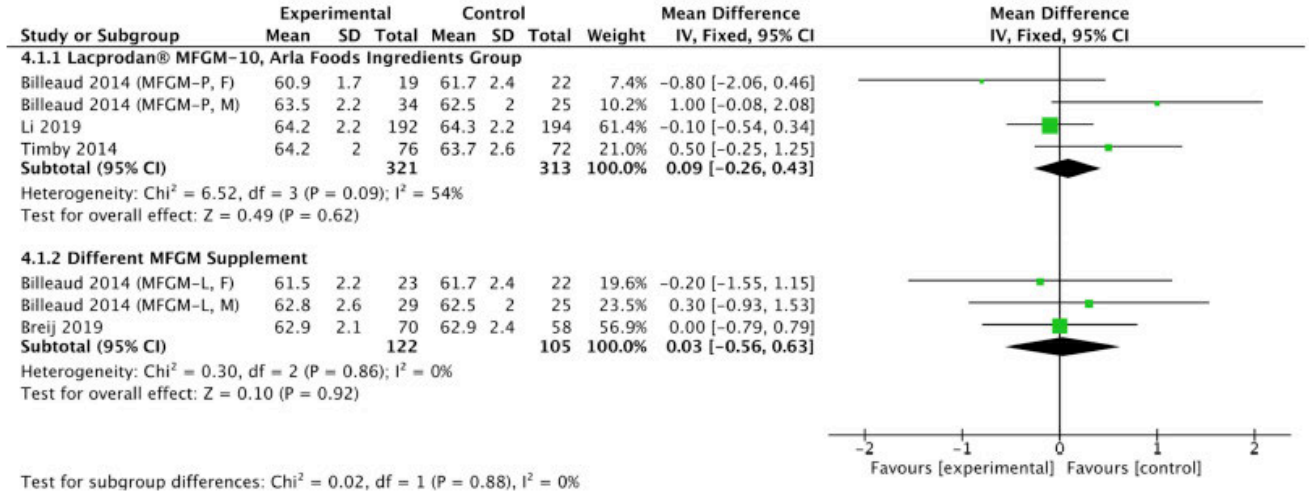
Figure 3-1 Meta-analyses of growth parameters at 4 months of age between children fed with standard formula or breastfed and formula supplemented with MFGM from Ambrożej et al. (2021)

(a) Weight comparing the experimental formulas (including MFGM) with the standard formulas;
(b) Weight comparing the experimental formulas (including MFGM) with breastfeeding;

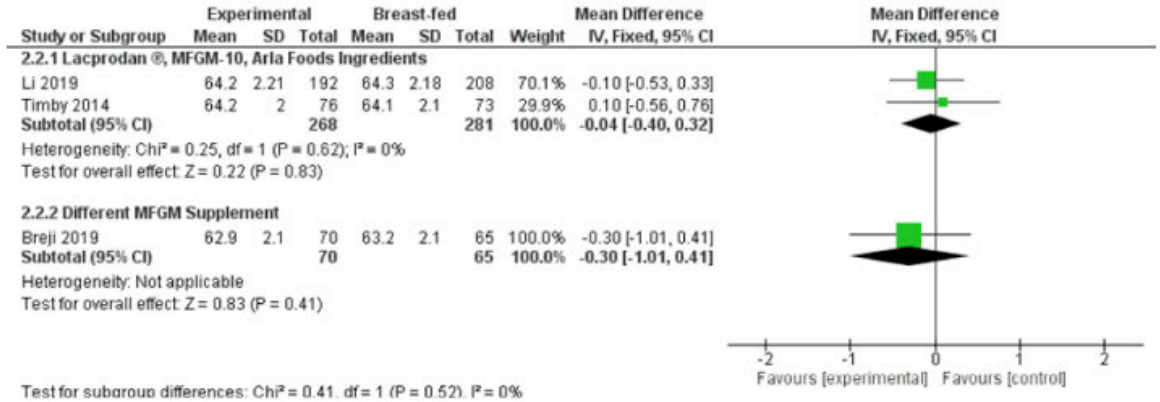


(c) Length comparing the experimental formulas (including MFGM) with the standard formulas;
(d) Length comparing the experimental formulas (including MFGM) with breastfeeding;

(c)

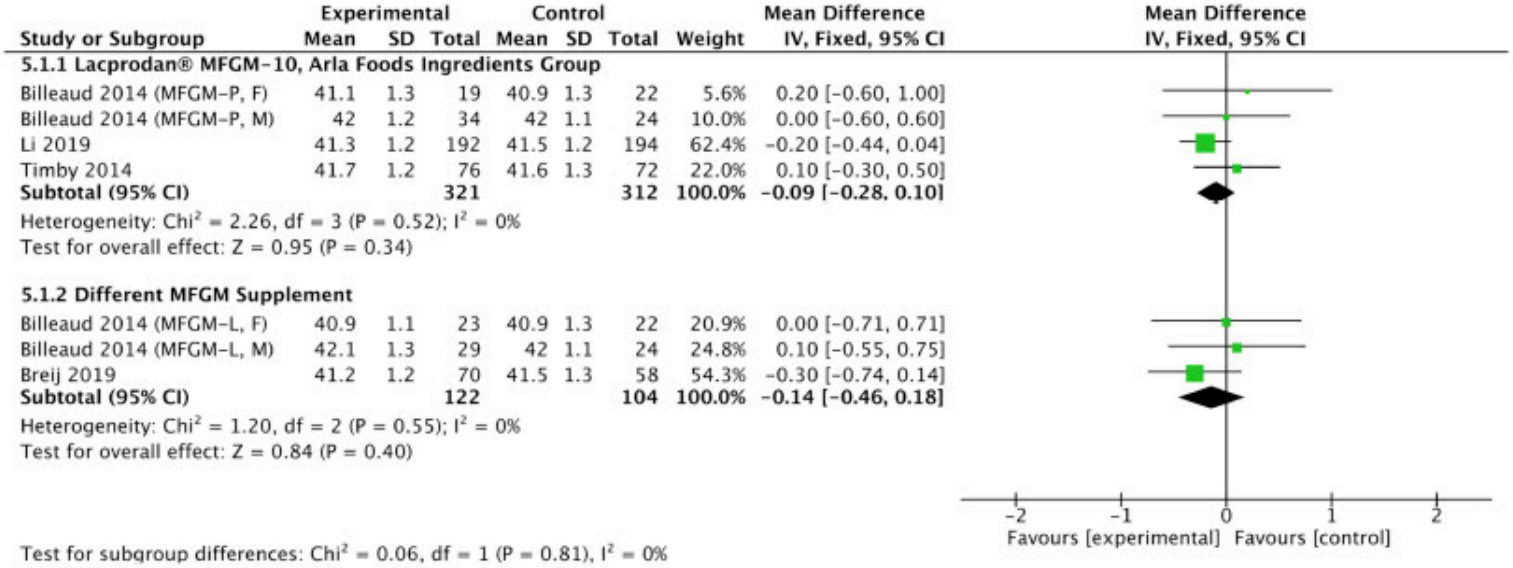


(d)

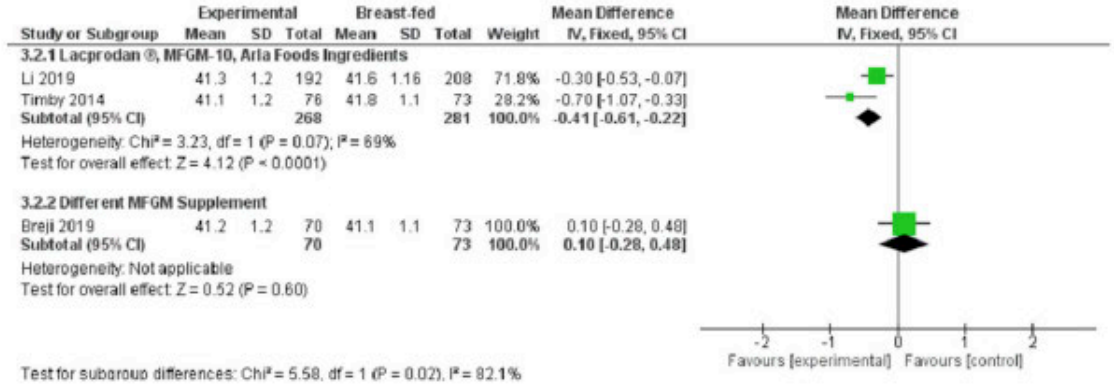


(e) Head circumference comparing the experimental formulas (including MFGM) with the standard formulas;
(f) Head circumference comparing the experimental formulas (including MFGM) with breastfeeding

(e)



(f)



3.2.3 Efficacy of the proposed compositional change – supporting neural development and cognitive function

Breastmilk is the preferred and recommended nutrition for infants, typically associated with better developmental outcomes of breast-fed compared to formula-fed infants. It provides all the essential nutrients for infant growth and development, together with an extensive assortment of bioactive components associated with digestion, absorption, gastrointestinal functions, growth, immune development and neurodevelopment (Demmelmair et al., 2017; Lönnerdal, 2014). Alterations in nutritional factors during early development can exert long term effects on growth, neural function and associated behaviours (Vickers et al., 2009).

This section outlines the evidence supporting the benefits of adding Lacprodan® MFGM-10 to infant formula products, compared to formula not containing added MFGM material, notably support for improved neural development and cognitive function.

3.2.3.1 Mechanistic action of MFGM relating to neural development and cognitive function

The exact mechanisms by which components of the MFGM and in particular the milk-derived polar lipids may modulate neurodevelopment are still largely unknown (Henriksen et al., 2021).

In the short term (up to 12 months of age) clinical trials of infant formulas that include an MFGM ingredient find cognitive benefits including attention, short term memory, language, visual processing and overall cognitive test scores (Cerdó et al., 2022; F. Li et al., 2019; Timby, Domellof, et al., 2014; Xia et al., 2021). Clinical studies using components of MFGM have also been shown to produce cognitive benefits, for example, gangliosides (Gurnida et al., 2012) and phospholipids, in particular sphingomyelin (Tanaka et al., 2013). This suggests that these components could play a role in the cognitive effects seen with MFGM supplementation.

Long-term follow-up studies of infant clinical trials using MFGM enriched infant formula show some lasting improvements in cognitive, language and behavioural scores (Colombo et al., 2023; Nieto-Ruiz, Diéguez, Sepúlveda-Valbuena, Catena, et al., 2020; Nieto-Ruiz, Diéguez, Sepúlveda-Valbuena, Herrmann, et al., 2020; Nieto-Ruiz et al., 2022; Lidewij Schipper et al., 2023). However, the results are more varied than the shorter-term cognitive results and not all studies find lasting effects into 6 years of age (Timby et al., 2021).

One proposed mechanism is improved myelination due to MFGM supplementation. Deoni et al. (2018) conducted a longitudinal study in infants using MRI to assess myelination from infancy to childhood. Throughout early neurodevelopment, myelination helps provide the foundation for brain connectivity and supports the emergence of cognitive and behavioral functioning. Early life nutrition is an important and modifiable factor that can shape myelination and, consequently, cognitive outcomes (Deoni et al., 2018). The study found that phosphatidylcholine and sphingomyelin (key components of MFGM) are highly associated with myelin development. Evidence is mounting of the relationship between dietary sphingomyelin, myelination and cognitive development (Albi et al., 2022; C. Jiang et al., 2022; Oshida et al., 2003; Y. Yuan et al., 2024). Further evidence for a role in myelination was found in a clinical trial that supplemented children with an alpha-lactalbumin enriched WPC with high levels of sphingomyelin and phospholipids. Increased myelination after 12 months of supplementation, lasted through to two years of age (Schneider et al., 2022; Schneider et

al., 2023). Supporting this proposed mechanism, MFGM supplementation has previously been shown to increase serum phospholipids, in particular sphingomyelin and phosphatidylcholine and a study using MFGM supplementation has also shown changes in auditory event related potentials that suggest improved neural circuit maturation and myelination at 24 months of age (Algarin et al., 2022). Preclinical studies also find improved memory and learning in animals receiving MFGM-enriched formula (L. R. Brink et al., 2019; L. R. Brink & Lonnerdal, 2018; Collins et al., 2022; O'Mahony et al., 2020; Zhang et al., 2023) as in the clinical studies mentioned above. The studies also find decreased anxiety and stress related effects (Collins et al., 2022; Mika et al., 2018; Mudd et al., 2016; O'Mahony et al., 2020) which could relate to the behavioural effects reported in clinical trials. Preclinical work finds that these cognitive effects are due to increased neurotransmitters and receptors important for learning and memory and increased markers of neuronal growth (L. R. Brink et al., 2019; L. R. Brink & Lonnerdal, 2018; Mika et al., 2018) which is in line with observations of increased connections in the brain (Waworuntu, Hanania, Boikess, Rex, & Berg, 2016), increased myelination (Zhang et al., 2023) and generally more brain maturation (Mudd et al., 2016). Two studies in pigs also report changes to the brain lipidome after MFGM consumption (Fraser et al., 2022; Oliveira et al., 2022). In particular Fraser et al. (2022) noted changes in the hippocampal lipidome, a brain region that plays a key role in learning and memory. Similar to outcomes in clinical studies, pigs consuming a phospholipid rich whey protein concentrate show increased levels of plasma phospholipids and sphingolipids (Henriksen et al., 2021).

A further putative mechanism involves the role of MFGM components in the structural modification of fat globules. This may impact intestinal lipid availability, digestion and absorption, altering lipid bioavailability and brain accretion of released PUFA's and other lipid molecules involved in neurodevelopment (L. Schipper et al., 2016). This is supported Gázquez et al. (2023) who reported MFGM may improve bioavailability of DHA, therefore some cognitive benefits may also be due to improved uptake of LC-PUFAs. Rodent studies have previously shown a relationship between dietary LC-PUFA intake, changes in brain lipid composition and behavioural outcome suggesting an effect of dietary lipids on brain function (Chung, Chen, & Su, 2008).

