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

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Supporting document 1

Risk and technical assessment – Application A1307

A1307 - Milk fat globule membrane as a nutritive substance in infant formula products

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Executive summary

Food Standards Australia New Zealand (FSANZ) received an application from Arla Foods Ingredients P/S to amend the Australia New Zealand Food Standards Code (the Code) to permit bovine milk fat globule membrane-enriched whey protein concentrate (MFGM-WPC) as a nutritive substance in infant formula products.

The applicant has provided evidence that the MFGM-WPC has beneficial attributes compared to standard bovine whey protein concentrate (WPC). WPC is permitted as an ingredient in infant formula products and has a history of safe use in Australia. MFGM-WPC is prepared from bovine milk using similar methods to WPC, with additional purification and concentration steps to isolate the milk-fat globule membrane fraction and produce two to four times higher concentrations of major membrane phospholipids.

FSANZ has compared the phospholipid composition of the MFGM-WPC with reported concentrations in human milk, and is satisfied that the phospholipid composition MFGM-WPC is sufficiently similar to human milk to be used in infant formula products.

Specifications for MFGM-WPC will be added to Schedule 3 of the Code. The applicant has provided sufficient evidence to demonstrate that the MFGM-WPC preparation would comply with those specifications when sold for use in infant formula products, and that sphingomyelin can be used as a marker to quantify the addition of MFGM-WPC in infant formula products.

The risk and technical assessment found that MFGM-WPC has an established history of safe use in many countries as an ingredient in infant formula products, with no case reports of adverse effects. MFGM-WPC has no more allergenic potential than other infant formula products based on bovine milk. FSANZ does not have concerns regarding the effect of MFGM-WPC in infant formula products on the absorption of other nutrients, nor were any adverse effects of MFGM-WPC on growth outcomes observed when compared to formula fed infants in studies up to a concentration of 5 g/L of MFGM-WPC. No additional microbiological safety risks arise from addition of MFGM-WPC to powdered infant formula products.

The dietary intake assessment estimated the intake of phospholipids from MFGM-WPC in infant formula and follow-on formula assuming the maximum use level proposed by the

applicant. Although higher than the estimated intakes of phospholipids by infants who consume mature human milk, estimated intakes of phospholipids from MFGM-WPC in infant formula and follow-on formula do not exceed estimated intakes assuming the regulatory limit of phospholipids specified in the Code.

FSANZ considered the evidence for the effect of MFGM-WPC in infant formula products on improved neural development and cognitive function in four human and five animal studies. Due to the limitations in the available data FSANZ concludes that MFGM-WPC supplemented infant formula may improve neural development and cognitive function in infants, but additional evidence would be required to make a definitive conclusion.

FSANZ also considered evidence for the effect of MFGM-WPC in infant formula products on improved development of the infant gut microbiota, anti-pathogenic effects, and immunomodulation effects for a formula-fed infant. FSANZ is satisfied that there is evidence the addition of MFGM-WPC to infant formula products could support the development of a gut microbiome that more closely resembles that of breastfed infants.

Considering the current labelling requirements for infant formula products in the Code, it is not anticipated that addition of MFGM-WPC to infant formula products will encourage more caregivers to formula feed instead of breastfeed.

Taken together, FSANZ is satisfied that MFGM-WPC is an appropriate source of phospholipids for inclusion of infant formula products and does not pose a safety risk to infants. While more data is needed to substantiate improved neural development and cognitive function compared to standard infant formula products, there is evidence MFGM could be beneficial for infant gut microbiota development.

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1 Introduction

Food Standards Australia New Zealand (FSANZ) received an application from Arla Foods Ingredients P/S to amend the Australia New Zealand Food Standards Code (the Code) to permit bovine milk fat globule membrane-enriched whey protein concentrate (MFGM-WPC) as a nutritive substance in infant formula products.

2 Food technology assessment

2.1 Introduction

The purpose of the food technology assessment is to provide food technology information on the applicant's MFGM-WPC, that is the subject of this application.

Schedule 29 of the Code includes a list of optional single component substances permitted for use as nutritive substances in infant formula products. In contrast to those substances that have been previously permitted, this application seeks to permit the use of a nutritive substance which takes the form of a preparation made up of various constituents, rather than a single component substance.

This food technology assessment compares the applicant's nutritive substance (i.e. MFGM-WPC) with standard WPC in relation to:

- their chemical identity
- their chemical and physical properties
- their manufacturing process and, in particular, whether that process introduces any safety concerns or impurities
- whether there are differences in how they are incorporated into the relevant food matrix (i.e. infant formula products).

Additionally, specifically for the nutritive substance, this food technology assessment will determine whether:

- there are relevant internationally recognised identity and purity specifications available
- FSANZ needs to develop and incorporate appropriate specifications in the Code
- the applicant has provided sufficient evidence to demonstrate that their preparation meets these specifications.

2.2 Chemical and physical properties

2.2.1 Background on bovine MFGM

MFGM-WPC is a commercial preparation of MFGM for addition to infant formula products (see section 4.3 for further details). MFGM is only found in milk, including bovine and human milk. The applicant proposes to produce its MFGM-WPC from bovine milk.

MFGM is a triglyceride-containing phospholipid vesicle of lactating mammary cells that travels to the plasma membrane and which allows fat to be transferred to milk in a homogenous solution (Dewettinick et al. 2008). After secretion from the endoplasmic reticulum milk fat globules are coated with a phospholipid monolayer (Pan et al. 2023). These cytoplasmic lipid droplets bud off with the plasma membrane phospholipid bilayer into the secreting fluid, forming a tri-layer membrane (Brink and Lönnerdal 2020). Several proteins then attach to the vesicle. MFGM is highly conserved across mammalian species

(Zou et al. 2013).

MFGM contains unique polar lipids and membrane-specific proteins. However the composition of MFGM is variable, depending on the methods used to isolate, purify and analyse its components (Dewettinick et al. 2008). The two main polar lipids in MFGM are glycerophospholipids and sphingolipids, which originate from the alveoli of mammary epithelial cells.

Glycerophospholipids contain a glycerol backbone with primarily two fatty acid tails and a charged phosphate group attached to either ethanolamine (phosphatidyl ethanolamine; PE), serine (phosphatidyl serine; PS), inositol (phosphatidyl inositol; PI) or choline (phosphatidyl choline; PC) (Brink and Lönnerdal 2020). The two main glycerophospholipids in MFGM are PC (35%) and PE (30%). Sphingolipids contain a ceramide backbone, with the major sphingolipid being sphingomyelin (SM; 25%), which has a myelin group attached to the charged phosphate. Other lipid components of MFGM include PI (5%), PS (3%), with glucosylceramide, lactosylceramide and gangliosides present in trace amounts (Deeth 1997; Danthine et al. 2000). Cholesterol is also present in the phospholipid membrane.

PC, PE, SM, PI and PS all contain a charged phosphate group and are collectively referred to in this assessment as phospholipids.

Approximately 25-70% of the MFGM is protein, depending on the source (Dewettinick et al. 2008), with protein from MFGM contributing 1-2% of total milk protein (Riccio 2004). Human MFGM has been found to contain 191 separate proteins including enzymes, immunoglobulins, secretory epithelial cytoplasmic proteins, and milk leukocyte proteins and skim milk constituents (Brink and Lönnerdal 2020).

2.2.2 Phospholipid composition of MFGM-WPC compared to human milk

The primary aim of the application is to produce a nutritive substance derived from bovine milk with levels of phospholipid and membrane proteins that more closely resemble those found in human milk.

Although breastfeeding is the recommended way to feed infants, a safe and nutritious substitute for human milk is needed for infants who are not breastfed. Infant formula products must have a nutrient composition to support normal growth and development when used as the sole or principal source of nutrition. FSANZ compared the composition of phospholipids in MFGM-WPC from the provided batch analysis with the phospholipid composition in human milk. The phospholipid composition of human milk was based on data identified from a search of PubMed[1] on 27 August 2024[2].

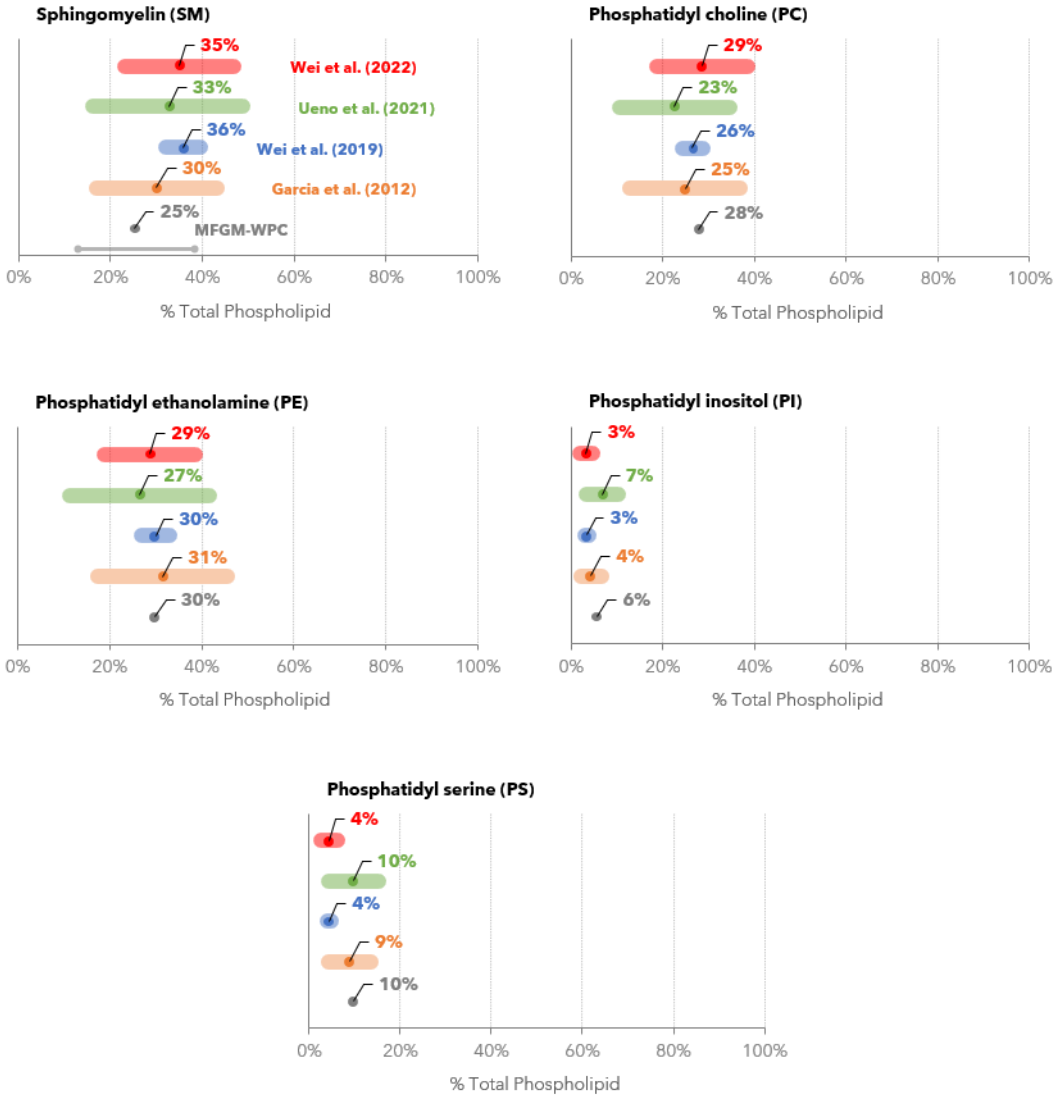
Only studies measuring each phospholipid concentration in complete human milk were considered. Studies that measured the composition of isolated human milk MFGMs were not considered to avoid the confounding effects that different methods of isolating human MFGMs may have on reported composition (Dewettinck et al. 2008). Additionally, only

[1] https://pubmed.ncbi.nlm.nih.gov/

[2] **Search term:** ("milk fat globule membrane"[Title/Abstract] OR MFGM[Title/Abstract] OR phospholipid[Title/Abstract] OR phosphatid*[Title/Abstract] OR sphingo*[Title/Abstract] OR glycolipid*[Title/Abstract] OR "polar lipid"[Title/Abstract] OR cerebro*[Title/Abstract] OR ganglio*[Title/Abstract] OR triglycer*[Title/Abstract] OR diacylglycer*[Title/Abstract]) AND (human[Title/Abstract] AND (breast[Title] OR lact*[Title] OR milk[Title] OR breastmilk[Title] OR breastfee*[Title])) AND (quanti*[Title/Abstract] OR level*[Title/Abstract] OR amount*[Title/Abstract] OR measur*[Title/Abstract] OR composit*[Title/Abstract]) NOT (Cancer[Title/Abstract] OR carcinoma[Title/Abstract] OR Disease[Title/Abstract] OR "cell line"[Title/Abstract] OR lactobacill*[Title/Abstract] OR therapy[Title] OR Proteom*[Title] OR synthesis[Title] OR storage[Title]) NOT (Review[Publication Type] OR Systematic Review[Publication Type])
Filters: Humans, English, Year 1994-today

studies where ^{31}P nuclear magnetic resonance (NMR) was used to quantify phospholipid classes were considered. The ^{31}P NMR method was chosen to remain consistent with the applicant's proposed specification and supplied data (see section 2.5), and recognised that there can be substantial variation in accuracy between different analytical methods used for lipid quantification (Wang and Zhou 2017).

Four publications were located that were directly comparable to the data supplied by the applicant for MFGM-WPC (Garcia et al. 2012; Wei et al. 2019; Ueno et al. 2021; Wei et al. 2022). Study details for these publications are presented in Appendix 1. The data extracted from these studies is presented in Figure 1, and compared to the batch analysis for MFGM-WPC supplied by the applicant (Table 2-14 of the applicant).



Dots and coloured bars represent mean and standard deviation from each study respectively. The proposed specification for the amount of sphingomyelin as a percentage of total phospholipid in MFGM-WPC is represented by the grey range at the bottom of the sphingomyelin comparison.

Figure 1 Published quantities of the five most abundant phospholipids in human milk, determined as a percentage of total phospholipid by ^{31}P NMR, compared to MFGM-WPC.

The composition of phospholipids in human milk shows a large degree of variation, both within and between studies (Figure 1). This is a recognised feature of human milk where

composition can change dramatically between individuals, as well as with changes in diet, lactation cycle and time of sample collection (Venkat et al. 2022). The SM mean is lower in MFGM-WPC when compared to the mean values for human milk, consistent with known interspecies variation between human milk and the bovine source of MFGM-WPC (Garcia et al. 2012; Wei et al. 2022). Overall however, the comparison between MFGM-WPC and human milk shows there is general similarity in phospholipid composition.

Given the nature of MFGM-WPC as an ingredient used in infant formula products, there are a number of limitations in comparing MFGM-WPC with human milk. Notably:

- The comparison does not account for other sources of phospholipid that may be used in the formulation of an infant formula product.

- The total contribution of other constituents in MFGM-WPC, such as protein, triglycerides, cholesterol, and other fatty acids were not included in the comparison. These ingredients are managed through existing compositional requirements for fat and protein in Standard 2.9.1.

- The ^{31}P NMR method for quantifying phospholipid classes does not enable differences in the attached fatty acid chains to be considered. Phospholipid composition is therefore compared based on the headgroup.

Notwithstanding these limitations, and the known phospholipid composition differences between human and bovine milk, as well as inter- and intra-individual variation in phospholipid composition; FSANZ is satisfied the phospholipid composition MFGM-WPC is sufficiently similar to human milk to be used as a source of phospholipids in infant formula products.

2.2.3 Comparison of the applicant's MFGM-WPC with standard WPC

Bovine MFGM comprises approximately 70% of the same whey proteins found in standard WPC. However MFGM-WPC differs from standard WPC as it contains two to four times higher concentrations of major membrane lipid components such as phospholipids and membrane proteins.

Since MFGM is comprised of various components it does not have a unique chemical name, a Chemical Abstract System (CAS) registry number or a structural formula.

The important chemical compositional differences between MFGM-WPC and the comparable WPC produced by the applicant highlights the enrichment of certain phospholipids noted above, as listed in Table 1. The five listed phospholipids make up greater than 98% of total phospholipids in both MFGM-WPC and standard WPC. In addition, the proportion of these phospholipids as a proportion of total fat is similar between MFGM-WPC and standard WPC.

Table 1 Compositional comparison between the applicant's MFGM-WPC and standard WPC (adapted from Table 2-14 of the application)

Analyte	MFGM-WPC %	WPC %	Ratio (MFGM-WPC:WPC)
Total fat	18.6	5.5	3.4
Total phospholipids	6.7	1.85	3.6
sphingomyelin (SM)	1.69	0.46	3.7
Phosphatidyl ethanolamine (PE)	2.0	0.46	4.3

Phosphatidyl choline (PC)	1.86	0.50	3.8
Phosphatidyl serine (PS)	0.65	0.180	3.6
Phosphatidyl inositol (PI)	0.38	0.136	2.7

Separately, the protein and amino acid profiles of MFGM-WPC compared to standard WPC are relatively similar. There are no unique proteins specific to MFGM-WPC compared to WPC.

