Vitenskapskomiteen for mattrygghet
Norwegian Scientific Committee for Food Safety

Assessment of benefits and risks of probiotics in processed cereal-based baby foods

Bifidobacterium lactis Bb12

Opinion of the Panel on Nutrition, Dietetic products, Novel food and Allergy and Panel on Biological Hazards of the Norwegian Scientific Committee for Food Safety

14.05.10

ISBN: 978-82-8082-360-1

VKM Report 2010: 17

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Acknowledgements

The Norwegian Scientific Committee for Food Safety (Vitenskapskomiteen for mattrygghet, VKM) has appointed an *ad hoc*-group consisting of both VKM members and external experts to answer the request from the Norwegian Food Safety Authority. The members of the *ad hoc*-group are acknowledged for their valuable work on this opinion.

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1. SUMMARY

1.1. Summary

The Norwegian Scientific Committee for Food Safety (VKM) has appointed an *ad hoc*-group of experts to answer a request from the Norwegian Food Safety Authority regarding benefit and risk assessment of *B. lactis* Bb12 in baby foods focusing on the age groups 4-6 months, 6-12 months and 1-3 years. This assessment is based on the literature provided by the notifier as well as that found by a MEDLINE search.

An notification for use of processed cereal-based baby foods (from now on called cereals) intended for infants and small children supplemented with the microorganism *Bifidobacterium lactis* (*B. lactis*) Bb12 in Norway initiated this work.

Studies of potential hazards and positive health effects from cereals containing *B. lactis* Bb12 intended for infants and young children have not been reported in the available literature. However, reports on safety of and positive health effects from infant and follow on formula supplemented with *B. lactis* Bb12 are available and have been assessed by VKM. In most of these clinical studies *B. lactis* Bb12 was administered in combination with other probiotic strains.

Clinical studies report no serious adverse events of infant formula supplemented with *B. lactis* Bb12. The effect of long term daily consumption of such supplemented formula by the actual age groups is not known.

A few studies have demonstrated some effect of supplementing baby food with probiotics, including *B. lactis* Bb12, on diarrhoea and atopic eczema while other studies do not show such effects. Thus, the scientific evidence for a favourable effect of supplementing formula or solid food with *B. lactis* Bb12, is weak and in some cases lacking.

There are no studies demonstrating a positive effect of cereals supplemented with *B. lactis* Bb12 intended for infants and small children.

Several health claims related to probiotics have been assessed by EFSA, including claims on reduction of gastro-intestinal discomfort, normal functioning of the alimentary tract, building of the natural intestinal barrier, improvement of the general immunity, mental and cognitive developments of children and immune system of children during growth. In the opinions so far, EFSA has concluded that a cause and effect relationship has not been established between the consumption of the probiotic containing products and the claimed effect. None of the products assessed so far contained *B. lactis* Bb12 (1 November 2009).

Commercially produced cereals are frequent given to infants and small children in Norway from an early age and this is particularly important for the establishment of the intestinal bacterial flora and the development of the intestinal mucosal immune system. According to the notifier, one portion (25gram) of the cereal powder contains 1 x 10^9 *B. lactis* Bb12 in monoculture. Taking into consideration that the daily intake is often greater than one portion of cereals, even in infants below 6 months of age, this would represent a daily intake of 1-2 x 10^9 cfu *B. lactis* Bb12 for an infant 4-6 months and even more in infants above 6 months. If a considerable amount of the *B. lactis* Bb12 survives the transport to the small intestine, it

would represent a dominating and monocultural supply, often several times a day, to the small intestine.
The immaturity and vulnerability of the intestinal microbiota and the immune system makes the two lowest age groups, 4 – 6 and 6 – 12 months, at the highest risk of unwanted health effects due to the daily intake of probiotics.

1.2. Norsk sammendrag

Vitenskapskomiteen for mattrygghet (VKM) har på oppdrag fra Mattilsynet utarbeidet en nytte- og risikovurdering av *B. lactis* Bb12 med fokus på aldersgruppene 4-6 måneder, 6-12 måneder og 1-3 år. For å besvare oppdraget nedsatte VKM en *ad hoc*-gruppe. Vurderingen er basert på gjennomgang av litteratur tilsendt fra søker og MEDLINE litteratursøk.

Bakgrunnen for oppdraget er en søknad om tilsetning av *B. lactis* Bb12 til barnemat i Norge.

Det er ikke funnet kliniske studier med *B. lactis* Bb12 tilsatt barnegrøt i den tilgjengelige litteraturen. Nytte- og risikovurderingen baserer seg derfor på studier der *B. lactis* Bb12 er tilsatt i morsmelkerstatning eller tilskuddsblandinger. Det skal imidlertid bemerkes at i de fleste av disse studiene var *B. lactis* Bb12 tilsatt i kombinasjon med andre probiotiske stammer.

Det er ikke rapportert om alvorlige negative helseeffekter fra morsmelkerstatning med *B. lactis* Bb12 i de kliniske studiene som har vært gjennomgått. Langtidseffekter av et daglig inntak av morsmelkerstatning tilsatt *B. lactis* Bb12 er imidlertid ikke studert for de aktuelle aldersgruppene.

Noen studier har vist en viss effekt av barnemat med probiotika, også *B. lactis* Bb12, ved diare og atopisk eksem, mens andre studier ikke har vist effekt. Således er vitenskapelige bevis for positive effekter svak eller mangler helt.

Ingen studier viste positiv effekt av grøt tilsatt *B. lactis* Bb12 i de aktuelle aldersgruppene.

Flere helsepåstander knyttet til produkter tilsatt probiotiske bakterier har vært vurdert av EFSA, herunder påstander om reduksjon i gastrointestinale plager, normal funksjon i fordøyelsessystemet, etablering av naturlig barriere i tarm, styrking av immunforsvar, mental og kognitiv utvikling hos barn og utvikling av immunsystem hos barn. Så langt har EFSA konkludert med at det ikke er dokumentert noen årsakssammenheng mellom inntak av probiotika og de påståtte effektene. Ingen av produktene som hittil er vurdert i EFSA er tilsatt *B. lactis* Bb12 (1 november 2009).

Bruk av kommersielt produsert barnegrøt er utbredt i Norge og starter i tidlig alder, og er spesielt viktig for etablering av bakterieflora i tarmen og utvikling av immunsystemet. Ifølge søker inneholder en porsjon (25 gram) grøtpulver 1 x 10^9 *B. lactis* Bb12 i monokultur. I de aktuelle aldersgruppene er det daglige grøtinntaket ofte større enn en porsjon, selv hos spedbarn under 6 måneder. Den daglige eksponeringen for *B. lactis* Bb12 fra de angjeldende produkter vil derfor kunne bli 1-2 x 10^9 cfu for spedbarn 4-6 måneder, og enda større hos spedbarn over 6 måneder. Dersom en vesentlig andel *B. lactis* Bb12 overlever transport fram til tynntarmen, så vil dette medføre en betydelig og monokulturell belastning ofte flere ganger daglig.

Langvarig tilførsel av *B.lactis* Bb 12 (1 x 10.9 cfu per portion) i monokultur for de yngste aldersgruppene (de under 12 måneder) kan ha hittil ukjente helseeffekter.
De to laveste aldersgruppene, altså 4-6 måneder og 6-12 måneder, er i den mest umodne og sårbare fasen når det gjelder etablering av bakterieflora i tarmen og utvikling av immunsystem, og vil derfor også være de gruppene med høyest risiko for eventuelle negative helseeffekter fra daglig inntak av probiotika.