Another potential mechanism proposed is that in addition to a direct effect of MFGM lipids on brain development, they may also exert indirect effects on the brain via the gut–brain axis (Fil et al., 2019). Bioactive lipids are able to affect gut microbiota composition and in turn influence gut-brain signaling by different mechanisms including modulation of neural, immune and endocrine pathways (Baptista, Sun, Carter, & Buford, 2020). In clinical trials, MFGM has been shown to impact gut microbiota (He et al. 2019, Lee et al. 2020, Zhao et al 2022, Chichlowski et al. 2021, Cerdo et al 2022).

3.2.3.2 Evidence from intervention studies in infants

Early infancy represents a significant and critical period to secure optimal brain development. The infant brain undergoes significant development from birth throughout the first years of life. The development includes both structural and organisational elements ensuring proper functionality. During this time, a rapid increase in brain growth, along with many new connections in the brain and a process called myelination to strengthen and mature these new connections occurs. Optimal nutrition is required to provide the right building blocks to ensure this significant development. Several studies report exclusive breast feeding is positively associated with cognitive abilities early in

life, suggesting that human milk contains components that support brain development (J. W. Anderson, Johnstone, & Remley, 1999; Pereyra-Elías, Quigley, & Carson, 2022) .

One component of breast milk found only in low amounts in infant formula is the milk fat globule membrane. This compound provides many essential components required for brain development including but not limited to sphingolipids, phospholipids, cholesterol, proteins, fatty acids, and glycoproteins (Davies et al., 2022). Human milk supplies the rapid developing brain with these components to ensure appropriate brain development and there is considerable evidence the milk fat globule membrane contributes to this supply of essential components for neurodevelopment (Lauren R. Brink & Lönnerdal, 2020).

A literature search (Section 2.1.2.1, Figure 2-1) identified a total of sixteen (16) publications relating to seven (7) prospective intervention studies in healthy term infants investigating neurodevelopmental and cognitive outcomes. These key attributes of these studies are summarised in

Table *3-3* , and discussed in more detail below.

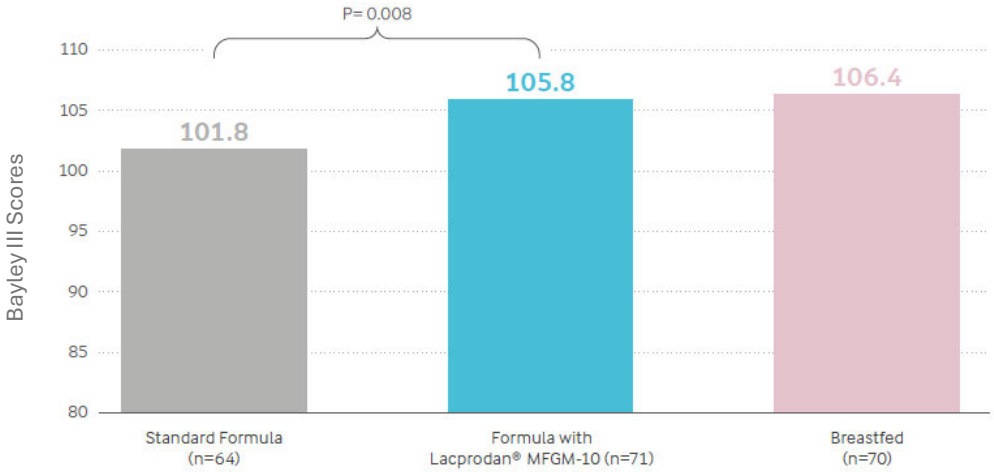
In a prospective, randomised, double-blind, controlled clinical intervention study (TUMME) (Timby et al. 2014a), 160 healthy term infants were randomly assigned to one of two formulas -an experimental formula enriched in Lacprodan® MFGM-10 (EF, n=80, 73 completed the study) and a standard formula (SF, n=80, 68 completed the study). A BFR reference group (BFR, n=80, 72 completed the study) was also included. The level of protein originating from Lacprodan® MFGM-10 in the formula was 5.4 g/100 g final formula. With a reconstitution rate of 114 g powder/L the concentration of Lacprodan® MFGM-10 in the final liquid formula was 6 g/L. The EF had lower energy (60 versus 66 kcal/100 mL) and protein level (1.20 versus 1.27 g/100 mL) compared to the SF.

The primary outcomes of this study were weight and health at 6 months of age and psychological assessment using the Bayley Scale of Infant Development (BSID), Third Edition, carried out at 12 months of age. The infants were enrolled at an age of 44 ±11 days (EF), 47 ± 10 days (SF), and 48 ± 5 days (BFR). The infants received the formulas from inclusion to 4 months of age, at which time weaning foods could be introduced. Infants continued to receive study formulas to 6 months of age.

At 12 months of age, the cognitive score (Bayley - III), was significantly higher in the EF group than in the SF group (105.8 ± 9.2 compared with 101.8 ± 8.0; $p = 0.008$) but was not significantly different from that in the BFR group (106.4 ± 9.5; $p = 0.73$) (Figure 3-2). Timby, Domellof, et al. (2014) concluded that supplementation of formula with MFGM (as Lacprodan® MFGM-10) narrows the gap in cognitive development between BF and formula-fed infants.

In a follow up study of the original TUMME cohort at 6.5 years of age, cognitive and executive functions were assessed using the Wechsler Intelligence Scale for Children 4th Edition IWISC-IV), Brown Attention-Deficit Disorder Scales for Children and Adolescents (Brown-ADD), and Quantified Behaviour (Qb) tests, and behaviour using the Child Behaviour Checklist (CHCL) and Teacher's Report Form (TRF) (Timby et al., 2021). Of the children enrolled in the original study, 58 (73%), 56 (70%) and 64 (80) of the EF, SF and BFR groups were still in the study and completed the psychological assessments at 6.5 years.

Figure 3-2 Primary cognitive outcome scores of groups at 12 months of age



Supplemental data showed the BFR group had higher scores in full scale IQ, verbal comprehension, perceptual reasoning, and working memory from WISC-IV than the EF and SF groups pooled together. In addition, the proportion of children with a borderline indication of affective problems in the Child Behaviour Checklist (CBCL) was lower in the BFR group than in the EF and SF groups pooled together, whereas there were no differences between the BFR and formula-fed groups in any of the other problem areas of the CBCL or in any of the problem areas of the TRF. Timby et al. (2021) speculated that as environmental factors other than early nutrition and genetics also influence neurodevelopment and may explain why the early differences between EF and SF groups did not persist. In addition, the authors noted the study was underpowered to detect the difference in cognitive scores between groups observed at 6.5 years of age. The study team concluded the consumption of a low-energy protein formula with bovine MFGM as infants, had no effect on neurodevelopment in children at 6.5 years (Timby et al., 2021).

In a multi-centre study in China, 451 healthy term infants were enrolled into a randomised, double-blind, controlled trial to evaluate neurodevelopmental outcomes at 1 year of age (F. Li et al., 2019). Participants were randomised to receive either a standard formula (SF) (n = 228) or a similar formula with added Lacprodan® MFGM-10 (5 g/L) and bovine lactoferrin (bLf) (0.6 g/L) (MFGM+LF; n=223). No breastfed comparator group was included in the study. Participants received the study formulas exclusively from randomisation at day 10-14 through day 120. Complementary feeding was permitted from day 120 in combination with the infant formula through day 180. A corresponding follow-on formula was fed during the interval from day 180 through day 365, resulting in a final intervention period of 12 months. Participants were eligible to continue in the study and complete neurodevelopmental testing at days 365 and 545 even if study formula consumption was discontinued after 180 days of age. The primary outcome was the Bayley-III cognitive composite score at day 365, and the study was powered to detect a 5-point difference in the Bayley-III cognitive composite score.

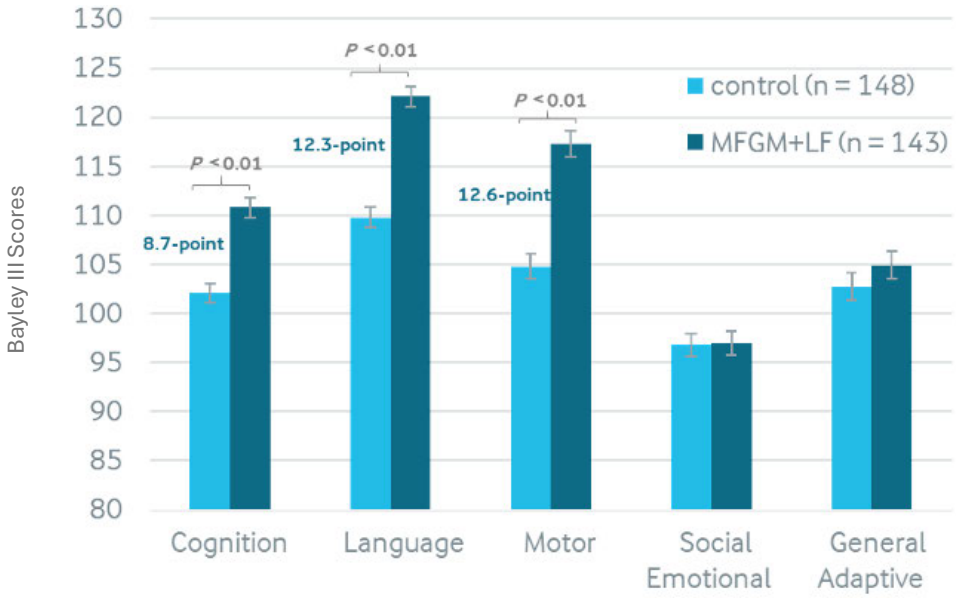
As shown in Figure 3-3, the cognitive outcome measures showed that the MFGM+LF group had higher mean cognitive (111.0 ± 0.9 vs 102.3 ± 0.9; an 8.7-point difference), language (122.6 ± 0.9 vs 110.3±

0.9; a 12.3-point difference), and motor (118.3 ± 1.2 vs 105.7 ± 1.2; a 12.6-point difference) scores (P < 0.001) at 12 months compared to the control group.

Along with evaluation of the Bayley-III cognitive scores at day 545, other secondary neurodevelopmental outcomes included Ages & Stages Questionnaire (ASQ), MacArthur-Bates Communicative Development Inventories (CDI), and Carey Toddler Temperament Scales (TTS). The ASQ was completed at days 120, 180, and 275. The CDI, TTS, and Single Object Free Play Task were conducted at days 365 and 545.

Higher ASQ scores for the MFGM +bLf groups at day 120 were statistically significant, although higher scores for the experimental formula persisted through day 275, they were not significant. No difference in CDI scores were observed at day 365, however several measures were significantly higher in the MGFM + bLf group at day 545 (F. Li et al., 2019) .

Figure 3-3 Bayley III composite scores at 12 months of age



This study suggests that infants receiving infant formula products enriched in MFGM and lactoferrin had an accelerated neurodevelopmental profile compared to the control formula-fed infants (F. Li et al., 2019). Although the individual contributions of MFGM and lactoferrin were not evaluated, it should be noted that lactoferrin has not been shown to impact cognitive development on its own (Miyakawa, Oda, & Tanaka, 2022).

Colombo et al. (2023) reported on a 5.5 years follow-up to the study of F. Li et al. (2019). One hundred and sixteen (n = 116) children were included in the follow-up study (standard formula n=59, MFGM enriched formula n = 57). Using the Wechsler Preschool & Primary Scale of Intelligence, fourth edition higher overall IQ (98.7 ± 1.4 vs 93.5 ± 1.5; p = 0.012), higher processing speed (107.1 ± 1.4 vs 100.0 ± 1.4; p < 0.001) and higher visual spatial scores (100.6 ± 1.7 vs 95.3 ± 1.7; p = 0.027) was observed in the MFGM + bLf group as compared to the standard infant formula group (Colombo et al., 2023). No differences were found in working memory, fluid reasoning or verbal comprehension. On the dimensional change card sort (DCCS), a test of cognitive flexibility, they also found that the MFGM + bLf group had higher scores in the most challenging phase of this test (7.4 ± 0.27 vs 6.5 ± 0.28, p = 0.013). Finally, with the Stroop test, (a test of attention, inhibitory control, and processing speed), they found the MFGM + bLf group had higher scores than the standard infant formula group (15.6 ± 0.4 vs

13.2 ± 0.4, p < 0.001). No group differences in the Child behaviour Checklist score were observed between groups. Overall, the authors show lasting improvements in cognitive scores in the MFGM group at 5.5 years of age.

The COGNIS study (A Neurocognitive and Immunological Study of a New Formula for Healthy Infants) was aimed to evaluate the effects of a new infant formula enriched with bioactive components on child neurocognitive, growth, and immunological development, compared to those infants who received a standard infant formula or were breastfed (Nieto-Ruiz et al., 2019). The COGNIS study was designed as a prospective, randomized double-blind, nutritional intervention study that would allow ongoing follow-up (Salas Lorenzo et al., 2019). At the baseline visit for the study 220 healthy term infants were enrolled, including n = 50 breast-fed infants as the BF control. Infants receiving formula were randomised to receive either standard infant formula (SF) (n=85) or an enriched infant formula (EF) (n=85) which included MFGM (10% of total protein content (wt:wt)), synbiotics, LC-PUFAs (long chain polyunsaturated fatty acids), gangliosides, sialic acid and nucleotides. Infants received infant formula from randomisation (0-2 months) until 6 months, after which they received a corresponding follow-on formula from 6-18 months of age. The enriched infant formula was supplemented with bioactive compounds, including MFGM components (10% of total protein content (wt:wt)), synbiotics (mix of fructooligosaccharides (FOS) and inulin (ratio 1:1); *Bifidobacterium infantis* IM1 and *Lactobacillus rhamnosus* LCS-742), LC-PUFAs (AA and DHA), gangliosides, nucleotides and sialic acid. Children in the COGNIS study cohort have participated in follow-up studies from 2.5 though 6 years of age.

