Vitenskapskomiteen for mat og miljø
Norwegian Scientific Committee for Food and Environment

VKM Protocol 2023

Protocol for risk assessment of *Bifidobacterium breve* M-16V in infant formula

From the Panel on Nutrition, Dietetic Products, Novel Food and Allergy of the Norwegian Scientific Committee for Food and Environment

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The Panel on Nutrition, Dietetic Products, Novel Food and Allergy of the Norwegian Scientific Committee for Food and Environment

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Protocol for the risk assessment of *Bifidobacterium breve* M-16V in infant formula

Preparation of the protocol

The Norwegian Scientific Committee for Food and Environment (Vitenskapskomiteen for mat og miljø, VKM) appointed a project group that drafted the protocol. The project group consisted of four VKM members and two VKM staff. The Panel on Nutrition, Dietetic Products, Novel Food, and Allergy assessed and approved the protocol.

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The authors have contributed to the opinion in a way that fulfils the authorship principles of VKM (VKM, 2019). The principles reflect the collaborative nature of the work, and the authors have contributed as members of the project group and/or the VKM Panel on Nutrition, Dietetic Products, Novel Food, and Allergy.

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Persons working for VKM, either as appointed members of the Committee or as external experts, do this by virtue of their scientific expertise, not as representatives for their employers or third-party interests. The Civil Services Act instructions on legal competence apply for all work prepared by VKM.

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Key words: VKM, risk assessment, Norwegian Scientific Committee for Food and Environment, Norwegian Food Safety Authority, probiotic, prebiotic, *Bifidobacterium breve* M16-V, infants, infant formula

Abbreviations and glossary

Abbreviations

AMR	Antimicrobial resistance
B. Breve	*Bifidobacterium breve*
CFU	Colony forming unit
EFSA	European Food Safety Authority
EU	European Union
GRAS	Generally Recognized As Safe
lcGOS	Long-chain fructo-oligosaccharides
LBW	Low birth weight
NEC	Necrotizing enterocolitis
NFSA	Norwegian Food Safety Authority
QPS	Qualified Presumption of Safety
scGOS	Short-chain galacto-oligosaccharides
SLR	Systematic literature review
ToR	Terms of Reference
VKM	Norwegian Scientific Committee for Food and Environment
WHO	World Health Organization

Glossary

Glossary term	
Colony forming unit	A unit of measurement used to determine the number of bacterial cells in a sample.
Colonization: transient, permanent	Colonization means infection and is the first stage of microbial infection by the establishment of the pathogen at the appropriate portal of entry. For a reduced number of authors, colonization and infection remain two different processes. All multicellular organisms are colonized to some degree by extrinsic organisms, and the vast majority of these exist in either a mutualistic or commensal relationship with the host. The difference between an infection and colonization is often only a matter of circumstance. Non-pathogenic organisms can become pathogenic given specific conditions, and even the most virulent organism requires certain circumstances to cause a compromising infection. (Dani, 2014) See also definition of infection.
Commensal bacteria	Commensal bacteria are microbes that reside on either surface of the body or at mucosa without harming human health. The relationship neither benefits from the other or provokes any harm.
Complementary foods	The first solid and semi-solid foods given to infants when human milk or infant formula is no longer sufficient to meet nutritional needs

Glossary term	
Dysbiosis	"... a compositional and functional alteration in the microbiota that is driven by a set of environmental and host-related factors that perturb the microbial ecosystem to an extent that exceeds its resistance and resilience capabilities." Levy et al. 2017.
Generally Recognized As Safe (GRAS)	GRAS is an American Food and Drug Administration (FDA) designation that a chemical or substance added to food is considered safe by experts, and so is exempted from the usual Federal Food, Drug, and Cosmetic Act (FFDCA) food additive tolerance requirements.
Homeostasis	Homeostasis defined as balanced microbial ecosystem. It refers to the ability of an organism to maintain the internal environment of the body within limits that allow it to survive.
Hypoallergenic	Do not contain allergens, and/or unlikely to cause allergic reaction.
Infant	Child younger than 12 months.
Infection	When germs enter the body, increase in number, and cause a reaction of the body (US Centers for Disease Control and Prevention: https://www.cdc.gov/infectioncontrol/spread/index.html. Page last updated: January 7, 2016).
Microbiome	The microbiome is the collection of all microbes (e.g. bacteria, fungi, viruses) and their genes and biomolecules that are part of a given environment.
Microbiota	Collective term for microbial community (i.e., any type of microorganism) that may be found within a given environment.
Neonate	Infant less than four weeks old.
Novel Foods and Food Ingredients:	Novel foods are foods and food ingredients that have not been used for human consumption to a significant degree within the European Community before 15 May 1997. With regards to probiotic products, the use of a new probiotic strain, that has not previously been consumed (to a significant degree) in the EU, would require a novel foods assessment. The use of a novel food matrix including an established probiotic strain may also require novel foods assessment.
Prebiotics	A prebiotic is a selectively fermented food ingredient that results in specific changes in the composition and/or activity of the gastrointestinal microbiota, thus conferring benefit(s) upon host health.
Premature infant	Infant born before 37 weeks gestation.
Probiotics	Live microorganisms, which when administered in adequate amounts confer a health benefit on the host (FAO/WHO 2001).
Qualified Presumption of Safety	The QPS approach is a system similar in concept and purpose to the GRAS definition used in the USA but has been modified to take account of the different regulatory practices in Europe. With respect to probiotics, QPS represents a possible route to harmonisation of

Glossary term	
	approaches for the safety assessment of microorganisms used in feed/food production.
Synbiotics	The term synbiotic is used for a product that contains both probiotics and prebiotics. Since the word alludes to synergism, this term should be reserved for products in which the prebiotic compound selectively favours the probiotic component.
Virulence factor	Virulence factors are microbe-associated molecules that are required for a microbe to cause disease while infecting eukaryotic hosts such as humans.

1 Introduction

1.1 Scope and structure of the protocol

This document outlines the protocol for the "Risk assessment of *Bifidobacterium breve* M-16V in infant formula". The protocol addresses the requestor's needs of both i) a general scientific risk assessment and ii) a risk assessment of two specific products in an application to the Norwegian Food Safety Authority (NFSA).

The current protocol was drafted by a project group appointed by the Norwegian Scientific Committee for Food and Environment (VKM) and has been reviewed and endorsed by an appointed interdisciplinary approval group. The protocol will not be published prior to the risk assessment to protect the applicant from public disclosure during the application process.

1.2 Background as provided by the Norwegian Food Safety Authority

The Norwegian Scientific Committee for Food and Environment (VKM) has previously published several benefit and risk assessments of bacterial strains with probiotic effect in products for infants and toddlers.

In these reports, VKM concludes, among other things, that long-term intake of the relevant bacteria in monoculture for the youngest age groups (those under 12 months) may have unknown health effects. Furthermore, the lowest age groups, i.e. four to six months and six to 12 months, are in the most immature and vulnerable phase when it comes to establishing bacterial flora in the intestine and developing the immune system. It will therefore be those age groups with the highest risk of any negative health effects from daily intake of probiotics.

All foods sold on the Norwegian market shall, pursuant to Section 16 of the Food Law, be safe. According to the preparatory works of the Law, stricter safety requirements are imposed on food products aimed to vulnerable groups in the population, such as infants and young children. There are also requirements in the legislation that these foods must be suitable for the relevant group of children. Based on some of the above-mentioned risk assessments, the Norwegian Food Safety Authority has therefore found it necessary to impose a ban on the sale of certain products for infants and toddlers containing probiotic bacteria.