2. BACKGROUND

In 2008, the Norwegian Food Safety Authority (Mattilsynet) received a notification of two processed cereal-based baby foods (from now on called cereals) intended for infants and small children supplemented with the microorganism *Bifidobacterium lactis* (*B. lactis*) Bb12 and prebiotics in the form of oligosaccharides and inulin.

In April 2009, the Norwegian Food Safety Authority requested the Norwegian Scientific Committee for Food Safety to make an assessment of the benefits and risks of the two cereal products, and an *ad hoc*-group of experts was appointed with the mandate to draft an assessment.

The notifier has provided documentation on the origin of the bacterial strain *B. lactis* Bb12, and the manufacturing process. A dossier containing documentation on microbial and chemical safety is included in the notification.

The addition of probiotics of different bacterial species and strains to regular foods, including infant formulas and baby foods, is increasing.

VKM has previously (2007 and 2005) published two assessments of the use of *Lactobacillus rhamnosus* (LGG) as an ingredient in infant formula and baby foods, and in 2009 a benefit and risk assessment of the use of probiotics for patients in hospitals (Opinion of the Steering Committee of the Norwegian Scientific Committee for Food Safety, 2009). Furthermore, the European Food Safety Authority has assessed and dismissed several health claims and nutrition claims related to probiotics. These reports and opinions have been valuable background documents.

3. TERMS OF REFERENCE

Translated from the Norwegian terms of reference[1]:

1. What benefit can infants/children in the following age groups gain from baby food containing probiotics? Assess the product in question with focus on *Bifidobacterium lactis* Bb12.
    a. infants 4- 6 months
    b. infants 6-12 months
    c. children 1-3 years

2. Are there any contraindications regarding the use of baby food containing probiotics for infants/children in the following age group? Assess the product in question with focus on *Bifidobacterium lactis* Bb12.
    a. infants 4- 6 months
    b. infants 6-12 months
    c. children 1-3years

3. Is there a risk that the inclusion of probiotics in baby food can lead to an increased development of bacterial resistance to antimicrobials? Assess the product in question with focus on *Bifidobacterium lactis* Bb12.

[1] Norwegian terms of reference are listed in Appendix I

4. Is the addition of probiotics to baby food likely to have any impact on the development of allergy in infants/children in the following age groups? Assess the product in question with focus on *Bifidobacterium lactis* Bb12.

a. infants 4- 6 months
b. infants 6-12 months
c. children 1-3 years

5. What influence can the amount (daily intake) of probiotics consumed by infants/children in the following age groups have on possible negative effects?
Assess the product in question with focus on *Bifidobacterium lactis* Bb12.

a. infants 4- 6 months
b. infants 6-12 months
c. children 1-3 years

The Norwegian Food Safety Authority is concerned that recommendations regarding limitations for intake of cereals with added probiotics will not be followed by the consumer. The Norwegian Food Safety Authority therefore finds it important that factors relating to daily intakes are made very clear.

6. What is the significance of mixing the product with breast milk/infant formula?

7. What significance does the addition of prebiotics have?

- Type of prebiotic?
- Amount of prebiotic?
- Type of prebiotic in relation to the type of food/other ingredients in the product?

4. INFORMATION PROVIDED BY THE NOTIFIER

According to the information received from the notifier, *B. lactis* Bb12 culture is added to the powder as a defined mixture, PP017[2]. Maltodextrin is used as a carrier and the amount added is minimum 3 x 10^{10} cfu/g (colony forming units per gram) with target 4 x 10^{10} cfu/g. The mixture PP017 constitutes 0.1% of the instant cereal powder.

4.1. Food/constituent as stated by notifier

The two instant cereal powders described in the notification are *Rice cereal with carrot* and *Oat cereal with prunes*. Neither of the products contains milk and the instant cereal powders should be mixed with breast milk or infant formula. The text below is a free translation of the Norwegian labelling text suggested by the notifier and from the notifier's dossier. The Norwegian labelling text is given in Appendix II.

Rice cereal with carrot: From age 4 months
"This cereal is based on rice. Rice does not contain gluten and has a calming effect on the stomach. The cereal is especially good for sensitive stomachs. In order to give a nutritionally complete meal, the cereal should be mixed with breast milk or infant formula. The cereal is not added sugar and contains only the natural carbohydrates present in the raw ingredients."

[2] The notifier has recently changed mixture from PP011 to PP017. The amount of added *B. lactis* has increased.

"The powder consists of 92% rice flour, 2.6% carrot powder. In addition, the powder contains oligofructose[3], corn starch, maltodextrin, inulin, *bifidus lactis*[4], vanillin, minerals (Ca, Zn and Fe) and vitamins (A, D, C, E, thiamine, niacin, B_6 and folic acid). The powder can contain traces of milk[5]. The raw ingredients do not contain gluten."

Oat cereal with prunes: From age 6 months

"Prunes have a laxative effect on the stomach. In order to give a nutritionally complete meal, the cereal should be mixed with breast milk or infant formula. The cereal is not added sugar and contains only the natural carbohydrates present in the raw ingredients."

"The powder contains 46% oat flour, wheat flour[6], 8% prunes. In addition, the powder contains oligofructose[3], inulin, *bifidus lactis*[4], maltodextrin, vanillin, minerals (Ca, Zn and Fe) and vitamins (A, D, C, E, thiamine, niacin, B_6 and folic acid). Can contain traces of milk[5]."

The nutritional information for both products is tabulated according to portion (when mixed with formula milk) and also according to 100 g powder.

Instructions for use for both products are as follows: "Prepare by warming 1.5 dl breast milk or infant formula to ca 37°C. Add cereal powder (ca. 4 dessert spoons, or ca. 0.5dl) to the desired consistency. If water is used to make this cereal, it will not be nutritionally complete. Ask for advice at your health clinic.
Expiry date: see top of package. Store dry and not above room temperature; the opened package should be used within 2 months. Keep the bag tightly closed."

4.2. Wording of the health claims as proposed by the notifier[7]

Foods containing probiotics are commonly marketed with different nutritional and health claims. Health claims related to probiotics are currently and continuously assessed by EFSA. So far none of the health claims related to probiotics have been accepted as sufficiently documented by EFSA, see list of EFSA opinions in 5.1. Data sources. The claimed health effects of the two products in question are similar to those assessed by EFSA for other probiotic bacteria. It is not the mandate of this report to evaluate the health claims related to the products as these health claims are assessed by EFSA.

Rice cereal with carrot: From age 4 months

"Balances the stomach"

Oat cereal with prunes: From 6 months

"For constipation"

The following labelling claims are suggested by the notifier for both products: "This cereal contains *Bifidus*-BL which are good bacteria that have a positive influence on the gut microbiota. The cereal also contains Prebio 1, a mixture of special fibres that in a simple way act as the foods for the good bacteria. The advantage of using this cereal is that it balances the little stomach, and thereby influences the whole body and the little child will feel comfort."