[redacted]

Nieto-Ruiz et al. (2019) reported on visual function (VF), an indicator of neurodevelopment, in infants in the COGNIS study at 3 and 12 months of age. Visual function was measured using cortical visual evoked potentials using electromyography, a technique where a fitted cap with electrodes is placed on the head to measure electrical potentials from the brain. The percentage of infants that responded to different binocular frequencies was determined according to intervention group.

At 12 months of age, SF and EF infants presented prolonged latencies and lower amplitudes in the P100 wave than BF infants. In the EF group, a higher percentage of infants presented response at 7 ½' of arc compared to 3 months of age; a similar proportion of BF and EF infants presented responses at 7 ½' of arc at 12 months of age. Although it cannot be determined if one ingredient was responsible for the observed effects or if it is a combination of them all, the authors conclude that early nutritional intervention with bioactive compounds could narrow the gap in growth and neurodevelopment between breastfed and formula-fed infants (Nieto-Ruiz et al., 2019).

The effects of the enriched formula on child behaviour and psycho-emotional disorders up to 2.5 years of age was investigated in a subset of the original COGNIS cohort using the Child Behaviour Checklist (CBCL) at 18 months (SF, n = 47; EF, n = 48; BFR, n = 37) and 2.5 years (SF, n = 29; EF, n = 41; BFR, n = 33) of age (Nieto-Ruiz, Diéguez, Sepúlveda-Valbuena, Herrmann, et al., 2020). The association between type of feed and CBCL scores categorised into normal, borderline, and pathological outcomes suggested there was no association of feeding type at 18 months and outcome category. However, at 2.5 years, SF-fed children were classified more frequently as borderline on internalising problems than BFR children (SF: 24.1%; BFR: 3.0%; p = 0.042). The EF-fed

children less frequently presented clinical pathological affective problems compared to SF-fed children at 2.5 years old (EF: 0.0%; SF: 13.8%; p = 0.026). Overall, the percentage of EF children who were classified as normal behaviour was more similar to that of the BFR children. The effects of feeding type were also compared to CBCL scores. Again, no difference was observed between groups at 18 months. At 2.5 years of age children in the SF group had higher scores in internalising (p = 0.035) and total problems (p = 0.017), as well as ADHD (p = 0.039), compared to those who were breastfed. Children in the EF or BFR groups had lower scores in externalising problems (p = 0.005) than SF-fed children. Overall, CBCL scores did not differ between children who received EF and BFR infants. Further analyses showed the significant differences between groups did not remain after adjustment for maternal educational level, socioeconomic status and place of residence (Nieto-Ruiz, Diéguez, Sepúlveda-Valbuena, Herrmann, et al., 2020). Further analysis of the data using a longitudinal model for behaviour development up to 2.5 years again showed significant differences between the SF and EF groups in internalising (p = 0.047), total (p = 0.044), ADHD (p = 0.036), and oppositional defiant problems (p = 0.003). Similar score increases were observed between EF and BFR groups, and SF children also showed significantly higher increases in scores for externalising problems (p = 0.026) compared to EF or BFR groups. Overall, this study suggests that the formula containing MFGM, as a component in an enriched formula, may have a beneficial effect on behavioural development through to 2.5 years of age, with no major behavioural differences between infants consuming that formula and breast-fed infants.

Language development in was assessed at 4 years of age in 122 children who were a part of the COGNIS study cohort (Campoy, Nieto-Ruiz, Arias, et al., 2018; Nieto-Ruiz, Diéguez, Sepúlveda-Valbuena, Catena, et al., 2020). One hundred and twenty-two (N = 122) children were available for assessment at the 4-year follow-up (SF, n = 46; EF, n = 43; BFR, n = 33). Language development was assessed using the Oral Language Task of Navarra-Revised (PLON-R). ANCOVA, chi-square test, and logistic regression models were performed. Children in the enriched formula group were less likely to be rated as delayed or need to improve than the standard formula group (32.6% vs 60.9%, p = 0.02) in language use. The enriched formula group also scored better on spontaneous language use compared to the standard formula group, with children in the SF group being more at risk of suffering language development progress than children in the BFR group. Nieto-Ruiz, Diéguez, Sepúlveda-Valbuena, Catena, et al. (2020) concluded that at 4 years, the enriched formula was associated with beneficial long-term effects on language development.

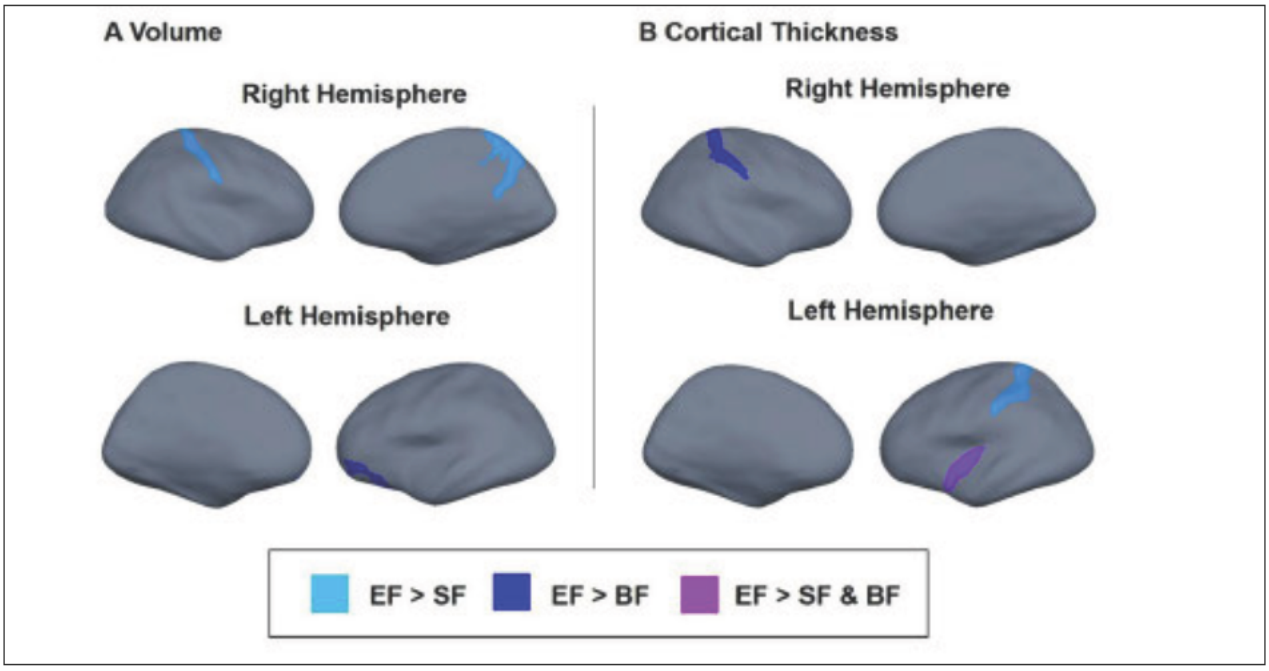
Cerdó et al. (2022) explored the association of gut microbiota maturation with neurodevelopment at 12 months and 4 years of age in children within the COGNIS cohort. Infant neurodevelopment was assessed at 12 months using the BSID-III and at 4 years PLON-R (Nieto-Ruiz, Diéguez, Sepúlveda-Valbuena, Catena, et al., 2020). Maturation of the gut microbiota was determined using genomic DNA isolated from faecal samples collected from participants at 1, 6, 12, and 18 months of age. Three (3) distinct age-associated microbial enterotypes were identified with the trajectory and rate of the shift between enterotypes strongly associated with type of feeding. Within the EF group, there was a distinct split in the microbiota maturation with a fast trajectory more similar to the SF group, and a slow trajectory more similar to the BFR group. EF infants with slow trajectories were more often in-home reared and born by vaginal delivery to mothers with pre-pregnancy lean BMI (Cerdó et al., 2022). At 12 months of age, infants in the EF group with fast trajectories had significantly higher language (p = 0.015) and expressive language (p = 0.014) scores than infants in the BFR group, with no differences observed between the slow trajectory EF group and BFR group. At 4 years of age, PLON-R

assessments showed no significant differences in language performance between gut microbiota maturation groups and BF. Cerdó et al. (2022) concluded that feeding the enriched formula, containing MFGM together with probiotics, prebiotics and LC-PUFA, in a specific infant environment supported probiotic growth and retarded gut microbiota maturation patterns and resulted in similar neurodevelopmental outcomes to breast-fed infants.

Nieto-Ruiz et al. (2022) followed up with 108 children (SF, n = 37; EF, n = 39; BFR, n = 32) from the original COGNIS study at six (6) years of age for further neurocognitive testing and assessment of brain structure using magnetic resonance imaging (MRI). No difference in outcomes between the infant formula groups on the Kaufman Brief Intelligence Test (K-BIT) or the PLON-R. However, the enriched formula group did show a higher IQ and vocabulary than the breastfed reference group on the K-BIT. On the Computerized Battery for Neuropsychological Evaluation of Children, the EF group showed better performance on an attention task (continuous performance, errors of commission) than the BFR group.

At 6 years of age differences in some aspects of brain structure were observed between groups. Analysis of MRI output (Figure 3-4) showed children in the EF group had greater volumes in parietal regions than the SF group (p = 0.002). Children in the EF group also had greater cortical thickness in the insular (p = 0.012) and temporal (p = 0.027) regions than those in the SF or BFR groups.

Figure 3-4 Differences in brain volume(A) and cortical thickness (B) between children in the COGNIS study at 6 years of ageFigure 3-2



(from Nieto-Ruiz et al. (2022))

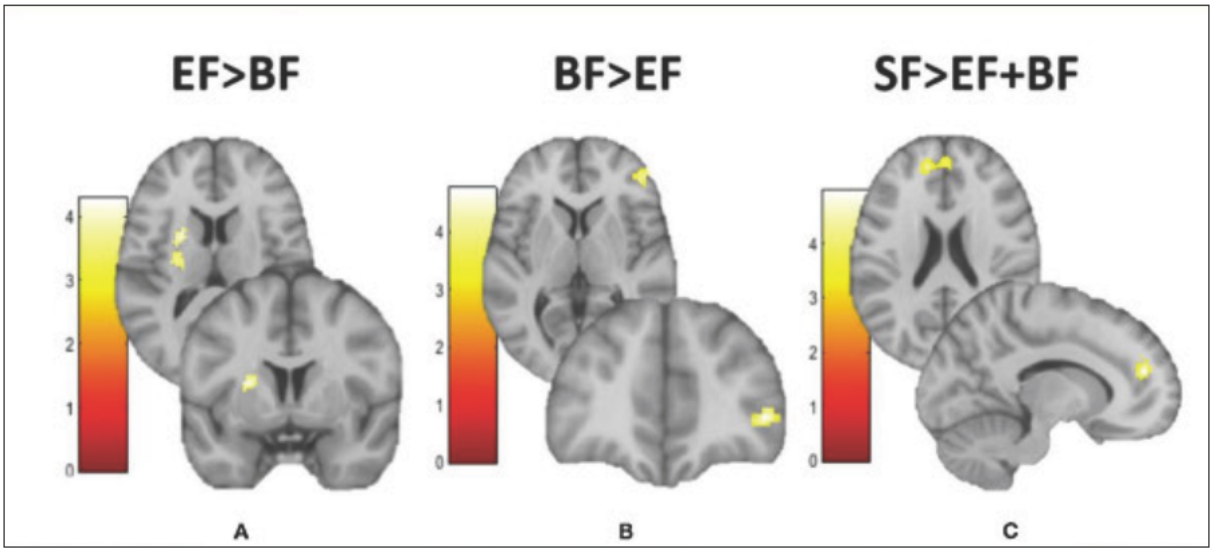
Analysis adjusted by smoking during pregnancy, maternal age, familiar socioeconomic status, age, and sex of the children. Brain volume analysis was also corrected for total brain volume. Experimental infant formula (EF) > Standard infant formula (SF) (light blue); EF > BF (dark blue); and EF > SF and EF > BF (purple). SF, Standard infant formula; EF, Experimental infant formula; BF, breastfed infants.

Increased brain volume provides a greater number of neurons and neural connections, supporting improved cognitive functioning, whilst thicker cortical regions are associated with increased neural density and connectivity, facilitating more efficient information processing. Specific brain regions

showing differences in volume and cortical thickness, such as the parietal, frontal, insular, and temporal regions, are involved in various cognitive processes. The positive association between brain structure and cognitive performance suggests that the structural differences in the brain contribute to improved cognitive development in terms of language (verbal comprehension) and executive function (working memory) (Nieto-Ruiz et al., 2022).

A further exploration of brain function in children participating in the COGNIS study at 6 years of age was reported by Diéguez et al. (2022). Differences in brain function in different regions of the brain were observed using MRI. Compared to the EF-fed group, BFR children showed higher functional connectivity (FC) between the medial hypothalamus (MH) and the inferior frontal gyrus (IFG), as well as lower FC between the MH and the left putamen extending to the middle insula (Figure 3-5). Moreover, those children in groups fed with EF and BF showed lower FC between the MH and the anterior cingulate cortex (ACC) in comparison with children fed with SF (Figure 3-5). These areas are key regions within the salience network, which is involved in processing salience stimuli, eating motivation, and hedonic-driven desire to consume food (Diéguez et al., 2022).