2.2.4 Incorporation into food matrices

The applicant states the physical attributes of the MFGM-WPC are similar to standard WPC therefore it can be used and added to food, including infant formula products, in a similar way. It is a free flowing spray dried powder of homogenous composition with a similar particle size distribution to standard WPC.

2.3 Manufacturing process

The manufacturing process for MFGM-WPC is similar to the well-characterised processes for standard WPC. They are both produced from bovine whey streams which are by-products from raw skim milk used to produce cheese or casein. The modifications used to produce MFGM-WPC instead of the standard WPC relate to two additional filtration steps and an additional concentration step. These filtration and concentration steps increase the concentration of whey protein components and reduce other components such as lactose, minerals and water. The filtration steps are conducted using separate ultrafiltration and microfiltration units. After the two filtration steps are completed the whey stream is concentrated using reverse osmosis which removes some of the water in the stream.

The additional filtration and concentration steps are the reasons why MFGM-WPC contains higher concentrations of whey lipids compared to WPC.

The resultant liquid-filtered and concentrated whey stream undergoes additional pasteurisation, and is then sprayed dried to produce a powder, which is sieved and bagged in a similar manner to standard WPC.

A schematic of the manufacturing process for the production of MFGM-WPC is provided in Figure 2 (taken from Figure 2-10 of the application), with the additional steps compared to the production of WPC being one ultra filtration step and one microfiltration step followed by reverse osmosis steps.

The purity of the final MFGM-WPC powder is confirmed by the proposed specification as detailed in section 2.4 below. The relevant impurity parameters are those of heavy metals (arsenic, cadmium, lead and mercury) as well as microbiological limits.

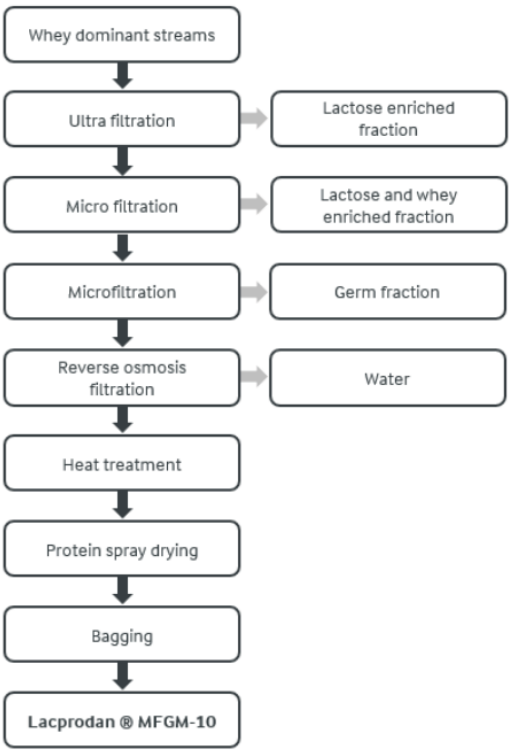


Figure 2 Schematic of the production process for MFGM-WPC

2.3.1 Stability results

Data provided in the application demonstrate that, like other WPC, MFGM-WPC powder is stabile over 18 months at ambient conditions (21°C and 45±5% relative humidity). Parameters including colour, water activity, pH, peroxide values, together with several sensory properties were analysed at intervals throughout the 18 month period and used to evaluate stability. All parameters assessed remained within acceptance limits up to 18 months of storage.

Additional data was provided by the applicant assessing the stability of the MFGM-WPC powder incorporated into infant formula products powders and packaged into usual conventional cans to replicate commercial infant formula and follow-on formula products. The stability of macronutrients, key fatty acids, and vitamins present in these products was assessed for two different storage conditions: (a) 25°C and 60% relative humidity; and (b) 30°C and 65% relative humidity for at least 17 to 24 months storage. The results support the conclusion that MFGM-WPC added to powdered infant formula products is stable for up to 24 months for both the storage conditions. The data assessed for the above nutrients indicated their concentrations were consistent with the label shelf life claim of 24 months.

The oxidative stability of the lipid components of the powdered infant formula products with MFGM-WPC were studied as lipids are prone to oxidation. Stability was examined under accelerated storage condition of 40°C and 75% relative humidity, with results collected for 8 months rather than for 24 months for the two other scenarios described at (a) and (b) above. The conclusions of this study is that low levels of oxidative damage occurred under the storage conditions tested.

A study was conducted by the applicant to assess the stability of the phospholipid SM in infant formula products which, as noted in earlier sections of this report, is present at higher concentrations in MFGM-WPC compared to standard WPC. The study was conducted for both infant formula and follow-on formula products stored using condition (a), out to 18 months storage. The conclusion of these data is that SM concentration does not change with storage conditions of up to 18 months when stored at 25°C and 65% humidity. This provides confirmatory evidence that the SM concentration in infant formula products is stable and can be used as an analytical marker for the addition of MFGM-WPC compared to other WPC to infant formula products.

2.4 Specifications

Under the Code, paragraph 1.1.1—15(1)(c) and subsection 1.1.1—15(2) require that a substance used as a nutritive substance must comply with any relevant specification set out in Schedule 3 – Identity and Purity.

Schedule 3 does not include a specification for MFGM-WPC. Therefore, a new specification needs to be created and added to Schedule 3. The applicant proposed an in-house specification for identity and purity with which their MFGM-WPC would need to comply. Analytical results for four non-consecutive batches were provided to FSANZ as confidential commercial information (CCI) and therefore full details cannot be disclosed. However, results indicate the applicant's MFGM-WPC meets the proposed specification.

A summarised version of the applicant's specification, which FSANZ is proposing be included in Schedule 3, is provided in Table 2. FSANZ proposes to only include the analytes considered important for a regulatory specification for identity and purity reasons. FSANZ omitted certain analytes proposed by the applicant as these were not considered necessary to ensure purity and safety.

Table 2 Proposed specification for milkfat globule membrane whey protein concentrate

Analyte	Specification
Name of nutritive substance	Milkfat globule membrane whey protein concentrate preparation
Appearance	Off-white powder
Total protein (%)	69.0 - 76.0
Lactose (%)	≤ 2.0
Fat (%)	16.0 – 22.0
Phospholipids (%)	6.0 – 10.0
Sphingomyelin (%)	1.3 – 2.3
Ash (%)	<3.0
Moisture (%)	<5.0
Arsenic (mg/kg)	≤0.2
Cadmium (mg/kg)	≤0.1
Lead (mg/kg)	≤0.05
Mercury (mg/kg)	≤0.02
Total plate count (incubated 30°C) (cfu/g)	≤10000

Analyte	Specification
Total plate count (incubated 55°C) (cfu/g)	≤1000
Bacillus cereus (cfu/g)	<50
Sulphite-reducing *Clostridia* (cfu/g)	<10
Enterobacteriaceae (cfu/g)	<10
Coagulase-positive *staphylococci* (cfu/g)	Absent/1 g
Yeast and moulds (cfu/g)	<10

There are specific food safety microbiological limits for powdered infant formula and powdered follow-on formula within the table to section 4 in Schedule 27 (S27—4). These microbiological limits are for *Cronobacter* (not detected in 10 g) and *Salmonella* (not detected in 25 g). Therefore FSANZ has not established a microbiological limit for *Salmonella* (as proposed by the applicant), even though the nutritive substance will be added at low levels into infant formula products.

2.5 Analytical methods for detection

As noted in earlier sections it is the increased concentrations of phospholipids and sphingolipids in MFGM-WPC compared to standard WPC that can assist in establishing the presence and quantity of the nutritive substance added to infant formula products.

The applicant's preferred method for analysing phospholipids employs a specialised analytical technique, specifically, ^{31}P NMR. This method is favoured by some laboratories for determining phospholipids, including SM, in milk-based matrices. Phospholipids can also be quantified using High Performance Liquid Chromatography with tandem mass spectroscopy (HPLC MS/MS), which is another specialised analytical method.

The Association of Office of Analytical Chemists (AOAC) has Standard Method Performance Requirements (SMPRs®) for the analytical determination of phospholipids in food products including infant formula products. This is AOAC SMPR® 2021.017 (AOAC International, 2022).

Due to the complexity of the MFGM-WPC preparation the applicant has proposed using SM as a marker lipid for the nutritive substance preparation. This is proposed to be used to check and quantify that MFGM-WPC has been incorporated into the infant formula products. The reasons for using SM as the marker are that:

- it is one of the major phospholipids present in MFGM-WPC
- there are standardised analytical methods available to analyse for it in both the MFGM-WPC and final infant formula products
- it is present at very low levels in standard WPC, and not in vegetable oils or lecithin and so can be quantified if present in infant formula products as coming from the MFGM-WPC
- levels in infant formula products, even if made from whole milk rather than standard WPC, are still much less than those in infant formula products with added MFGM-WPC.

2.6 Food technology conclusions

FSANZ concludes that the applicant's MFGM-WPC is similar to standard WPC but with some important compositional differences. MFGM-WPC is manufactured in a similar way to standard WPC except it undergoes additional purification and concentration steps. These additional steps result in the production of MFGM-WPC which contains two to four times higher concentrations of major membrane lipid components such as phospholipids and

sphingolipids compared to standard WPC. SM has been determined to be a useful analytical marker to differentiate as well as quantify the addition of MFGM-WPC to food products such as infant formula products.

The physical attributes of MFGM-WPC are similar to standard WPC and therefore it can be used and added to food, including infant formula products, in a similar way. Infant formula products powders containing MFGM-WPC powder are stable when stored similar to WPC prepared infant formula products, consistent with the label statement of 24 months shelf life.

Schedule 3 – Identity and purity of the Code does not contain a relevant specification for MFGM-WPC. Therefore, a specification is required to be written into Schedule 3. Analyses provided by the applicant indicate its MFGM-WPC meets this specification. There are various analytical methods available to analyse and quantify MFGM-WPC added to food including infant formula products, ensuring its efficacy.

3 Safety assessment

3.1 Toxicology assessment

3.1.1 History of safe use

The MFGM-WPC that is the subject of this application has a history of safe use that includes 15 years of consumption in infant and follow-on foods in the European Union. It is currently on the market in Argentina, Bulgaria, Brazil, Canada, China, Colombia, Czechia, Denmark, Ecuador, Finland, Hong Kong, India, Indonesia, Japan, Latvia, Lithuania, Malaysia, Mexico, Nigeria, Norway, Panama, Peru, Philippines, Poland, Portugal, Russia, Singapore, South Korea, Spain, Sri Lanka, Sweden, Taiwan, Thailand, the USA, and Vietnam (Figure 3).

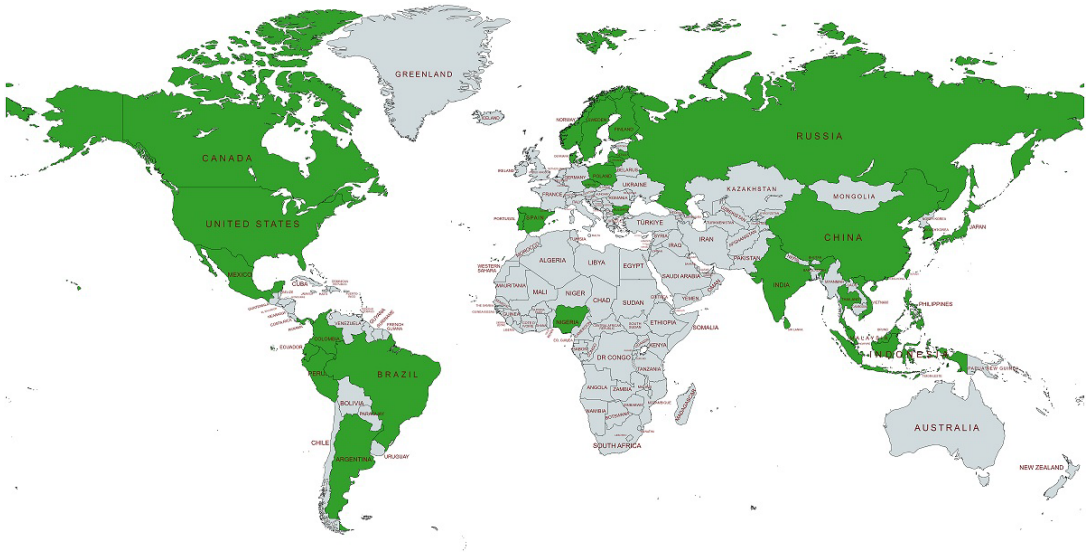


Figure 3 Countries in which MFGM-WPC is currently available as an ingredient in infant formula products. (Map created using https://www.mapchart.net)

3.1.2 Information on metabolism

The MFGM-WPC contains a variety of biological molecules, as described in Section 2. These molecules are the same as those normally found in MFGM of human milk and/or bovine milk and would be expected to be metabolised in same way. The metabolic fates of important

components of MFGM are briefly summarised below.

3.1.3 Glycerophospholipids

MFGM represents a major source of the total phospholipid content of milk (Lee et al. 2018a). Dietary phospholipids are not absorbed intact but broken down by several lipases in the gastrointestinal lumen (Nilsson and Duan 2019) to produce lyso-phospholipids, as well as other metabolites, all of which are absorbed into the systemic circulation. There is negligible excretion of intact phospholipids in the faeces.

3.1.4 Sphingomyelin

SM is not absorbed intact, but is hydrolysed in the brush border of enterocytes by alkaline sphingomyelinase to produce ceramide (Duan and Nilsson 2000; Nilsson et al. 2021), which is further hydrolysed by neutral ceramidase to sphingosine and free fatty acids, which are the metabolites that are absorbed into the systemic circulation (Nilsson and Duan 2019). Hydrolysis of SM mainly occurs in the distal jejunum. Intact SM may be found in the colon and faeces (Liu et al 2000).

3.1.5 Gangliosides

The gangliosides in milk are taken up intact by enterocytes in the small intestine (McJarrow et al. 2009). Some intact gangliosides may be found in the faeces of infants (Larson et al. 1990).

3.1.6 MFGM Proteins

In vitro studies using enzymes found in the gastrointestinal tract support the conclusion that proteins in MFGM are available to proteolysis in the gastrointestinal tract (Kobylka and Carraway 1973; Ye et al. 2010; Vanderghem et al. 2011; Ye et al. 2011; Le et al. 2012). Peptides from milk proteins can be isolated from the faeces of infants (Beverly et al. 2020) and these may include peptides from MFGM proteins.

3.1.7 Studies in animals

The applicant provided confidential details of a recent (2024) report of literature searches for safety/toxicity studies of MFGM in animals. The searches were well-designed and comprehensive but did not identify any relevant studies. FSANZ also conducted searches for toxicity studies of MFGM on PubMed and EBSCO, and did not identify any relevant studies.

The application included several animal studies designed to investigate the beneficial effects of dietary MFGM or MFGM-WPC on neurological development. No evidence of adverse effects of MFGM supplementation were reported in any of these studies. The studies are summarised in Section 4.

3.1.8 Studies in humans

The application included studies conducted in human infants in support of the potential beneficial effects of MFGM-WPC. Only one of these studies, that of Billeaud et al. (2014), showed a significantly higher rate of an adverse observation, specifically eczema, in the infants supplied with a protein-rich fraction of MFGM (MFGM-P), compared to the control group. There are reasons to interpret this finding as unrelated to MFGM consumption. The study was not designed to assess risk of eczema, but to measure growth, and lacked statistical power for assessing risk of eczema. A much larger study would be required to assess treatment-related effects on eczema. Consistent with this, the overall incidence of

eczema in the study was lower than the expected background level. The finding concerning eczema was identified only on post-hoc analysis of parental reports, daily reports and physician-reported data. The authors themselves remarked that “caution is … warranted in extrapolating this finding”. Timby et al. (2017a) reviewed three double-blind randomised controlled trials of MFGM supplementation in early infancy, including that of Billeaud et al. (2014) and a study they themselves had conducted (Timby et al. 2014a, 2014b, 2015), and described the finding of increased incidence of eczema as uncertain on grounds including limited number of observations, lack of a systematic eczema scoring system, and no increased incidence of eczema in their own study.

3.1.9 Potential for allergenicity

Production of the MFGM-WPC that is the subject of this application does not introduce any processing aids and does not include processing methods that would be anticipated to alter the chemical or physical properties of the proteins, when compared to those in other dairy products. The potential for allergenicity of infant formula that includes this MFGM-WPC is anticipated to be similar to that of other bovine milk-derived infant formula products.

This product is not suitable for inclusion in infant formula for infants with cow’s milk protein allergy and is not intended for that purpose.

3.1.10 Safety assessment reports prepared by international agencies or other national government agencies

No safety assessment reports by other agencies were found.

3.1.11 Safety assessment conclusions

MFGM-WPC has an established history of safe use in many countries, with no case reports of adverse effects. The metabolism of the major classes of biological molecules in MFGM is understood. No toxicity studies in experimental animals were found in comprehensive literature searches. Increased incidence of eczema in only one of a number of studies of MFGM supplementation of infants is unlikely to be related to the supplementation. MFGM-WPC is not considered to carry greater allergenic potential than other infant formula products based on bovine milk. With the exception of infants with milk protein allergies, no public health and safety concerns were identified in the assessment of the MFGM-WPC that is the subject of this application.