Food for special medical purposes is regulated by Regulation of 10 January 2014 No. 21 on foods for specific groups implementing Regulation (EU) 2016/128 into Norwegian law. Hypoallergenic nutritional products aimed at infants with, for example cow's milk allergy, fall under the provisions of these regulations. In these regulations, *infants* are defined as children under twelve months of age and *toddlers* are defined as children of one to three years of age.

A business operator wishes to market two products containing the bacterial strain *Bifidobacterium breve* M-16V on the Norwegian market. The products are defined as “for the dietary management of cow's milk allergy” and are registered as *foods for special medical purposes*. The business operator points out that both products are market in many countries in Europe. The business operator has submitted documentation about the bacterial strain, in addition to documentation that they believe supports that the products are safe and suitable for use in products intended for infants.

Considering the requirements regarding safety and suitability, the Norwegian Food Safety Authority requests VKM for a risk assessment of *Bifidobacterium breve* M-16V when added to infant formula in general, and when added to two specific hypoallergenic formula.

2 Terms of reference (ToR) as provided by the Norwegian Food Safety Authority

The NFSA asks VKM to answer the following questions:

1. Are there any health risks of giving infant formulas containing *Bifidobacterium breve M-16V* to infants 0-12 months:
    a. As a full diet
    b. As a supplementary diet

2. Are there any health risk of giving two specific hypoallergenic infant formulas containing *Bifidobacterium breve M-16V* (marketed as foods for special medical purposes) to infants with cow milk allergy and/or other food allergies and related allergic conditions:
    a. PEPTI SYNEO™ as full diet from 0-6 months
    b. PEPTI SYNEO™ as supplementary diet from 6-12 months
    c. NEOCATE SYNEO™ as full diet from 0-12 months

2.1 Interpretation of terms of reference

The Terms of Reference (ToR) of the mandate have been interpreted by VKM project group as follows:

1) ToR1 is a general scientific risk assessment of the safety of oral intake of B. breve M-16V through infant formula. The formula may be for general use, or special medical purposes (e.g. hypoallergenic), and may or may not contain prebiotics.

2) ToR2 is a risk assessment of two specific symbiotic products in an application to the NFSA. *B. breve* M16-V is added with prebiotics to hypoallergenic infant formula for the management of cow's milk allergy and other allergic conditions. The products are regulated as food for special medical purposes.

Depending on intended use of a probiotic (medication vs. food or dietary supplement), regulations differ. Infant formula and foods for special medical purposes that contain probiotics, such as certain hypoallergenic infant formula, are regulated as foods for specific groups, and will be risk assessed in line with guidelines for probiotics in foods. Reference to the dietary management of diseases, disorders, or medical conditions for which the food is intended should not be considered as attribution of the property of preventing, treating, or curing a human disease (Regulation (EU) No 609/2013). VKM will not weigh benefits against risks, a common procedure when assessing medications. However, it is assumed that uses of *B. breve* M16-V in critically ill infants and in intensive care settings, as medication or supplement (e.g. powder used in enteral feeding regimens), can inform on the safety of the probiotic when used in infant formula.

2.2 Limitation/delimitations

2.2.1 Common to both risk assessments

- Infants are defined as children aged 0 to 12 months as in the ToR from the NFSA.

- The risk assessment is performed for *B. breve* M-16V as monoculture, not in probiotic mixtures with other strains, but *B. breve* M-16V alone in symbiotic mixtures will be included.

- Potential beneficial effects of *B. breve* M-16V are not included in the ToR from the NFSA and will not be evaluated.

2.2.2 General risk assessment

- The general risk assessment is performed for oral or enteral intake of *B. breve* M-16V for infants of any gestational age.

2.2.3 Product specific risk assessment

- It is assumed that hypoallergenic infant formula for cowmilk allergy, is used for children with clinical allergy who do not receive any human milk. The manufacturer has declared that the recommended use of the products is after full consideration of all feeding options, including breastfeeding with maternal elimination diet.

- Cow milk, cow milk products or human milk are not considered as complementary foods for children receiving hypoallergenic infant formula due to cow-milk allergy.

- The health risks of *B. breve* M16-V in specific products are assessed for intended use and under the assumption that the products are prepared and used according to the manufacturer's instructions. Use is contraindicated in premature infants or infants who are immunocompromised.

- The health risks are limited to effects of *B. breve* M-16V, and not use of liquid formula as a full diet for prolonged periods (e.g. potential effects on oral motor skills or speech-language development).

3 Background provided by VKM

3.1 Preliminary scoping search

To help draft the current protocol, an initial scoping search for guidance documents on probiotics and literature (not systematic or exhaustive) on *B. Breve* M-16V alone and in combination with infant formula was performed using different search engines (Google Scholar, Open Alex) and indexed databases (PubMed, Epistemonicos, and the Cochrane database). Literature on the development of the infant gut microbiota, and on the safety of probiotics in general was also collected. Identified review studies suggest that much of the published literature on *B. Breve* M-16V is related to complications in premature- and low birth weight (LBW) infants (e.g. necrotizing enterocolitis and late-onset sepsis) or infants with allergic conditions (atopic dermatitis and cow's milk allergy) (Athalye-Jape et al., 2018; Wong et al., 2019), with fewer studies in infants without any specified medical condition.

3.2 Probiotics

3.2.1 Definition

A widely adopted definition of "probiotics" is "live microorganisms, which when administered in adequate amounts confer a health benefit on the host" (FAO/WHO 2001; Hill et al., 2014). Probiotics (bacteria, yeasts, or fungi) can be administered via different routes (oral, intravaginal, or topical) and are not limited to human use (companion animals, livestock, fish). In this assessment, the term "probiotics" is used to describe food-grade commercial microorganisms, although within the EU the term "probiotic" on a food label require an authorized health claim. Commercial probiotics can be used as ingredients in functional foods, infant formula, and porridge, and can be taken or administered as a dietary supplement. So far, no health claim has been approved for probiotic containing foods (source: EU Register of Health Claims).

3.2.2 Potential mechanisms

Several major mechanisms have been suggested to be involved in the functions of probiotics (Suez et al., 2019). These mechanisms include immunomodulation (through effects on expression of immune-related genes, inflammatory pathway activity and immune marker levels), direct and indirect pathogen antagonism, improved barrier function, and other mechanisms such as the ability to deconjugate bile acid, degrade lactose and complex carbohydrates, affect signalling to the enteric and central nervous systems, and modulate the indigenous microbiome. The mechanisms of action of probiotics are not completely understood and some remain debated, including the capacity of the administered microorganisms to colonize the host gastrointestinal mucosal surface (stably or transiently), and the degree of interaction with the host microbiome (Suez et al., 2019). Not all probiotics are thought to function in the same manner. Strain-specificity of probiotic effects is an important cornerstone principle of probiotic science, although certain mechanisms (Sanders et al., 2018) and genomic traits (Rodriguez and Martiny, 2020) may be shared between groups of bacteria at the species, or genus level.

3.3 Regulatory status

Many probiotics added to foods have regulatory status as safe, such as GRAS (generally regarded as safe) status in the United States, and QPS (Qualified Presumption of Safety) status in the European Union. Some products may be regulated for foods for special medical purposes such as hypoallergenic infant formula. The safety profile of probiotics in foods (such as lactic acid bacteria and bifidobacteria) is mainly based on the history of safe use, and on observations noted in clinical trials assessing probiotics efficacy, rather than safety, as the main outcome (Bafeta et al, 2018; Suez et al., 2019).