[3] The amount of prebiotics should be stated
[4] *Bifidus lactis* is not the correct nomenclature. Should be changed to *Bifidobacterium lactis* Bb12
[5] This claim seems meaningless as the cereal powders can be mixed with cow's milk based infant formula
[6] Presumably ca. 40%, not stated
[7] Free translation of Norwegian packaging text. Norwegian labelling is given in Appendix II

On the notifier's Danish website:
Rice cereal with carrot and Oat cereal with prunes are marketed with several health claims. The Danish text is given in Appendix III. The following health claims are freely translated from the Danish text: "In addition to contributing to a healthy gut flora, probiotics may also strengthen the immune system and thereby reduce risk of infections, shorten treatment time of diarrhoea and regulate constipation. Probiotics can be said to make up a "barrier" against bad bacteria and protect the body from infections. Studies also show that probiotics may reduce the risk of some allergic diseases."

5. LITERATURE

5.1. Data sources

Data sources are articles (see Appendix IV) and reports submitted by the notifier.

The following reports have been provided by the notifier:

- Probiotics for pediatric health.
- Safety Dossier *B. lactis*

Other relevant background papers used in this assessment are previous opinions on probiotics from VKM and the published opinions from EFSA on health claims related to probiotics (none of the claims previously assessed are related to *B. lactis* Bb12):

- The use of probiotics for patients in hospitals. A benefit and risk assessment (Halvorsen *et al.*, 2009).
- Risk assessment on use of *Lactobacillus rhamnosus* (LGG) as an ingredient in infant formula and baby foods (II). VKM 2007.
- Risk assessment on use of *Lactobacillus rhamnosus* (LGG) as an ingredient in infant formula and baby foods. VKM 2005.
- Scientific Opinion on the substantiation of health claims related to non-characterised microorganisms pursuant to Article 13(1) of Regulation (EC) No 1924/20061. EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) (EFSA, 2009).
- Scientific substantiation of a health claim related to LGG® MAX and reduction of gastro-intestinal discomfort pursuant to Article 13(5) of Regulation (EC) No 1924/20061. Scientific Opinion of the Panel on Dietetic Products, Nutrition and Allergies, 30 October 2008.
- Scientific substantiation of a health claim related to LACTORAL (a combination of three probiotic strains: *Lactobacillus plantarum*, *Lactobacillus rhamnosus*, *Bifidobacterium longum*) and normal functioning of the alimentary tract pursuant to Article 14 of Regulation (EC) No 1924/20061. Scientific Opinion of the Panel on Dietetic Products, Nutrition and Allergies, 28 October 2008.
- Scientific substantiation of a health claim related to regulat®.pro.kid BRAIN and mental and cognitive developments of children pursuant to Article 14 of Regulation (EC) No 1924/20061. Scientific Opinion of the Panel on Dietetic Products, Nutrition and Allergies, 2 October 2008.
- Scientific substantiation of a health claim related to regulat®.pro.kid IMMUN and immune system of children during growth pursuant to Article 14 of Regulation (EC) No

1924/20061. Scientific Opinion of the Panel on Dietetic Products, Nutrition and Allergies, 2 October 2008.

In addition literature searches were performed in the MEDLINE database 1966-2009 and on the World Wide Web using search keywords such as *B. lactis* Bb12 AND probiotics.

5.2. Data extraction

All the articles and reports provided by the notifier have been assessed. From the MEDLINE search articles in English, Norwegian, Danish and Swedish regarding the use of *B. lactis* Bb12 as probiotics in various products were assessed. Articles and reports investigating *B. lactis* Bb12 in infant formula and baby food and preferably in the relevant age group (4months to 3 years) have been considered sufficiently relevant, and included in this report.

ASSESSMENT

6. HAZARD IDENTIFICATION AND CHARACTERISATION

6.1. Genus/species/strain

6.1.1. Identification of the bacteria

The species *B. lactis* is a non-sporulating, anaerobic, non-motile, Gram-positive Y-shaped bacterium.

The strain *B. lactis* Bb12 exhibits a fermentation profile (API 32A gallery, BioMerieux, F) claimed to be typical for this species.

B. lactis Bb12 is deposited at the German Culture Collection (DSM) (Braunschweig, Germany) under the number DSM-20215 and was originally designated as *B. bifidium* of human origin. Following new classification in 1997, the Bb12 strain was found to be identical with *B. lactis* (DSM-10140) and thus identified as *B. lactis*.

6.1.2. Determination of the presence of plasmids, Insertion Sequence element (IS-elements), transposons, integrons or other transposable elements

B. lactis Bb12 does not contain plasmids. According to information from the notifier, the complete 2.0 Mb genome has been sequenced and compared against the reference database in order to identify the presence of specific DNA sequences. Only one hit was retrieved, which corresponds to *tetW,* a gene involved in tetracycline resistance in microorganisms. The gene was chromosomally located. In *B. lactis* Bb12, *tetW* was found to be adjacent to an insertion sequence (IS), which is a transposable element. Extended analysis of the flanking DNA sequences showed that the gene was not contained in a mobile element.

6.1.3. Antimicrobial resistance properties of *B. lactis* Bb12

A potential long term effect of the administration of probiotics is the spread of antimicrobial resistance genes to pathogenic bacteria (Courvalin, 2006). According to the guidelines for probiotics in food (FAO, 2002), it is recommended that use of probiotic bacteria should be

restricted to those strains that do not harbour transmissible drug resistance genes encoding resistance to clinically used drugs.

According to the information from the notifier, *B. lactis* Bb12 is susceptible to many broad spectrum and Gram-positive-specific antibiotics, but insensitive to many aminoglycosides.

In *B. lactis* Bb12, the *tetW* gene mediates ribosomal protection against tetracycline. Several studies have shown the presence of *tetW* gene in various species of *Bifidobacterium* (Scott *et al.*, 2000; Masco *et al.*, 2006).

A sequence similarity of >99.9% was reported between the *tetW* gene of a rumen isolate of *Butyrivibrio fibriosolven* and that of *Bifidobacterium longum* isolated from human, suggesting the possible gene transfer between these species from animals and humans (Scott *et al.*, 2000). However, while the *tetW* gene in *Butyrivibrio fibriosolven* was associated with the conjugative transposons (*TnB1230*), (Melville *et al.*, 2004), the *tetW* gene in *B. lactis* was not shown to be linked with any transposable elements (Scott *et al.*, 2000). We are not aware of any data which shows the association between the *tetW* in *B. lactis* Bb12 with any transposable elements.

The review article of Salyers *et al*., summarises the literature which shows that transfer of the resistance gene *tetW* between Gram-positive and Gram-negative bacteria can occur in the mammalian colon and in the other environmental sites (Salyers *et al.*, 2004).

6.1.4. Pathogenic criteria

Translocation: It has been shown that some probiotic strains may be able to translocate. However, a search in PubMed Nov 3 2009 gave no publication in which this question was addressed for *B. lactis* Bb12, indicating that it might not have been investigated.

Platelet aggregation: The possibility that probiotics may be able to aggregate platelets *in vivo* was recently commented upon (Halvorsen *et al.*, 2009). To the best of our knowledge, there are no reports of investigations into whether *B. lactis* Bb12 is able to aggregate platelets *in vitro* or *in vivo*.