Figure 3-5 Differences between study groups in the resting-state functional connectivity of the medial hypothalamus



from (Diéguez et al., 2022).

Colour bars represent the connectivity intensity value or t-value. (A) EF > BF: MH-putamen extending to insula; (B) BFR EF: MH-IFG; (C) SF > EF + BFR: MH-dorsal ACC. ACC, anterior cingulate cortex; BFR, breastfeeding; EF, experimental infant formula; IFG, inferior frontal gyrus; MH, medial hypothalamus; SF, standard infant formula.

This study provides further evidence that an enriched formula containing MFGM may contribute to brain development in terms of specific regions of hypothalamic functional connectivity developed are more similar to those of breast-fed infants and this may impact healthy eating patterns in later life.

In summary, the COGNIS clinical trial has demonstrated persistent cognitive and behavioural effects of an infant formula enriched with MFGM. However, it should be noted that these effects cannot necessarily be contributed to MFGM alone as there were other ingredients and the individual and/or synergistic effects between them were not assessed.

Participants in a randomised, double blind controlled study of Peruvian 6 to 11 month old infants received a complementary food with the recommended dietary allowance (RDA) of micronutrients

and a protein source as either whey protein concentrate (Lacprodan®-MFGM-10, source of MFGM) or skim milk powder (Control) (Zavaleta et al., 2011) and were followed up at 14 years of age and assessed for the effects of the MFGM intervention on executive functions (Lazarte et al., 2022) and cognitive development (Lazarte, Garcia, Lonnerdal, Slupsky, Murguia-Peniche, Heckmann, & Kvistgaard, 2021). Of the original study cohort (n=499), 398 adolescents (79.8%) were located for follow-up.

Lazarte et al. (2022) assessed executive functions using the Cambridge Neuropsychological Test Automated Battery (CANTAB). CANTAB tasks provide a comprehensive computerized assessment of cognitive abilities, including executive functions. For this study, 4 executive functions were assessed through CANTAB: cognitive flexibility, control inhibition, visuospatial memory, and spatial working memory. Whilst gender showed a significant effect on each of the 4 executive functions, with boys performing better than girls, there was a significant effect the feeding group ($p = 0.036$) on strategic working memory with those in the MFGM supplemented group performing better than control group. This follow-up study suggests infants receiving MFGM had significant advantages on a strategic working memory task at 14 years of age, even when important covariates were appropriately controlled (Lazarte et al., 2022). These data add to the mounting evidence that feeding components from MFGM may have lasting and meaningful effects on neurocognitive outcomes.

Cognitive development was assessed in the follow-up cohort using the Wechsler Intelligence Scales for Children (Fourth Edition, WISC-IV; Spanish language version [Mexico]) is comprised of 13 subtests grouped in four composite scores (Verbal Comprehension, Perceptual Reasoning, Working Memory, and Processing Speed), together with the Full Scale Intelligence Quotient (FSIQ) determined by the sum of 10 of the subtests (Lazarte, Garcia, Lonnerdal, Slupsky, Murguia-Peniche, Heckmann, & Kvistgaard, 2021). No statistically significant differences in mean WISC-IV composite scores or FSIQ scores were detected between study groups suggesting infants having MFGM have similar outcomes to those receiving standard complementary food when evaluated at 14 years.

In China, a prospective, multi-centre, double blind randomised trial was conducted to evaluate the effects of a MFGM enriched infant formula on the neurodevelopmental outcomes of healthy term Chinese infants (Xia et al., 2021). At baseline 418 infants were enrolled with a breast-fed reference group (BFR, n = 206) and randomisation to either the MFGM enriched formula (EF, n = 108) or standard formula (SF, n = 104) of the remaining infants. Infant formula (birth – 6 months) and follow-on formula (6 – 12 months) were provided with the key compositional difference between EF and SF being the enrichment of the EF with MFGM-rich ingredient (SureStart™ MFGM Lipid 100; NZMP, Fonterra) that provided minimum ganglioside concentrations of 17.9 mg/100 g (infant formula) and 16.9 mg/100 g (follow-on formula).

Cognitive function was assessed using the Bayley-III test at 6 and 12 months. At 6 months there were no differences in any of the Bayley-III composite scores between the formula-fed groups (p values ranged between 0.16 and 0.95). Across the range of parameters evaluated the BFR sored significantly higher than the SF group, but not the EF group.

At 12 months the Bayley-III social emotional and adaptive behaviour composite scores were 3.50 (95% CI 0.03 to 6.79, p= 0.048) and 5.62 (95% CI 1.78 to 9.38, p = 0.004) points higher in the EF than in the SF group. The cognitive score was 2.86 points higher in the MF group than in the SF group, the difference was not statistically significant (p = 0.08). All composite scores of the BFR group were higher than those for MF and the SF groups at 12 months (Xia et al., 2021). There was no difference

in composite attention scores between the formula-fed groups at 12 months ($p > 0.05$), however for short-term memory, the mean score for EF (102.15±1.87) was significantly higher than for SF (95.29±1.86) at 12 months (95% CI 1.40 to 12.33, p=0.002). Xia et al. (2021) concluded the MFGM-enriched formula improved some measures of cognitive development in Chinese infants and putatively attributed this to the ganglioside content.

In a Chilean study, Algarin et al. (2022) used a range of neurophysical outcomes to assess cognitive maturation in infants at 24 months. The infants were a subset of the cohort in a double blind, randomised controlled trial that included a BFR group, n = 235, and infants randomised to receive either a SF (n = 174) or a formula enriched with 5 g/L of Lacprodan® MFGM-10 (n = 173) through to 12 months of age. Neurophysiologic syllable sound perception was evaluated at 24 months of age by testing the difference in auditory related potentials familiar and unfamiliar sounding syllables, using electroencephalographic recordings collected in children using a [geodesic] sensor net (128 scalp sites). The auditory event-related potential (ERP) was collected in 122 children at day of life 730 (SF, n=42; EF, n=35; BFR, n=45). Infants who received the EF showed lower event related potential amplitude compared to BFR ($p < 0.04$) and shorter latency for unfamiliar stimuli than both the BFR group ($p < 0.03$) and SF group ($p < 0.003$). This difference may reflect a higher degree of neural circuit maturation and improved myelination resulting from the consumption of MFGM enriched formula (Algarin et al., 2022).

Lidewij Schipper et al. (2023) reported on a follow-up study in infants through to 5 years of age who participated in the Mercurius study (Breij et al., 2019). The study aimed to determine if the composition and structural properties of the lipid droplets in infant formula could influence cognitive performance in childhood. Analysis of erythrocyte fatty acids at 3-months of age showed some differences between the formula groups and the breastfed group, particularly for the LCPUFA's. The fatty acid profile of breastmilk in the original study was not measured and therefore some differences may have been largely related to the oil composition of the formula products rather than the influence of MFGM. For example, the erythrocyte alpha-linolenic acid (ALA, 18:3n-3) levels were higher in both formula-fed groups compared to the breastfed group. On the other hand changes in LCPUFA uptake had previously been shown to be influenced by the presence of MFGM and the potentially the lipid droplet size (Lidewij Schipper, van Dijk, & van der Beek, 2020), and LCPUFA to influence cognitive outcomes in childhood (Colombo et al., 2013). The cognitive function of children in the follow-up study was assessed using the National Institutes of Health Toolbox Early Childhood Cognition Battery (NHITB-CB) at 3, 4, and 5 years of age. No group differences were found at 3 or 4 years of age. At 5 years of age the enriched formula had higher scores than the standard formula ($p = 0.021$) on the DCCS task, a test of cognitive flexibility. The enriched formula group as comparable to the breastfed group in the highest reached levels on the Flanker Inhibitory Control and Attention (FICA) test for inhibitory control and selective attention. These results were more similar to the breastfed infants. Lidewij Schipper et al. (2023) concluded that 3–4 months exposure during infancy to an IF that more closes resemble human milk in lipid composition as well as structural properties of lipid droplets may positively affect cognitive outcomes during childhood. And that the effects may be mediated by differences in LCPUFA incorporation in tissue membranes during early life.

The outcomes of these clinical trials are also backed up by other clinical observations. For example that ingredients with MFGM components such as those used in Schneider et al. (2022) were attributed to increased myelination in 6 month old infants. Myelination is a process key to establishing strong and lasting connections between neurons and is associated with better cognitive function. As well,

similar improved cognitive scores have been found in pilot studies of infant formulas containing the key components of MFGM; phospholipids (Gurnida et al., 2012) and sphingomyelin (Tanaka et al., 2013).

Together the body of clinical evidence shows at long-term follow-ups of infant clinical trials using MFGM enriched infant formula show some lasting improvements in cognitive and behavioural scores are observed. However, the results are more varied than the shorter-term cognitive results and not all studies find lasting effects into 6 years of age. Although there is variation in the length of dosing and types of cognitive tests, generally with MFGM enriched infant formulas are associated with improved cognitive scores and potentially better social, emotional, and behaviour traits. No studies reported worse performance than those receiving a standard formula.

Table 3-3 Intervention studies assessing the benefits of MFGM on neurodevelopment and cognition in infants

References	Objectives	Study design	Country	Study population, ages at baseline and number	Study groups and intervention	Summary of findings	Significant of findings
Timby, Domellof, et al. (2014) (TUMME Study)	To test the hypothesis that feeding an infant formula with reduced energy and protein densities and supplemented with MFGM reduces differences in cognitive development and early growth between formula-fed and breastfed infants.	Prospective, double-blind, randomised controlled trial	Sweden	160 healthy term infants, 2 months of age, randomly assigned to the experimental or control formula (n=80 per group) A breastfed reference (BFR) group (n = 80) N recruited = 80 in each group N in final analysis = 73 experimental formula, 68 standard formula, 72 breastfed. Dropouts were mostly "no cause given" or "moved from study site" (n=12). Most common causes of discontinued intervention were cows milk allergy (n=3) and gastrointestinal symptoms (n=2)	**Experimental formula:** MFGM-supplemented, low-energy, low-protein experimental formula (EF) (6 g MFGM/L) Lacprodan® MFGM-10; AFI, Denmark **Control formula:** a standard formula (SF) **Breastfed reference group** The energy and protein contents of the EF and SF were 60 and 66 kcal/100 mL and 1.20 and 1.27 g/100 mL, respectively. Duration: from 2 months until 6 months of age	At 12 months of age, the cognitive score on the Bayley -III was significantly higher in the EF group than in the SF group (P=0.008), but was not significantly different from that in the BFR group (P=0.73). Motor scores on the r-III were comparable among the 3 groups. Verbal scores were significantly higher in the BFR group compared to the EF (P=0.025) and SF groups (P=0.029). The EF group ingested larger volumes of formula than did the SF group (P=0.022), fully compensating for the lower energy density. No significant differences in linear growth, weight gain, body mass index, percentage body fat, or head circumference were found between the EF and SF groups	Evidence for beneficial health effect of MFGM by improving cognitive outcomes at 12 months of age

References	Objectives	Study design	Country	Study population, ages at baseline and number	Study groups and intervention	Summary of findings	Significant of findings
Timby et al. (2021) (TUMME study)	To evaluate neurodevelopment, growth, and plasma cholesterol status at 6 and 6.5 y of age in the same study population.			Of the original cohort: n=58 experimental formula, n=56 standard formula and n=64 breastfed.		At 6.5 years of age no difference was found between formula groups on the Weschler Intelligence Scale for Children fourth edition, the Brown Attention-Deficit Disorder Scales for Children and Adolescents, Quantified Behavior tests, and behavior using the Child Behavior Checklist and Teacher's Report Form . There were no differences between the formula groups in weight, length, or head or abdominal circumferences, nor in plasma concentrations of homocysteine, lipids, insulin, or glucose.	Evidence that infants given MFGM for four months have similar cognitive outcomes as infants given standard formula when tested at 6.5 years of age.
F. Li et al. (2019)	To evaluate neurodevelopment, growth, and health outcomes in infants receiving bovine milk fat globule membrane (MFGM) and lactoferrin in infant formula	Randomised, double-blind, controlled, multi-centre, parallel group	China	Healthy term infants <14 days of age Enrolled: Control, n= 228; MFGM + Lf, n = 223 Day 365: Control, n= 148; MFGM + Lf, n = 144 Day 545: Control, n= 88; MFGM + Lf, n = 95	**Control formula** (stage 1 & 2) **Test formula with MFGM** (5g/L Lacprodan® MFGM-10, AFI, Denmark) + 0.6 g/L lactoferrin (Lf) (stage 1 & 2) Exclusive formula feeding to day 120 Stage 1 formula to day 180, stage 2 formula to day 365	**Primary:** Bayley-III cognitive composite mean ±SE score at day 365 significantly higher for the MFGM + LF vs the control group (111.0± 0.9 vs 102.3± 0.9; p <0.001). **Secondary:** No statistically significant differences in weight growth or other anthropometric measures by gender between groups. Formula intake and tolerance similar between groups. Significantly less adverse events in the MFGM + Lf group.	Evidence for beneficial health effect of MFGM by improving cognitive outcomes at 12 months of age

References	Objectives	Study design	Country	Study population, ages at baseline and number	Study groups and intervention	Summary of findings	Significant of findings
						Ages and Stages Questionnaire scores significantly higher for MFGM + Lf group at day 120, persisting through day 275. No differences in MacArthur-Bates Communicative Developmnet Inventories scores at day 365, but significantly higher at day 545 in MFGM + Lf group.	
Colombo et al. (2023)	To evaluate neurodevelopmental outcomes at 5.5 years of age in this same study population			116 infants enrolled and completed assessments (Control: 59, MFGM+LF: 57).		**Primary Outcomes:** Weschler Intelligence Scale for Children fourth edition composite scores for Visual Spatial (5 point difference, p=0.027), Processing Speed (7 point difference, p<0.001), and Full Scale IQ (5 point difference, p=0.012) were significantly higher for MFGM+LF vs Control. **Secondary outcomes:** Stroop Task scores were significantly higher in MFGM+LF vs control. Using the Dimensional Change Card Sort test, a test of cognitive flexibility, they found higher scores (P=0.013) in the border phase (the most complex/challenging phase) and more children passed the border phase (32% vs 12%; P=0.039) for MFGM vs control. No group differences in the Child Behaviour Checklist score were detected.	Evidence for beneficial health effect of MFGM by improving cognitive outcomes also at 5.5 years of age.