3.4 Use in infants

Infant are considered a vulnerable group when assessing potential health risks of probiotics. In newborn children, a commensal flora has not yet been established. Establishment of the GI microbiome is considered an essential developmental process, and is influenced by multiple factors, including maternal factors, host genetics, mode of delivery, gestational age, type of infant feeding (breastfeeding or formula feeding), antibiotics use, and environmental factors (Tamburini et al., 2016; Milani et al., 2017).

The relative importance of these factors remains debated and reported influence on the microbiome are somewhat inconsistent.

Probiotics are administered on a large scale to different groups of infants in attempts to reduce neonatal morbidities in preterm infants, such as necrotizing enterocolitis (NEC) and late-onset sepsis (van den Akker et al., 2020) and to manage allergic conditions (e.g. cow's milk allergy or atopic dermatitis). The purpose of giving probiotics to healthy infants may be to relieve gastrointestinal symptoms and reduce risk of infectious diseases and antibiotics use (Indrio et al., 2022). Differences in the gut microbiome of breast-fed and formula fed infants, has motivated food manufacturers to add probiotics to infant formula, to compensate for the bacteria transmitted by breast feeding. The development of the infant gut microbiome, as well as the effects of breast-feeding vs. formula feeding, are described in more detail below.

It has been hypothesized that in infants, probiotic microorganisms could become primary colonizers that remain long-term, perhaps even for life (FAO/WHO, 2001), with largely unknown health consequences. Disruptions in the development of the gut microbiome have been linked to health issues later in life, such as asthma and allergies, irritable bowel disease, overweight/obesity, and diabetes (Tamburini et al., 2016) although a role of probiotic infant formula in this process has not been established.

4 Development of the infant gut microbiome during the first year of life

Prior to birth the foetal gastrointestinal (GI) tract is, by most, thought to be a sterile environment, with microbial colonization commencing during the birthing process, and first exposure strongly dependent upon the mode of delivery (vaginal or C-section). The early infant gut is not a fully anaerobic environment, and successful colonizers during the first weeks of life usually include facultative anaerobic bacteria like *Staphylococcus*, *Streptococcus*, *Klebsiella* and *Escherichia coli*. These early groups are often transient colonizers that are not necessarily adapted to the more developed gut environment, and they usually decline in abundance during the first year of life. However, they have an important function in using up available oxygen in the early gut, and thus they contribute to the rapid establishment of the anaerobic environment favoured by more gut-adapted taxa. An important group of early colonizers, largely perceived as symbionts, is the anaerobic genus *Bifidobacterium* (phylum Actinomycetota), represented by species like longum, bifidum, breve and infantis. While *Bifidobacterium* spp. are commonly found as part of the adult gut microbiome, they are particularly prevalent in breast-feeding infants, where they are able to break down human milk oligosaccharides (HMOs) that are not metabolizable by the host and produce short chain fatty acids (SCFAs) that are beneficial to the development of the maturing gut. After weaning, abundances of Bifidobacterium spp. normally decline. Although the gut microbiome is generally not considered to reach an adult-like composition before around the age of three years, approaching the first birthday the microbiome is normally dominated by bacterial taxa highly adapted to the gut, like the genera Bacteroides and Prevotella (phylum Bacteroidota) and the diverse families *Oscillospiraceae* and *Lachnospiraceae* (phylum *Bacillota*). These taxa are capable of metabolizing a wide range of energy substrates, and often play important roles in host physiology and health. The transition to a more mature microbiome composition is associated with a general increase in microbiome taxonomic diversity.

4.1 Effects of breast-feeding vs. formula feeding on the infant gut microbiome

A large prospective study published by Stewart at al. (2018) based on the TEDDY study found that breast-feeding was the single most important factor in explaining variations in microbiome composition in infants aged 3-14 months. E.g. human milk was found to be a much more potent determinant of microbiome development than mode of delivery, and receipt of breast milk was predominantly linked with elevated abundances of Bifidobacterium spp. A study of Swedish infants (Bäckhed et al., 2015) found that the microbiomes of babies that received both formula and human milk tended to reach a mature-like constellation earlier than in those fed exclusively human milk.

A few studies have looked explicitly at the effects of human milk vs. formula on the microbiome of infants born to term. A 2011 study (Bezirtzoglou et al., 2011) found that *Bifidobacterium* spp. were enriched in breast fed relative to formula fed infants, while the formula fed group was enriched for *Bacteroides* spp. (Praveen et al., 2015) found that *B. breve* and bifidum were reduced in formula fed relative to breast fed infants, while *B. longum* and *Ruminococcus gnavus* showed a relative increase in formula fed infants. The authors also reported increased microbiome diversity in formula fed infants, possibly reflecting a relatively early shift toward a more mature microbial community. A 2020 study compared three groups of infants, one exclusively breast-fed and two fed exclusively on one of two kinds of formula, over the first four months of life (Ma et al., 2020). This study did not find major effects on microbiome diversity, nor did it find consistent effects on taxon composition, although

abundance of *Bifidobacterium* spp. was reduced in one of the formula-fed groups relative to breast-fed infants.

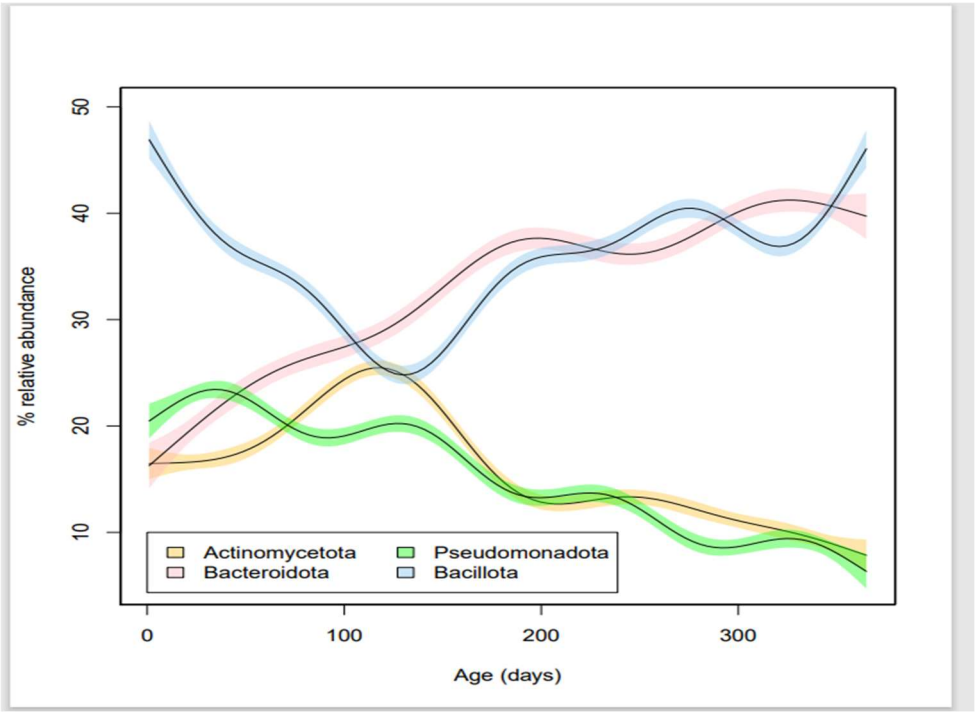

Figure 1: Relative abundance of the four main bacterial phyla of the infant gut microbiome during the first year of life. The black lines are smooth terms from generalized additive models, based on 2684 faecal samples collected from 12 healthy born-to-term infants from birth until the first birthday. The shaded bands indicate the phylum and represent 95% confidence limits of the fitted models. Data are from a study by de Muinck and Trosvik 2018 (Nature Communication; Individuality and convergence of the infant gut microbiota during the first year of life). Phylum designations in the legend box follow the 2021 revision: Actinomycetota (formerly Actionbacteria), Bacteroidota (Bacteroidetes), Pseudomonadota (Proteobacteria), Bacillota (Firmicutes).