6.2. Adaptive properties of *B. lactis* Bb12 in the gastrointestinal tract and effect on epithelial cells

Some *in vitro* tests on *B. lactis* Bb12 have shown that this strain shows tolerance for acid and bile salts at concentrations relevant to the environment in the stomach and duodenum. In addition, *in vitro* adherence to intestinal mucin isolated from children has been shown. However, we have found no reports where *in vivo* studies support the clinical relevance of these findings.

6.2.1. Resistance to gastric acidity

Following ingestion, the first major potentially inhibitory or fatal hurdle for bacteria in foods is the low pH in the stomach. The pH may be as low as 2 in fasting conditions but higher following ingestion of food.

B. lactis Bb12 was found to survive *in vitro* exposure to pH 3.0 with less than 1 log reduction after 15 hours. However, a 4 log reduction in cells was observed after 15 hours exposure to

pH 2.5. Fifteen hours is an excessive period to test acid tolerance as it does not have relevance to the *in vivo* situation. Similar results were obtained for both freshly grown and freeze-dried cultures (Saarela *et al.*, 2005).

Five strains of *Bifidobacterium* were studied by Vernazza *et al.* who found that *B. lactis* Bb12 showed good acid tolerance whereas the other strains quickly died under test conditions of pH 2 and 3 (Vernazza *et al.*, 2006).

To our knowledge, there are no *in vivo* studies regarding survival of *B. lactis* Bb12 in the acid environment of the infant stomach.

6.2.2. Bile salt resistance

In the duodenum, bile salts are excreted into the lumen and can also exert an antibacterial effect. Bile salts may be inhibitory or destructive to bacterial cells. *In vitro* testing of tolerance to bile salts is usually carried out by addition of various levels of bile salts to a suitable growth medium (Saarela *et al.*, 2005; Vernazza *et al.*, 2006).

To our knowledge, there are no *in vivo* studies regarding survival of *B. lactis* Bb12 in the bile salt environment of the infant duodenum.

6.2.3. Adherence to cell lines and human epithelial cells

The ability to adhere to intestinal surfaces is thought to be important for the efficacy of probiotic strains, and is claimed to be one of the main criteria for selecting such strains.

To our knowledge, there are no *in vivo* studies regarding adherence of *B. lactis* Bb12 to infant intestinal epithelial cells.

6.3. Health effects of intake of *B. lactis* Bb12

In the terms of reference, the Norwegian Food Safety Authority has requested an assessment of probiotics with focus on *B. lactis* Bb12 in baby foods intended for infants and small children (4 months to 3 years). EFSA has recently published an opinion on health claims and non-characterised microorganisms. EFSA has decided to use the following criteria for characterisation of food constituents that are microorganisms, which are the subject of health claims:

- Species identification by DNA-DNA hybridization or 16S rRNA sequence analysis.
- Strain identification by DNA macrorestriction followed by PFGE, RAPD, ARDRA or other internationally accepted genetic typing molecular methods.

Only when these two criteria were fulfilled, was the microorganism considered to be sufficiently characterised. In the case of combination of several microorganisms, EFSA considers that if one microorganism used in the combination is not sufficiently characterised, the combination proposed is not sufficiently characterised.

Health effects and adverse events are considered to be species and strain specific and only studies investigating the strain *B. lactis* Bb12 are relevant in assessing benefits and risks from

the products in question. Moreover, the food matrix may be of importance when assessing the safety. Baby foods can have many different matrixes, and none of the studies including *B. lactis* Bb12 has studied cereals as matrix.

Only articles and reports investigating *B. lactis* Bb12 and preferably in the relevant age group (4 months to 3 years) have been considered sufficiently relevant to be included in this report.

6.3.1. Safety studies

Few studies have focused on the safety of daily intake of *B. lactis* Bb12 or other probiotic bacteria by infants and small children. However, Saavedra *et al*., assigned infants and small children aged 3-24 months to receive a milk based formula containing *B. lactis* Bb12 and *S. thermophilus* 1 x 10^6 cfu/g or 1 x 10^7 each for 210 +/- 127 days (Saavedra *et al.*, 2004). The formulas were well tolerated and were regarded safe as it resulted in adequate growth and development of the children. In addition, a reduced incidence of colic or bowel irritability and a lower antibiotic use in the study groups compared with a control group on formula without probiotics was demonstrated. The study is hardly relevant as the formulas used contain a combination of two probiotics; furthermore, the studied products were not cereal powders.

Weizman *et al*., conducted a pilot study in which formula-fed infants, 3-65 days old, received a formula containing *B. lactis* Bb12, *L. reuteri* or no supplementation for 4 weeks (Weizman *et al.*, 2005). No adverse effects on infant growth, stooling habits or infant behaviour were observed. The supplemented formulas were well tolerated. The study, however, is not directly relevant for the age group 4 months to 3 years.

Seventy-two formula-fed full term infants received a formula containing *B. lactis* Bb12 in combination with long-chain polyunsaturated fatty acids from birth until 7 months. A control group (n=70) received a standard formula (Gibson *et al.*, 2009). No differences were seen in weight gain, body length, head circumference or body mass index (BMI). The reported adverse events including symptoms and signs involving the digestive system, intestinal infectious disease, candidiasis, dermatitis and respiratory infections were similarly distributed in the two groups, while frequency of feeding problems was significantly lower in the experimental group.

There are several studies of *B. lactis* Bb12 on effect parameters, such as the impact on infectious diseases or allergies, which report that no adverse effects were observed. These studies were not, however, designed to reveal adverse effects.

The notifier provided an overview by Prof. J Saavedra, along with the notification documentation. This article summarises current knowledge on intestinal microbiota and the impact of probiotic bacteria. It is stated that there have been no documented cases of infections with bifidobacteria used in food products and that studies on children and infants indicate a beneficial effect with respect to acute and antibiotic-associated diarrhoea. No adverse effects have been reported.

In safety studies, as well as in pharmaceutical or nutritional studies, the value of any claim is strengthened if the component under study is administered in a form identical to that intended for the market. None of the studies cited above used *B. lactis* Bb12 together with prebiotics in cereals. Thus the actual products in question have not been studied regarding safety.

It has been shown in several studies over the last 10-12 years that many intestinal bacteria, including some probiotics, can alter gene expression in enterocytes and that such gene alterations might be of functional importance, *in vivo* (Nurmi *et al.*, 2005). These alterations might influence the diversity of the microbiota in the intestine, as well as other unwanted side effects. There should be concern about possible long term effects of a mono-bacterial dietary supplement to the age-group in question. However, we are not aware of any *in vivo* studies explicitly concerning the ability of *B. lactis* Bb12 to influence gene expression of epithelial cells, which would be an indication of a potential mechanism of action.

6.3.1.1 Summary safety studies

No serious adverse events are reported, but neither has the effect of long term intake of a single bacterial strain been studied. Furthermore, cereals supplemented with *B. lactis* Bb12 intended for infants and toddlers have not been studied regarding safety. We are not aware of any *in vivo* studies explicitly concerning the ability of *B. lactis* Bb12 to influence gene expression of epithelial cells.

6.3.2 Studies on beneficial effects

6.3.2.1 Effect on development of allergies and atopic eczema

Several studies have been published where the effect of different strains of probiotic bacteria on allergic sensitization, atopic eczema and asthma has been discussed. Isolauri *et al*. studied extensively hydrolyzed formula containing *B. lactis* Bb12 or *Lactobacillus GG* administered to infants with early onset atopic eczema at weaning (mean age 4.6 months) (Isolauri *et al.*, 2000). After two months, eczema was significantly more under control in the study groups compared to placebo.