References	Objectives	Study design	Country	Study population, ages at baseline and number	Study groups and intervention	Summary of findings	Significant of findings
Campoy, Nieto-Ruiz, Arias, et al. (2018) (abstract) Nieto-Ruiz, Diéguez, Sepúlveda-Valbuena, Catena, et al. (2020)a (COGNIS study)	To analyse the long-term effects of a new infant formula enriched with bioactive compounds on healthy children's language development at four years old.	Prospective, randomized double-blind, nutritional intervention study	Spain	Healthy term infants >2 months of age **Standard formula** (n=85) **Enriched infant formula** (EF, n=85) **Breastfed reference group** n= 50 Exclusively breastfed for at least 2 months, were included between 0–6 months of age Up to 18 months of life, a total of 40 infants were excluded in the SF and EF groups as follows: 24 were excluded in the SF group (1 infant due to perinatal hypoxia, 1 infant had growth deficiency, 15 infants did not take the infant formula, 2 had colic of the infant, 3 were excluded due to lactose intolerance, 1 infant due to digestive surgical intervention, and 1 infant su ered hydrocephalus); 16 infants were excluded in the	Infants received infant formula from randomization (0-2 months) until 6 months, after which they received a corresponding follow-on formula from 6-18 months of age Standard infant formula: (Stage 1 and follow on) Enriched formula included MFGM (10% of total protein content (wt:wt), synbiotics, LC-PUFAs, gangliosides, sialic acid and nucleotides Duration: from 2 to 18 month of age	Oral Language Task of Navarra-Revised (PLON-R) testing at 4 years of age found that EF children had higher scores in use of language (p=0.033) oral spontaneous expression than SF children (p=0.014). SF children were more frequently categorized into "need to improve and delayed" in the use of language than EF children (p=0.020)	Evidence for beneficial health effect of MFGM by improving language outcomes at 4 years of age.

References	Objectives	Study design	Country	Study population, ages at baseline and number	Study groups and intervention	Summary of findings	Significant of findings
				EF group (2 infants presented growth deficiency, 2 infants lactose intolerance, 11 infants did not take the infant formula, and 1 was excluded due to epileptic seizure). Furthermore, one infant of the BF group was excluded because he/she was not breastfed This follow-up study involves a subset of those children. Standard formula n =46, Enriched formula n = 43 Breastfed n = 33.			
Nieto-Ruiz et al 2019	To analyze the influence of a new enriched-infant formula with bioactive compounds on growth, neurodevelopment, and visual function (VF) in healthy infants during their first 18 months of life.			This follow-up study involves a subset of those children. Standard formula n =48, Enriched formula n = 56 breastfed n = 37.		Neurodevelopment was assessed by general movements at 2, 3 and 4months. No differences were found between groups. Visual function, as a measure of brain maturation, was measured by cortical visual evoked potentials at 3 and 12 months. Formula fed infants had longer latencies and lower amplitudes in the P100 wave than BF infants at 3 months of age ($p<0.01$ for all arcs). The EF group had a higher percentage of infants that responded at 7 ½ ' of	Evidence that MFGM results in more similar visual function to breastfed infants at 12 months of age

References	Objectives	Study design	Country	Study population, ages at baseline and number	Study groups and intervention	Summary of findings	Significant of findings
						arc at 12 months compared to 3 months of age (p=0.001). A similar proportion of BF and EF infants responded to the 7 ½ ' arc at 12 months of age and higher than that of SF infants (p=0.03)	
Nieto-Ruiz, Diéguez, Sepúlveda-Valbuena, Herrmann, et al. (2020)b (COGNIS study)	To analyze the effects of a bioactive nutrients-enriched-infant formula on children's behavior up to 2.5 years, compared to a standard infant formula or breastfeeding			This follow-up study involves a subset of those children. Standard formula n =29, Enriched formula n = 41 breastfed n = 33.		Using the Child Behaviour Checklist it was found that EF children aged 2.5 years presented fewer pathological affective problems than SF children (p=0.026) Rates of externalizing problems were increased in SF infants compared to EF and BF infants (p=0.005). SF children presented higher scores in internalizing (p = 0.035) and total problems (p = 0.017), as well as ADHD (p = 0.039), compared to those who were breastfed. No differences in behaviour between formula groups was found at 18 months.	Evidence for beneficial health effect of MFGM by improving behavioural outcomes at 2.5 years of age.
Nieto-Ruiz et al. (2022) (COGNIS study)	To analyse the long-term effects of an experimental infant formula (EF) on neurocognitive function and brain structure in healthy children aged 6 years compared to those			This follow-up study involves a subset of those children. Standard formula n =37 Enriched formula n = 39 Breastfed n = 32.		Using the Kauffman Brief Intelligence test EF children had higher vocabulary scores (p=022) and higher IQ scores (p=0.031) than BF children. Using the Computerized batter for neuropsychological evaluation of	Evidence for beneficial health effect of MFGM by improving language and cognitive outcomes at 6 years of age.

References	Objectives	Study design	Country	Study population, ages at baseline and number	Study groups and intervention	Summary of findings	Significant of findings
	fed with a standard infant formula or breastfed.					children (BENCI) they found that EF and SF children had better performance in an attention task than BF children (p=0.001). No difference was found between the groups in language using the PLON-R test. Magnetic Resonance Imaging (MRI) at 6 years old showed that EF children had greater volumes in the left orbital cortex than BF children (p=0.012) EF children also presented greater volumes in parietal regions than SF children (p=0.002). Additionally, greater cortical thickness in the insular (p=0.012), and temporal (p=0.027) areas were found in children from the EF group than those fed with SF or BF groups. Further correlation analyses suggest that higher volumes and cortical thickness of different parietal and frontal regions are associated with better cognitive development in terms of language (verbal comprehension) and executive function (working memory).	

References	Objectives	Study design	Country	Study population, ages at baseline and number	Study groups and intervention	Summary of findings	Significant of findings
Cerdó et al. (2022) (COGNIS study)	To compare the dynamics of gut microbiota maturation and explored its association with neurodevelopment at 12 months and 4 years of age in infants fed standard or enriched infant formulas.			This follow-up study involves a subset of those children. Standard formula n =48 Enriched formula n = 56 Breastfed n = 37.		Microbiota maturation in EF split into a fast trajectory similar to the SF group, and a slow trajectory similar to the BF group. EF infants with slow trajectories were more often in home reared and born by vaginal delivery to mothers with pre-pregnancy lean BMI. At 12 months of age, language (p=0.015) and expressive language scores (p=0.014) were significantly higher in EF infants with fast trajectories than in BF. Neurodevelopmental outcomes were similar between EF infants with slow trajectories and BF at 12 months and 4 years of age.	Evidence for beneficial health effect of MFGM by improving language outcomes and giving similar cognitive outcomes as breastfed infants at 12 months of age.
Diéguez et al. (2022) (COGNIS study)	To assess long-term differences of hypothalamic functional connectivity, assessed at 6 years old.			This follow-up study involves a subset of those children. Standard formula n =22 Enriched formula n = 20 Breastfed n = 20.		At 6 years of age, groups fed with EF and BF showed lower functional connectivity between the medial hypothalamus (MH) and the anterior cingulate cortex (ACC) in comparison with SF-fed children. Moreover, the BF children group showed lower functional connectivity between the MH and the left putamen extending to the middle insula, and higher functional connectivity between the MH and the inferior frontal gyrus (IFG) compared to the EF-fed children group. These areas are key regions within the salience network, which is involved in processing salience stimuli, eating motivation, and hedonic-driven desire to consume food.	Evidence of potential health benefit of MFGM showing more similar brain development to breastfed infants at 6 years of age which may impact healthy eating choices later in life.

References	Objectives	Study design	Country	Study population, ages at baseline and number	Study groups and intervention	Summary of findings	Significant of findings
						In addition, BF children showed lower mean glucose levels compared to SF-fed children at 6 years old. EF was no significantly different than either the SF or EF groups.	
Lazarte, Garcia, Lonnerdal, Slupsky, Murguia-Peniche, Heckmann, and Kvistgaard (2021) (abstract)	To evaluate the effects of early nutrition on cognitive outcomes at 14 years of age	Randomized, double-blind controlled study	Peru	Infants; 6-11 months n=499 in original study n=398 in this follow up study and 386 completed testing.	Complementary food (40 g/d) divided into 2 servings **Control Group:** complementary food with skim milk (n=246) **MFGM Group:** complementary food enriched with MFGM protein fraction (Lacprodan®, Arla Foods Ingredients, Viby, Denmark; n=253) Duration: Daily, for 6 months	Using the Weschler Intelligence Scales for Children (WISC-IV), at 14 years of age there were no differences between the groups. Using the Full Scale Intelligence Quotient there were also no differences.	Evidence that infants given MFGM have similar cognitive outcomes as infants given standard complementary food when tested at 14 years of age.
Lazarte et al. (2022) (abstract)	To evaluate the effects of early nutrition on executive function at 14 years of age					Cambridge Neuropsychological Test Automated Battery (CANTAB) used to assess cognitive flexibility, control inhibition, visuospatial memory, and spatial working memory. Significant differences observed on	Evidence that infants given MFGM have significant advantage for aspects of working memory of those that did not when at 14 years of age supports the potential for long term neurocognitive outcomes.
Xia et al. (2021)	To evaluate neurodevelopment and growth of healthy term infants fed formula supplemented with MFGM	Prospective, multi-center, double-blind, randomized Trial	China	Healthy term infant >14 days of age N= 212 infants **Standard infant formula** (n=104) **Enriched infant formula** (n=108). **Breastfed reference group** n= 206	Formulas were made with the same macro and micronutrient composition. A stage 1 formula was used for 0-6 months and a follow-on formula given from 6-12 months. **Control formula** (stage 1 & 2) **Test formula with MFGM** (Fonterra, NZ) with a minimum ganglioside concentration of	Primary outcomes: Using the Bayley-III at 12 months of age they found that the EF group had better social emotional (a 3.5-point difference, p=0.048) and general adaptive behaviour (a 5.62-point difference, p=0.004) than the SF group. Cognitive scores were 2.8 points higher in the EF group than the SF but this was only a trend (p=0.08)	Evidence for beneficial health effect of MFGM by improving social emotional, behavioural and attention outcomes at 12 months of age

References	Objectives	Study design	Country	Study population, ages at baseline and number	Study groups and intervention	Summary of findings	Significant of findings
				61 infants dropped out. There was no significant difference in dropout rate among the formula-fed groups. The most common reason for discontinuation was related to formula intolerance, as evidenced by constipation (EF, n=3; SF, n=5), vomiting (SF, n=1), and allergic reaction (SF, n=1). The most common reason for withdrawing from the breastfed reference group was the perception of insufficient milk production. Other reasons were loss of contact, voluntary withdrawal, and inability to follow protocols.	17.9mg/100g (first formula) and 16.9 mg/100g (follow-on formula) Duration: 0 to 12 months of age.	Secondary outcomes: The EF and BF infants had better attention scores at 6 months of age and better short-term memory scores at 12 months. At 4 months, serum gangliosides were significantly higher in EF and BFR than SF (95% CI 0.64 to 13.02; p=0.025). No differences were found in measures of growth between formula groups.	
Lidewij Schipper et al. (2023)	To evaluate the effects of an infant formula mimicking human milk lipid composition and milk fat globule structure on childhood cognitive performance.	Randomized, double-blind, controlled, prospective, multi-country trial	Netherlands, Belgium, France and Singapore	Healthy term infant <35 days of age **Standard formula:** n=108 **Enriched formula:**	The formulas were similar in energy content, total lipid content and n-3 and -6 PUFA composition Standard formula with vegetable oil. Lipid droplets were small (~0.5 um)	Cognitive outcomes were tested at 3, 4 and 5 years of age using the National Institutes of Health Toolbox Early Childhood Cognition Battery (NHITB-CB).	Evidence for beneficial health effect of MFGM by improving cognitive outcomes at 5 years of age

References	Objectives	Study design	Country	Study population, ages at baseline and number	Study groups and intervention	Summary of findings	Significant of findings
				N=115 **Breastfed reference group:** n=88 Duration: 0-17 weeks of age	Enriched formula: the vegetable oil was partially replaced by dairy lipids (48%), and milk PL derived from bovine MFGM were added. Lipid droplets in this formula were large (mode diameter of ~3–5 um)	No group differences were found at 3 or 4 years of age. The only group difference found due to formula group was at 5 years of age where the enriched formula had higher scores than the standard formula (p= 0.021) on the Dimensional Change card sort task, a test of cognitive flexibility. These results were more similar to the breastfed infants.	
Algarin et al. (2022) (ChiNuT)	To assess growth and tolerance in infants receiving an enriched infant formula.	Double-blind randomized, controlled trial	Chile	Healthy infants < 120 days of age **Standard formula:** (n=174) **Enriched formula:** (n=173) **Breastfed reference group:** (n=235) A subset of children (n=122) underwent neurophysiological testing Standard n=42, Enriched n=35, Breatfed n=45	**Control formula:** a standard infant formula **Test formula with MFGM** (5g/L Lacprodan® MFGM-10, AFI, Denmark) Duration: 0 to 12 months of age	**Primary Outcomes:** Growth and tolerance not reported in this abstract **Secondary outcomes:** At 24 months of age children were tested for neurophysiologic syllable sound perception. They tested the difference in auditory related potentials when listening to familiar, native but unfamiliar and foreign sounding syllables. Infant who received enriched formula showed lower event related potential amplitude (p=0.04 vs breastfed) and shorter latency for native unfamiliar stimuli (p=0.03 vs breastfed and p=0.003 vs standard formula). These changes may reflect a higher degree of neural circuit maturation and improved myelination.	Evidence for beneficial health effect of MFGM by improving outcomes reflecting neural circuit maturation and myelination at 24 months of age.