5 Bifidobacterium breve (B. breve) M-16V

The species *B. breve* is a non-sporulating, anaerobic, non-motile, Gram-positive Y-shaped bacterium which lives symbiotically in the intestines of humans and is found in breast milk.

B. breve M-16V is a commercial strain of bacteria owned and distributed by the Japanese company Morinaga Milk Industry Co., Ltd. Therefore, the origins and manufacturing process for *B. breve* M-16V will not vary by brand of infant formula. *B. breve* M-16V is often added to infant formula with a prebiotic mixture, e.g. short-chain (sc) galacto-oligosaccharides (GOS) and long-chain (lc) fructo-oligosaccharides (FOS), or scFOS with lcFOS to achieve a synbiotic effect. *B. breve* M-16V has been available in Japan since 1976 (https://morinagamilk-ingredients.com/probiotics/m16v/).

The strain was originally deposited with the Belgian Co-ordinated Collections of Microorganisms (BCCM: https://bccm.belspo.be/) with LMG accession number 23729 but has been moved to the NITE Patent Microorganisms Depositary (NPMD) at the National Institute of Technology and Evaluation in Japan (https://www.nite.go.jp/en/nbrc/patent/npmd/index.html) with accession number NITE BP-02622.

6 General risk assessment – (ToR 1)

In accordance with EFSA's guidance on protocol development for generic scientific assessments (EFSA, 2022), the general risk assessment of *B. Breve* M-16V has been translated into assessment questions and sub-questions.

Hazard identification is the process to determine whether exposure to *B. Breve* M-16V has the potential to harm human health, short term, or long term. Hazard characterization is the evaluation of the nature of the adverse health effect(s), such as a dose-response relationship, sub-populations at increased risk, and effect modifiers. Exposure assessment is the evaluation of likely intake of the probiotic bacteria and other substances of interest (e.g. prebiotics). Risk characterization will be performed based on the outcome of the hazard characterization and exposure assessment to give an estimate (qualitative or quantitative) of the risk and severity of adverse health effects. If possible, a quantitative statement will be made about the comparison of estimated exposure to a reference value or hazard characterization vale. Figure 2 illustrates the structure of the risk assessment.

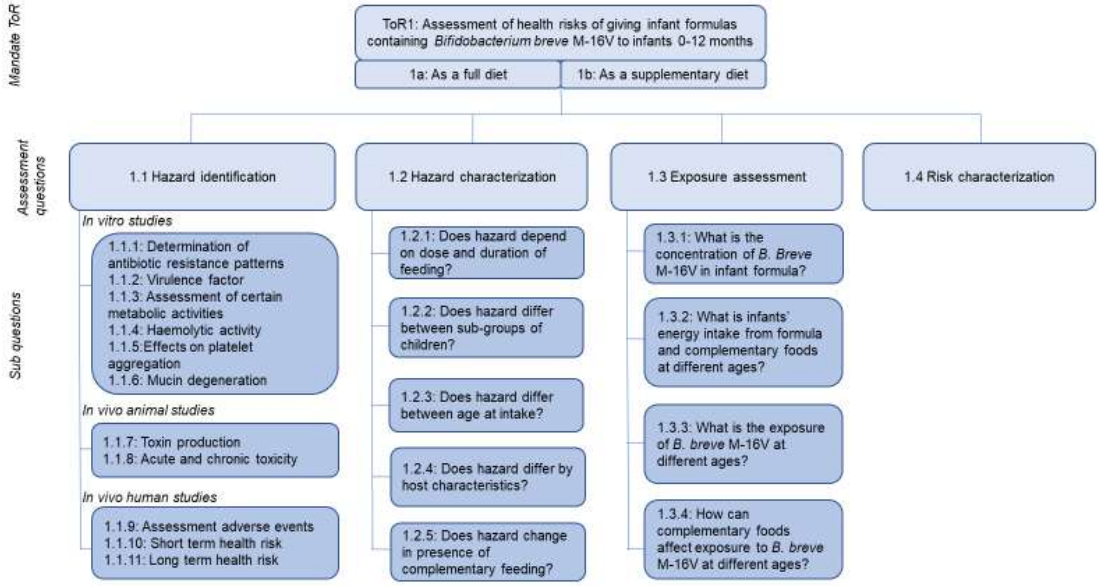

Figure 2: The current risk assessment based on EFSA´s guidance on protocol development for generic scientific assessments (EFSA 2020).

6.1 Hazard identification

According to the FAO/WHO guideline for the evaluation of probiotics in foods (FAO/WHO 2001, level 1.1 in Figure 2), no adverse effects related to probiotic administration should be experienced when food is considered. Further, bacteria which contain transmissible drug resistance genes, should not be used in foods. Evaluations of the safety of probiotics should include studies in humans and be based on intended use of products.

The following safety characteristics of *B. Breve* M-16V will be assessed:

- Based on in vitro studies:
    - Antimicrobial resistance patterns and whether resistance genes are located on, or associated with, mobile genetic elements (transposons, integrons or plasmids located genes).

  - Virulence factors
  - Certain metabolic activities (production of D-lactate, biogenic amines, ammonia production, bile salt deconjugation, azoreductase or nitroreductase activity)
  - Haemolytic activity
  - Effects on platelet aggregation or viability
  - Adherence to mucus and/or human epithelial cells and cell lines

- Based on in vivo safety in animal studies
  - Toxin production, when the strain under evaluation belongs to a species that is known to produce toxins of relevance to mammals
  - Acute, subacute, and chronic animal toxicity after oral administration of the probiotic
  -
- Based on human studies
  - Assessment of adverse events
  - Short term health risks
  - Long-term health risks

VKM defines short-term health risks as those occurring during intake, and long-term health risks as occurring after cessation of intake.

Based on the scoping search for literature (Indrio et al., 2022; Upadhyay et al., 2020; Athalye-Jape et al., 2018) some expected study outcomes are

  - probiotic sepsis (considered an adverse event)
  - other infections
  - gastrointestinal symptoms (e.g. vomiting, diarrhoea, cholic, flatulence)
  - infant growth/anthropometrics
  - neurodevelopment
  - medication use (e.g. antibiotics)
  - duration of hospitalizations
  - non-clinical outcomes such as dysbiosis/changes in intestinal or faecal microbiome, and changes in immune- and inflammation parameters

Of note, the question of long-term health risk will be challenging to address as there seems to be few follow-up studies of children who have consumed infant formula containing *B. breve* M-16V. However, such studies will be searched for by VKM (se Section on Methods and Literature search).

6.2 Hazard characterization

If health risks (hazard) of intake of *B. breve* M-16V are identified under level 1.1, they will need to be characterized in more detail (level 1.2 in Figure 2).

The following sub-questions will be addressed:

- Does hazard depend on dose and duration of feeding with *B. breve* M16-V?
- Does the hazard differ for sub-groups of infants, such as infants born pre-term, and children with allergies?
- Does the hazard differ by age at intake (0-12 months)?
- Does the hazard differ by host characteristics or antibiotic use?
- Does the hazard change in the presence of prebiotics or complementary foods?