Rautava *et al*. investigated formula fed infants under 2 months of age (Rautava *et al.*, 2009). The infants received formula supplemented with *Lactobacillus* GG and *B. lactis* Bb12, or a placebo, daily until 12 months. Cow's milk specific IgA was enhanced at 7 months, but not at 3 or 12 months, in infants receiving formula with Bb12 and LGG. The authors speculate that this may be achieved via stimulation of the innate immune system through an increased production of sCD14. The study provides insight in possible mechanisms through which probiotics may promote immunological maturation in infancy. There was no apparent effect on the risk of sensitization to cow's milk or other dietary antigens. In another study *B. lactis* Bb12 was not able to induce tolerance in infants with cow's milk allergy (Hol *et al.*, 2008).

6.3.2.2. Effect on infections

Weizman *et al*. studied the effect of *B. lactis* Bb12 on infections in children in daycare centres. A slight effect on diarrhoea was found, no effect on respiratory illnesses (Weizman et al, 2005). A multicenter, prospective, double blind study in residential children's care centres on the preventive effect of *B. lactis* Bb12 on acute infectious diarrhoea in infants <8 months of age was performed (Chouraqui *et al.*, 2004). The number of infants in the study was small (too small to avoid a considerable risk of false negative results). The infants received at least 1.5×10^8 cfu/day (46 infants) or placebo (54 infants). No significant difference was found between the groups regarding the cumulative incidence of diarrhoea or diarrhoeal episodes. However, the authors state that "a significantly lower daily probability of diarrhoea in infants receiving Bb12" was found.

Another multicenter study evaluated the effect of a milk product containing *B. lactis* Bb12 and a mixture of prebiotics on the incidence of diarrhoea in children 1-3 years attending day care centres. The children consuming the supplemented milk for five months experienced 20% reduction in the number of days with four or more stools per day (Gibson *et al.*, 2009). In an effect study of *B. lactis* Bb12 and *Streptococcus thermophilus* on antibiotic associated diarrhoea (Correa NB. *et al.*, 2005) demonstrated a lower incidence of diarrhoea in the study group compared with placebo.

Mao *et al*. investigated infants with severe acute diarrhoea by administering *B. lactis* Bb12 and *S. thermophilus* -supplemented formula after initial rehydration (Mao *et al.*, 2008). No influence on the duration of diarrhoea or the average number of stools/day was seen. A slightly higher probability of reduction of rotavirus shedding was seen in the probiotic group. A protective effect on rotavirus diarrhoea has been demonstrated (Phuapradit *et al.*, 1999; Gibson *et al.*, 2009). Nopchinda also demonstrated better growth from 6-12 months in the group of Thai children receiving *B. lactis* Bb12 supplemented formula.

The effect of feeding infants baby food/formula supplemented with a mixture of *B. lactis* Bb12 and *L. rhamnosus* GG from before 2 months and until 12 months of age was investigated (Rautava *et al.*, 2009). A significant lower incidence of acute otitis media (AOM) and a lower incidence of antibiotic use were demonstrated in the probiotic group compared with a group of infants given non-supplemented standard formula.
Further studies are needed to confirm the results observed in this study. It is known that AOM is a self-limiting disease and resolves spontaneously in 80% of the cases without any treatment.

There are few *in vivo* studies which report antimicrobial activity of *B. lactis* Bb12 against pathogenic bacteria (Weizman et al., 2005; Chouraqui *et al.*, 2004).

6.3.2.3. Beneficial effects - summary
Some evidence of beneficial effects of *B. lactis* Bb12 on diarrhoea in hospitalized children with infectious diarrhoea, especially rotavirus-associated diarrhoea, has been reported. Furthermore a possible demonstration of protection against antibiotic associated diarrhoea. A positive effect on atopic eczema in infants has been demonstrated in one study, but the treated numbers were small. No clinical studies have been performed with cereals containing *B. lactis* Bb12.

7. EXPOSURE ASSESSMENT

According to a reported dose-response study with *B. lactis* Bb12 in healthy young adults, there was a dose-dependent recovery of *B. lactis* in the faeces. A daily intake of 10^8 cfu/day resulted in the recovery of *B. lactis* Bb12 in only 3 of the 15 participants taking this dosage, compared to 13 of another group of 15 participants taking 10^{11} cfu/day. An intake of 10^{10} cfu/day was the lowest dose giving a statistically significant chance of recovering viable *B. lactis* Bb12 from the faeces (Larsen *et al.*, 2006). Similar studies have not been done with infants. Furthermore, the recovery of the probiotic bacteria in faeces may not be representative of the microflora in the different parts of the intestine.

According to the notifier, one portion (25gram) of the cereal powder contains 10^9 cfu *B. lactis* Bb12 in monoculture. This is less than what is sufficient for recovery of probiotic bacteria in

faeces in adults, according to the study mentioned above. However, one portion of the product each day might still be sufficient to be a transient, but dominating part of the microflora in some part of the intestine.

If a considerable part of the *B. lactis* Bb12 survives the transport to the small intestine, it would represent a dominating bacterial supply often several times a day to the small intestine. The use of commercially produced cereals is frequent in infants and small children in Norway and starts at an early age. This time is of special importance for the establishment of the intestinal bacterial flora and the tightly interconnected development of intestinal functions and the intestinal immune system, which comprises the largest part of the immune system. A dominating and monocultural supply several times a day could influence the relatively (in comparison with the colon) scarce bacterial flora in the small intestine, an important site for the intestinal immune system. This may have impact on the future incidence of immune mediated diseases.

In a consumption survey of infants in Norway (at 6 months), 1% of the infants were introduced to solid food before the age of 3 months, and 11% before the age of 4 months. Before the age of 5 months maize-, rice or sorghum cereals were the types of solid foods introduced to most infants (41%). (Spedkost 6 mnd). Published 2008 by Helsedirektoratet, Mattilsynet og Universitetet i Oslo)

By the age of 6 months, 87% of the infants were given cereals. Among these, the average number of cereal meals was 1.7 per day. Most infants (86%) were given commercially produced cereals. Among these the average intake was 161 g/day (ready to eat). Intake at the 95th percentile was 200g/day (ready to eat) (consumers only).

In consumption survey with older infants (at 12 months), 82% of the infants were still given commercially produced cereals, and the average daily intake in those infants was 265 g (ready to eat). Intake at the 95th percentile was 750g/day (ready to eat) (consumers only). (Spedkost 12 mnd published 2009 by Helsedirektoratet, Mattilsynet og Universitetet i Oslo)

At the age of 24 months 17% of the children still eat commercially produced cereals, the average daily intake in those children was 146g (ready to eat), the intake at the 95th percentile was 400 g/day (ready to eat) (consumers only).(Småbarnskost 2 år). Pubilshed 2009 by Helsedirektoratet, Mattilsynet og Universitetet i Oslo

According to the information provided by the notifier, 25 grams cereal powder contains 10^9 cfu (1 portion as stated by the notifier) *B. lactis* Bb12.