3.2 Effect of MFGM-WPC on absorption of other nutrients

To determine if MFGM-WPC inhibits or modifies the absorption of other nutrients, FSANZ undertook a search in PubMed on 3 October 2024[3]. No relevant studies were identified. However six studies from this search were identified that investigated the effect of components of MFGM including phospholipids and sphingomyelin on nutrient absorption (Nyberg et al. 2000; Duivenvoorden et al. 2006; Kamili et al. 2010; Ramprasath et al. 2013; Le Barz et al. 2021; Vors et al. 2020).

Overall, the available evidence indicates that phospholipids can inhibit cholesterol absorption, (with some evidence of a decrease in triglyceride absorption) in adults and rats. A randomised control trial (RCT) in 58 postmenopausal women reported that phospholipid-supplementation (3 g and 5 g/day) for four weeks decreased plasma total cholesterol at both concentrations and decreased plasma triglyceride concentrations at 5 g/day (Vors et al.

[3] **Search terms:** (“milk fat globule OR milk fat globule membrane OR MFGM”) AND ("antinutrient" or "antinutritional" or "anti-nutrient" or "anti-nutritional" or "absorbed" or "absorption")

2020) but did not result in significantly different fasting serum sphingomyelin, phospholipid or ceramide compared to controls (Le Barz et al. 2021). An animal study by Kamili et al. (2010) reported that mice fed a diet containing 1.2% wt/wt phospholipid for 3 to 5 weeks resulted in a significant decrease in intestinal cholesterol uptake. Another study reported that dietary sphingomyelin inhibited cholesterol absorption in rats (Nyberg et al. 2000), while Duivenvoorden et al. (2006) found that consumption of sphingolipids decreased plasma cholesterol and triglyceride concentrations in mice. However an RCT by Ramprasath et al. (2013) in 10 healthy adults who consumed a controlled diet with 1 g/day sphingomyelin for 14 days found no significant difference on serum cholesterol or triglyceride concentrations compared to the non-supplemented diet.

No Nutrient Reference Values exist for infants for the consumption of cholesterol or triglycerides therefore FSANZ has no concerns regarding any potential effect of these components of MFGM-WPC on absorption of these nutrients (NHMRC and MoH 2006).

FSANZ notes that phospholipids are permitted in infant formula products and are present in human milk. No Nutrient Reference Values exist for infants for the consumption of cholesterol or triglycerides. Therefore based on the available evidence FSANZ does not have concerns regarding any potential effect of phospholipids present in MFGM-WPC on the absorption of cholesterol or triglycerides (NHMRC and MoH 2006).

3.3 Growth Assessment

3.3.1 Effect on infant growth

The objective of the assessment was to determine the effect, if any, of the addition of the applicant's MFGM-WPC (at concentrations of 4 to 7 g/L) to infant formula products on infant growth in formula-fed infants.

The applicant provided six studies that investigated the effect on growth of MFGM-WPC or an MFGM-rich ingredient in infant formula products that were used in the body of evidence (Billeaud et al. 2014; Timby et al. 2014a; Li et al. 2019b; Xia et al. 2021; Jaramillo-Ospina et al. 2022; Jiang et al. 2022c). One additional systematic review and meta-analysis was provided (Ambrożej et al. 2021), however all relevant studies within the paper were already provided by the applicant. FSANZ conducted a literature search[4] in PubMed on 16 August 2024 and no additional relevant studies were identified. Studies excluded from assessment are listed in Table A2.1.

The six included publications reported results from five parallel, double-blinded RCTs. Two publications reported results from the same trial (Xia et al. 2021; Jiang et al. 2022c). Growth endpoints reported in studies included weight, length and head circumference (HC) gain per day or month, mean weight, length, body mass index (BMI) and HC, weight-for-age (WAZ), length-for-age (LAZ), BMI-for-age (BAZ) and HC-for-age (HCZ) z-scores and percentage body fat over a minimum three-month study period. Where relevant, results in this assessment are reported as mean and standard deviation (SD) or 95% confidence intervals (CI). Table A2.2 summarises key study characteristics.

3.3.2 Discussion

Six studies measured the effect of consuming infant formula containing either Lacprodan® MFGM-10 MFGM-WPC (Billeaud et al. 2014; Timby et al. 2014a; Li et al. 2019b; Jaramillo-

[4] **Search terms:** "milk fat globule or milk fat globule membrane" and "milk or breast or formula" and "anthropometric or weight or growth or development" and "child or infant or baby or maternal"

Ospina et al. 2022) or a MFGM-rich ingredient (Billeaud et al. 2014; Xia et al. 2021; Jiang et al. 2022c) for at least 4 months on infant growth in five clinical trials. Two studies used EF containing 5 g/L MFGM-WPC (Li et al. 2019b; Jaramillo-Ospina et al. 2022), however Timby et al. (2014a) reported the concentration of MFGM-WPC as 4% (wt:wt) total protein which precluded estimating the concentration in g/L. Two trials did not report the concentration of MFGM-rich products (Billeaud et al. 2014; Xia et al. 2021; Jiang et al. 2022c) and therefore the outcomes are of limited relevance for the assessment. The composition of the MFGM-rich product was ambiguous and FSANZ cannot conclude the exact relationship to the applicant's product due to proprietary information (Billeaud et al. 2014; Xia et al. 2021; Jiang et al. 2022c). Age at enrolment ranged from 14 ± 3 to 81.4 ± 25.4 days.

No significant differences in weight gain, WAZ, mean weight, LAZ, mean length, BAZ or BMI were reported between EF and SF groups at any timepoint between enrolment and up to 24 months (Billeaud et al. 2014; Timby et al. 2014a; Li et al. 2019b; Xia et al. 2021; Jiang et al. 2022c; Jaramillo-Ospina et al. 2022), with HC data being similar in the majority of studies and timepoints.

In three studies no significant differences in mean weight, length or HC measures or WAZ, LAZ and HCZ scores were observed in BFR, EF and SF groups at the majority of timepoints (Timby et al. 2014a; Xia et al. 2021; Jiang et al. 2022c). However in one study WAZ, LAZ but not HCZ scores were significantly greater in the BFR groups until age 2 years (Jaramillo-Ospina et al. 2022). Birth weight was greater in the BFR group compared to infant formula groups in one study, but mean weight was not significantly different between groups by 12 months (Timby et al. 2014a). In one study mean weight was significantly greater at birth and until 4 months of age in the BFR group compared to infant formula groups but not at any other time point (Li et al. 2019b).

FSANZ has noted several limitations in the body of evidence including three studies that did not provide the concentration of MFGM-WPC or MFGM-rich ingredient in the EF (Billeaud et al. 2014; Xia et al. 2021; Jiang et al. 2022c), the test period commencing after one month of age (Timby et al. 2014a; Jaramillo-Ospina et al. 2022), missing error margins (Li et al. 2019b), greater than 20% infant dropout in some (Xia et al. 2021; Jaramillo-Ospina et al. 2022; Jiang et al. 2022c), or all study arms (Billeaud et al. 2014) and not controlling for significant differences in characteristics at enrolment (Li et al. 2019b). Two MFGM-rich products were added to EF and their relationship to the applicant's product is uncertain. Some differences in vitamin and amino acid content between EF and SF were also noting including 14–34% variation for vitamins B2, B4, B7, B9 and B12, cysteine and arginine, despite authors reporting that EF were derived from the same SF with the addition of MFGM-WPC (Billeaud et al. 2014; Timby et al. 2014a; Li et al. 2019b). One study used a formula enriched with MFGM-WPC with reduced total protein and energy compared to SF (Timby et al. 2014a), however differences were no more than 10% between formulas in this study.

3.3.3 Conclusion

The applicant has requested the addition of MFGM-WPC to infant formula products at a concentration of 4 to 7 g/L. Based on the body of evidence, formula enriched with MFGM-WPC at a concentration up to 5 g/L is unlikely to affect growth of infants when compared to SF between 14 days and 12 months of age. FSANZ is unable to make any conclusions on the effect, if any, of MFGM-WPC on growth at concentrations above 5 g/L due to a lack of available evidence.

3.4 Microbiology assessment

The objective of this assessment is to review the microbiological safety of the addition of MFGM-WPC to infant formula products.

The production process described by the applicant includes controls to reduce the risk of microbial contamination. The whey dominant streams used to produce MFGM-WPC are by-products of cheese or casein production from pasteurised milk. Before the manufacture of MFGM-WPC the whey dominant streams are stored at 5°C to control microbial growth. Selective separation by micro- and ultra-filtration is used to generate the MFGM enriched whey stream. It is then further concentrated by reverse osmosis. This material is then heat treated at 60-70°C for approximately 20 seconds prior to being spray dried to achieve the final product. Each batch is also tested against microbiological specifications prior to release of the product to customers. Additionally, the implementation of quality control systems in accordance with cGMP, and application of HACCP principles as stated by the applicant will contribute to reducing microbiological risks.

The applicant stated MFGM-WPC is primarily destined for wet blended infant formula but may also be used in the manufacture of dry blended infant formula products. Due to differences in production processes, the microbiological safety of dry blend formulas relies on the microbiological quality of individual ingredients and strict hygiene during blending. As stated by Codex Alimentarius Commission (CAC) (2008), although the primary responsibility lies with the manufacturers of dry blended formulas to ensure the microbiological safety for their intended use, there is a continuum of effective control measures that need to be performed by other parties, including manufacturers of ingredients, to assure the safety. This includes manufacturers of ingredients destined for use in dry blending employing good manufacturing and good hygienic practices and implementing HACCP systems (CAC, 2008). The applicant has stated that these processes are implemented, and information provided to FSANZ confirmed the applicant has additional controls in place to meet more stringent microbiological limits, including for *Cronobacter sakazakii*, if requested by infant formula manufactures.

3.4.1 Conclusion

No additional microbiological safety risks arise from addition of MFGM-WPC to powdered infant formula products or its preparation and consumption beyond those encountered with infant formula products that are not supplemented with MFGM-WPC.

3.5 Dietary intake assessment

3.5.1 Objective of the dietary intake assessment

The objective of this dietary intake assessment is to estimate the dietary intake of phospholipids from the proposed use of MFGM-WPC in infant formula, follow-on formula and Special Medical Purpose Products for infants (SMPPi).

3.5.2 Approach for the dietary intake assessment

Dietary intake assessments require data on the concentrations of the chemical of interest in the foods requested, as well as any naturally occurring sources and any current permissions for additions to food; and consumption data for the foods which are usually collected through a national nutrition survey. As there are no national consumption data for Australian children younger than two years of age, the dietary intakes of phospholipids for this assessment were estimated using: (1) the maximum proposed use level of MFGM-WPC and the highest specification for phospholipids in MFGM-WPC; and (2) model diets for infants aged 3 months and 9 months.

Dietary intakes of phospholipids from human milk, and from infant formula and follow-on formula assuming the regulatory limit in the Code (72 mg/100 kJ), were also estimated for

comparative purposes.

A summary of the general FSANZ approach to conducting the dietary intake assessment for this application is in Appendix 3. A detailed discussion of the FSANZ methodology and approach to conducting dietary intake assessments is set out in *Principles and Practices of Dietary Exposure Assessment for Food Regulatory Purposes* (FSANZ 2009).

3.5.2.1 Concentrations of phospholipids

Concentrations of phospholipids from the proposed use of MFGM-WPC in infant formula, follow-on formula and Special Medical Purpose Products for infants

The application seeks permission to add MFGM-WPC to infant formula products (as prepared or ready-to-feed) at a maximum use level of 7 g/L. The proposed specification for phospholipids in MFGM-WPC preparation is 6.0 – 10.0% (see Section 2.4 of this report), with the applicant's MFGM-WPC containing a total phospholipid concentration of 6.7% (see Table 1 in this report).

The food categories requested in the application proposed to contain MFGM-WPC and the maximum concentrations of phospholipids from MFGM-WPC (in g/L and g/kg) assuming the proposed maximum use level of MFGM-WPC in infant formula products and the maximum specification for phospholipids in MFGM-WPC are listed in Table 3 below.

Table 3 Maximum concentrations of phospholipids from MFGM-WPC in infant formula products[1]

Food	Maximum concentration of phospholipids from MFGM-WPC	
	g/L	**g/kg**
Infant formula (as prepared or ready-to-feed)	0.7	0.67
Follow-on formula (as prepared or ready-to-feed)	0.7	0.67
Special Medical Purpose Products for infants (as prepared or ready-to-feed)	0.7	0.67

[1] Calculated using the maximum proposed use level of MFGM-WPC in infant formula (7 g/L), the maximum specification for phospholipids in MFGM-WPC (10%), and the density of infant formula (1 L prepared infant/follow-on formula is equivalent to 1.050 kg (FSANZ 2016)).

Concentration of phospholipids in mature human milk

As described in Section 2.2.2 of this report, the phospholipid composition of human milk is highly variable. In a recent systematic review, the range of mean phospholipid concentrations in mature human milk from mothers of full-term infants (considering full-breast studies only, concentrations described/calculated in mg/100 mL or mg/100 g, including different regions and not differentiated for detection methods) was 14.7 – 26.0 mg/100 mL (Venkat et al. 2024). The higher concentration in this range was from a study of human milk lipids of Chinese mothers, where the median and SD of total phospholipids from mature milk (16 days – 8 months) of mothers in the Suzhou region was 26.02 ± 11.3 mg/100 mL (Giuffrida et al 2016). These data are consistent with previously reported naturally occurring levels of phospholipids in human milk of approximately 25 mg/100 mL (EFSA 2020, FSANZ 2021). For the purpose of this assessment, this median concentration from Giuffrida et al. (2016) as reported by Venkat et al. (2024) was used to estimate dietary intake of phospholipids from human milk.

Concentration of phospholipids from the Code

In the Code, there is a restriction on the total phospholipid content in infant formula, follow-on formula and SMPPi of 72 mg/100 kJ. The intent of the restriction is to ensure phospholipids are not added to infant formula products at levels above those naturally occurring in milk and does not reflect an existing permission for phospholipid use as a nutritive substance (FSANZ 2023a). This regulatory limit (72 mg/100 kJ) will be used to estimate the dietary intake of total phospholipids from infant formula and follow-on formula.

3.5.2.2 Consumption data used

The hazard identification and characterisation did not identify any population sub-groups for which there were specific safety considerations in relation to the intake of phospholipids. The population groups that are used for the dietary intake assessment are:

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

Model diets were used for the population groups 3 months and 9 months, to represent the consumption of infant formula or follow-on formula (where appropriate) and human milk for these groups. A set of model diets was not established for infants consuming SMPPi. A description of how the model diets were constructed, and the justification for not including a model diet for infants consuming SMPPi is in Appendix 3.

3.5.2.3 Assumptions and limitations of the dietary intake assessment

The aim of the dietary intake assessment was to make the most realistic estimation of dietary intakes of phospholipids from MFGM-WPC and human milk as possible. However, where significant uncertainties in the data existed, conservative assumptions were generally used to ensure that the estimated dietary intake was not an underestimate of intake.

Assumptions made in the dietary intake assessment included:

- All infant formula and follow-on formula containing phospholipids from MFGM-WPC do so at the maximum concentration specified in Table 3 in this report
- there is 100% market penetration of the infant formula and follow-on formula containing phospholipids from MFGM-WPC

- all human milk contains phospholipids at 26.0 mg/100 mL
- 1 litre of infant formula and follow-on formula equals 1.050 kg
- 1 litre of human milk equals 1.04 kg
- infants aged 3 months exclusively consume infant formula/human milk
- infants aged 9 months consume follow-on formula/human milk
- consumption of foods as outlined in the model diets represent current food consumption amounts for Australian infants aged 3 months and 9 months
- there is no contribution to phospholipid intakes through foods and beverages other than from MFGM-WPC in infant formula and follow-on formula, and from human milk
- there is no contribution to phospholipid intakes through the use of complimentary or other medicines.

In addition to the specific assumptions made in relation to this dietary intake assessment, there are several limitations in comparing the estimated dietary intake of phospholipids from infant formula and follow-on formula to those from human milk:

- the estimated dietary intake of phospholipids from human milk does not account for the natural variation in phospholipid concentration levels reported in the literature
- the estimated dietary intake of phospholipids from MFGM-WPC in infant formula and follow-on formula does not account for other sources of phospholipids (such as lecithin, vegetable oils, milk fat) that may be used in the formulation of an infant formula product (also noted in Section 2.2.2 of this report)
- the regulatory limit of phospholipids in the Code (72 mg/100 kJ, equivalent to 2 g/L), although aligned with international regulations (FSANZ 2021) is higher than reported naturally occurring levels in human milk.

3.5.3 Estimated dietary intakes of phospholipids

3.5.3.1 Estimated dietary intakes of phospholipids from human milk

When it is assumed that infants aged <12 months are consuming human milk (and no infant formula or follow-on formula), the estimated mean and 90th percentile (P90) intakes of phospholipids from human milk are 0.19 g/day and 0.38 g/day for 3 month old infants and 0.13 g/day and 0.26 g/day for 9 month old infants.

On a grams per kilogram body weight per day basis, the estimated mean and P90 dietary intakes of phospholipids from human milk are 0.03 g/kg bw/day and 0.06 g/kg bw/day for 3 month old infants and 0.014 g/kg bw/day and 0.029 g/kg bw/day for 9 month old infants.