3.2.3.3 Supporting evidence from studies in animal models

A literature review (Section 2.1.2.2) was completed to identify preclinical studies that specifically included measures of neural development and/or cognitive outcomes in neonatal animals. Studies without these outcomes were excluded. The criteria for neonatal rodents (rats and mice) equates to study doses administered by post-natal day 10. A number of other studies with MFGM products in pregnant animals (e.g. Q. Yuan, Gong, Du, Li, & Mao, 2022), juvenile/young adult animals (e.g. Mika et al., 2018; O'Mahony et al., 2020; Waworuntu et al., 2016) and adult/aged animals (e.g. Davies et al., 2022; Y. Li et al., 2023) provide supportive information for other age ranges and putative mechanisms. Studies with MFGM products or products similar to MFGM have been included.

3.2.3.3.1 Studies in rats

Vickers et al. (2009) investigated the potential effects of an MFGM preparation on learning behaviour, and postnatal growth and development neonatal rats was. Neonatal male Wistar rats were supplemented with a "complex milk lipid (CML) preparation", a type of MFGM product. Rats were given either a high (1%) or low (0.2%) dose of CML via oral gavage from postnatal day 10 until weaning. Water was used as a control. After weaning through to postnatal day 80 they received a gel supplementation to the standard chow. They tested rats for cognitive performance in the Morris Water Maze (MWM), a test of spatial memory and the Novel Object Recognition Test (NORT), a test of novelty recognition and found that CML supplementation improved the performance on both tests ($p<0.05$ for MWM and $p<0.02$ for NORT). There was no effect of supplementation on operant learning.

Another study in rats was conducted by Guan et al. (2015) testing a complex milk lipid beta serum concentrate (BSC). Male rats were given a gel to supplement their food with either BSC or a blank control gel from postnatal day 10-70. Spatial memory was tested using the MWM and anxiety with a dark-light box and elevated plus maze. BSC supplementation improved spatial memory as seen by decreased latency to the platform in the MWM but had no effect on measures of anxiety. BSC supplementation also increased striatal dopamine terminals and hippocampal glutamate receptors.

L. R. Brink and Lonnerdal (2018) tested Lacprodan® MFGM-10 supplementation in a growth restriction model in rats. Rats were either in a normal litter (n=10 pups) or a growth restricted litter (n=16 pups). From postnatal day 2-21 rats received either MFGM-10 or non-fat milk via oral gavage. Cognitive testing using the T maze and passive avoidance tests found that while growth restricted animals had lower scores on the passive avoidance tests, MFGM supplementation prevented this reduction. In both normal and restricted growth animals, MFGM supplementation increased genes involved in brain function including brain-derived neurotrophic factor and St8 alpha-N-acetyl-neuraminide alpha-2,8-sialytransferase 4.

Moukarzel et al. (2018) tested the effects of bovine MFGM supplementation on reflex development and on brain lipid and metabolite composition in rats using the "pup in a cup" model. From postnatal day 5-18, rats received, via canula, either control formula or formula supplemented with Lacprodan® MFGM-10 from AFI. MFGM supplementation reduced the gap in maturation age between mother-reared and standard formula fed groups for measures of motor reflexes. MFGM supplementation also narrowed the difference in brain phospholipid and metabolite composition between mother-reared and standard formula fed groups.

L. R. Brink et al. (2019) again used a growth restriction rat model to investigate the effects of various MFGM components on cognitive outcomes. In this study growth restricted rat pups received one of 5 treatments from postnatal day 2-21: (a) Lacprodan® MFGM-10, (b) bovine phospholipid concentrate

(PL), (c) sialic acid (SIA) at 200 mg/kg body weight (bw) SIA100, (d) SIA at 2 mg/kg bw and (e) non-fat milk as control. This was essentially to test MFGM as whole compared to some of the individual components. The rats underwent behavioural tests including the T maze, NORT and MWM. Gene expression in the hippocampus was also assessed. L. R. Brink et al. (2019) found that MFGM-10 supplementation had higher T-maze scores than the SIA group (p = 0.01). At PD14, supplementation upregulated gene expression. At PD21 MFGM-10 group had higher BDNF, ST8Sia4 and Drd1 expression than control. There was little effect of supplementation of gene expression in adulthood. Only the phospholipid group had higher drd1 expression than the control. No other group differences were found in other behavioural tests or stereology. The authors note that compared to its individual components, MFGM had the largest impact on neurodevelopment through upregulation of genes and improved T-maze scores.

The effect of a phospholipid enriched whey protein concentrate (containing 10% lipids) vs a control formula (containing 35% corn oil, 50% soybean oil and 15% cocoa butter) on the brain lipidome was assess by Oliveira et al. (2022). Rats received this formula from postnatal day 7-21 via a feeding tube. The researchers found that phospholipid supplementation had significant spatial and temporal effect on specific fatty esters, glycerophosphocholines, glycerophosphoethanolamines, and phosphosphingolipid of the brain lipidome which could contribute to general brain growth.

Collins et al. (2022) used a maternal separation rat model to test the effects of Lacprodan® MFGM-10 supplementation on visceral pain and cognition. Rats were randomized to either be in a maternal separation (MS) group or a control group (no separation; NS) and to receive either a control food or an MFGM enriched food, resulting in four groups. NS-control, NS-MFGM, MS-control and MS-MFGM. Food was given in the form of pellets to the pregnant dam two days prior to birth and continued throughout the postnatal period. At weaning offspring then received the same food until the end of the experiment. They found that maternal separation resulted in visceral hypersensitivity and that this was ameliorated by MFGM supplementation. They also tested cognition using the NORT and the MWM. There was no effect of MFGM supplementation on the NORT but it did improve performance on the MWM.

3.2.3.3.2 Studies in pigs

Numerous studies have also been conducted in piglets. Mudd et al. (2016) supplemented full term piglets from postnatal day 2-31 with Lacprodan® MFGM-10 from AFI. The experimental formula included polydextrose (1.2 g/100 g diet), galactooligosaccharides (3.5 g/100 g diet), bovine lactoferrin (0.3 g/100 g diet), and Lacprodan® MFGM-10, 2.5 g/100 g diet). Learning and memory was tested using the T-maze and brain imaging was conducted using MRI. In the T maze, piglets given the experimental formula exhibited greater latency in acquisition and reversal of choice and the authors suggest that this may indicate that the experimental diet reduced impulsivity and/or anxiety in the piglets. Brain imaging showed no overall volumetric differences between groups. However, the piglets receiving the experimental formula had lower diffusivity in the internal capsule and a decrease in grey matter as compared to piglets receiving the control formula. The authors suggest that the decrease in grey matter could suggest more brain maturation due to increased axonal pruning.

Fil et al. (2019) supplemented full term piglets from postnatal day 2-31 with high (5g/L) or low (2.5 g/L) dose of Lacprodan® MFGM-10, and compared these pigs to ones receiving a control formula with no added MFGM. They tested cognition with the NORT and conducted brain imaging. They found few

group differences in brain volumes and water diffusivity and cognitive testing using the NORT. MFGM supplementation did increase serum cholesterol.

Henriksen et al. (2021) used a preterm piglet model (90% of term) to assess the cognitive effects of a phospholipid rich whey protein concentrate [redacted] or an extracellular vesicle enriched whey protein concentrate as compared to soy lecithin. MRI was used for brain imaging, plasma lipidomics analysis to understand changes in plasma lipids, and the T-maze for short term memory evaluation. They found improved hippocampal maturation in the two treatment groups as compared to the soy group as shown my lower mean diffusivity in the hippocampus. They also found increased plasma phospholipids and sphingolipids in the treatment group. No difference in hippocampal lipid composition or short-term memory were observed between the groups.

Fraser et al. (2022) analysed the brain lipidome of full-term piglets that received either a control, a low dose (4%) MFGM or a high dose (8%) MFGM formula from postnatal day 10-21. MFGM consumption did not significantly alter the lipidome in most brain regions, regardless of dose, compared to the control infant formula. However, in the hippocampus an increase in 16 triglyceride species due to MFGM supplementation was identified.

Zhang et al. (2023)supplemented full term piglets from postnatal day 2-31 with a high (6.09 g MFGM per 100g diet), medium (4.64 g MFGM per 100g diet), low (1.74 g MFGM per 100g diet) dose of Lacprodan®MFGM-10 compared to a control formula. Brain imaging using MRI was completed, with learning and memory assessed using the T-maze, and mRNA and protein expression in the hippocampus and prefrontal cortex measured. MFGM supplementation improved accuracy on the T-maze, with the low dose MFGM group having the best performance. As well, the fractional anisotropy in the left and right hippocampus of piglets in the low dose MFGM group was significantly higher than in the other three groups and this correlated with performance on the T-maze. MFGM supplementation also increased expression of BDNF.

Gázquez et al. (2023) evaluated the effect of MFGM plus milkfat added to infant formula on DHA availability in a suckling piglet model. Five (5) experimental groups were used to look at the difference in the lipid and phospholipid profile of the formula: L1 receiving vegetable fat and palm oil. L2 receiving canola oil. L3 receiving milk fat, canola oil and 1% MFGM. L4 receiving canola oil and 1% MFGM. L5 receiving milk fat, canola oil and 2% MFGM. The MFGM product was Lacprodan® MFGM-10 from AFI. Piglets in group L1-3 received the formula from postnatal day 5-21 and group L4/5 from postnatal day 3-21. It was found that L3 (milk fat and MFGM) increased DHA and LC-PUFA n-3 in the liver total fatty acids and in the jejunum compared to other formulas. When a higher dose of MFGM was use (group L5) then DHA was found both in peripheral tissues and plasma phospholipids. The authors conclude that MFGM supplementation may increase DHA availability of infant formulas.

Together these studies provide emerging evident the consumption of MFGM can impact the lipid and phospholipid composition of both plasma and brain, together with influence on brain structure, and that these changes may be associated with neurodevelopmental and cognitive changes observed in neonatal populations consuming MFGM.

Table 3-4 Preclinical studies on neurodevelopment and cognition

Reference	Animal model	Study groups	Dose and duration of treatment	Method of data collection	Study Outcomes
Vickers et al. (2009)	Growing Wistar male rats (n=96 at weaning; n=32/group at post weaning)	Control: chow Low CML High CML CML Mixed: Gavage–preweaning Dietary–postweaning	Study diets: Preweaning (gavage): - Control: water Low CML: 0.2% (w/w) intake High CML: 1% (w/w) of intake Post weaning: Control: Chow Low CML: chow, 0.2 CML gel High CML: chow, 1% CML gel CML fat: 5.9% GG (GM3, 0.7%; GD3, 5.2%), 50.6% PL. Source: milk-derived Dose duration from PD10-80	Behavioral tests: - MWM (n=16) - NORT (n=16) Operant learning techniques (n=48) Physical & chemical measures: Plasma lipids Brain GG & PL	CML supplementation significantly increased linear growth rate ($P<0.05$), and the improved growth trajectory was not attributed to the added usable energy of the nutritional supplement containing the CML or related to changes in body composition as quantified by dual- energy x-ray absorptiometry scanning. Compared to control groups, high CML groups had significant improvements in novelty recognition and spatial memory (during acquisition phase), with no differences in operant testing. Brain concentrations of total or individual species of PLs and GGs did not differ between control and treatment groups. **Summary:** The levels of lipids in the CML, in particular GGs, were in a physiologic range & within human milk range. Supplementation with a CML rich in PLs and GGs had positive growth and learning behavioral effects in young normal growing rats.
Guan et al. (2015)	Male Wistar rats (n=32, 16 per group)	Control: Blank gelatin Experimental: gelatin formulated with cream-derived complex lipid ingredient beta serum	The dose of BSC was 5.0 mg/g/day, which was calculated daily based on the body weight of the individual rat. Dose duration: PD10-70	Memory and anxiety-like behaviors were evaluated using the Morris water maze, dark–light boxes, and elevated plus maze tests. Neuroplasticity and white matter were measured using immunohistochemical staining.	No change on tests of anxiety (Dark-Light Box and Elevated plus maze) For the Morris Water Maze, the overall performance in seven-day acquisition trials was similar between the groups. Compared with the control group, BSC supplementation reduced the latency to the platform during day one of the acquisition tests. Supplementation improved memory by showing reduced latency and improved path efficiency to the platform quadrant, and smaller initial heading error from the platform zone.