When taking into consideration that the daily intake often is greater than one portion even in infants below 6 months of age, this would represent a daily intake of 1-2 x 10^9 cfu *B. lactis* Bb12 in infant 4-6 months and even more in infants above 6 months. Thus cereals containing probiotic bacteria would constitute a daily intake of high monocultural bacterial load.

8. RISK CHARACTERISATION

B. lactis Bb12 does not contain plasmids. The *tetW* was found to be adjacent to an insertion sequence (IS), which is a transposable element. Extended analysis of the flanking DNA sequences showed, however, that the gene was not contained in a mobile element.

Although it has been shown that some probiotic strains may be able to translocate, this has never been investigated for *B. lactis* Bb12.

Whether and to what extent *B. lactis* Bb12 is able to perform platelet aggregation *in vivo* is unknown.

To our knowledge, there are no *in vivo* studies regarding adherence to infant intestinal epithelial cells.

In clinical studies, no serious adverse events from *B. lactis* Bb12 are reported, but the effect of a high long term intake of one single bacterial strain has not been studied.

In safety studies, as well as in pharmaceutical or nutritional studies, the value of any claim is strengthened if the component under study is administered in a form identical to that intended for the market. None of the studies cited above used *B. lactis* Bb12 together with prebiotics in cereals. Thus the actual products in question have not been studied regarding safety.

The mammalian small intestine does not contain hydrolases that can split oligosaccharides and inulin, whereas such hydrolases are present in most intestinal microorganisms often in a very specific and strain-related set-up. It has been demonstrated that *B. lactis* Bb12 cannot utilize high molecule inulin, and its capability to hydrolyze oligosaccharides is variable (Vernazza *et al.*, 2006). As the types and amount of oligosaccharide and inulin in the product are not given, it is not possible for the Panel to evaluate the possible risk factors. Additionally, it might be mentioned that prunes are well known for having a laxative effect. The effect of laxatives on establishment of more physiological relevant stool habits in the age groups 4 months to 3 years has not been evaluated by the Panel.

Some evidence of beneficial effects of *B. lactis* Bb12 on diarrhoea in hospitalized children with infectious diarrhoea, especially rotavirus-associated diarrhoea as well as antibiotic associated diarrhoea have been reported. Furthermore, a positive effect on atopic eczema in infants has been demonstrated in one study of *B. lactis* Bb12, but the finding is not reproduced in other studies. However, no clinical studies have been performed with cereals containing *B. lactis* Bb12.

Several of the papers assessed, but not specifically commented upon, show apparent positive effects as indicated by some of the cytokines produced and the immune markers studied (Christensen et al, 2006; Amarri S. *et al.*, 2006; Rautava S. *et al.*, 2006)**.** However, due to the complexity of the immune system, studies showing a favourable shift in one or two markers are not sufficient to demonstrate an overall benefit of the supplementation. It is important to note that the infant's diet comprises a restricted variety of foods which often are taken several times a day during a period of life when a stable intestinal flora is not yet established. The establishment of a normal intestinal microbiota takes at least two years and thus the intake of large numbers of probiotic bacteria in monoculture during the first years of life may greatly influence this process.

Taking into consideration that the daily intake often is greater than one portion of cereals even in infants below 6 months of age, this would represent a daily intake at 1-2 x 10^9 cfu *B. lactis* Bb12 in infant 4-6 months and even more in infants above 6 months. If a considerable amount

of the *B. lactis* Bb12 survives the transport to the small intestine, it would represent a dominating and monocultural supply, often several times a day, to the small intestine.

From birth to 24 months, and especially after weaning, more than 1000 bacterial species are normally established in the intestinal tract. This individual intestinal microbiota is continuously influencing the host and the host's immune system, establishing physiological functions and defence mechanisms. The immaturity and vulnerability of the intestinal microbiota and the immune system makes the two lowest age groups at the highest risk of unwanted health effects of the daily intake of probiotics. A daily intake of probiotics may have negative effect on establishment of intestinal bacterial flora and development of intestinal functions and mucosal immune system in all of the three different age groups. Possible long term effects of a year long monocultural supply in these age groups, have to the best of our knowledge, not been evaluated by the notifier.

In infants and small children, cereals represent a substantial part of their daily diet. Consumption of cereals supplemented with one specific bacterial strain taken 1–3 times a day will lead to the ingestion of large numbers of that probiotic strain several times a day. This situation is entirely different from the consumption of e.g. a probiotic yoghurt by an adult, where it would represent only a small part of the total diet. In comparing these circumstances it is obvious that the challenge to the resident flora is likely to be much greater in infants and small children than in adults. Additionally, the counter-challenge from the resident microbiota is likely to be less in small children than in adults, and this could allow greater proliferation of the given probiotic strain.

As long as long time effects of cereals supplemented with a single strain of probiotic bacteria in high numbers are unknown, we suggest that such supplementation could be denoted a large scale uncontrolled clinical trial with until now virtually unknown health effects, especially for the youngest age group in question: those under 12 months of age.

The beneficial effects of foods containing probiotics on human health, are increasingly being promoted by health professionals (FAO, 2002). It has been claimed that probiotic microorganisms can play an important role in immunological, digestive and respiratory functions and could have a significant effect in alleviating infectious and allergic disease in children. Several health claims related to probiotics have been assessed by EFSA, including claims on reduction of gastro-intestinal discomfort, normal functioning of the alimentary tract, building of the natural intestinal barrier, improvement of the general immunity, mental and cognitive developments of children and immune system of children during growth. In the opinions so far, EFSA has concluded that a cause and effect relationship has not been established between the consumption of the probiotic containing products and the claimed effect. None of the products assessed so far contained *B. lactis* Bb12 (1 November 2009).

The claimed health effects of the product in question are similar to those assessed by EFSA for other probiotic bacteria. According to EFSA's register for questions, assessment of several health claims connected to *B. lactis* Bb12 (such as "maintain normal blood cholesterol and natural immune function") is in progress. It is not the mandate of this report to evaluate the health claims related to the products as these health claims are assessed by EFSA.

According to the notifier, probiotic bacteria have been added to human foods for many years. The strain *B. lactis* Bb12 has been added to infant formula and follow-on formula since 1991 without adverse effects being reported to the production company. However, we are not aware

of any studies designed to unveil any possible long term effects, positive or negative, following ingestion of foods supplemented with probiotic bacteria by infants. Furthermore, the supplementation of cereals with probiotics has not been studied, as in the available literature probiotic bacteria in capsules or added to formula have been used.

9. ANSWER TO THE QUESTIONS

1. What benefit can infants/children in the following age groups gain from baby food containing probiotics? Assess the product in question with focus on *Bifidobacterium lactis* Bb12.

a. infants 4-6 months
b. infants 6-12 months
c. children 1-3 years

a. Some studies have demonstrated a favourable effect of supplementation of baby food with probiotics, including *B. lactis* Bb12, on diarrhoea and atopic eczema while other studies do not. Thus, the scientific evidence for a favourable effect of supplementing formula or solid food with *B. lactis* Bb12, for infants 4–6 months, is weak and in some cases lacking.

   There are no studies demonstrating a positive effect of cereals supplemented with *B. lactis* Bb12 intended for infants 4-6 months.

b. Some studies have demonstrated a favourable effect of supplementation of baby food with probiotics, including *B. lactis* Bb12, on diarrhoea and atopic eczema while other studies do not. Thus, the scientific evidence for a favourable effect of supplementing formula or solid food with *B. lactis* Bb12, for infants 6-12 months, is weak and in most cases lacking.