6.3 Exposure assessment

To evaluate potential exposure to *B. breve* M-16V though infant formula in Norwegian infants, the following questions will be addressed (level 1.3 in Figure 2):

- What is the concentration range of *B. breve* M-16V (cfu/100 ml) in ready to eat infant formula?
- How much of infants' energy intake is provided by infant formula and complementary foods at different ages (0-12 months)?
- What is the exposure of *B. breve* M-16V at different ages (0-12 months) given estimates of concentration and formula intake?
- When infant formula is used as supplementary diet, how can complementary foods affect exposure to *B. breve* M-16V?

6.4 Risk characterization

No sub-questions have been specified for the risk characterization (level 1.4 in Figure 2).

7 Product specific risk assessment – (ToR 2)

If no clear health risks are identified in the general risk assessment (ToR 1), VKM will continue with the product specific risk assessment.

The assessment will be based on the general risk assessment, considering additional documentation on the product composition (food matrix, concentration of *B. breve* M-16V), and intended use as a food for medical product for infants with clinical allergy, for specific age intervals.

The applicant has provided documentation on the product information and product safety according to VKM's Guidelines for assessment of safety aspects of probiotic (food) products (VKM, 2014).

7.1 Product description includes:

- Recommended storage condition (time, temperature, exposure to air and relative humidity)
- Number of viable probiotic bacteria per gram of product/per serving
- If mixed with water or milk: number of bacteria per gram of the ready-to-eat product or per serving
- Daily intake/number of recommended doses daily
- Food matrix (according to ordinary regulations for food)
- Shelf life (if affected by opening the packaging, give the shelf life after opening)

7.2 Product safety includes:

- Control systems for ensuring the integrity, purity and stability of the culture
- Method for determining the number of viable cells (agar used, incubation parameters, etc)
- Control procedures implemented to determine viability during storage –
- Antagonistic/synergistic effects (if any).
- Should the product or should the product not be consumed with other foodstuffs or therapeutic agents?
- Are there any known deleterious health effects if consumed with other foods or medical products?

8 Method specification and information sources

This part of the protocol will give a description of how the four steps of risk assessment will be answered for ToR 1 and ToR 2: (i) hazard identification, (ii) hazard characterization, (iii) exposure assessment, and (iv) risk characterization (Figure 2).

8.1 Hazard identification and characterization-methods

To answer assessments questions of levels 1.1 and 1.2 in the risk assessment (Figure 2), VKM will rely on the following sources of information:

a) Documentation in GRAS notices for *B. breve* M-16V provided by the NFSA
b) Documentation from the applicant
c) The most recent QPS status of *B. breve* M-16V for microorganisms intentionally added to food or feed as notified to EFSA (Koutsoumanis et al., 2023)
d) Risk Group (RG) classification in the WHO system
e) Systematic literature review performed by VKM

For *B. breve* M-16V, there is established documented use in infants over time. For basic safety characteristics of *B. breve* M-16V (listed in Section on Hazard identification) assessed in vitro or in vivo in animals, VKM will rely on sources a) to d).

Documentation from the business operator (information source b) will be evaluated by the project group according to the VKM's guidelines for assessment of safety aspects of probiotic (food) products (VKM 2014). If the documentation is found to be incomplete or unclear, VKM may prepare a list of questions to be sent back to the applicant through the NFSA.

To assess health risk based on human studies, VKM will do a systematic literature search as described below, complemented with citation searching for human studies in sources a) and b) for completeness.

8.2 Literature review – human health effects

The protocol for the current literature review has been prepared using elements from the PRISMA-P (Preferred Reporting Items for Systematic review and Meta-Analysis Protocols) 2015 checklist: recommended items to address in a systematic review protocol (Moher et al., 2015).

8.2.1 Rationale and objective for review

VKM will review the scientific literature for evidence of health risks in relation to the use of the probiotic strain *B. breve* M-16V in infant formula or in enteral feeding, independently of the literature provided by the applicant, the manufacturer of the products being evaluated, and the patent holder of B. breve M-16V.

The scoping search for literature by VKM identified three previous strain-specific reviews of B. breve M-16V, of which two were termed systematic literature reviews (SLRs). The most recent SLR includes studies of children with cow's milk protein allergy (CMPA) and is affiliated with the manufacturer of the products being assessed (Sorensen et al., 2021). The second SLR summarizes studies of premature and LBW infants given B. breve M-16V through enteral feeding (Athalye-Jape et al., 2018). The third review focuses on potential beneficial effects B. breve M-16V on premature birth complications and immune-mediated disorders in infants and is affiliated with the patent holder Morinaga Milk Industry (Wong et al., 2019). A fourth SLR was found that includes a wide range of probiotics and symbiotics in infant formula, and one primary study of B. breve M-16V. Thus, this SLR does not qualify as a review of B. breve M-16V (Indrio et al., 2022). VKM finds that a new systematic literature search and evidence synthesis is justified. The objective is to identify and characterize potential adverse effects and health risks of oral intake of *B. breve* M-16V during infancy.

8.2.2 Eligibility criteria

The systematic literature search will include both review studies and primary studies of oral intake of *B. breve* M-16V as monoculture that also address safety or health risk. The eligibility criteria for human studies to be included in the general risk assessment are included in Table 1.

Table 1 Overview of eligibility criteria for human studies in general risk assessment

Criterion	Inclusion	Exclusion
Study design	Case reports of adverse events, randomized controlled trials (RCTs) and observational studies	Cross-sectional studies
Population	Infants age 0-12 months, including vulnerable groups (e.g. premature, or low birth weight infants)	Infants treated medically with immunosuppression or with chemotherapy, or treated surgically for intestinal/digestive conditions
Intervention or exposure	Intake (oral or enteral) of *B. Breve* M-16V as monoculture, alone or in synbiotic mixtures, in infant formula or as supplement or medical use	Probiotic mixtures with other strains, prebiotics only, postbiotics
Comparator	Infant feeding (oral or enteral) without probiotics, may contain prebiotics	
Outcome	Outcomes measured or reported in relation to safety or health risks, short term, and long term (see text below)	Outcomes with unclear health risk (changes in faecal microflora/microbiome composition, stool characteristics, markers of immunomodulation and inflammation, C-reactive protein), bacteremia without sepsis
Language	No restriction if few studies need translation from languages other than English or Scandinavian.	Studies that cannot be machine translated, if translation is required
Date	No limitation (inception to search date)	
Type of literature	Previous review studies, systematic or non-systematic, and primary studies in peer-reviewed journals	

Study design: Case reports of adverse events, randomized controlled trials (RCTs), and observational studies with control for potential confounders, are considered suitable to address health risks and will be included. Cross-sectional designs will be excluded.

Population: Probiotic products are often contraindicated in immunosuppressed individuals but are widely administered for complications in infants born prematurely and/or with a low birth weight (LBW) who also have an immature or weakened immune system (van den Akker et al., 2020). Excluding these infants will probably exclude a large part of the literature that may inform on safety. Therefore, studies in premature and/or LBW infants and other subgroups will be included in the general risk assessment although the products to be evaluated are contraindicated in the premature. The generalizability from vulnerable groups to other infants needs to be discussed (see Section on “Assumptions and Generalizability)”. Infants with allergic conditions will be included, as the products to be evaluated are hypoallergenic formula. Infants with medically induced immunosuppression, and infants undergoing intestinal surgery will be excluded.