Reference	Animal model	Study groups	Dose and duration of treatment	Method of data collection	Study Outcomes
		concentrate (BSC) Rats were hand fed from PN day 10 to PN day 21 (weaning day) and were individually cage fed thereafter until PN day 69.			Supplemented rats showed an increase in striatal dopamine terminals and hippocampal glutamate receptors. **Summary**: Complex milk lipid supplementation improved learning and memory, independent from anxiety. It also increased dopamine and glutamate in specific brain areas.
Mudd et al. (2016)	Full term piglet (n=24, 12 per group).	Control: control formula Experimental: formula with MFGM and lactoferrin	Experimental formula included with polydextrose (1.2 g/100 g diet), galactooligosaccharides (3.5 g/100 g diet), bovine lactoferrin (0.3 g/100 g diet), and Lacprodan® MFGM-10, 2.5 g/100 g diet) Dose duration: PD2-31	T-maze for neurodevelopment, MRI for brain imaging and brain tissue samples collected for mRNA expression	Diffusion tensor imaging revealed differences in radial ($P = 0.032$) and mean ($P = 0.028$) diffusivities in the internal capsule, where control piglets had higher rates of diffusion. Voxel-based morphometry indicated larger ($P < 0.05$) differences in cortical gray and white matter concentrations, with control piglets having larger tissue clusters in these regions In the T maze, piglets given the experimental formula exhibited greater latency in acquisition and reversal of choice. No difference in BDNF expression **Summary**: Piglets given experimental formula had brain changes that may suggestion more advanced brain maturation.
L. R. Brink and Lonnerdal (2018)	Sprague-Dawley rat litters were cross-fostered in random manner on PD2 to adjust litter size to either normal (n=10 pups/dam) or restricted (n=16 pups/dam)	Normal growth, Control Normal growth, Experimental Restricted growth, control	Diets administered by oral gavage. The mean volume of supplement (50 mg/ml) increased from 8 ul on PD 2 to 52 ul on PD 21. Control: Non fat milk Experimental: and Lacprodan® MFGM-10	Effects of growth restriction or dietary supplementation with MFGM on growth, cognitive function and gene expression in brain. T maze and passive avoidance for cognitive development. RT-qPCR for gene expression. Immunoblotting for protein expression.	There was a large effect of litter size when controlling for treatment, in which restricted rats were on average 5.88 g smaller than the normal rats, and MFGM groups weighed on average 1 g more than controls fed non-fat milk Rats who were growth restricted through litter size manipulation and received supplementation with MFGM had higher behavioral scores measured by T-maze as compared to pups who were growth restricted and received non fat milk. Females in the growth restricted group performed worse on T-maze and passive avoidance) than non-restricted females.

Reference	Animal model	Study groups	Dose and duration of treatment	Method of data collection	Study Outcomes
	growth. (n=52 total)	Restricted growth, experimental	Dose duration: PD 2 to 13 or 21 (weaning).		BDNF showed increased expression due to MFGM supplementation in both the normal and restricted growth animals. GluR-1, glucagon-like peptide 1 receptor and St8 alpha-N-acetyl-neuraminide alpha-2,8-sialyltransferase 4 all showed increased gene expression due to MFGM supplementation in normal growth animals and significant decreases due to restricted growth. There were no significant differences in myelin basic protein or dopamine receptor 1 expression among groups. **Summary**: MFGM upregulated genes involved in brain and cognitive development and improves cognitive performance on the T maze.
Moukarzel et al. (2018)	Sprague-Dawley rats, "pup in a cup" model (n= at least 6 per group)	Control: standard formula Experimental: Formula supplemented with Lacprodan® MFGM-10 Reference: Dam fed	Rat pups were fed by canula Experimental formula had 6g/L Lacprodan® MFGM-10. Dose duration: PD 5-18	Measures of physical feature development and reflex development. Brain lipid and metabolite composition.	No difference in brain or body weights. MFGM supplementation reduced the gap in maturation age between mother-reared and standard formula-fed groups for the ear and eyelid twitch, negative geotaxis and cliff avoidance reflexes. Significant differences in brain phospholipid and metabolite composition were found at d13 and/or d18 between mother-reared and standard formula-fed groups, including a higher phosphatidylcholine:phosphatidylethanolamine ratio, and higher phosphatidylserine, glycerol-3 phosphate, and glutamine in mother-reared compared to formula-fed pups. Adding MFGM to formula narrowed these differences. **Summary**: MFGM supplementation promotes reflex development and alters brain phospholipid and metabolite composition
Fil et al. (2019)	Full term piglets (n = 18 per group from across16 litters; n=54 total)	Control Low MFGM (2.5 g/L) High MFGM (5 g/L)	Formula contained Lacprodan® MFGM-10. Dose duration: PD 2 to 31.	Effects of added MFGM on behavioral testing, selective blood and tissue analyses and magnetic resonance imaging in young pigs	No group differences in body weight gain or milk intake were observed. MFGM increased serum lipoprotein cholesterol, but there were no group differences in early brain cholesterol concentrations, macrostructure, microstructure, or recognition memory of pigs at 31 days of age.

Reference	Animal model	Study groups	Dose and duration of treatment	Method of data collection	Study Outcomes
					Summary: MFGM was well-tolerated and supported growth. It also increased serum lipoprotein but no changes were found in the brain.
L. R. Brink et al. (2019)	Sprague-Dawley rat pups using a growth restriction model.	One of five treatments: (a) bovine MFGM Control: non-fat milk Experimental groups: Lacprodan® MFGM-10.: 100 mg/kg body weight Phospholipid concentrate (product name PL-20): 100 mg/kg body weight SIA 100 (sialic acid): 200 mg/kg body weight SIA (sialic acid): 2 mg/kg body weight	Pups were randomized, cross-fostered into litters of 17 pups per dam to produce growth restriction. Supplementation was given by oral gavage Dose duration: PD 2 to 21.	Effects of growth restriction and dietary supplementation with MFGM on growth, behavior and hippocampal gene expression. Behavioral tests were performed at adulthood: T-Maze Spontaneous Alternation, Novel Object Recognition and Morris Water Maze.	Growth was not different by treatment. The MFGM group exhibited higher T-maze scores compared to the SIA group (P=.01), but not the control or other groups. In the Novel Object Recognition test the Sia100 group was the only group to have an increased tendency to visit the novel object compared to control (P=.02) . No differences due to supplementation were found in the Morris Water Maze or nonbiased stereology. At PD14, supplementation did upregulate gene expression. MFGM, phospholipid and SIA100 groups had higher BDNF, GLuR-1 and ST8Sia4 expression than the control. At PD21 MFGM had higher BDNF, ST8Sia4 and Drd1 expression than control. Phospholipid, Sia100 and Sia groups also had increased Drd1 expression at PD21. There was little effect of supplementation of gene expression in adulthood. Only the phospholipid group had higher drd1 expression than the control. **Summary**: MFGM had an impact on neurodevelopmental through up-regulation of genes and demonstrated an improved T-Maze scores compared to the SIA group
Henriksen et al. (2021)	Preterm (90%) piglets (n=74, Soy n=25, EV n=24, PL n=25)	Soy lecithin diet (SL)	Milk formula diets provided by Arla Food Ingredients	Plasma lipidomic, MRI, behaviour tested with novel object recognition test and T maze.	The main differences of plasma lipidomics analysis were increased levels of some sphingolipids, and lipid molecules with odd-chain (17:1, 19:1, 19:3) as well as mono- and polyunsaturated fatty acyl chains (16:1, 20:1, 20:3) in the WPC-A-EV and WPC-PL groups

Reference	Animal model	Study groups	Dose and duration of treatment	Method of data collection	Study Outcomes
		Phospholipid enriched diet (PL) Extracellular vesicle enriched diet (EV)	Dose duration: PD1-19		Diffusor tensor imaging measurements of mean diffusivity in the hippocampus were lower for EV and PL groups compared to SL indicating improved hippocampal maturation. No differences in hippocampal lipid composition or short-term memory were observed between groups **Summary**: Enrichment with PL and EVs increases plasma phospholipids and potentially leads to improved hippocampal maturation
Oliveira et al. (2022)	Growing male Wistar rats (n=32, 16 in each group)	Control: oil (35% corn oil, 50% soybean oil, and 15% cocoa butter) Experimental: PL extract emulsion (PL extracted from alpha-lactalbumin-enriched whey protein concentrate (WPC) containing 10% lipids, (37% phospholipids and 15% sphingomyelin))	3.3 mg/10 µl/g body weight of PL extract emulsion suspended in water or oil blend as control was given via a feeding tube. Dose duration: PD7-21	matrix-assisted laser desorption ionization as a mass spectrometry imaging (MALDI-MSI) to assess brain lipidome	Among the molecular ion peaks whose levels were significantly increased by PL supplementation in the whole brain, 39 lipids were annotated, which belonged to fatty acyl, glycerophospholipid, and sphingolipid categories. The key intermediate molecule in glycerophospholipids biosynthesis species, phosphatidic acid [PA(38:5) and PA(38:3)], and its conversion product, cytidine diphosphate-diacylglycerol (CDP-DG) such as CDP-DG(40:7)), were also increased in the total brain following PL supplementation. In specific brain regions, the effect of PL was prominent for a specific set of lipids comprising fatty esters [e.g., CAR(16:0) and CAR(16:2)], glycerophosphocholines [e.g., PC(O-36:1)|PC(P-36:0) and LPC(O-14:1)], ether-phosphoethanolamines (plasmalogen) [e.g., PE(O-40:1)|PE(P-40:0) and PE(O-40:2)|PE(P-40:3)] and phosphosphingolipids [e.g., PE-Cer(t40:1)]. **Summary:** PL supplementation had significant spatial and temporal effect on specific fatty esters, glycerophosphocholines, glycerophosphoethanolamines, and phosphosphingolipids.
Fraser et al. (2022)	Full term piglets (n=23, control n=7, MFGM low =8, MFGM high =8)	Control infant formula Experimental groups:	MFGM was from NZMP MFGM Lipid 100 (Fonterra). Dose duration: PD10-31	Untargeted liquid chromatography-mass spectrometry lipidomics	MFGM consumption did not significantly alter the lipidome in most brain regions, regardless of dose, compared to the control infant formula. Unless you use less stringent tests and then there are 30 lipids in the hippocampus of the high dose group that are difference. There is a decrease in 16 triacylglyceride species in the hippocampus in

Reference	Animal model	Study groups	Dose and duration of treatment	Method of data collection	Study Outcomes
		Enriched infant formulas with Low MFGM (4%) or High MFGM (8%)			the high dose group and an increase in 6 phosphatidycholines in MFGM group. **Summary:** MFGM may have an effect on the lipidomics of the hippocampus, but not on overall brain lipidomics.
Collins et al. (2022)	Growing male Sprague-Dawley rats and using a maternal separation (MS) model	NS-Control: Control diet without MS MS-Control: Control diet with MS NS-MFGM: Experimental diet with MFGM-10 and without MS MS-MFGM: Experimental diet with MFGM-10 and MS	Maternal separation was conducted from postnatal day 2-12. Diets were provided in the form of food pellets. This started two days before birth, where pregnant dams would have eaten the food and then offspring continued to receive this same food after weaning until the end of the experiment. Experimental diet contained 15.9 g/kg Lacprodan® MFGM-10. Dose duration: PD -2 until this end of testing	Behaviour was tested with the Novel Object Recognition Test and the Morris Water Maze. Visceral sensitivity was tested by colorectal distension. Immunohistochemistry of colon. Intestinal permeability tests.	MS resulted in visceral hypersensitivity, which was ameliorated to a greater extent by supplementation with MFGM. No effect of MS or MFGM on the intestinal permeability. No effect of MS or MFGM on the Novel object recognition test MFGM supplementation improved performance on the Morris Water Maze No effects of MS were observed on enteric neuronal or glial networks in early life or adulthood, however an increase in the immunoreactivity of βIII-tubulin in adult colonic myenteric ganglia was noted in the MFGM intervention non-separated group. **Summary:** MFGM supplementation ameliorated maternal separation induced visceral hypersensitivity and improved performance on a test of spatial memory.
Zhang et al. (2023)	Full term 48 hours old piglets (n=80, n= 20 per group)	Control diet Experimental groups: High MFGM (6.09g/100g) Medium MFGM (4.6 g/100g)	Formulas contained Lacprodan® MFGM-10. Dose duration: PD 2-31	T maze, MRI, mRNA and protein expression	The MFGM supplemented diet significantly improved the accuracy of the piglets in the T-maze test, with the MFGM-L group exhibiting the best performance. MRI showed no volumetric differences in the gray and white matter between the groups. However, the fractional anisotropy in the left and right hippocampus of piglets in the MFGM-L group was significantly higher than in the other three groups.

Reference	Animal model	Study groups	Dose and duration of treatment	Method of data collection	Study Outcomes
		Low MFGM (1.74g/100g)			There was a strong correlation between the accuracy of the T-maze test and hippocampal fractional anisotropy. The MFGM supplemented diet increased the expression of BDNF in the cerebral cortex in a dose-dependent manner. However, the changes in BDNF were not consistent with the results of the T-maze test. **Summary**: MFGM supplementation improved learning and memory, and this correlated with increased white matter integrity in the hippocampus. However, the effects of MFGM were not always dose-dependent.
Gázquez et al. (2023)	Full term 5 day old piglets (n= 36, L1 n=8, L2 n=8, L3 n=8, L4 n=7, L5 n=5)	Group L1 vegetable fat and palm oil. Group L2. Canola oil. Group L3 milk fat + canola oil + 1% MFGM (2g/L). Group L4 canola oil + 1% MFGM, Group L5 milk fat + Canola oil + 2% MFGM	Group L3, 4 and 5 formulas contained Lacprodan® MFGM-10. All formulas contained 0.2% DHA and 0.2% arachidonic acid. Dose duration: day 5-21 (17 days) for L1-L3 and day 3-21 for L4 and L5.	Intestine samples used for fatty acid analysis and histology/immunoflourescence	DHA levels were similar among the groups in both total fatty acids and plasma phospholipids (PL). However, MFGM (Group L3) increased significantly the proportion of DHA and LC-PUFA n-3 in liver total fatty acids, jejunum, and also in jejunum PL respect to the other formulas. There were no changes in gut histology, cell proliferation, apoptosis, or brain DHA content. In Experiment 2, higher MFGM dose was used (group L5). Then, higher DHA was not only found in peripheral tissues of MFGM (Group L5) piglets but also in plasma PL, while a similar trend was observed in cortex PL **Summary**: MFGM supplementation may improve bioavailability of DHA.