Further details are presented in Table 4.

Table 4 Estimated dietary intakes of phospholipids from human milk for infants aged 3 months and 9 months

	Unit	3 months	9 months
Recommended energy intake[1]	kJ/kg bw/day	343	330
P50 body weight[2]	kg	6.4	8.9
Recommended energy intake	kJ/day	2195	2937
100% energy requirements[3]	g/day	2195	n/a
50% energy requirements[3]	g/day	n/a	1469
Mean dietary intake of phospholipids from human milk[4]	g/day	0.19	0.13
	g/kg bw/day	0.03	0.014
P90 dietary intake phospholipids from human milk[4]	g/day	0.38	0.26
	g/kg bw/day	0.06	0.029

[1] United Nations University et al. 2004.
[2] World Health Organization 2006.
[3] Energy content of human milk is 286 kJ/100 g (FSANZ, 2016).
[4] Concentration of phospholipids used in calculation is 0.26 g/L (Venkat et al. 2024) and 1 L of human milk is equivalent to 1.04 kg (FSANZ 2016).

3.5.3.2 Estimated dietary intakes of phospholipids from MFGM-WPC in infant formula and follow-on formula

The estimated mean and P90 intakes of phospholipids from MFGM-WPC in infant formula are 0.55 g/day and 1.1 g/day for 3 month old infants, and in follow-on formula are 0.37 g/day and 0.74 g/day for 9 month old infants.

On a grams per kilogram body weight per day basis, the estimated mean and P90 dietary intakes of phospholipids from MFGM-WPC in infant formula are 0.086 g/kg bw/day and 0.17 g/kg bw/day for 3 month old infants, and from MFGM-WPC in follow-on formula are 0.042 g/kg bw/day and 0.083 g/kg bw/day for 9 month old infants (see Table 5).

Table 5 Estimated dietary intakes of phospholipids from MFGM-WPC in infant and follow-on formula for infants aged 3 months and 9 months

	Unit	3 months	9 months
Recommended energy intake[1]	kJ/kg bw/day	343	330
P50 body weight[2]	kg	6.4	8.9
Recommended energy intake	kJ/day	2195	2937
100% energy requirements[3]	g/day	2195	n/a
50% energy requirements[3]		n/a	1469
Mean dietary intake of phospholipids from infant/follow-on formula[4]	g/day	0.55	0.37
	g/kg bw/day	0.086	0.042
P90 dietary intake phospholipids from infant/follow-on formula[4]	g/day	1.1	0.74
	g/kg bw/day	0.17	0.083

[1] United Nations University et al. 2004.
[2] World Health Organization 2006.
[3] Energy content of infant/follow-on formula is 264 kJ/100 g (FSANZ, 2016).
[4] Concentration of phospholipids in infant formula and follow-on formula is 0.7 g/L and 1 L infant formula/follow-on formula is equivalent to 1.05 kg (FSANZ 2016).

3.5.3.3 Estimated dietary intakes of total phospholipids from infant formula and follow-on-formula assuming the regulatory limit in the Code

The estimated mean and P90 intakes of total phospholipids from infant formula assuming the regulatory limit in the Code are 1.6 g/day and 3.2 g/day for 3 month old infants, and from follow-on formula are 1.1 g/day and 2.1 g/day for 9 month old infants.

On a grams per kilogram body weight per day basis, the estimated mean and P90 dietary intakes of phospholipids from infant formula are 0.25 g/kg bw/day and 0.49 g/kg bw/day for 3 month old infants, and from follow-on formula are 0.12 g/kg bw/day and 0.24 g/kg bw/day for 9 month old infants (see Table 6).

Table 6 Estimated dietary intakes of total phospholipids in infant and follow-on formula assuming the regulatory limit in the Code

	Unit	3 months	9 months
Recommended energy intake[1]	kJ/kg bw/day	343	330
P50 body weight[2]	kg	6.4	8.9
Recommended energy intake	kJ/day	2195	2937
100% energy requirements	g/day	2195	n/a
50% energy requirements		n/a	1469
Mean dietary intake of phospholipids from infant/follow-on formula[3]	g/day	1.6	1.1
	g/kg bw/day	0.25	0.12
P90 dietary intake phospholipids from infant/follow-on formula[3]	g/day	3.2	2.1
	g/kg bw/day	0.49	0.24

[1] United Nations University et al. 2004.
[2] World Health Organization 2006.
[3] The regulatory limit of phospholipids infant formula and follow-on formula in the Code is 72 mg/100 kJ.

3.5.4 Conclusion

Based on the maximum proposed use level of MFGM-WPC proposed by the applicant and the maximum specification for phospholipids in MFGM-WPC, the estimated mean and P90 intakes of phospholipids from MFGM-WPC in infant formula and follow-on formula range between 0.37 and 1.1 g/day. These intakes are higher than the estimated mean and P90 intakes of phospholipids from mature human milk (0.13 to 0.38 g/day), and do not exceed the estimated intakes of total phospholipids from infant formula and follow-on formula assuming the regulatory limit of phospholipids in the Code (1.1 to 3.2 g/day).

4 Beneficial Health Effect Assessment

4.1 Nutrition benefit assessment

4.1.1 Evidence for the effect of MFGM-WPC on improved neural development and cognitive function

The applicant provided forty five studies to support the proposed beneficial effect from MFGM-WPC of improved neural development and cognitive function. FSANZ reviewed all of the papers and included nine studies in the body of evidence, including four human studies (Tanaka et al. 2013; Timby et al. 2014a; Timby et al. 2021; Xia et al. 2021) and five animal studies (Schipper et al. 2016; Brink et al. 2019; Fil et al. 2019; O'Mahony et al. 2020; Collins et al. 2022). Thirty six studies were excluded for following reasons (Table A2.1):

- Endpoints not relevant to the current assessment
- Inappropriate controls
- Endpoints cannot be directly attributed to MFGM – additional substances in test formula

- Abstracts without sufficient detail (no full publication)
- Review papers.

FSANZ undertook a literature search in PubMed on 19 August 2024 to identify any additional relevant studies[5], but none were identified.

4.1.2 Human Studies

Two double-blinded randomised controlled infant feeding trials (three studies) measured the effect of MFGM-supplemented infant formula on cognitive function of infants using instruments that measure cognitive deficit or intelligence in infants or small children (Timby et al. 2014a; Timby et al. 2021; Xia et al. 2021). Of these, one was a substitution study, decreasing the content of fat and protein and adding MFGM-WPC (Timby et al. 2014a; 2021). One study altered the phospholipid composition of infant formula (Tanaka et al. 2013).

The study by Timby et al. (2014a) was described previously in section 3.3.2 and additional details are in Table A2.2. Briefly, infants consumed EF containing MFGM (of unknown concentration) or SF from enrolment to 6 months of age. No significant differences in background characteristics were reported between the two infant formula groups. The Bayley Scales of Infant and Toddler Development - Third Edition (Bayley-III) was used to measure infant cognition at 12 months. The Bayley-III cognitive test is considered the gold standard for identifying developmental delay in children aged 16 days to 42 months, where delay is indicated (e.g. congenital abnormalities, neonatal complications) by comparing abilities to normative age-matched children. It is not designed to measure intelligence as it does not assume that a measure of ability at one time point will predict later ability (Bayley 2006; Anderson and Burnett 2017; Del Rosario et al. 2021). Scores are usually expressed as standard scores with a mean of 100 and SD of 15. The average range is considered to be 85 to 115, with a score of less than 85 potentially indicating developmental delays. Each domain is scored separately, to understand a developmental profile (Bayley 2009).

The cognitive score was significantly increased in the EF group compared to SF group, but not compared to the BFR group at 12 months[6]. The verbal and motor scores were not significantly different between the EF and SF groups ($p > 0.05$; Table A4.1).

In a follow-up study with the same cohort, the Wechsler Intelligence Scale for Children 4th Edition (WISC-IV) test was used to determine differences in intelligence between the two infant formula groups at 6.5 years, with analysis of the ITT population (Timby et al. 2021; Wechsler 2003). Due to the time elapsed the percentage of participants that could be assessed was lower than the number required when power calculations were undertaken, 73% and 70% in EF and SF groups respectively. No statistically significant difference in full scale IQ, verbal comprehension, perceptual reasoning, working memory or processing speed between infant formula groups was reported ($p > 0.05$; Table A4.1).

A similarly designed study was undertaken by Xia et al. (2021) in four centres in China. Details of the study are provided in section 3.3.2. Briefly, infants in the EF group consumed infant formula products with added bovine MFGM-rich ingredient (further details and concentration not provided) from enrolment to age 12 months. Cognitive function was measured using the Bayley-III test. No significant differences were observed in any of the individual scores (cognitive, language, motor, social emotional, general adaptive) between the infant formula groups at 6 months ($p > 0.05$, adjusted for maternal age, parental education and family income; Table A4.1), although the overall difference between infant

[5] **Search term:** (((milk fat globule or milk fat globule membrane) AND (milk or breast or formula)) AND (cognitive or cognition or child development or neuropsychological tests)) AND (child or infant or baby)

[6] EF: 105.8 ± 9.2 (SD) vs SF: 101.8 ± 8.0, $p = 0.008$ adjusted for parental age, years of education and smoking; BFR: 106.4 ± 9.5 vs EF (adjusted $p = 0.35$); vs SF (adjusted $p = 0.029$)

formula and BFR groups was significantly greater for cognitive score and motor score (p =0.05 and p<0.001 respectively). At 12 months, no significant differences were observed between the infant formula groups for cognitive score, language or motor skills (adjusted p >0.05). However the social emotional and general adaptive scores were significantly greater in the EF compared to SF groups[7] with a significant overall difference for general adaptive score (adjusted p = 0.01). However the relevance of these scores to improved neural development or cognitive function is unclear.

A pilot double-blinded randomised controlled study by Tanaka et al. (2013) studied the effect of infant formula containing different concentrations of phospholipid on neurobehavioural development in very low birthweight (< 1500 g) premature infants. The EF and SF compositions differed only in the concentration of individual phospholipids, with total phospholipid remaining constant[8] (Table A4.1). No BFR group was included in the study. Although the study cannot be used to determine the effect of MFGM-WPC on cognitive function it could provide some evidence on the effect of changes in phospholipid composition in infant formula on cognitive function.

The Bayley-II test was used to measure neurobehavioural development at 6, 12 and 18 months. The test is a predecessor to the Bayley-III test and consists of three components including the Mental Development Index (MDI) that measures cognitive function, Psychomotor Development Index (PDI) and Behaviour Rating Scale (BRS) which assesses interest, attention and social skills including orientation, emotional and motor skills. No significant differences were observed at 6, 12 or 18 months in the MDI or PDI scores (p > 0.05; Table A4.1).

Orientation and emotional outcomes were significantly greater at all measured timepoints in the EF compared to SF[9]. Motor quality was also greater in the EF compared to SF at 12 and 18 months but not at 6 months[10].

The Fagan test of infant intelligence was also undertaken in infants at 3, 6 , 9 and 12 months (27, 29, 39, 52 weeks) of corrected age using the Fagan Test Kit (Infantest, Cleveland Ohio, USA), which measures novelty preference rates. At 12 months the EF group had a significantly greater score than the SF group[11]. No significant difference was observed between groups at 3, 6, and 9 months (Table A4.1).

Results from four Fagan cognitive testing sessions are used to create a composite novelty score which may indicate a potential for developmental delay. The authors of the instrument did not discuss the relevance of individual scores and therefore conclusions from findings in the present study are uncertain (Fagan and Shepard 1986).

Being a pilot study statistical power was not discussed and the authors noted the need to undertake multivariate analysis to eliminate confounding including duration of breastfeeding, maternal food intake and socioeconomic status.

4.1.3 Animal Studies

FSANZ considered five animal studies in the body of evidence for the effect of MFGM-WPC

[7] social emotional EF: 94.18 ± 1.48 vs SF: 90.68 ± 1.48, adjusted p=0.048; general adaptive EF: 95.73 ± 1.56 vs SF: 90.11 ± 1.55 adjusted p=0.004
[8] SM: 20% vs 13%, PC: 22% vs 29% and PS:16% vs 20% in EF and SF respectively
[9] Orientation: 76.7 ± 16.3 vs 47.5 ± 19.8, 65.1 ± 8.0 vs 44.5 ± 26.1, 73.2 ± 21.8 vs 44.3 ± 13.0, p < 0.01; Emotional: 71.0 ± 2.36 vs 50 ± 24.1 p < 0.05, 69.5 ± 20.7 vs 43.1 ± 6.9 p < 0.01, 69.4 ± 20.7 vs 50 ± 12.3 p < 0.01 at 6, 12 and 18 months respectively
[10] 80.9 ± 26.2 vs 48.0 ± 18.4; 74.2 ± 30.0 vs 39.7 ± 8.0; at 12 and 18 months respectively, both p < 0.01)
[11] 50.8 ± 4.8 (SE) vs 44.2 ± 6.2; p < 0.01

or its components on cognitive outcomes in mice (Schipper et al. 2016), pigs (Fil et al. 2019) and rats (Brink et al. 2019; O'Mahony et al. 2020; Collins et al. 2022). Duration of experimental diets were from 21 days to 14 weeks. Several behavioural tests were used including Novel Object Recognition (NOR; all studies) that measures memory and cognitive function, Morris Water maze (O'Mahony et al. 2020; Collins et al. 2022) and T-maze (Schipper et al. 2016; Brink et al. 2019) that assess spatial learning (Morris 1981; Wenk 1998; Bevins and Besheer 2006; Deacon and Rawlins 2006; Vorhees and Williams 2006). In addition, Barnes maze, Radial arm maze, open field test and spontaneous behaviour tests were undertaken in one study (Schipper et al. 2016). Experimental diets contained MFGM-WPC in four studies (Brink et al. 2019; Fil et al. 2019; O'Mahony et al. 2020; Collins et al. 2022) and one study used phospholipid supplementation only (Schipper et al. 2016).

All of the animal studies in the body of evidence used NOR testing. The four studies with MFGM-WPC supplementation reported a non-significant difference in NOR scores between animals fed the test and control diets ($p > 0.05$; Table A4.2). One study that supplemented diets with phospholipids reported a significantly greater NOR score at day 78[12] but not at day 35, however novel object placement test results were not statistically significant on day 35 or 78 in the same study (Schipper et al. 2016).

Two studies undertook T-maze testing (Schipper et al. 2016; Brink et al. 2019). Brink et al. (2019) reported a significant increase in test scores in the MFGM-WPC group compared to the control (sialic acid[13]) group[14]. Schipper et al. (2016) reported a significant increase in percentage alternation on day 36 or 37[15] but not on day 79 or 80. Two studies undertook water maze testing and reported a significantly lower time to reach the platform on day 1 and day 4 (but not on day 2 or 3) in the maternally separated rats consuming MFGM-WPC but not any days in the non-separated rats (Collins et al. 2022). Similar results were reported in the study by O'Mahony et al. (2020), with no difference in effect in the non-separated rats consuming MFGM-supplemented diet compared to non-supplemented diets over 4 days (Table A4.2). The MFGM-consuming maternally-separated rats completed the maze faster on day 2 compared to the non-supplemented rats[16] but not on day 1, 2 or 4.

4.1.4 Conclusion

FSANZ considered the evidence for the effect of MFGM-WPC in infant formula products on improved neural development and cognitive function in four human and five animal studies.

One study reported that cognitive scores in infants fed formula containing MFGM-WPC for over 4 months were significantly greater than those fed standard formula, but motor and verbal scores were similar. However the Bayley-III instrument used in the study identifies developmental delay in young children and does not predict cognitive ability above normal ranges. Therefore no conclusions relating to improved cognitive function can be drawn from these results. However it is noted that the scores for cognition in the MFGM-WPC group were similar to the BFR group. When the same cohort had a cognitive (IQ) assessment at age 6 years no significant difference in intelligence was identified.

Another study using the Bayley-III instrument reported greater social emotional and general adaptive scores in infants that consumed infant formula containing a MFGM-rich ingredient at some timepoints, with no significant differences in cognitive, language or motor scores. However neither of the human MFGM studies provided sufficient detail to determine the final concentration of MFGM ingredients in the EF.

[12] 0.48 ± 0.11 vs 0.05 ± 0.16; $p = 0.038$
[13] The author states the sialic acid group contains the same concentration of sialic acid as the MFMG group
[14] 7.41 ± 1.48 (SD) vs 6.10 ± 1.95; $p = 0.03$
[15] 87.1 ± 2.92 vs 74.2 ± 4.87; $p = 0.037$
[16] 187.0 ± 23.6 vs 275.1 ± 22.4; $p < 0.05$

A study that measured the effect of infant formula with different phospholipid composition on infant development also reported increased scores in some parameters at some timepoints. Five animal studies that measured the effects of MFGM-WPC or phospholipids on behavioural outcomes in animals reported improved scores for some tests and at some measured timepoints.

The body of evidence shows limited evidence that infant formula products supplemented with MFGM-WPC or MFGM-rich ingredients supports improves neural development and cognitive function in infants. Some studies report an increase in a subset of measured developmental scores in the MFGM-fed infants compared to controls however the biological relevance of these results is not clear because the instruments do not measure cognitive ability above normal levels. Studies in animals had similar findings. Due to the limitations in the available evidence FSANZ concludes that MFGM-WPC supplemented infant formula products may improve neural development and cognitive function in infants, but additional data is required to make a definitive conclusion.