Intervention or exposure: the main intervention/exposure of interest is infant feeding with formula containing *B. breve* M-16V as monoculture. Symbiotic blends with *B. breve* M-16V will be included, but not mixtures of multiple probiotic strains. Formula feeding does not have to be exclusive. In premature/LBW infants, *B. breve* M-16V administered as a supplement, orally or enterally, in formula, expressed breast milk or human donor milk is considered relevant. When evaluating the safety of symbiotic blends in general, the separate effects of prebiotics and *B. breve* M-16V are also considered important.

Comparator: The comparator should be the same formula, but without *B. breve* M-16V, or in the case of premature infants, similar feeding but without probiotics.

Outcomes: Health risk is a wide term. A hybrid approach to reviewing health risk will be used (Peryer et al., 2023). This approach allows expected outcomes (see Section on Hazard identification) as well as outcomes that cannot be pre-specified. This is to identify any unanticipated and rare adverse effects. Outcomes considered to pose an unclear health risk, will be excluded from summaries. Examples are changes in faecal microbiota composition, stool characteristics, changes in markers of immunomodulation and inflammation, and bacteraemia without sepsis.

The librarian performed a test search in MEDLINE (Appendix 1) at the protocol stage. In line with the described approach to reviewing health risk, no search terms or filters will be used for health outcomes, or the comparator. It was decided to expand the search to all bifidobacteria to capture studies of *B. breve* M-16V that do not mention this specific strain in the title or abstract, based on some known examples from the scoping search and product application. The final search strategy will be developed for multiple databases by the research librarian.

8.2.3 **Study selection**

Member of the project group will independently, in pairs of two, screen the identified records in two steps, first by 1) titles and abstracts, and 2) as full text articles, against eligibility criteria. Disagreements will be resolved by consensus or by consulting a third author. The first screening will be performed using Rayyan software for blinding.

All studies of infant feeding containing probiotic bifidobacteria will be evaluated in full text if this is necessary to determine if *B. breve* M-16V has been investigated.

8.2.4 **Internal validity of studies/risk of bias**

The internal validity of included review- or primary studies will be formally assessed using established risk of bias (RoB) instruments, if time and resources are sufficient for the number of studies identified. If the number of studies is found to be too high, studies of intake of infant formula with *B. breve* M-16V will be prioritized.

Authors will independently, in pairs of two, perform the risk of bias evaluation. Disagreements will be resolved by consensus or by consulting a third author.

Of note, it is expected that part of the scientific evidence will be funded by the patent holder of *B. breve* M-16V or the manufacturer of the products being evaluated, and that study authors may have industry affiliations. VKM will not exclude these studies, but quality assess all studies by the same criteria. Studies with unspecified safety outcomes and/or only authors' reports of no adverse events without supporting result presentations, will be judged to be of low quality/high risk of bias.

8.2.5 **Data extraction and synthesis of review studies**

Data will be extracted from review studies that systematically summarizes one or more safety outcomes in relation to infant intake of *B. breve* M-16V. Review studies that are not systematic or have a different study objective than the VKM literature search (e.g. broader with regard to probiotic strains) will only be screened for eligible primary studies.

The planned data extraction for systematic review studies includes bibliographic data, study objective, inclusion/exclusion criteria, infant population characteristics, feeding regimen (product/manufacturer, formulation, concentration, duration) for *B. Breve* M-16 V including control/comparator group, safety or health risk summarized, if safety or health risk assessment was stated as a primary objective or not, the total number of studies, total number of cases, summary estimate and measures of between study-heterogeneity if provided (e.g. I^2 and/or P-value for test of heterogeneity), results from any meta-dose response analyses, overall author conclusion per outcome, including publication bias, tool used for grading the quality of primary studies, and the overall grading of the meta-evidence if reported.

8.3 **Data extraction and synthesis of primary studies**

The planned data extraction for primary studies includes bibliographic data, study objective (and if safety or health risk was a primary outcome), inclusion/exclusion criteria, study design, infant population characteristics, setting, (e.g. hospital/intensive care, or outpatient), indication for probiotic treatment, feeding regimen (product/manufacturer, formulation, concentration, duration), study sample (numbers randomized to intervention or control group, and numbers analysed), compliance, feeding regimen (dose/amount given and duration), comparator/control group, any co-treatments (e.g. use of antibiotics), type of outcome/safety assessment (e.g. self-reported tolerance, clinical examination, blood tests) and time points, reported results, if results were stated or supported by a presentation of results, conflict of interest (COI) statement, including corporate study funding or authorship, and trial registration number. Results will be summarized by indication for probiotic use (e.g. NEC, allergic conditions).

How to address confidence in the cumulative evidence and overall grading of the evidence is not specified in the current protocol.

9 Exposure assessment – methods (1.3)

Daily and total exposure to M-16V in infants will depend on the intake of infant formula and complementary foods at different ages and bodyweight.

Scenarios for intake of infant formula and complementary foods in Norwegian infants will be estimated using National recommendations for infant nutrition (National Directorate of Health, 2016) and the WHO child growth standards (https://www.who.int/tools/child-growth-standards/standards/weight-for-age).

National recommendations provide information on breast feeding, formula feeding and the introduction of complementary foods. Complementary food is recommended from six months, and not before four months of age. The National recommendations also provide information on mean recommended daily intake of energy and nutrients to infants 0-12 months (HDIR, 2016).

The national survey on infant nutrition, Spedkost 3, provides information on the diet of infants from birth to 12 months in Norway, age of introduction of complementary foods, and type of foods given. The most common introduction time point for complementary food is four months of age. Cereal porridge, mashed potato, vegetables, and fruits are the most commonly complementary foods eaten (Myhre et al., 2020).

Concentrations of *B. Breve* M-16V in infant formula provided by the applicant will be used to calculate exposure (Documentation check list). Based on energy requirements in infants and the concentrations of *B. breve* M16-V (CFUs) and prebiotics in infant formula, the number of bacteria per volume of the ready-to-eat product or per serving can be calculated.

Exposure to *B. breve* M-16V will be estimated for different mean energy intakes for the 3rd and 97th percentiles of weight for age at different ages for both genders, and for different proportions of energy intake from formula and complementary foods.

Example:

A 3-month girl infant with bodyweight of 5 kg (97th percentile of weight for age), a recommended energy intake of 98 kcal/kg/day and a concentration of 66 kcal/100 ml of energy and 14,4 x 10^7-10^8 cfu/100 ml of *B. breve* M16-V in a ready-to-eat formula (Pepti Syneo/Neocate Syneo) gives:

Recommended energy per day: 5 kg × 98 kcal = 490 kcal

Total volume formula needed per day: 490 kcal/66 kcal = 740 mL

Total exposure of *B. breve* M16-V per day: (14.4 × 10^7– 10^8 cfu/100 mL) x 7.4

10 Risk characterization – methods (1.4)

To characterize risk in the general assessment, the anticipated exposure to *B. Breve* M-16V from infant formula in Norwegian infants will be compared to the range of exposure in the identified literature on hazard identification.

10.1 Assumptions and generalizability

10.1.1 Strain specificity

In this assessment, it is assumed that the health effects of bacteria are strain-specific, meaning that evidence of any health risks identified for *B. Breve* M-16V cannot be generalized to another strain, or vice versa, without careful consideration of any shared properties, e.g. genomic similarities (Rodriguez and Martiny, 2020) that may affect the ability to adapt to the intestinal environment, and shared mechanisms of action.