   There are no studies that demonstrate a positive effect of cereals supplemented with *B. lactis* Bb12 intended for infants 6-12 months.

c. One study demonstrated a reduced incidence of diarrhoea in children 1-3 years attending day-care centres. The formulation used was a milk drink containing prebiotics and *B. lactis* Bb12.

   There are few studies on this age group and to our knowledge no studies demonstrating a positive effect of cereals supplemented with *B. lactis* Bb12 intended for children 1-3 years have been performed.

2. Are there any contraindications regarding the use of baby food containing probiotics for infants/children in the following age group? Assess the product in question with focus on *Bifidobacterium lactis* Bb12.

a. infants 4-6 months
b. infants 6-12 months
c. children 1-3 years

No special contraindications for use have been mentioned in the assessed literature.

However, especially in the two youngest age groups (a and b) there are important and unanswered questions about the health effects.

According to the notifier one portion (25gram) of the cereal powder contains 1 x 10^9 *B. lactis* Bb12 in pure culture. If a considerable amount of the *B. lactis* Bb12 survives the transport to the small intestine, it would represent a dominating and monocultural supply, often several times a day, to the small intestine. The use of commercially produced cereals is frequent in infants and small children in Norway and starts in an early age with especial importance for the establishment of the intestinal bacterial flora and the tightly interconnected development of the intestinal mucosal immune system. Thus, making an intentional addition to cereals of a "probiotic" bacteria in high numbers (1 x 10^9 cfu per portion), would in our opinion make it an unique large scale uncontrolled clinical trial. The strain has, until now, virtually unknown health effects, especially for the youngest age group in question - those under 12 months of age.

3. Is there a risk that the inclusion of probiotics in baby food can lead to an increased development of bacterial resistance to antimicrobials? Assess the product in question with focus on *Bifidobacterium lactis* Bb12.

Consumption of probiotic microororganism *B. lactis* Bb12 that harbour gene encoding resistance against tetracycline (*tetW*) may increase the risk of the transfer of such genes to the resident microbiota and pathogenic bacteria and hence increase development of bacterial resistance. High similarity has been observed between *tetW* gene in bacteria of human and environment origin and *B. lactis* Bb12. This suggests the spread of tetracycline resistance gene (*tetW*) between bacteria of various origins. However, the transfer of tetracycline resistance gene (*tetW)* to other bacteria as a consequence of consumption of Bb12 has not been studied.

4. Is the addition of probiotics to baby food likely to have any impact on the development of allergy in infants/children in the following age groups? Assess the product in question with focus on *Bifidobacterium lactis* Bb12.

a. infants 4-6 months
b. infants 6-12 months
c. children 1-3 years

a. There are some studies reporting a favourable effect on atopic eczema when infant formula supplemented with probiotics, including *B. lactis* Bb12, was administered from early infancy, or during weaning from 4-6 months. However, other studies could not reproduce this effect. No favourable effect on allergic sensitization has been reported, whereas a number of studies suggest to the contrary, an increased tendency to sensitization.

   The demonstration of diminishing or increasing allergic sensitization is not carried out for *B. lactis* Bb12.

   There are no studies that demonstrate a positive effect of cereals supplemented with *B. lactis* Bb12 intended for infants 4–6 months.

b. There are some studies reporting a favourable effect on atopic eczema when formula supplemented with probiotics, including *B. lactis* Bb12, was administered from early infancy, or during weaning from 6-12 months, other studies could not reproduce this effect. No favourable effect on allergic sensitization has been reported, a number of studies suggest to the contrary, an increased tendency to sensitization but these studies do not comprise *B. lactis* Bb12.

   There are no studies that demonstrate a positive effect of cereals supplemented with *B. lactis* Bb12 intended for infants 6–12 months.

c. There is one study reporting a significant reduction of clinical symptoms of atopic dermatitis in atopic children 1- 3 years receiving probiotic supplemented food. The probiotic in question, however, was a combination of two *Lactobacillus* strains. The effect of *B. lactis* Bb12 on allergic disease administered in cereals to children 1-3 years has not been studied.

5. What influence can the amount (daily intake) of probiotics consumed by infants/children in the following age groups have on possible negative effects? Assess the product in question with focus on *Bifidobacterium lactis* Bb12.

a. infants 4-6 months
b. infants 6-12 months
c. children 1-3 years

a. infants 4-6 months
   At the age of 6 months 87% of the infants receive commercially produced cereals, and the average intake is 161 g/day (ready to eat). Intake at the 95th percentile was 200g/day (ready to eat) (consumers only), and average number of cereal meals was 1.7 per day. The present dominant role of commercially produced cereals in the daily diet reported for this age group in Norway indicates that the introduction of cereals supplemented with *B. lactis* Bb12 may have the potential for negative health effects in this immunologically vulnerable age group. See below.

b. infants 6-12 months
   At the age of 12 months 82% of the infants receive commercially produced cereals, and the average daily intake was 265 g (ready to eat). Intake at the 95th percentile was 750g/day (ready to eat) (consumers only). The present dominant role of commercially produced cereals in the daily diets reported also for this age group in Norway indicates that the introduction of cereals supplemented with *B. lactis* Bb12 may have the potential for negative health effects in this immunologically vulnerable age group. See below.

c. infants 1-3 years
   At the age of 12 months 82% of the infants receive commercially produced cereals, and the average daily intake was 265 g (ready to eat). Intake at the 95th percentile was 750g/day (ready to eat) (consumers only). At the age of 24 months 17% of the children still eat commercially produced cereals, and the average daily intake in those children was 146g (ready to eat). The intake at the 95th percentile was 400 g/day (ready to eat) (consumers only). The present limited role of

commercially produced cereals in the daily diet reported for this age group in Norway indicate that the introduction of cereals supplemented with *B. lactis* Bb12 may probably have only a limited potential for negative health effects in this immunologically less vulnerable age group. See below.

The relative intake of commercially produced cereals per kg body weight and also the high consumption of cereals compared to other foods, including other foods containing probiotics, is considerably less at 12 months and even far less at 24 and 36 months than in the younger groups.

A daily intake of probiotics may have negative effect on establishment of intestinal bacterial flora and development of intestinal mucosal immune system in all of the three different age groups. The age groups 4-6 months and 6-12 months are in the most vulnerable period for establishment of intestinal bacterial flora and development of intestinal functions and mucosal immune system. The high intake of cereals in infants aged 4-12 months and the fact that the immune system is considerably more immature at this age, points to a higher risk for negative effects of supplementing baby food with probiotics in the age groups 4-6 months and 6-12 months (a and b).

According to the information provided by the notifier, 25 grams cereal powder (1 portion) contains 1 x 10^9 cfu *B. lactis* Bb12.

When taking into consideration that the daily intake often is greater than one portion even in infants below 6 months of age, this would represent a daily intake at 1-2 x 10^9 cfu *B. lactis* Bb12 in infant 4-6 months and even more in infants above 6 months. This might be sufficient to colonize some parts of the intestine. If a considerable amount of the *B. lactis* Bb12 survives the transport to the small intestine, it would represent a dominating and monocultural supply, often several times a day, to the small intestine.