4.2 Microbiological benefit assessment

The objective of this assessment is to review reported health benefits of the addition of MFGM-WPC to infant formula products on the development of the gut microbiota, in terms of composition (bifidogenic), anti-pathogenic, and immunomodulation effects for a formula-fed infant.

The literature around the effects of isolated, whole MFGM is lacking. Consequently, the majority of studies mentioned below used either a mixture of MFGM and another substance or the constituents of MFGM (Thum et al. 2022).

4.2.1 Introduction

FSANZ has previously described microbiological benefits of an additive to infant formula as those effects that may promote gut microbiota development in formula fed infants that is more closely aligned to that of breastfed infants (FSANZ 2023b).

The effect MFGM and its components have on gut microbiota and immune health has been demonstrated through *in vitro* and *in vivo* mechanistic studies, animal studies and human intervention studies, as reviewed by Nie et al. (2024) and Fontecha et al. (2020). MFGM is comprised of phospholipids, sphingolipids, and membrane-bound proteins that have been shown to work independently or in tandem to affect the body and its microbiota (Nie et al. 2024).

4.2.2 Bifidogenic effects

Tojo et al. (2014) reviewed the critical role of intestinal microbiota, focusing on *Bifidobacterium*, in health and development. *Bifidobacterium* is the predominant bacterial genus present in the gut microbiome of healthy breastfed infants. Colonisation of the gastrointestinal tract starts at birth with bacteria derived from the mother during birth, through breastfeeding and the environment. The gut microbiota composition is highly individual, but predominantly comprises Bifidobacteria and Lactobacilli in the first few weeks of life. Breastfeeding promotes a microbiota rich in Bifidobacteria in infants that has been linked to healthier immune development and providing competitive based protection from infection by pathogenic bacteria. It has been noted that altered or delayed colonisation is observed in clinical cases of inflammatory conditions of the gut or other related immune disorders.

The gut microbiota of exclusively formula-fed infants has been characterised by a microflora

profile that more closely resembles the digestive tract of adults. This is reported to be due to the lack of selective growth-promoting factors found in breast milk that favour beneficial infant-specific bacteria like *Bifidobacterium* (Tojo et al. 2014). In contrast studies show that the microbiota of formula-fed infants enriched with MFGM more closely resemble the microbiota of breastfed infants (Borewicz and Bruck 2024; Chen et al. 2024).

Zhao et al. (2022) investigated the effect of MFGM on microbiota and metabolism. RNA analysis and changes in stool composition were utilised to draw conclusions about microbiota development in infants. The MFGM components actadherin, sialic acid and phospholipid were found to have positive influence on the growth of *Bifidobacterium longum* subsp. Infantis. Actadherin, sialic acid and phospholipid were also negatively correlated with *Veillonella*, *Escherichia* and *Shigella* growth. The proposed mechanism behind this is the altered expression of the genes 2-hydroxyacid dehydrogenase, 4-hydroxy-tetrahydrodipicolinate synthase and ABC transporter ATP-binding protein. Zhao et al. (2022) reported that these genes significantly enriched the glyoxylate and metabolism pathways driving *Bifidobacterium* growth.

Chen et al. (2024) examined the impact fortified formula, including both 1,3-dioleoyl-2-palmitoylglycero and MFGM, had on infant gut microbiota by comparing a breast milk group, fortified formula group, and a regular formula group. 16S rDNA acquired from stool samples at 1-, 4- and 6-months of age were compared to determine gut microbiota diversity. At all three time points, the gut microbiota composition of the fortified formula group and breastfed group was very similar. The fortified formula group and breastfed group both had a higher abundance of *Bifidobacterium* compared to the regular formula group. As the time periods progressed, the microbiota of the regular formula group approached the composition of the fortified and breastfed groups, with *Bifidobacterium* being the dominant genus. At 6 months there was no significant differences in *Bifidobacterium* abundance between the three groups. The mechanisms behind the microbiota composition and the effects of MFGM or 1,3-dioleoyl-2-palmitoylglycero added separately were not assessed in this study.

Chichlowski et al. (2021) assessed the effect infant formula with added bovine MFGM (specifically Lacprodan's MFGM-10) and lactoferrin had on the microbiota and metabolite profiles of 4monthold infants. Healthy term infants were randomly assigned the formula with MFGM and lactoferrin or a control formula with no added MFGM or lactoferrin as their only source of nutrition. Stool samples were collected at the beginning of the study and at day 120. Bacterial microbiota diversity was quantified via 16S rRNA gene sequencing. Alpha (within-sample) and beta (between-sample) diversity was reported. Chicholwski et al. (2021) found that diet had no significant effect on alpha diversity in the study showing that MFGM + lactoferrin enriched infant formula did not significantly change the stool microbiota diversity from the control. However, two species of *Bacteroides* were found to have increased abundance in the MFGM + lactoferrin group: *B. uniformis* and *B. plebeius*. At 4 months no significant differences between the groups and metabolite profiles were observed. However, stool lactate increased in the MFGM + lactoferrin group. Lactate is produced by *Bifidobacteria*, *Bacteroides*, *Enterococci*, and *Streptococci,* primary colonisers of the infant gut (Hofman et al. 2022). These bacteria are typically more prevalent in breastfed infants, which is associated with beneficial gut colonisation in early infancy (Louis et al. 2022).

4.2.3 Anti-pathogenic effects

Attachment of bacteria and viruses to the epithelial cells lining the gut is the first step for colonisation or invasion, which is dependent on common target carbohydrate structures on the host cell surface (Guri et al. 2012). Prevention of bacterial adhesion is now regarded as a promising strategy for reducing infectious disease (Douellou et al. 2017).

Kvistgaard et al. (2004) investigated the inhibitory effects of bovine milk constituents on

rotavirus *in vitro,* focusing on understanding the antiviral mechanisms of specific milk proteins. Rotavirus is a pathogen responsible for mortality in children under five. Previous research has shown a mucin complex containing MFGM proteins such as MUC1 and lactadherin can significantly inhibit rotavirus replication (Newburg et al. 1998, Yolken et al. 1992). Lactadherin has been hypothesised to be responsible for the effects of the mucin complex. Kvistgaard et al. (2004) used embryonic monkey kidney cell line MA104 as a model, growing cells to 80% confluence before inoculating them with rotavirus in the presence or absence (control) of milk proteins. The cells were incubated for an hour before being rinsed and incubated for 18-22 hours in standard infection conditions to allow for viral replication. Infection rates were then assessed using immunoperoxidase staining to quantify rotavirus-infected cells. The study found 6.3 µg (protein/mL) of bovine protein MUC1 decreased rotavirus infectivity rate significantly compared to the control. However, pure bovine lactadherin showed no measurable inhibitory effect on rotavirus infectivity. This result does not agree with the results of Sato et al. (2023), who observed that bovine lactadherin levels were strongly associated with MFGM's anti-rotavirus activity. Sato et al. (2023) and Andersen et al. (2000) both demonstrated that bovine lactadherin binds to integrin αvβ3, a known rotavirus receptor. The reason behind this discrepancy in the data is unknown.

Enterohemorrhagic *Escherichia coli* (EHEC) is a pathogen associated with gastroenteritis, enterocolitis, bloody diarrhea and represents a serious health concern. EHEC adhesion to the epithelial cells of the human intestine is a critical step to establishing infection. Previous studies suggest that oligosaccharides from bovine milk glycans are similar to host epithelial cell receptors and prevent bacterial pathogen adhesion by acting as decoys (Bao et al. 2007; Weinborn et al. 2020). The *in vitro* study by Douëllou et al. (2018) examined the effects of MFG on EHEC adhesion using a co-culture of human intestinal cell lines (Caco-2 and HT29-MTX). Raw milk rich in MFG significantly reduced EHEC adhesion to the intestinal cells compared to UHT skimmed milk lacking MFG, suggesting MFG also acts as a decoy or blocks bacterial attachment sites. Additionally, the *in vivo* study used a mouse model to further test the effectiveness of MFGM-components on reducing EHEC binding (Douëllou et al. 2018). The mice were fed cheese containing either 40% fat (rich in MFMG components) or 0% fat. When mice were infected with EHEC through the oral route the 0% fat cheese mice had EHEC detected in their faeces one day post feeding. EHEC was detected in the 40% cheese mice two days post feeding. Although Douëllou et al. (2018) did not investigate the exact mechanisms involved, these findings suggest that MFGM components delay EHEC binding to the intestinal cells, providing evidence for a protective role against pathogenic colonisation.

Guri et al. (2012) examined the effect of milk fat globules on *Salmonella* Enteritidis. *Salmonella* Enteritidis is an important foodborne pathogen that is known to use specific binding mechanisms and plays a vital role in biofilm formation. The attachment of *Salmonella* Enteritidis was evaluated by incubating the pathogenic bacteria with milk fat globules in the colon cancer cell line, HT-29. The HT-29 line was chosen because it has been extensively characterised as a relevant model for the tissue it was derived from. When a milk fat globule suspension was added to the cells before inoculation with *Salmonella*, a statistically significant decrease in bacterial binding was observed. Similarly, the exposure of the cells to fat globules before infection inhibited the internalization of *Salmonella*. Although the exact mechanisms of inhibition were not investigated in this experiment the results show that having MFGM present before introduction of *Salmonella* Enteritidis reduces its binding potential. This result does not agree with results of Sprong et al. (2012) below. The methodology described below was also applied to *Salmonella* Enteritidis. A diet high in MFGM fed to rats was found to have no effect on colonisation or binding potential.

Sprong et al. (2012) compared the effects between a low MFGM diet and a high MFGM diet on the colonisation of *Listeria monocytogenes in vivo*. Skimmed milk powder (low MFGM) and sweet buttermilk powder (high MFGM) were manufactured from bovine milk to form the

rat's diets. After 14 days of the diet the rats were orally dosed with *L. monocytogenes*. Faecal samples were collected three days pre- and post-infection to assess colonisation. Faecal excretion *of L. monocytogenes* was significantly lower in rats fed the high MFGM diet compared to those on the low MFGM diet, indicating reduced intestinal colonisation by the pathogen. The study also examined the impact of MFGM on pathogen translocation by measuring *L. monocytogenes* levels in extra-intestinal organs as an indicator of bacterial spread beyond the gut. Compared to the low MFGM diet, the high MFGM diet significantly decreased levels of *L. monocytogenes* in the proximal small intestine, caecum and colon. These findings suggest that MFGM components in the diet can limit both the intestinal colonization and systemic translocation of *L. monocytogenes*.

4.2.4 Immunomodulation effects

The gastrointestinal immune system of neonates is immature and human milk, in addition to nutrition from other sources, is purported to regulate immune-related homeostasis and provide a protective mechanism for inducing tolerance to antigens (Duijts et al. 2010). Cell culture experiments and studies conducted in mammalian models demonstrate that ingested MFGM displays a complex array of effects on the host innate and adaptive immune responses (Lee et al. 2018a; Mohamed et al. 2022).

Jiang et al. (2022b) used two models to demonstrate MFGM's effects on intestinal differentiation. In one model, rat pups were supplemented orally with MFGM for 20 days post birth to evaluate its effects in early life intestinal barrier development. In the second model, differentiated Caco-2 cells, used as a human intestinal epithelial cell model, were exposed to MFGM to assess its effects on human intestinal barrier permeability and tight junction proteins. Expression of differentiation markers and tight junction proteins were analysed through qRT-PCR as a measurement of MFGM's effect on differentiation of the intestines. Analysis of the rat model results showed transcription of the intestinal differentiation markers Cdx1 and Cdx2 and the tight junction proteins claudin-2, claudin-4, occludin, ZO-1 and ZO-2 were positively affected by the presence of MFGM across the intestinal system. The Caco-2 cells were subjected to transepithelial electrical resistance (TEER) measurement. TEER is a measure of how permeable a barrier is. At increasing dosages of MFGM the TEER value increased indicating that MFGM markedly decreases permeability of human intestinal epithelial cells. To understand how the permeability of the intestinal barrier was decreasing, qRT-PCR was used to identify any increase in transcription of tight junction proteins. Similarly to the rat model, an increase in transcription of key components for intestinal barrier development including claudin-2, claudin-4, occludin, ZO-1, and ZO-2 was found. Jiang et al. (2022b) concluded that orally ingested MFGM may support intestinal development by activating multiple cellular signalling pathways, leading to the upregulation of tight junction proteins and enhancing intestinal barrier function.

Bhinder et al. (2017) investigated whether supplementation of formula with MFGM could normalise intestinal development in neonates. Using artificial rearing, rat pups were exclusively fed formula from 5 days post birth receiving either a control formula with vegetable based fats or an identical formula where MFGM provided the fat content. A group fed mother's milk served as a positive control. Measurements at 10 days after the experiment started showed the MFGM formula group had similar villus length and crypt depth to the mother's milk control at two different sites in the intestines and the colon. In comparison, the control formula displayed significantly shorter crypt depths at all these sites. Paneth cells, goblet cells and enteroendocrine cells were also tested for as a marker of immunomodulation. The MFGM group also had Paneth, goblet, and enteroendocrine cell numbers comparable to the mother's milk group, with significantly higher counts of Paneth and goblet cells than the control. These results suggest that addition of MFGM to formula may create an intestinal environment more similar to those seen in breastfed pups, with

potential benefits for gut immunity and development.

Brønnum et al. (2005) explored how the bovine sourced gangliosides GD3 and GM3 differentially inhibited dendritic cell maturation and effector functionalities in vitro. GD3 and GM3 are the two predominant gangliosides of human milk. Their concentration is altered during lactation. After birth GD3 is the most abundant ganglioside species, whereas GM3 becomes dominant after the first month of lactation or in mature milk. *In vitro* gangliosides have been found to exhibit immune-modulating activity on various immune competent cells. To explore the effect of GD3 and GM3 on dendritic cells, mouse bone marrow-derived dendritic cells with GD3 and GM3 were incubated together during lipopolysaccharide induced maturation. Cytokine levels were measured via ELISA kits to determine how GD3 and GM3 impacted dendritic cell maturation. The results showed that GD3 suppresses the production of IL-6, IL-10, IL-12 and TNF-α, whereas GM3 is only effective in reducing IL-10 and IL-12 production. Brønnum et al. (2005) hypothesised the more inhibitory effects of GD3 could potentially hamper antigen presentation in newborn infants and thus avoids excessive immune responses against harmless antigens during the first weeks of life.

4.2.5 Conclusion

The beneficial effects of MFGM have been documented in the literature from the early 2000's, with *in vitro* and *in vivo* animal studies along with clinical trials building a credible evidence base on the health effects of MFGM used to supplement infant formula products. Although the exact mechanisms and pathways by which MFGM impacts *Bifidobacterium* expression are unknown, the evidence shows that MFGM and its constituents support increased abundance of infant-specific beneficial bacteria including *Bifidobacterium*. The data suggests MFGM in milk may provide protection from infection by pathogenic microorganisms which a newborn infant could be exposed to. Additionally, there is evidence that MFGM may play a role in immunomodulation including inflammatory suppression and facilitating appropriate immune responses. MFGM gangliosides suppress interleukin production as a potential mechanism to prevent excessive immune responses in newborns. In animal models, MFGM has been shown to have a role in supporting intestinal development by triggering various pathways. Infants fed with human milk are considered to be the gold standard for infant health and development. The above studies provide evidence that infant formula supplemented with MFGM may support the development of a microbiota that more closely resembles that of breastfed infants.

5 Conclusions

After reviewing the data supplied by the applicant and available data in the scientific literature, FSANZ is satisfied that MFGM-WPC is an appropriate source of phospholipids for inclusion in infant formula products and does not pose a safety risk to infants. While more data is needed to substantiate improved neural development and cognitive function compared to standard infant formula products, there is evidence MFGM could be beneficial for infant gut microbiota development.

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Appendix 1. Supporting information for phospholipid composition

Table A1.1 Publications used to compare phospholipid composition between human milk and MFGM-WPC

Publication, sample population and lactation stage	Objectives and analytical methods	Extracted lipid classes (± SD) as a % of total PL[1]	Notes
Wei et al. (2022) 6 healthy mothers, following term birth (Wuxi, China) 2 and 3 months post birth	Study objective was to compare PL composition and molecular chemistry between human milk and other mammalian species (cow, goat, yak, donkey). ^{31}P NMR (PL Conc.); Folch's Extraction (total lipid)	SM: 34.9% (± 11.9) [2] PC: 28.5% (± 10.0) PE: 28.7% (± 9.9) PI: 3.3% (± 1.5) PS: 4.5% (± 1.8)	Unclear if this study is using human milk samples collected as part of the Wei et al. (2019) study.
Ueno et al. (2021) 20 healthy mothers (Japan) *Time post-birth not reported*	Study objective was to compare DHA content of human milk with the mother's diet. ^{31}P NMR (PL Conc.); Gas chromatography (FA)	SM: 32.7% (± 16.5) [2,3] PC: 22.6% (± 11.9) PE: 26.5% (± 15.2) PI: 6.8% (± 3.5) PS: 9.8% (± 5.5)	The grouped values have been extracted for this comparison.
Wei et al. (2019) 6 healthy mothers, following term birth (Wuxi, China) 0-3 months post birth	Study objective was to compare PL composition between human milk at different lactation stages and commercially available infant formula products. ^{31}P NMR (PL Conc.) Röse-Gottlieb Extraction (FA)	SM: 35.8% (±3.7) [2] PC: 26.4% (±2.3) PE: 30.0% (±3.0) PI: 3.4% (±0.4) PS: 4.3% (±0.5)	The mean of the reported 30, 60, and 90 day time points was used for comparison.
Garcia et al. (2012) 22 mothers (France) *Time post-birth not reported*	Study objective was to establish ^{31}P NMR methodology to sensitively measure PL composition in mammalian milk. ^{31}P NMR (PL Conc.) Folch's Extraction (total lipid)	SM: 30.0% (±13.1) [2] PC: 24.8% (±12.0) PE: 31.5% (±14.2) PI: 4.3% (±2.2) PS: 8.8% (±4.6)	Human milk samples were compared with bovine milk using same ^{31}P NMR methodology, which showed bovine milk had lower SM (20.6 % ±15.0~) as a % of PL in comparison.