10.1.2 Generalizability from evidence in premature infants

Premature infants are immune compromised and have high rates of mortality, septicaemia, and gastrointestinal morbidities, such as necrotizing enterocolitis. They often have low birth weight and undergo complex medical treatments that require close supervision in neonatal intensive care unit settings. Treatments may involve parenteral nutrition and use of antibiotics. Probiotics may be administered as drops or combined with human milk or formula designed for premature infants. If *B. Breve* M-16V is found to be safe in premature infants, it can be reasonably assumed that *B. Breve* M-16V may also be safe in other infants, including those with cow's milk allergy. However, if the literature reveals adverse effects of *B. Breve* M-16V in premature infants, those findings will be considered, but may not be generalized to other groups of infants who are not immune compromised.

10.2 Surrogates for long-term health risks

In case of too limited evidence from human studies to draw conclusions regarding long-term health risks of consuming infant formula with B. Breve M-16V, the VKM project group has considered evaluating the potential for such health risks using other evidence as surrogates. The potential to cause long-term health effects may depend on the potential of *B. breve* M-16V to colonize the gut mucosa, the requirement for and availability of nutrients, and the ability to interact with the host and the indigenous microbiota or microbiome. However, the scoping search for literature indicates long-standing debates and uncertainties related to the proposed mechanisms of action of probiotic bacteria (see Section on “Potential mechanisms”, and Suez et al., 2019), such as the capacity of different probiotic bacteria to colonize the host gastrointestinal mucosal surface (transiently or permanently), and whether microbiome modulation and /or immune imprinting could have unfavourable effects, such as increased risk of immune-mediated diseases later in life. Therefore, the project group has decided to not review mechanistic evidence and may not be able to conclude on the long-term health risk in the current assessment.

11 Uncertainties

Based on the scoping search and clinical documentation provided by the applicant, uncertainty stemming from limited evidence is expected in the following areas and assessment questions:

- Long-term health risks of *B. breve* M-16V from intake of infant formula
- How the activity of *B. breve* M-16V may change in the presence of complementary foods acting as prebiotics (e.g. dietary fibres) or other probiotic bacteria from foods such as the Biola yoghurt drink on the Norwegian market

Appendix 1: test literature search

The research question the literature search is intended to answer			
Are there any health risks of giving infant formula containing Bifidobacterium breve M-16V to infants 0-12 months?			
The research question according to the PICO formula			
Population	**Intervention**	**Comparison**	**Outcome**
Infants (age 0-12 months) at the time of intervention/exposure	Infant formula containing the probiotic bacteria Bifidobacterium breve M-16V	Infant formula without probiotic bacteria	Health risks

The search strategy is presented below for Ovid MEDLINE(R) from inception to 09.08.2023

Database: Ovid MEDLINE(R) ALL <1946 to August 08, 2023>
Dato: 09.08.2023
Antall treff: 90

#	Searches	Results	Commentary
1	exp Infant/ or exp Pediatrics/ or Child Development/	1325103	
2	(pediatric* or paediatric* or perinatology or neonatology or infant? or suckling? or newborn? or "new born" or neonat* or "neo nat*" or toddler*).tw,kf.	1211698	
3	(((age or aged) adj ("1" or one or "3 month*" or "three month*" or "4 month*" or "four month*" or "5 month*" or "five month*" or "6 month*" or "six month*" or "7 month*" or "seven month*" or "8 month*" or "eight month*" or "9 month*" or "nine month*" or "10 month*" or "ten month*" or "11 month*" or "eleven month*" or "12 month*" or "twelve month*")) or (("1" or one) adj ("year* old" or "yr* old")) or (("3 month*" or "three month*" or "4 month*" or "four month*" or "5 month*" or "five month*" or "6 month*" or "six month*" or "7 month*" or "seven month*" or "8 month*" or "eight month*" or "9 month*" or "nine month*" or "10 month*" or "ten month*" or "11 month*" or "eleven month*" or "12 month*" or "twelve month*") adj old)).tw,kf.	90207	
4	or/1-3	1941241	

5	Infant Formula/ or Probiotics/ or Prebiotics/ or Synbiotics/	32290	
6	((infant or baby or feeding) adj3 (formula or formulas or formulae?)).tw,kf.	9751	
7	((formula or formulas or formulae? or artificial or synthetic) adj3 milk).tw,kf.	5520	
8	((amino acid or aminoacid) adj3 (formula or formulas or formulae?)).tw,kf.	470	
9	(probiotic? or prebiotic? or synbiotic? or "AAF-S").tw,kf.	47746	
10	or/5-9	65707	
11	Bifidobacterium breve/	179	
12	(breve adj3 ("M 16V" or M16V)).tw,kf.	71	
13	11 or 12	224	
14	4 and 10 and 13	90	

References

Athalye-Jape G., Rao S., Simmer K., Patole S. (2018) Bifidobacterium breve M-16V as a Probiotic for Preterm Infants: A Strain-Specific Systematic Review. JPEN J Parenter Enteral Nutr 42:677-688. DOI: 10.1177/0148607117722749.

Backhed F., Roswall J., Peng Y., Feng Q., Jia H., Kovatcheva-Datchary P., Li Y., Xia Y., Xie H., Zhong H., Khan M.T., Zhang J., Li J., Xiao L., Al-Aama J., Zhang D., Lee Y.S., Kotowska D., Colding C., Tremaroli V., Yin Y., Bergman S., Xu X., Madsen L., Kristiansen K., Dahlgren J., Wang J. (2015) Dynamics and Stabilization of the Human Gut Microbiome during the First Year of Life. Cell Host Microbe 17:690-703. DOI: 10.1016/j.chom.2015.04.004.

Bafeta A., Koh M., Riveros C., Ravaud P. (2018) Harms Reporting in Randomized Controlled Trials of Interventions Aimed at Modifying Microbiota: A Systematic Review. Ann Intern Med 169:240-247. DOI: 10.7326/M18-0343.

Bezirtzoglou E., Tsiotsias A., Welling G.W. (2011) Microbiota profile in feces of breast- and formula-fed newborns by using fluorescence in situ hybridization (FISH). Anaerobe 17:478-82. DOI: 10.1016/j.anaerobe.2011.03.009.

Bloem M. (2007) The 2006 WHO child growth standards: , British Medical Journal Publishing Group. pp. 705-706.

Dani A. (2014) Colonization and infection. Cent European J Urol 67:86-7. DOI: 10.5173/ceju.2014.01.art19.

de Muinck E.J., Trosvik P. (2018) Individuality and convergence of the infant gut microbiota during the first year of life. Nat Commun 9:2233. DOI: 10.1038/s41467-018-04641-7.

EFSA, Martino L., Aiassa E., Halldórsson T.I., Koutsoumanis P.K., Naegeli H., Baert K., Baldinelli F., Devos Y., Lodi F., Lostia A., Manini P., Merten C., Messens W., Rizzi V., Tarazona J., Titz A., Vos S. (2020) EFSA Supporting Publications - 2020 - - Draft framework for protocol development for EFSA s scientific assessments 2020:EN-1843. DOI: 10.2903/sp.efsa.2020.EN-1843.

FAO/WHO. (2001) Report of a Joint FAO/WHO Expert Consultation on Evaluation of Health and Nutritional Properties of Probiotics in Food Including Powder Milk with Live Lactic Acid Bacteria. In: Probiotics in food. Health and nutritional properties and guidelines for evaluation. FAO Food and Nutrition Paper 85. .