3.3 Information related to the dietary intake or dietary exposure to Lacprodan® MFGM-10

3.3.1 Data to enable dietary intake or exposure of the target population to be estimated

Infant formula, including IFPSDU may be the sole source of nutrition in formula-fed infants from birth to 6 months, with infants consuming a progressively more diverse diet from 6 months of age onwards.

The relative proportion of IFP containing Lacprodan® MFGM-10 or otherwise enriched with MFGM components in markets where it is used, is unknown and complex to estimate. An optimistic percentage of IFP and IFPSDU that may contain Lacprodan® MFGM-10 in the next 10 years would be 25% of all formula sold in Australia and New Zealand.

The number of infants fed IFP and IFPSDU (proposed to be updated to special medical purpose products for infants (SMPPi)) in Australia and New Zealand has recently been estimated (Supporting document 4 to FSANZ Proposal P1028 Infant Formula – Appendix B)[13]. This estimate reproduced below provides the latest and best available data on expected rates of formula consumption in Australia and New Zealand currently and projected out 10 years.

The Australian National Infant Feeding Survey in 2010-2011 found that in the day before the survey, approximately:

- 40% of infants aged 1 month old received non-human milk or infant formula products
- 55% of infants aged 6 months old received non-human milk or infant formula products

A similar pattern was discernible from New Zealand statistics. A 2007 report from the New Zealand Ministry of Health National Breastfeeding Advisory Committee found:

- 41% of infants were exclusively fed infant formula products at six months old
- 35% of infants were fed a combination of breast milk and infant formula at six months old

Therefore, it is likely that the population of infants likely fed infant formula products (exclusively or with breast milk) by six months of age is currently:

- 168,000 in Australia
- 45,000 in New Zealand

Over ten years, it is expected that the total number of infants fed formula (either exclusively, or in combination with breast milk) is expected to increase almost 10-fold to:

- 1.7 million in Australia
- 0.5 million in New Zealand

[13] https://www.foodstandards.gov.au/sites/default/files/food-standards-code/proposals/Documents/Supporting%20Document%204%20-%20Costs%20and%20benefits.pdf

Using the optimistic value of 25% of all formula fed in Australia and New Zealand in 10 years' time to contain Lacprodan® MFGM-10 (or be enriched with other MFGM components) potentially 550,000 infants annually may be exposed to it.

3.3.1.1 Estimated dietary exposure to Lacprodan® MFGM-10

Infants consuming existing milk-based infant formula products will be consuming lower levels of MFGM components that are present in milk, milk powder and WPC ingredients. The addition of Lacprodan® MFGM-10 will provide greater levels of dairy phospholipids, sphingomyelin, gangliosides and MFGM proteins, at levels that are closer to the levels present in human milk.

Lacprodan® MFGM-10 is added as an ingredient that principally delivers whey protein (as protein) with the added benefit of providing MFGM components to enrich the formula. This enrichment is quantified based on the level of SM present in the formula.

The basic premise of Lacprodan® MFGM-10 addition to formula is based on an addition rate of between 4 to 7 g/L of formula as consumed. This typically means Lacprodan® MFGM-10 may provide up to approximately 35% of the total protein in the formula.

The dietary exposure of infants to Lacprodan® MFGM-10 is based on exposure to the ingredient in its entirety (Table 3-5).

Table 3-5 Estimated mean intake of Lacprodan® MFGM-10 in formula fed infants at proposed maximum levels

	Mean intake of Lacprodan® MFGM-10 of infants consuming formula containing Lacprodan® MFGM-10 at maximum proposed level[a] (g/day)
Infants (Birth to 6 months)	5.6
Infants (6 to 12 months)	4.2

[a] *Based on maximum addition rate of 7 g/L Lacprodan® MFGM-10 and typical human milk intakes of 0.8L/day (birth to 6 months) and 0.6L/day (6 to 12 months)(Food Standards Australia New Zealand, 2016)*

Claumarchirant et al. (2016) estimated the average daily intakes of total phospholipids from breastmilk to be 104 and 165 mg day^{-1} at 0 and 12 months respectively, with a maximum average intake of 278 mg day-1 at the transitional stage between colostrum and mature milk. This is similar to the estimate of a mean PL intake to 140 mg day in a 4-week old exclusively breastfed infant (Giuffrida et al., 2013).

Total PL intake from infant formula ranged between 152 mg day^{-1} in a 0.5-month-oldreceiving a standard formula to 575 mg day^{-1} in a 5-month-old infant receiving an MFGM enriched formula. Irrespective of formula type, the average phospholipid intake from formula ranged from 244 to 366 mg day^{-1} at 0.5 and 6 months of age respectively (Claumarchirant et al., 2016).

Sphingomyelin intake ranged from 36 mg day^{-1} in a 0.5-month-old receiving a standard formula to 136 mg day^{-1} in a 5-month-old infant receiving an MFGM enriched formula. Average SM intakes, independent of formula type ranged from 54 to 88 mg day^{-1} at 0.5 and 6 months respectively. This agrees with and estimated intake of about 62 mg day-^{1} of SM in a term infant estimated by C. Garcia et al. (2012) based on the consumption of about 800 ml day-1 of human milk and the typical SM

content of human milk. Previously Motouri et al. (2003) had estimated based on animal models that infant intakes of SM should be between about 50 to 150 mg day^{-1}

3.3.2 Data on the recommended level of formula consumption for the target population

Daily maximum intake levels based on a typical feed guide for infant sold in Australia and New Zealand, is presented in Table 3-6Table 3-5. Applying the proposed maximum addition to infant formula of 7 g/L, exposure gradually increases over the first months of life, and peaks at 7.84 /day at 6 -8 months of age in exclusively formula-fed infants if feeding guide is followed. As older infants increasingly start consuming complementary food, their consumption of formula reduces due to the consumption of other foods, and hence so too will their exposure to Lacprodan® MFGM-10.

The estimated 90th percentile intake of Lacprodan® MFGM-10, based on daily energy intake by formula-fed infants is 1.367 g/kg bw/day (Ziegler, Fomon, & Carlson, 2003). The intended use level has been demonstrated to be safe and well tolerated in infant clinical studies described (Section 3.2.2). No safety concerns are expected for infants consuming infant formula and follow-on formula products containing Lacprodan® MFGM-10 at the intended level of use.

Table 3-6 Daily maximum intake of Lacprodan® MFGM-10 based on a typical feeding guide table for IFP in Australia and New Zealand

Age of infant	Water volume (mL)	Level scoops of powder[a]	Total volume per feed (mL)	Energy per feed /serve (kJ)	Number of feeds per day	Total energy per day (kJ)	Daily maximum intake of Lacprodan® MFGM-10 (g/day)[b]
Birth – 2 weeks	50	1	55	152	≤ 10	≤1520	3.85
2 – 4 weeks	100	2	110	304	6 - 7	1822 - 2125	5.39
1 – 2 months	150	3	165	455	5 - 6	2277 - 2732	6.93
3 – 4 months	150	3	165	455	5 - 6	2277 - 2732	6.93
5 – 6 months	200	4	220	607	4 - 5	2429 - 3036	7.7
6 – 8 months	200	4	224	618	3 - 5	1868 - 3114	7.84
9 – 12 months	200	4	224	618	3 - 4	1868 - 2491	6.72

[a] *Scoop weight is 7.3 g of powder*
[b] *Based on maximum rate Lacprodan® MFGM-10 at 7 g/L made up formula*

The maximum potential intake of SM based on the proposed upper limit of 7.5 mg /100kJ and the maximum energy intake of formula are shown in Table 3-6. These maximum levels are unlikely to be realised as it would require a formulation with maximum energy permissible and maximum addition rate of Lacprodan® MFGM-10. In a study comparing phospholipid intakes by infants consuming either a standard infant formula or MFGM-enriched formulas, the SM intakes ranged from 36 mg day^{-1} for a 0.5 month old infant consuming a standard formula through to 136 mg day^{-1} for a 5-month old infant consuming a MFGM-enriched formula (Claumarchirant et al., 2016). Average SM intakes, independent of formula type ranged from 54 to 88 mg day^{-1} at 0.5 and 6 months respectively. This

agrees with and estimated intake of about 62 mg day-[1] of SM in a term infant estimated by C. Garcia et al. (2012) based on the consumption of about 800 ml day-1 of human milk and the typical SM content of human milk. Previously Motouri et al. (2003) had estimated based on animal models that infant intakes of SM should be between about 50 to 150 mg day-[1].

Claumarchirant et al. (2016) noted the addition of MFGM to infant formula increased the SM level of formulas to levels similar to that in human milk.

Table 3-7 Maximum potential SM intake based on maximum energy intakes

Age of infant	Water volume (mL)	Number of feeds per day	Total volume per feed (mL)	Total energy per day (kJ)	Daily maximum intake of SM (mg/day)[a]
Birth – 2 weeks	50	≤ 10	55	≤1520	114
2 – 4 weeks	100	6 - 7	110	1822 - 2125	159
1 – 2 months	150	5 - 6	165	2277 - 2732	205
3 – 4 months	150	5 - 6	165	2277 - 2732	205
5 – 6 months	200	4 - 5	220	2429 - 3036	228
6 – 8 months	200	3 - 5	224	1868 - 3114	234
9 – 12 months	200	3 - 4	224	1868 - 2491	187

[a] *Based on proposed maximum SM content of formula of 7.5 mg/100kJ, at maximum formula intake.*

Claumarchirant et al. (2016) also estimated the average daily intakes of total phospholipids from breastmilk to be 104 and 165 mg day-[1] at 0 and 12 months respectively, with a maximum average intake of 278 mg day-1 at the transitional stage between colostrum and mature milk. This is similar to the estimate of a mean PL intake to 140 mg day in a 4-week old exclusively breastfed infant (Giuffrida et al., 2013).

Total PL intake from infant formula ranged between 152 mg day-[1] in a 0.5-month-oldreceiving a standard formula to 575 mg day-[1] in a 5-month-old infant receiving an MFGM enriched formula. Irrespective of formula type, the average phospholipid intake from formula ranged from 244 to 366 mg day-[1] at 0.5 and 6 months of age respectively (Claumarchirant et al., 2016). Whilst there is L variability in the PL content of both formula and human milk, of MFGM to formula will better align the PL composition of formula with that of human milk

3.3.3 Information related to exposure to the substance from other sources

Lacprodan® MFGM-10 is currently not available for use in other products that infants may consume once their diet becomes more diversified with the introduction of complementary feeding.

Aside from the exposure of infants to MFGM and components in human milk, infants may be exposed to low levels of these components from the consumption of non-enriched milk-based formulas and other milk-based products such as yoghurt as their diet becomes increasingly diversified.

3.4 Information related to labelling requirements under Part 2.9 of the Code

3.4.1 Information related to safety or nutritional impact of the proposed labelling change

The addition of Lacprodan® MFGM-10 to IFP does not create any significant safety or nutrition impact for the proposed labelling requirements. Clarity of the source (milk) and mandatory allergen labelling of the presence of milk ingredients in infant formula products address safety issues. As whey protein is the main component of Lacprodan® MFGM-10, the contribution to the total protein content of the formula may be significant and must be accounted for in the product formulation and stated protein content.

Parents who choose to formula-feed and are aware of MFGM may choose a formula containing MFGM (as Lacprodan® MFGM-10) and thereby replace a similar formula not containing MFGM. Arla Foods Ingredients P/S does not anticipate any nutritional concerns with this replacement seeing that any infant formula products sold in Australia and New Zealand must meet strict regulatory standards. Arla Foods Ingredients P/S also anticipates that parents and caregivers of infants, who are already formula-fed, may choose to change to a formula because of the addition of MFGM, as discussed in Section 2.6.2.

It is important to note that Standard 1.2.7-4 prohibits health and nutrition claims on Infant formula products. Furthermore, of nutritive substances can only be labelled as permitted by the FSC. Hence the inclusion of Lacprodan® MFGM-10 in iInfant formula products is only likely to be noted by those caregivers who pay attention to product composition when making a choice in formula selection, not a driver to initiate formula feeding.

3.4.2 Information to demonstrate the proposed labelling change will be understood by consumers

The inclusion of an appropriate name for Lacprodan® MGFM-10 in the ingredients list will assist consumers in understanding the nature of the ingredient and that it is added as a source of MFGM components. As detailed in Section 2.2.9.1 proposed options for the ingredient list that accurately reflect the nature of Lacprodan® MFGM-10 include:

- Lacprodan® MFGM-10 (**milk**)
- Whey protein phospholipid concentrate (**milk**)
- Phospholipid enriched whey protein concentrate (**milk**)
- Milk fat globule membrane enriched whey protein concentrate (**milk**)
- MFGM enriched whey protein concentrate (**milk**)
- Whey protein concentrate (containing milk fat globule membrane) (**milk**)
- Whey protein concentrate (containing MFGM) (**milk**)
- Whey protein concentrate (**milk**)* (* a source of MFGM)
- Whey protein concentrate (**milk**)* (* a source of milk fat globule membrane)

Consumers are generally familiar with the Nutrition Information Panel and will be able to identify the inclusion of sphingomyelin in the table, if it is included. The intent would be to link the SM to MFGM, e.g. Sphingomyelin (from MFGM). This general statement whilst linking to the added Lacprodan® MFGM-10 does not differentiate the natural (form other dairy ingredients) from the added MFGM (from Lacprodan® MFGM-10), but informs consumers the sphingomyelin is from MFGM.

3.5 Information related to internationally recognised standards, codes of practice, recommendations and guidelines

Please refer to Section 1.7 for the relevant information regarding international regulations related to the addition of MFGM ingredients including Lacprodan® MFGM-10.

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