[1] When determined, lyso, acyl-alkyl, and plasmalogen glycerophospholipids values have been combined with their corresponding diacyl.
[2] Values calculated using error propagation from the published values.
[3] Approximated from reported median and interquartile range using methods in Wan et al. (2014) and Luo et al. (2018).

DHA, docosahexaenoic acid; FA, fatty acid; NMR, nuclear magnetic resonance; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PL, phospholipids; PS, phosphatidylserine; SD, standard deviation of the mean; SM, sphingomyelin

Appendix 2. Supporting information for growth and benefit assessment

Table A2.1 Exclusion reasons for studies for nutritional growth and/or benefit assessments.

Study Reference	Reason for exclusion
Abrahamse-Berkeveld et al. 2024	Test formula is a complex mixture that is not MFGM (G)
Albi et al. 2022	Study outcome not directly related to proposed benefit (B)
Algarin et al. 2022	Conference abstract – insufficient detail (G, B)
Ambrożej et al. 2021	Review (G)
Best et al. 2023	Cannot isolate effect of MFGM (G)
Breij et al. 2019	Test formula is a complex mixture that is not MFGM (G)
Brink and Lönnerdal 2018	Inappropriate control (B)
Campoy et al. 2016	Conference abstract – insufficient detail (G)
Campoy et al. 2018a	Abstract – insufficient detail (G)
Campoy et al. 2018b	Abstract – insufficient detail (G, B)
Cerdó et al. 2022	Cannot isolate effect of MFGM (B)
Chicklowski et al. 2021	Cannot isolate effect of MFGM (G)
Colombo et al. 2023	Cannot isolate effect of MFGM (G, B)
Demmelmair et al. 2017	Review (B)
Deoni et al. 2018	Study does not test MFGM (B)
Dieguez et al. 2022	Cannot isolate effect of MFGM (G, B)
Dieguez et al. 2023	Cannot isolate effect of MFGM (G)
Fraser et al. 2022	Study outcome not directly related to proposed benefit (B)
Gould et al. 2024	Methods paper (G, B)
Grip et al. 2018	No growth outcomes reported (G)
Gurnida et al. 2012	Cannot isolate effect of MFGM (G, B)
He et al. 2019a,b	No growth outcomes reported (G)
Hedrick et al. 2021	Cannot isolate effect of MFGM (G)
Henriksen et al. 2021	Study outcome not directly related to proposed benefit (B)
Jaramillo-Ospina et al. 2023	No growth outcomes reported (G)
Jiang et al. 2022a	Review (B)
Lazarte et al. 2021a,b	Abstract – insufficient detail (G, B)
Lazarte et al. 2022	Abstract – insufficient detail (G, B)
Lazarte et al. 2023	Abstract – insufficient detail (G, B)
Lee et al. 2018b	Does not test MFGM in infant formula products (G)
Lee et al. 2021	No growth outcomes reported (G)
Li et al. 2019a	Cannot isolate effect of MFGM (G, B)
Li et al. 2021	No growth outcomes reported (G)
Lönnerdal 2014	Review (B)
Lorenzo et al. 2019	Does not study MFGM or components of MFGM (B)
Mika et al. 2018	Cannot isolate effect of MFGM (B)
Mudd et al. 2016	Cannot isolate effect of MFGM (B)
Nieto-Ruiz et al. 2017	Abstract – cannot isolate effect of MFGM (G)
Nieto-Ruiz et al. 2019	Cannot isolate effect of MFGM (G, B)
Nieto-Ruiz et al. 2020	Cannot isolate effect of MFGM (G, B)
Nieto-Ruiz et al. 2022	Cannot isolate effect of MFGM (G, B)
Oliveira et al. 2022	Study outcome not related to proposed benefit (B)

Study Reference	Reason for exclusion
Oshida et al. 2003	Study outcome not directly related to proposed benefit (B)
Poppitt et al. 2014	Does not study MFGM or components of MFGM (G)
Ren et al. 2024	Cannot isolate effect of MFGM (B)
Schipper et al. 2023	Test formula is a complex mixture that is not MFGM (G, B)
Schneider et al. 2022	Cannot isolate effect of MFGM (G, B)
Schneider et al. 2023	Cannot isolate effect of MFGM (G, B)
Shek et al. 2021	Test formula is a complex mixture that is not MFGM (G)
Tanaka et al. 2013	No growth outcomes reported (G)
Teoh et al. 2022	Test formula is a complex mixture that is not MFGM (G)
Timby et al. 2014b	No growth outcomes reported (G)
Timby et al. 2015	No growth outcomes reported (G)
Timby et al. 2017b	No growth outcomes reported (G)
Timby et al. 2021	Growth outcomes at 6y (same study as Timby et al. 2014a) (G)
Vickers et al. 2009	Test formula is a complex mixture that is not MFGM (B)
Waworuntu et al. 2016	Cannot isolate effect of MFGM, outcome not relevant (B)
Yuan et al. 2024	Review (B)
Zavaleta et al. 2011	Does not test MFGM in infant formula products (G, B)
Zhang et al. 2023	Study outcome not related to proposed benefit (B)

G growth; B benefit

Table A2.2 Summary of studies included in infant growth assessment.

Publication, study design, age and weight at enrolment and inclusion/ exclusion criteria	Objectives and differences in characteristics at enrolment	Sample size (% male), followed up (% retained) and reasons for dropout	Interventions and method	Results
Billeaud et al. 2014 (France and Italy; M/C) Double blind RCT Mean age at enrolment 13.9 ± 1.8 d (MFGM-P), 13.6 ± 2.0 d (MFGM-L) and 13.7 ± 1.8 d (SF) Mean wt at enrolment 3498 ± 451 g (MFGM-P), 3499 ± 345 g (MFGM-L) and 3494 ± 374 g (SF) Inclusion: healthy, full-term (≥37 weeks), singleton birth, birth wt 2.5–4.5 kg. Exclusion: breastfed past 14 d, significant illness affecting growth, antibiotic therapy, rehospitalisation >2 d out of first 14 d	Determine the difference in wt gain between 0 and 112 d (non-inferiority) between formula groups No differences in reported characteristics at enrolment (birth wt, length, HC, gest. age, sex, wt at enrolment, length, HC, age at enrolment, parental age, maternal alcohol and smoking intake)	Recruited: MFGM-P: 72 (56% male), MFGM-L: 70 (56% male), SF: 57 (54% male) Follow-up at 4 mo: MFGM-P: 47 (65%), MFGM-L: 52 (74%), SF: 45 (79%) Reasons for dropout: voluntary withdrawal (MFGM-L=13, MFGM-P=14, SF=8), lost to follow up (MFGM-L=6, MFGM-P=3, SF=1), gastrointestinal reflux, constipation or vomiting (MFGM-L=2, MFGM-P=1), major protocol deviation (SF=2), colic (MFGM-L=1, MFGM-P=1), family history of asthma (SF=1), bronchitis (MFGM-P=1), hypertrophic pyloric stenosis (MFGM-L=1)	NAN1 SF (total PL 220 mg/L, 1.9 g/100 kcal protein), SF with Lacprodan® MFGM-10 (MFGM-P*) (total PL 452 mg/L, 1.9 g/100 kcal protein) or SF with Fonterra MFGM (MFGM-L*) (total PL 647 mg/L, 2.0 g/100 kcal protein) fed ad libitum from enrolment to 112 d 3-d food diary prior to each visit; formula intake volume not reported; no complementary foods permitted ANOVA with gender and formula as fixed factors Analysis on ITT basis Power analysis: 52 per group required to detect non-inferiority margin of -3 g/d with 6.1 g/d SD and 80% power at 0.05 significance level. Enrolment target of 70 per group to account for dropouts. Study intervals: 14 ± 3 d, 56 ± 5 d, 84 ± 7 d and 112 ± 7 d	Difference in wt gain MFGM-P vs SF: -0.65 g/d, 95% CI: [-2.66, 1.36] MFGM-L vs SF: -0.80 g/d, 95% CI: [-2.81, 1.22] WAZ, LAZ and HCZ between -1.0 and +1.0 in all groups for study period
Timby et al. 2014a (Sweden) Double blind RCT	Determine the effect of MFGM supplementation on infant cognition and	Recruited: EF, SF, BFR: 80 per group, IF groups stratified for sex (50% male in IF groups)	BabySemp1 (Semper AB) SF or SF with decreased protein and energy with MFGM (Lacprodan® MFGM-	WAZ: EF, SF, BFR† Enrolment: 0.03, 95% CI: [-0.14, 0.18], -0.07, 95% CI: [-0.26, 0.11], 0.17, 95% CI: [0.00, 0.36] 4 mo: 0.18, 95% CI: [-0.03, 0.37], 0.11, 95% CI: [-0.13, 0.33], 0.15,

Publication, study design, age and weight at enrolment and inclusion/ exclusion criteria	Objectives and differences in characteristics at enrolment	Sample size (% male), followed up (% retained) and reasons for dropout	Interventions and method	Results
Mean age at enrolment 44 ± 11 d (EF), 47 ± 10 d (SF) and 48 ± 5 d (BFR) Wt at enrolment not reported; mean birth wt 3.53 ± 0.40 kg (EF), 3.44 ± 0.47 kg (SF) and 3.61 ± 0.37 kg (BFR) Inclusion: healthy, full-term (37–42 weeks) birth, birth wt 2.5–4.5 kg. Exclusion: chronic illness, not exclusively formula or breastfeeding at point of enrolment.	growth EF vs SF: greater number of gest. diabetes cases (6 vs 0, p = 0.029), lower paternal BMI (26.0 ± 3.8 vs 27.7 ± 4.8, p = 0.041) BFR vs FF: greater age at enrolment (p = 0.045), parental education (p < 0.001), maternal and paternal age (p = 0.012 and 0.021), gest. age (p = 0.033), birth wt (p = 0.036); lower number of maternal and paternal smokers (p = 0.015 and 0.022) and caesareans (p = 0.039), lower maternal pre-pregnancy BMI (p = 0.040).	Follow up at 12 mo: EF: 73 (91%), SF: 68 (85%), BFR: 72 (90%) Reasons for dropout: no cause/moved from study site (n=12), gastrointestinal symptoms (n=6). Reasons for discontinuation: cow's milk allergy (n=3), gastrointestinal symptoms (n=2)	10) providing 4% w/w total protein content from enrolment to 6 mo Completed 3-d food diary each mo until completion of study; formula intake 2–6 mo EF 876 ± 148 mL/d vs SF 810 ± 146 mL/d formula consumed, adjusted p = 0.018 (PP analysis); complementary foods from 4 to 6 mo Linear mixed model for growth outcomes, adjusted for EF vs SF (maternal wt gain during pregnancy, gestational diabetes, maternal and paternal BMI, smoking, chronic disease), unadjusted for FF vs BFR Analysis on ITT basis; cases with missing outcome data excluded A-priori power analysis: 63 per group required to detect a difference of 0.5 SD in WAZ with 80% power at a significance level of 0.05 at 6 and 12 mo. With an expected dropout rate of 25% 80 infants were recruited Study intervals: 46 ± 9 d, 4, 6	95% CI: [-0.03, 0.32] 6 mo: 0.43, 95% CI: [0.22, 0.63], 0.30, 95% CI: [0.08, 0.51], 0.26, 95% CI: [0.08, 0.43] 12 mo: 0.78, 95% CI: [0.56, 1.00], 0.62, 95% CI: [0.39, 0.83], 0.49, 95% CI: [0.31, 0.67] WAZ 0–12 mo: EF vs SF: p = 0.88; FF (EF + SF) vs BFR: p = 0.025 LAZ: EF, SF, BFR† Enrolment: 0.31, 95% CI: [0.07, 0.51], 0.11, 95% CI: [-0.10, 0.32], 0.52, 95% CI: [0.32, 0.71] 4 mo: 0.58, 95% CI: [0.38, 0.77], 0.36, 95% CI: [0.10, 0.61], 0.52, 95% CI: [0.31, 0.71] 6 mo: 0.66, 95% CI: [0.44, 0.90], 0.45, 95% CI: [0.20, 0.69], 0.44, 95% CI: [0.25, 0.64] 12 mo: 0.74, 95% CI: [0.49, 0.99], 0.47, 95% CI: [0.23, 0.70], 0.34, 95% CI: [0.14, 0.56] LAZ at enrolment FF vs BFR p < 0.05; LAZ 0–12 mo EF vs SF p = 0.76, FF vs BFR p = 0.003 BAZ and HCZ 0–12 mo EF vs SF: p = 0.92 (BAZ) and p = 0.51 (HCZ) FF vs BFR: not reported

Publication, study design, age and weight at enrolment and inclusion/ exclusion criteria	Objectives and differences in characteristics at enrolment	Sample size (% male), followed up (% retained) and reasons for dropout	Interventions and method	Results
			and 12 mo	
Li et al. 2019b (China; M/C) Double blind RCT Mean age at enrolment 21 ± 7 d Wt at enrolment not reported; mean birth wt 3279 ± 399 g (EF), 3298 ± 374 g (SF) and 3381 ± 314 g (BFR) Inclusion: Healthy, full-term (37–42 weeks) birth, birth wt 2.5–4.0 kg. Exclusion: chronic illness, disease affecting normal growth, antibiotic treatment, or fed formula with pre- or probiotics.	Determine the effect of formula containing MFGM or probiotic on infant growth and infection rates ‡ EF vs SF: no reported differences BFR vs FF: higher birth wt (p = 0.002) and maternal and paternal education (p < 0.013); fewer with any sibling (p = 0.001).	Recruited: EF: 192 (48% male), SF: 194 (51% male), BFR: 208 (47% male) Follow up at 12 mo: EF: 161 (83.9%), SF: 167 (86.1%), BFR: 179 (86.1%) Reasons for dropout: parent/caregiver decision without explanation (78%)	SF (Arla Foods) or SF containing Lacprodan® MFGM-10 (5 g/L) from enrolment until 4 mo (EF switched to SF for mo 5 and 6) Completed 3-d formula intake record each mo from enrolment to end of fifth mo; formula intake for EF 866 mL/d vs SF 876 mL/d during intervention period; complementary foods from 4 to 6 mo Independent sample t test for comparison of means with Bonferroni-adjusted p values Analysis on ITT basis, missing values with last value (continuous variables) or zero (categorical variables) Power analysis: 180 per group required to detect a 20% difference in infectious outcomes with 80% power at 0.05 significance level. With an expected dropout of 15-20% 200 infants per group were recruited. Study intervals: enrolment, 1, 2, 3, 4, 5, 6, 9 and 12 mo	Wt gain (g/d) 0–4 mo: EF, SF, BFR: 30.9, 31.7, 31.5 g/d, EF vs SF p = 0.508, no test EF vs BFR Wt gain (g/d) 5–12 mo: EF, SF, BFR: 11.4, 10.8, 10.3 g/d, EF vs SF p = 0.224, EF vs BFR p = 0.012 Mean wt: EF, SF, BFR 1 mo: 4.4, 4.4, 4.6 kg, EF vs SF p = 0.612; FF vs BFR p ≤ 0.041 2 mo: 5.6, 5.6, 5.8 kg, EF vs SF p = 0.999; FF vs BFR p ≤ 0.041 3 mo: 6.5, 6.6, 6.8 kg, EF vs SF p = 0.836; FF vs BFR p ≤ 0.041 4 mo: 7.2, 7.3, 7.5 kg, EF vs SF p = 0.999; FF vs BFR p ≤ 0.041 5 mo: 7.8, 7.8, 8.0 kg, EF vs SF p = 0.999 6 mo: 8.4 kg all groups, EF vs SF p = 0.999 9 mo: 9.4 kg all groups, EF vs SF p = 0.999 12 mo: 10.2, 10.1, 10.2 kg, EF vs SF p = 0.900 WAZ 0–12 mo: EF vs SF no significant difference, no test EF vs BFR Length gain (cm/d) 0–4 mo: EF, SF, BFR: 0.104, 0.107, 0.104 cm/d, EF vs SF p = 0.276, no test EF vs BFR Length gain (cm/d) 5–12 mo: EF, SF, BFR: 0.044, 0.045, 0.044 cm/d, EF vs SF p = 0.656, no test EF vs BFR Mean length: EF, SF, BFR 1 mo: 54.7, 54.4, 54.8 cm, EF vs SF p = 0.432 2 mo: 58.6, 58.5, 58.7 cm, EF vs SF p = 0.828 3 mo: 61.8, 61.7, 61.9 cm, EF vs SF p = 0.999 4 mo: 64.2, 64.3, 64.3 cm, EF vs SF p = 0.999 5 mo: 66.4, 66.6, 66.4 cm, EF vs SF p = 0.999 6 mo: 68.3, 68.6, 68.1 cm, EF vs SF p = 0.390 9 mo: 72.4, 72.6, 72.2 cm, EF vs SF p = 0.848 12 mo: 75.9, 76.2, 75.7 cm, EF vs SF p = 0.472 LAZ 0–12 mo: EF vs SF, FF vs BFR (p > 0.05)