HDIR. (2016) National guideline on infant nutrition: "Nasjonal faglig retningslinje for spedbarnsernæring" Oslo: Norwegian Directorate of Health (last updated 8. July 2021, accessed May 31. 2023). Available at: https://www.helsedirektoratet.no/retningslinjer/spedbarnsernaering.

Hill C., Guarner F., Reid G., Gibson G.R., Merenstein D.J., Pot B., Morelli L., Canani R.B., Flint H.J., Salminen S., Calder P.C., Sanders M.E. (2014) Expert consensus document. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nat Rev Gastroenterol Hepatol 11:506-14. DOI: 10.1038/nrgastro.2014.66.

Indrio F., Gutierrez Castrellon P., Vandenplas Y., Cagri Dinleyici E., Francavilla R., Mantovani M.P., Grillo A., Beghetti I., Corvaglia L., Aceti A. (2022) Health Effects of Infant Formula Supplemented with Probiotics or Synbiotics in Infants and Toddlers: Systematic Review with Network Meta-Analysis. Nutrients 14. DOI: 10.3390/nu14235175.

Koutsoumanis K., Allende A., Álvarez-Ordóñez A., Bolton D., Bover-Cid S., Chemaly M., de Cesare A., Hilbert F., Lindqvist R., Nauta M., Peixe L., Ru G., Simmons M., Skandamis P., Suffredini E., Cocconcelli P.S., Fernández Escámez P.S., Maradona M.P., Querol A., Sijtsma L., Suarez J.E., Sundh I., Vlak J., Barizzone F., Hempen M., Correia S., Herman L. (2023) EFSA Panel on Biological Hazards (BIOHAZ). Update of the list of qualified presumption of safety (QPS) recommended microorganisms intentionally added to food or feed as notified to EFSA. EFSA Journal 21. DOI: 10.2903/j.efsa.2023.7747.

Levy M., Kolodziejczyk A.A., Thaiss C.A., Elinav E. (2017) Dysbiosis and the immune system. Nat Rev Immunol 17:219-232. DOI: 10.1038/nri.2017.7.

Ma J., Li Z., Zhang W., Zhang C., Zhang Y., Mei H., Zhuo N., Wang H., Wang L., Wu D. (2020) Comparison of gut microbiota in exclusively breast-fed and formula-fed babies: a study of 91 term infants. Sci Rep 10:15792. DOI: 10.1038/s41598-020-72635-x.

Milani C., Duranti S., Bottacini F., Casey E., Turroni F., Mahony J., Belzer C., Delgado Palacio S., Arboleya Montes S., Mancabelli L., Lugli G.A., Rodriguez J.M., Bode L., de Vos W., Gueimonde M., Margolles A., van Sinderen D., Ventura M. (2017) The First Microbial Colonizers of the Human Gut: Composition, Activities, and Health Implications of the Infant Gut Microbiota. Microbiol Mol Biol Rev 81. DOI: 10.1128/MMBR.00036-17.

Moher D., Shamseer L., Clarke M., Ghersi D., Liberati A., Petticrew M., Shekelle P., Stewart L.A., Group P.-P. (2015) Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. Syst Rev 4:1. DOI: 10.1186/2046-4053-4-1.

Myhre, J. B., Andersen, L. F., & Kristiansen, A. L. (2020). *SPEDKOST 3. Landsomfattende undersøkelse av kostholdet blant spedbarn i Norge, 6 måneder [Norwegian]*. https://www.fhi.no/publ/2020/spedkost-3/

Peryer G., Golder S., Junqueira D., Vohra S., Loke Y.K. (2023) Chapter 19: Adverse effects, In: Higgins JPT, Thomas J, Chandler J, Cumpston M, Li T, Page MJ, Welch VA (editors). Cochrane Handbook for Systematic Reviews of Interventions version 6.4 (updated August 2023). www.training.cochrane.org/handbook.

Praveen P., Jordan F., Priami C., Morine M.J. (2015) The role of breast-feeding in infant immune system: a systems perspective on the intestinal microbiome. Microbiome 3:41. DOI: 10.1186/s40168-015-0104-7.

Rodriguez C.I., Martiny J.B.H. (2020) Evolutionary relationships among bifidobacteria and their hosts and environments. BMC Genomics 21:26. DOI: 10.1186/s12864-019-6435-1.

Sanders M.E., Benson A., Lebeer S., Merenstein D.J., Klaenhammer T.R. (2018) Shared mechanisms among probiotic taxa: implications for general probiotic claims. Curr Opin Biotechnol 49:207-216. DOI: 10.1016/j.copbio.2017.09.007.

Sorensen K., Cawood A.L., Gibson G.R., Cooke L.H., Stratton R.J. (2021) Amino Acid Formula Containing Synbiotics in Infants with Cow's Milk Protein Allergy: A Systematic Review and Meta-Analysis. Nutrients 13. DOI: 10.3390/nu13030935.

Stewart C.J., Ajami N.J., O'Brien J.L., Hutchinson D.S., Smith D.P., Wong M.C., Ross M.C., Lloyd R.E., Doddapaneni H., Metcalf G.A., Muzny D., Gibbs R.A., Vatanen T., Huttenhower C., Xavier R.J., Rewers M., Hagopian W., Toppari J., Ziegler A.G., She J.X., Akolkar B., Lernmark A., Hyoty H., Vehik K., Krischer J.P., Petrosino J.F. (2018) Temporal development of the gut microbiome in early childhood from the TEDDY study. Nature 562:583-588. DOI: 10.1038/s41586-018-0617-x.

Suez J., Zmora N., Segal E., Elinav E. (2019) The pros, cons, and many unknowns of probiotics. Nat Med 25:716-729. DOI: 10.1038/s41591-019-0439-x.

Tamboli C.P., Neut C., Desreumaux P., Colombel J.F. (2004) Dysbiosis in inflammatory bowel disease. Gut 53:1-4. DOI: 10.1136/gut.53.1.1.

Tamburini S., Shen N., Wu H.C., Clemente J.C. (2016) The microbiome in early life: implications for health outcomes. Nat Med 22:713-22. DOI: 10.1038/nm.4142.

Upadhyay R.P., Taneja S., Chowdhury R., Strand T.A., Bhandari N. (2020) Effect of prebiotic and probiotic supplementation on neurodevelopment in preterm very low birth weight

infants: findings from a meta-analysis. Pediatr Res 87:811-822. DOI: 10.1038/s41390-018-0211-9.

van den Akker C.H.P., van Goudoever J.B., Shamir R., Domellof M., Embleton N.D., Hojsak I., Lapillonne A., Mihatsch W.A., Berni Canani R., Bronsky J., Campoy C., Fewtrell M.S., Fidler Mis N., Guarino A., Hulst J.M., Indrio F., Kolacek S., Orel R., Vandenplas Y., Weizman Z., Szajewska H. (2020) Probiotics and Preterm Infants: A Position Paper by the European Society for Paediatric Gastroenterology Hepatology and Nutrition Committee on Nutrition and the European Society for Paediatric Gastroenterology Hepatology and Nutrition Working Group for Probiotics and Prebiotics. J Pediatr Gastroenterol Nutr 70:664-680. DOI: 10.1097/MPG.0000000000002655.

VKM. (2014) Guidelines for assessment of safety aspects of probiotic (food) products. Opinion of the Panel on Biological Hazards of the Norwegian Scientific Committee for Food Safety.

Wong C.B., Iwabuchi N., Xiao J.Z. (2019) Exploring the Science behind Bifidobacterium breve M-16V in Infant Health. Nutrients 11. DOI: 10.3390/nu11081724.