Intestinal bacterial flora and the tightly interconnected development of the intestinal mucosal immune system are established in early age, and the period between 4-12 months is of special importance. The immaturity and vulnerability of the intestinal flora and the immune system makes the two lowest age groups (a and b) at the highest risk of unwanted health effects of the daily intake of probiotics.

6. What is the significance of mixing the product with breast milk/infant formula?

The significance of mixing with breast milk or infant formula is that the product can be prepared for infants using the type of milk that the infant is otherwise receiving. Some infants are allergic to cow's milk, or in other respects have an allergic disposition. If the cereal powder was prepared using ordinary dried milk, then it would be unsuitable for several groups of infants.

7. What significance does the addition of prebiotics have?

- Type of prebiotic?

The products contain two prebiotic components, i.e. oligofructose and inulin. Neither of these can be hydrolyzed by the mammalian enzymes, but can be looked upon as "food" for

intestinal microbes. Thus, the addition of these two compounds will act upon the intestinal flora in the child, but this has not been investigated by the notifier.

- Amount of prebiotic?

The amount of prebiotic in the products is not declared.

- Type of prebiotic in relation to the type of food/other ingredients in the product?

We are not aware of any publications that have considered prebiotic addition to foods in connection with other ingredients used. One potential problem is the possible increased consistency in the product due to the addition of prebiotics, which could result in a lower energy density in the infant food.

Appendix I.

Norwegian terms of reference:

1. Hvilken nytte kan følgende grupper barn ha av å bruke barnemat tilsatt probiotika? Vurder med spesiell fokus på aktuelle produkter med *Bifidobacterium lactis* Bb12.
    a. spedbarn 4-6 mndr
    b. spedbarn 6-12 mndr
    c. barn fra 1-3 år

2. Er det noen kontraindikasjoner ved bruk av barnemat som inneholder probiotika for følgende grupper barn? Vurder med spesiell fokus på aktuelle produkter med *Bifidobacterium lactis* Bb12.
    a. spedbarn 4-6 mndr
    b. spedbarn 6-12 mndr
    c. barn fra 1-3 år

3. Er det fare for at bruken av probiotika i barnemat kan føre til en økt utvikling av resistens mot antimikrobielle substanser? Vurder med spesiell fokus på aktuelle produkter med *Bifidobacterium lactis* Bb12.

4. Hva betyr tilsetning av probiotika i forhold til allergi for følgende grupper barn? Vurder med spesiell fokus på aktuelle produkter med *Bifidobacterium lactis* Bb12.
    a. spedbarn 4-6 mndr
    b. spedbarn 6-12 mndr
    c. barn fra 1-3 år

5. Hva betyr mengden probiotika (døgndose) i forhold til eventuelle negative effekter for følgende grupper barn? Vurder med spesiell fokus på aktuelle produkter med *Bifidobacterium lactis* Bb12.
    a. spedbarn 4-6 mndr
    b. spedbarn 6-12 mndr
    c. barn fra 1-3 år

    Mattilsynet er i tvil om merking med mengdebegrensning på denne type produkt som inngår i basis mat for små barn vil følges av forbruker. Det er derfor viktig å få klare tilbakemeldinger for faktorer knyttet til døgndose.

6. Hvilken betydning har det at barnegrøten kan blandes med morsmelk/morsmelkerstatning?

7. Hvilken betydning har tilsetning av prebiotika?
    a. type prebiotika?
    b. mengde prebiotika?
    c. type prebiotika vs. type næringsmiddel/ andre ingredienser i produktet?

Appendix II. Påstander i forslag til merking av produktene på norsk

Risgrøt med gulrot (fra 4 mnd alder)
Balanserer magen.
Denne grøten inneholder *Bifidus*-BL som er gode bakterier og gir den lille magen en god tarmflora. Grøten inneholder også Prebio 1 som er en blanding av spesialfiber som på en enkel måte fungerer som mat for de gode bakteriene. Fordelen med grøten er at den er balanserende for den lille magen hvilket igjen betyr at den påvirker hele kroppen og gjør at det lille barnet føler seg vel tilpass.

Grøten passer ekstra godt for følsomme mager.

Havregrøt med svisker (fra 6 mnd alder)
Ved hard mage.
Denne grøten inneholder *Bifidus*-BL som er gode bakterier og gir den lille magen en god tarmflora. Grøten inneholder også Prebio 1 som er en blanding av spesialfiber som på en enkel måte fungerer som mat for de gode bakteriene. Fordelen med grøten er at den er balanserende for den lille magen hvilket igjen betyr at den påvirker hele kroppen og gjør at det lille barnet føler seg vel tilpass.

Svisker har en løsnende effekt på magen.

Appendix III. Danish text from the notifier's Danish website

Probiotika og spædbørn

Det er meget vigtigt for småbørnsforældre, at børnene er friske og har det godt. Her kan du læse om, hvad en god tarmflora kan betyde for barnets sundhed.

Bakteriefloraen i tarmen

Hos en nyfødt indeholder tarmen slet ingen bakterier, men allerede få timer efter fødslen etableres bakterier, der kommer både fra moren og fra omgivelserne. Når barnet er to år, er tarmens bakterieflora stort set den samme som hos voksne. Den indeholder en afbalanceret mængde af både "gode" og "dårlige" bakterier. Det er vigtigt at have en god balance og masser af bakterier for at opbygge en tyktarmsflora, der er modstandsdygtig over for sygdomsfremkaldende bakterier. En god balance i tarmen har også betydning for opbygningen af kroppens immunforsvar.

Hvad er Probiotika?

Probiotika er levende organismer, der tilsættes maden og kan påvirke sundheden positivt. Nogle af disse bakteriestammer findes naturligt i tyktarmen. For det meste drejer det sig om forskellige stammer af bifidobakterier og laktobaciller, der anvendes som probiotika. Ordet bakterier har måske en dårlig klang, men et tilskud af probiotika er faktisk positivt i mange tilfælde. Probiotika findes i forskellige madvarer, f.eks. nogle yoghurtprodukter og mælkedrikke, og kan hjælpe med til at forbedre balancen av forskjellige bakteriestammer i tarmkanalen.

Hvorfor anvendes Pobiotika?

Brystmælken giver dit barn den bedst mulige næring i de første 6 måneder. Takket være brystmælkens sammensætning får barnet en bakterieflora i tarmen, der hovedsagelig består af bifidobakterier, hvilket er positivt. Når barnet begynder at spise anden mad og møder nye miljøer, stiger antallet af andre bakteriestammer. Hvis du vælger et produkt med probiotika, kan du hjælpe barnet med at bibeholde og fremme en god bakteriebalance i tarmen. Probiotika bidrager ikke alene til en sund bakterieflora i tarmen – det kan også bidrage til at styrke immunforsvaret og dermed reducere risikoen for tarminfektioner, forkorte behandlingstiden ved diarré og regulere tarmen ved forstoppelse. Man kan sige, at det danner en "barriere" mod dårlige bakterier og på den måde beskytter kroppen mod infektioner. Forsøg har også vist, at probiotika kan reducere risikoen for nogle allergisygdomme. *Bifidus* BL er en probiotikastamme med det latinske navn *Bifidobacterium lactis*. *Bifidus* BL er en kendt og sikker probiotika, der kan regulere tarmfloraen.

Appendix IV. List of articles provided by the notifier

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