Publication, study design, age and weight at enrolment and inclusion/ exclusion criteria	Objectives and differences in characteristics at enrolment	Sample size (% male), followed up (% retained) and reasons for dropout	Interventions and method	Results
				HC gain 0–4 mo: EF, SF, BFR: 0.048, 0.050, 0.047 cm/d, EF vs SF p = 0.698, no test EF vs BFR HC gain 5–12 mo: EF, SF, BFR: 0.017 cm/d all groups, EF vs SF p = 0.112, no test EF vs BFR
Xia et al. 2021; Jiang et al. 2022c (China; M/C) Double blind RCT Age at recruitment <14 d Mean birth wt: 3.21 ± 0.36 kg (EF), 3.24 ± 0.39 kg (SF) and 3.28 ± 0.33 kg (BFR); overall p = 0.18 Inclusion criteria: healthy, full-term infants, birth wt 2.5–4.0 kg, Apgar score ≥ 7, IF groups: intending to formula feed > 60%; BFR group: intending to breast feed > 90% for first 6 months Exclusion: obvious birth defect or genetic disease	Determine effect of IF containing MFGM on neurodevelopment, growth at 6 and 12 mo. EF vs SF: lower maternal wt (p = 0.045)	Recruited: EF: 108, SF: 104, BFR: 206 Follow up at 12 mo: EF: 92 (85.2%), SF: 83 (79.8%), BFR: 182 (88.3%) Reasons for dropout: lost to follow up (EF=10, BFR=1), breastfeeding in FF (EF=4, SF=6), voluntary withdrawal (EF=1, SF=4, BFR=4), insufficient breast milk in BFR (n=7), constipation (EF=2, SF=5), distrust (EF=4, SF=2), refusal (EF=3, SF=2, BFR=1), maternal infection in BFR (n=2), vomiting (EF=1, SF=1), allergy (SF=1). No statistically significant difference in dropout rate between groups (p > 0.05)	SF (Fonterra Ltd. PL 39.4 IF and 36.5 mg/100 mL FOF) or Fonterra SureStart™* (PL 71.5 IF and 75.5 mg/100 mL FOF) as IF from enrolment to 6 mo and FOF from 6–12 mo 24-hour recall of formula intake at each visit; mean formula intake at 4 mo 967 ± 271 mL/d (EF) and 937 ± 351 mL/d (SF) (adjusted p = 0.65 from 42 d to 12 mo); complementary foods from 4 mo with >60% intake from formula ANOVA adjusted for group and sex as fixed factor, group by sex interaction, site as random factor Analysis on ITT basis Power analysis: 88 infants per formula group required to detect 0.5 SD difference in neurodevelopment outcomes at 12 mo with 90% power and 0.05 significance level. To allow 25% dropout, 120 infants required per formula	Xia et al. 2021† WAZ: overall time trend for EF vs SF in males (p = 0.60) and females (p = 0.57); EF, SF, BFR: Females at 4 mo: 0.27 ± 0.12, 0.36 ± 0.13, 0.61 ± 0.09, overall p = 0.024 LAZ: overall time trend for EF vs SF in males (p = 0.90) and females (p = 0.98). HCZ: overall time trend for EF vs SF in males (p = 0.30) and females (p = 0.82); EF, SF, BFR: Males at 6 mo: -0.01 ± 0.16, 0.10 ± 0.17, -0.25 ± 0.10, overall p = 0.016 Males at 8 mo: 0.10 ± 0.13, 0.32 ± 0.16, -0.18 ± 0.08, overall p = 0.027 BAZ: overall time trend for EF vs SF in males (p = 0.53) and females (p = 0.34). Jiang et al. 2022c Wt gain (g/d) enrolment to 4 mo: EF, SF, BFR: 33.0 ± 6.07, 32.4 ± 5.99 and 31.4 ± 5.63 g/d, EF vs SF p = 0.63, overall p = 0.09 Length gain (mm/mo) enrolment to 4 mo: EF, SF, BFR: 36.5 ± 4.96, 35.9 ± 5.36 and 35.9 ± 5.16 mm/mo, EF vs SF p = 1.00, overall p = 0.51 HC gain (mm/mo) enrolment to 4 mo: EF, SF, BFR: 17.0 ± 3.47, 18.3 ± 9.72 and 17.7 ± 3.98 mm/mo, EF vs SF p = 1.00, overall p = 0.18

Publication, study design, age and weight at enrolment and inclusion/ exclusion criteria	Objectives and differences in characteristics at enrolment	Sample size (% male), followed up (% retained) and reasons for dropout	Interventions and method	Results
			group with 200 BFR. Study intervals: enrolment, 42 d, 4, 6 and 12 mo (all ± 5 d)	
Jaramillo-Ospina et al. 2022 (Chile) Double blind RCT Mean age at enrolment 84.2 ± 26.3 d (EF), 83.5 ± 26.1 d (SF), 77.8 ± 23.9 d (BFR); overall 81.4 ± 25.4 d Mean wt at enrolment 5.89 ± 0.91 kg (EF), 5.97 ± 0.93 kg (SF), 5.84 ± 0.94 kg (BFR), p > 0.05 Inclusion criteria§: healthy, full-term infants, birth wt 2.5–4.5 kg with history of normal growth <120 d. Exclusion criteria: complementary feeding, history of underlying condition affecting normal growth, feeding difficulties or intolerance,	Determine the effect of MFGM-supplemented formula on infant growth, body composition and safety to 730 d EF vs SF: lower WAZ (p = 0.035) BFR vs EF: higher gest. age (p = 0.037), WAZ (p = 0.018), LAZ (p = 0.011), HCZ (p = 0.033), maternal education (p < 0.001); lower age at enrolment (p = 0.006), compliance at 12 mo (p = 0.002), pre-pregnancy and infant enrolment maternal BMI (p < 0.001).	Recruited: EF 173 (54.9% male); SF 174 (51.1% male); BFR 235 (46.4% male) Follow up: 180, 365 and 730 d: EF 161 (93.1%), 150 (86.7%), 144 (83.2%); SF 164 (94.3%), 153 (87.9%), 147 (84.4%); BFR 223 (94.9%), 199 (84.7%), 187 (79.6%) Reasons for dropout: voluntary withdrawal (EF=14, SF=11, BFR=36), lost to follow up (EF=10, SF=11, BFR=12), gastrointestinal symptoms (EF=4, SF=3), exclusion criteria (EF=1, SF=2)	SF or formula with Lacprodan® MFGM-10 (5 g/L) from <120 d to 365 d Monthly call for feeding compliance through 365 d; formula intake volume not reported; complementary foods from 180 d Adjusted multiple linear regression for growth outcomes with all BAZ, LAZ, HCZ and WAZ estimated from WHO growth standards Analysis on ITT basis for compliance, excluded if withdrawn from study Power analysis from pilot growth data: 120 infants per group required to detect 0.8 kg or kg/m^2 difference in wt (12.5 ± 1.6 kg) or BMI (17.5 ± 1.5) and 1.5 cm in length (86.3 ± 3.0) at 730 d with 80% power and a 0.05 significance level. Study intervals: enrolment, 180, 365 and 730 ± 15 d	Mean wt: EF, SF, BFR 180 d: 8.00, 95% CI: [7.92, 8.08], 7.94, 95% CI: [7.86, 8.02], 7.78, 95% CI: [7.71, 7.85] kg; EF vs SF p = 0.272, EF vs BFR p < 0.001 365 d: 10.1, 95% CI: [10.0, 10.3], 10.05, 95% CI: [9.93, 10.2], 9.56, 95% CI: [9.45, 9.66] kg; EF vs SF p = 0.299, EF vs BFR p < 0.001 730 d: 13.0, 95% CI: [12.8, 13.2], 12.9, 95% CI: [12.7, 13.1], 12.1, 95% CI: [12.0, 12.3] kg; EF vs SF p = 0.590, EF vs BFR p < 0.001 WAZ: EF, SF, BFR 180 d: 0.44, 95% CI: [0.36, 0.53], 0.38, 95% CI: [0.30, 0.46], 0.16, 95% CI: [0.09, 0.23]; EF vs SF p = 0.278, EF vs BFR p < 0.001 365 d: 0.67, 95% CI: [0.57, 0.76], 0.61, 95% CI: [0.51, 0.71], 0.16, 95% CI: [0.07, 0.25]; EF vs SF p = 0.406, EF vs BFR p < 0.001 730 d: 0.71, 95% CI: [0.58, 0.83], 0.67, 95% CI: [0.55, 0.79], 0.18, 95% CI: [0.07, 0.28]; EF vs SF p = 0.665, EF vs BFR p < 0.001 Mean length: EF, SF, BFR 180 d: 66.6, 95% CI: [66.4, 66.8], 66.5, 95% CI: [66.3, 66.7], 66.2, 95% CI: [66.0, 66.3] cm; EF vs SF p = 0.522, EF vs BFR p = 0.001 365 d: 74.8, 95% CI: [74.5, 75.1], 74.7, 95% CI: [74.4, 75.0], 74.1, 95% CI: [73.8, 74.3] cm; EF vs SF p = 0.675, EF vs BFR p < 0.001 730 d: 87.7, 95% CI: [87.3, 88.1], 87.6, 95% CI: [87.2, 87.9], 86.3, 95% CI: [86.0, 86.7] cm; EF vs SF p = 0.597, EF vs BFR p < 0.001 LAZ: EF, SF, BFR 180 d: 0.08, 95% CI: [-0.01, 0.17], 0.05, 95% CI: [-0.03, 0.14], -0.18, 95% CI: [-0.26, -0.11]; EF vs SF 0.664, EF vs BFR p < 0.001 365 d: -0.09, 95% CI: [-0.21, 0.03], -0.11, 95% CI: [-0.23, 0.01], -0.38, 95% CI: [-0.48, -0.28]; EF vs SF p 0.782, EF vs BFR p < 0.001 730 d: 0.28, 95% CI: [0.17, 0.40], 0.24, 95% CI: [0.12, 0.36], -0.15, 95% CI: [-0.25, 0.04]; EF vs SF p 0.600, EF vs BFR p < 0.001 Mean HC: EF, SF, BFR

Publication, study design, age and weight at enrolment and inclusion/ exclusion criteria	Objectives and differences in characteristics at enrolment	Sample size (% male), followed up (% retained) and reasons for dropout	Interventions and method	Results
immunodeficiency, maternal illiteracy.				180 d: 43.4, 95% CI: [43.3, 43.5], 43.2, 95% CI: [43.1, 43.3], 43.3, 95% CI: [43.1, 43.3] cm; EF vs SF p = 0.006, EF vs BFR p = 0.239 365 d: 46.3, 95% CI: [46.2, 46.5], 46.2, 95% CI: [46.1, 46.4], 46.3, 95% CI: [46.1, 46.4] cm; EF vs SF p = 0.370, EF vs BFR p = 0.494 730 d: 49.1, 95% CI: [48.9, 49.2], 49.0, 95% CI: [48.8, 49.1], 48.9, 95% CI: [48.7, 49.0] cm; EF vs SF p = 0.353, EF vs BFR p = 0.041 HCZ: EF, SF, BFR 180 d: 0.59, 95% CI: [0.50, 0.68], 0.41, 95% CI: [0.32, 0.50], 0.47, 95% CI: [0.40, 0.55]; EF vs SF p = 0.005, EF vs BFR p = 0.057 365 d: 0.60, 95% CI: [0.49, 0.70], 0.54, 95% CI: [0.44, 0.65], 0.56, 95% CI: [0.46, 0.65]; EF vs SF p = 0.480, EF vs BFR p = 0.569 730 d: 0.96, 95% CI: [0.85, 1.07], 0.90, 95% CI: [0.79, 1.01], 0.83, 95% CI: [0.73, 0.93]; EF vs SF p = 0.445, EF vs BFR p = 0.105

ANOVA: analysis of variance; BAZ: BMI-for-age z-score; HC: head circumference; HCZ: HC-for-age z-score; LAZ: length-for-age z-score; WAZ: weight-for-age z-score; IF: infant formula; FOF: follow-on formula; BF: breastfed; BFR: breastfed reference; EF: experimental formula (test); FF: formula-fed (EF and SF groups); SF: standard formula (control); d: day; M/C: multicentre; MFGM: milk-fat globule membrane; mo: month; ITT: intent-to-treat; PP: per protocol; PL: phospholipid; RCT: randomised control trial; wt: weight. * Concentration of MFGM not reported in Billeaud et al. 2014, Xia et al. 2021 and Jiang et al. 2022c; † Data extracted from graphs using webplotdigitizer where possible and raw data not provided; ‡ Li et al. 2019b included an additional EF arm containing *Lactobacillus paracasei* ssp. *paracasei* strain F19 (1×108 cfu/L), however this is not discussed in this assessment; § Inclusion and exclusion criteria reported in protocol paper for the study, Toro-Campos et al. 2020.

Appendix 3. How the infant model diets were constructed

Infants aged 3 and 9 months

As there are no data available from the 2011-12 Australian National Nutrition and Physical Activity Survey (NNPAS), model diets were constructed to estimate the dietary intakes of phospholipids for infants aged 3 months and 9 months.

As the 3 month and 9 month old infant model diets are based on mean food consumption amounts only, a distribution of food consumption was not available, and hence, a distribution of phospholipid intakes was not able to be produced. Therefore, the 90th percentile dietary intakes were estimated using the calculation shown in Equation 1.

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

90^{th} percentile exposure = mean exposure x 2

(World Health Organization et al.,1985)

The energy content of human milk and infant formula is required for the calculation of the dietary intake of phospholipids in the model diets for 3 month and 9 month old infants. AUSNUT 2011-13 (the nutrient dataset for the 2011-12 NNPAS) is the latest survey specific nutrient data set published for Australian foods. In this dataset, the energy content of Milk, human/breast, mature, fluid is 286 kJ/100 g and for Infant formula, 6-12 months, prepared with water is 264 kJ/100 g (FSANZ, 2016). A set of model diets were developed using the AUSNUT energy contents for human milk and infant formula in the calculation of the dietary intake of phospholipids for 3 month and 9 month old infants.

A set of model diets was not established for infants consuming Special Medical Purpose Product for infants (SMPPi) as the energy and/or fluid requirements can vary depending on the medical conditions of the infant. Additionally, the energy content of the various SMPPi can be variable. The assessment of A1155 (FSANZ 2019) included an examination of products, including formulas for premature infants, formulas for use by infants with inborn errors of metabolism, and formulas for use by infants with severe food allergies, which found the range of energy contents was 269 – 415 kJ/100 g. If an infant consuming SMPPi has similar energy requirements to those used in the model infant diets and their specific formula has a similar energy content to that used in the model diets, then their intake of phospholipids from MFGM-WPC is anticipated to be similar to that outlined in the assessment for this application. If an infant consuming SMPPi has similar energy requirements to those used in the model infant diets and their specific formula has a higher energy content to that used in the model diets, then their intake of phospholipids from MFGM-WPC is anticipated to be similar to or lower than that outlined in this assessment.

Infants aged 3 months

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

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

Infants aged 9 months

By the age of 9 months, infants are consuming a mixed diet of solids and follow-on formula/ human milk. The model diet was constructed based on recommended energy intakes, mean body weight and the proportion of milk and solid foods in the diet for a 9 month old infant. The recommended energy intake for a 9 month old boy (330 kJ/kg bw/day) (United Nations University et al. 2004) and the 50th percentile weight (8.9 kg) (World Health Organization 2006) for the same age and sex was used as the basis for the model diet. The body weight of 8.9 kg was used to estimate dietary intakes for 9 month old infants on a body weight basis. It was assumed that 50% of energy intake was derived from follow-on formula/human milk and 50% from solids and other fluids (Butte et al, 2004; Hitchcock 1986; Pan American Health Organization, 2003).

Appendix 4. Supporting information for nutrition benefit assessment

Table A4.1 Properties of human studies included in benefit assessment

Publication, study design and age at recruitment *	**Objectives and differences in background characteristics[%]**	**Sample size, Followed-up Reasons for dropout**	**Interventions and Method**	**Results**
Timby et al. 2014a Details in Table A2.2	Details in Table A2.2	Details in Table A2.2	Details for intervention in Table A2.2 Bayley-III cognitive test Analysis on ITT basis A-priori power analysis: 63 per group required to detect a difference of 0.5 SD in cognitive test with 80% power at a significance level of 0.05 at 12 months. With an expected dropout rate of 25% 80 infants were recruited.	Cognitive score: EF: 105.8 ± 9.2 (SD) vs SF: 101.8 ± 8.0, p = 0.008 adjusted for parental age, years of education and smoking; BFR: 106.4 ± 9.5 vs EF (adjusted p = 0.35); vs SF (adjusted p = 0.029) Motor score: EF: 98.6 ± 9.3 vs SF: 98.2 ± 9.0, adjusted p = 0.81; BFR: 100.2 ± 7.2 vs EF (adjusted p = 0.24); vs SF (adjusted p = 0.34) Verbal score: EF: 102.6 ± 10.4 vs SF: 102.5 ± 8.9, adjusted p = 0.92; BFR: 106.7 ± 10.7 vs EF (adjusted p = 0.025); vs SF (adjusted p = 0.029)
Timby et al. (2021) (Sweden) Study design described in Timby et al. (2014a) in Table A2.2	See Timby et al. (2014a) in Table A2.2	Randomisation: see Timby et al. (2014a) in Table A2.2 Follow-up at 6.5 years: EF: 58 (73%), SF: 56 (70%), BFR: 64 (80%)	Wechsler Intelligence Scale for Children 4th Edition (WISC-IV) Analysis on ITT basis Comparison of means done by independent-samples t test	Full Scale IQ: EF: 93.8 ± 11.2 (SD) vs SF: 92.5 ± 11.5 p =0.55 adjusted for gestational age; BFR: 98.7 ± 9.4 (no comparison to individual IF groups undertaken) Verbal comprehension: EF: 98.0 ± 8.5, SF: 98.5 ± 10.0 adjusted p= 0.69; BFR: 102.4 ± 8.2 Perceptual reasoning: EF: 98.7 ± 13.4, SF: 96.3 ± 12.7 adjusted p= 0.26; BFR: 103.5 ± 12.4 Working memory: EF: 87.1 ± 11.0, SF: 85.7 ± 12.2 adjusted p= 0.57; BFR: 91.4 ± 9.0 Processing speed: EF: 94.3 ± 13.7, SF: 93.0 ± 11.4 adjusted p= 0.52; BFR: 93.7 ± 11.4

Xia et al. 2021 (China) Details in Table A2.2	Details in Table A2.2	Details in Table A2.2	Details for intervention in Table A2.2 Bayley-III cognitive test, with attention and short-term memory test analysed using method for young Chinese children undertaken at 12 months (primary outcome) and 6 months Power analysis as per Timby et al. (2014a); details in Table A2.2	12 months: Cognitive score: EF: 97.6 ± 1.35, SF: 94.8 ± 1.34 p =0.08 adjusted for maternal age, parental education family income and blood trace elements; BFR: 97.5 ± 1.18 Overall adjusted p=0.14 Language: EF: 95.2 ± 1.00, SF: 94.8 ± 0.99 adjusted p= 0.87; BFR: 96.3 ± 0.87. Overall adjusted p=0.38 Motor: EF: 92.4 ± 1.10, SF: 91.5 ± 1.10 adjusted p=0.49, BFR: 92.3 ± 0.97. Overall adjusted p = 0.75 Social emotional: EF: 94.18 ± 1.48, SF: 90.68 ± 1.48 adjusted p=0.048**,** BFR: 93.74 ± 1.30. Overall adjusted p=0.10 General adaptive: EF: 95.73 ± 1.56, SF: 90.11 ± 1.55 adjusted p=0.004, BFR: 92.19 ± 1.37. Overall adjusted p=0.01 6 months: Cognitive score: EF: 91.6 ± 1.17 (SE) vs SF: 89.7 ± 1.23 p =0.21 adjusted for maternal age, parental education and family income; BFR: 93.3 ± 0.95 Overall adjusted p=0.05 Language: EF: 90.5 ± 0.60, SF: 89.3 ± 0.63 adjusted p= 0.16; BFR: 90.5 ± 0.49. Overall adjusted p=0.24 Motor: EF: 86.2 ± 1.45, SF: 85.1 ± 1.53 adjusted p=0.53, BFR: 92.6 ± 1.18. Overall adjusted p<0.001 Social emotional: EF: 90.0 ± 1.63, SF: 89.6 ± 1.71 adjusted p=0.79, BFR: 93.6 ± 1.32. Overall adjusted p=0.08 General adaptive: EF: 97.1 ± 1.10, SF: 96.9 ± 1.15 adjusted p=0.83, BFR: 98.6 ± 0.89. Overall adjusted p = 0.40

Tanaka et al. 2013 (Japan) Double blind RCT Age at recruitment: Infants admitted to neonatal intensive care unit. Gestational age at recruitment EF: 29.1 ± 2.1 (SE) w; SF: 30.1 ± 2.3 w	Pilot study to measure the effect of sphingomyelin-fortified IF on neurobehavioural development in very low birth weight premature infants Baseline characteristics with no significant difference between IF groups: maternal age, gestational age at birth, birth measurements and pregnancy and birth complications Baseline characteristics measured but results not reported: race, parental education, birth order and parity	24 infants randomised; EF:12, SF: 12; 24 followed up.	EF and SF compositions only differed in percentage of individual PLs in total PL: **%** EF SF PE 18 20 PC 22 29 SM 20 13 PS 16 20 PI 12 11 Bayley-II cognitive test Fagan test of infant intelligence Colombo sustained attention test Evaluations undertaken at 3, 6, 12 and 18 months	Bayley-II 6 months: Mental Development Index (MDI) : EF: 89.1 ± 13.6 (SE); SF: 90.3 ± 15.0 p > 0.05 Psychomotor Development Index (PDI) : EF: 97.8 ± 15.1; SF: 91.1 ± 8.6 p > 0.05 Orientation: EF: 76.7 ± 16.3, SF: 47.5 ± 19.8 p < 0.01 Emotional: EF: 71.0 ± 2.36, SF: 50 ± 24.1 p < 0.05 Motor quality: EF: 62.8 ± 28.3, SF: 63.7 ± 22.8 p > 0.05 Total: EF: 62.8 ± 28.3, SF: 53.6 ± 21.4 p > 0.05 12 months: MDI : EF: 97.8 ± 15.1; SF: 91.1 ± 8.6 p > 0.05 PDI : EF: 93.8 ± 15.1; SF: 88.8 ± 9.0 p > 0.05 Orientation: EF: 65.1 ± 8.0, SF: 44.5 ± 26.1 p < 0.01 Emotional: EF: 69.5 ± 20.7, SF: 43.1 ± 6.9 p < 0.01 Motor quality: EF: 80.9 ± 26.2, SF: 48.0 ± 18.4 p < 0.01 Total: EF: 76 ± 18.5, SF: 47.5 ± 9.4 p < 0.01 18 months: MDI : EF: 95.9 ± 9.2; SF: 91.8 ± 9.4 p > 0.05 PDI : EF: 97.1 ± 3.9; SF: 93.7 ± 18.4 p > 0.05 , Orientation: EF: 73.2 ± 21.8, SF: 44.3 ± 13.0 p < 0.01 Emotional: EF: 69.4 ± 20.7, SF: 50 ± 12.3 p < 0.01 Motor quality: EF: 74.2 ± 30.0, SF: 39.7 ± 8.0 p < 0.01 Total: EF: 76.3 ± 25.9, SF: 51.2 ± 13.5 p < 0.05 Fagan Test: 3 months: EF:50.3 ± 9.1 (SE)%, SF: 50.1 ± 3.8; p > 0.05 6 months: EF:50.3 ± 5.6 %, SF: 48.2 ± 3.3; p > 0.05 9 months: EF:49.9 ± 5.3 %, SF: 46.5 ± 7.2; p > 0.05 12 months: EF:50.8 ± 4.8 (SE)%, SF: 44.2 ± 6.2; p 0.01

* RCT: randomised controlled trial; EF: experimental formula; SF: standard formula; BFR: breastfed reference; d: days; w:weeks; IF: infant formula; wt weight; SE standard error; PL: phospholipid; PE: phosphatidyl ethanolamine; PC: phosphatidyl choline; SM: sphingomyelin; PS: phosphatidyl serine; PI: phosphatidylinositol amine; DHA docosahexaenoic acid; ARA arachidonic acid; ITT intention to treat

+ Data extracted from graphs using Webplotdigitizer where possible and raw data not provided.

& SEM could not be determined from graph

% Only relevant objectives, study arms and results are described

Table A4.2 Properties of animal studies included in benefit assessment

Publication, study design and age at recruitment *	Objectives%	Sample size	Interventions and Endpoint Method	Results
Schipper et al. 2016	IF containing phospholipid-coated lipid droplets affects specific cognitive behaviours in healthy male mice	ED: 14. SD: 13 (male) On day 2 litters were randomly assigned to 6 pups/dam	Standard diet (SD) and experimental diet (ED): AIN-93G with added fat from IF products; similar total lipid and FA composition. ED contained phospholipids (4 g/kg) and was processed to create phospholipid-coated lipid droplets Diets were consumed in addition to milk from dam from day 16-21, and diet only from day 21-44 Novel object (and placement) recognition postnatal day 35/ 78 T-maze: day 36/7, 79/80 Barnes maze: day 42/3 Radial-arm maze: day 101 Open field test: day 35/78 Spontaneous behaviour test: day 73-79 No power analysis provided	Novel object recognition (NOR) and Novel object placement (NOP): Discrimination Index: Day 35 NOR: ED: 0.33 ± 0.21 (SEM) vs SD: -0.04 ± -0.20; p > 0.05 NOP: ED: -0.1 ± 0.2 vs SD: 0.2 ± 0.2; p > 0.05 Day 78 NOR: ED: 0.48 ± 0.11 vs SD: 0.05 ± 0.16; p = 0.038 NOP: ED: 0.1 ± 0.2 vs SD: 0.2 ± 0.2; p > 0.05 T-Maze Spontaneous alternation % Day 36-37: ED: 87.1 ± 2.92 vs SD: 74.2 ± 4.87; p = 0.037 Day 79-80: ED: 76.6 ± 4.62 vs SD: 75.4 ± 3.16; p > 0.05 Barnes Maze No difference in latency to reach target hole between diet groups during probe trial Visits to target zone/total visits Probe trial day 42/3: ED: 0.12 ± 0.01 vs SD: 0.11 ± 0.01; p > 0.05 Reversal trial day 43: ED: 0.17 ± 0.02 vs SD: 0.20 ± 0.02; p > 0.05 Radial-arm maze Working memory errors (re-enters previously visited arms)/total visits Day 101: ED: 77.26 ± 4.21 vs SD: 75.79 ± 2.95; p > 0.05 Reference memory index (visits to baited arms/total visits) Day 101: ED: 0.48 ± 0.04 vs SD: 0.49 ± 0.02; p > 0.05 Open field test and Spontaneous behaviour Used as control tests. Total distance moved and time spent in the centre was similar between diet groups at both time points. No differences in spontaneous behaviour were reported. No statistical analysis provided for either test. Open field test Day 35 Total distance 44.5 ± 25.1 m vs 42.5 ± 23.8 m Time in centre: 112 ± 11 sec vs 126 ± 16 sec Day 78 Total distance 55.1 ± 31.9 m vs 56.8 ± 37.6 m Time in centre: 28 ± 4 sec vs 29 ± 6 sec

Brink et al. 2019	Investigate which components of MFGM drives neurological development of rats	T-maze and NOR: 30 per group (15 male) Morris Water Maze:16 per group (16 male) Cross-fostered litters on day 2	Supplements of Lacprodan® MFGM-10 (100 mg/kg bw) or sialic acid (SIA; 2 mg/kg bw) via oral gavage day 2-21 when weaned. Behavioural testing on day 50 T-maze Novel object recognition Water maze No power analysis provided	T-maze Score MFGM vs SIA (One way ANOVA with Kruskal-Wallis rank test) [%]**:** 7.41 ± 1.48 (SD) vs 6.10 ± 1.95; p = 0.03. NOR ratio of visit frequence: 0.51 ± 0.07 vs 0.53 ± 0.08; p > 0.05 Total distance travelled: 2590 ± 704 vs 2491 ± 885 cm; p > 0.05 No difference between groups was identified in water maze for velocity, distance moved or latency (p > 0.05). Details could not be determined
Fil et al. 2019	Evaluate the effect of dietary MFGM on neurodevelopment in young pigs	Young pigs 0.25 mg/L group: n=11; 0.5 mg/L group: n=15; control group n=17	Milk replacer formula (Mead Johnson Nutrition) containing 0, 2.5 or 5 g/L Lacprodan® MFGM-10 until day 30/31. Start date of formula not provided Object recognition memory testing: day 29 No power analysis provided	Mixed design ANOVA with post-hoc Tukey adjustment, fixed effects of diet and postnatal age. Pigs receiving dietary MFGM did not show a novelty preference compared to control-fed pigs. Mean recognition index: MFGM 2.5: 0.59 ± 0.061 (SE), 73% > 0.5 p = 0.096 MFGM 5.0: 0.55 ± 0.074 , 67% > 0.5 p = 0.265 Control: 0.59 ± 0.048, 59% > 0.5 p = 0.047
Collins et al. 2022 Single blinded randomised trial	Effect of MFGM supplementation on signalling pathways in maternal-separated and non-separated rats	Male rats n=11-12 per group Non separated (NS)-control, NS-MFGM, Maternal separated (MS)-control, MS-MFGM	SD: AIN-93G diet with DHA/ARA oil 5.3 g/kg; ED: standard diet + whey protein concentrate MFGM-10 15.9 g/kg Maternal separation: Day 2-12 Novel object recognition: day 70 Morris water maze: day 77	Water maze - Time to reach platform (min) in ED vs SD Mixed design ANOVA with trial date as repeated measure factor and diet as independent factor[+] NS rats Day 1: 0.89 ± 0.11 (SEM) vs 1.2 ± 0.11; p = 0.06 Day 2: 0.39 ± 0.06 vs 0.48 ± 0.05; p > 0.05 Day 3: 0.33 ± 0.04 vs 0.24 ± 0.04; p > 0.05 Day 4: 0.22 ± 0.06 vs 0.14 ± 0.00; p > 0.05 MS rats Day 1: 0.95 ± 0.11 (SEM) vs 1.23 ± 0.10; p < 0.05 Day 2: 0.55 ± 0.14 vs 0.67 ± 0.12; p > 0.05 Day 3: 0.30 ± 0.03 vs 0.32 ± 0.06; p > 0.05 Day 4: 0.14 ± 0.02 vs 0.25 ± 0.04; p < 0.05 Two-way Anova found no effect of diet F(1,41) = 1.018, p = 0.32,

			No power analysis provided	or of diet x early life stress effect F(1,41) = 0.098, p =0.76 on the discrimination index in the novel object recognition test
O'Mahony et al. 2020	Determine the long term benefits of feeding prebiotic blend with and without MFGM to male rats subjected to early life MS.	Male rats n=12 per group Non separated (NS)-control, NS-MFGM Maternal separated (MS)-control, MS-MFGM	Standard diet: casein 200g/kg; l-lysine 3 g/kg; corn starch 392 g/kg; maltodextrin 132 g/kg; sucrose 100 g/kg; lactose monohydrate 7.5 g/kg; soya bean oil 64.7 g/kg; cellulose 50 g/kg; mineral mix (without Ca and P) 13.4 g/kg; calcium carbonate 7.2 g/kg; calcium phosphate dibasic 7.0 g/kg; vitamin mix AIN93VX 15 g/kg; choline bitartrate 2.5 g/kg; vitamin K1 2 mg/kg; antioxidant 14 mg/kg; DHA/ARA oil 5.3 g/kg Experimental diet: standard diet + 15.9 g/kg MFGM-10 Maternal separation Day 2-12 Novel object recognition (Bevins and Besheer (2006): week 8 (3 days) Morris water maze: week 9 and 10 No power analysis provided	Water maze - Time to reach platform (sec) in ED vs SD[+] NS rats Day 1: 369.7 ± 18.7 (SEM) vs 294.9[&]; p > 0.05 Day 2: 236.8 ± 34.9 vs 249.2; p > 0.05 Day 3: 152.4 ± 15.0 vs 121.3; p > 0.05 Day 4: 81.8 ± 6.23 vs 84.3; p > 0.05 MS rats Day 1: 318.2 ± 29.8 (SEM) vs 279.7 ± 26.1; p > 0.05 Day 2: 187.0 ± 23.6 vs 275.1 ± 22.4; p < 0.05 Day 3: 137.7 ± 13.7 vs 161.3 ± 22.4 ; p > 0.05 Day 4: 108.3 ± 9.9 vs 85.9 ± 28.6; p > 0.05 No effect from dietary intervention on novel object recognition was found. Data not provided

* RCT: randomised controlled trial; EF: experimental formula; SF: standard formula; BFR: breastfed reference; d: days; w:weeks; IF: infant formula; wt weight; SE standard error; PL: phospholipid; PE: phosphatidyl ethanolamine; PC: phosphatidyl choline; SM: sphingomyelin; PS: phosphatidyl serine; PI: phosphatidylinositol amine; DHA docosahexaenoic acid; ARA arachidonic acid; ITT intention to treat

[+] Data extracted from graphs using Webplotdigitizer where possible and raw data not provided.

[&] SEM could not be determined from graph

[%] Only relevant objectives, study arms and results are describe