Revised Opinion on the health benefits of infant and follow-on formula with "probiotics" as ingredients

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Some manufacturers of infant and follow-on formulae offer their products with probiotics as ingredients. These are bacterial strains that are said to have positive effects on the health of infants. The manufacturers claim, for example, that when babies are fed with these products, fewer infections occur.

The German Federal Institute for Risk Assessment (BfR) has assessed the safety and benefits of infant and follow-on formulae containing bacterial strains currently used in Germany in infant formulae for healthy infants.

The BfR comes to the conclusion that for some of the bacterial strains very few studies have been carried out with healthy infants. Despite this, the currently available study results do not provide any indications of adverse effects in healthy infants. From the BfR's point of view, further data from well-planned and controlled intervention studies are still desirable in order to be able to draw reliable conclusions about the safety of these microorganisms for routine use in infant formula.

The BfR also points out that based on the available data, no health benefits can be derived from using infant and follow-on formulae containing the assessed bacterial strains. Infant formulae, to which so-called "probiotic" bacteria have been added, therefore have no advantage for the nutrition **of healthy infants** compared to similar products without such additions.

1 Subject of the assessment

The BfR was asked to assess the safety, as well as any benefits that could possibly be expected, of infant formulae, to which so-called "probiotic" bacteria have been added.

According to information available to the BfR (as of July 2020), infant and follow-on formulae containing the following (strains of) bacteria are currently in circulation in Germany:

- *Lactobacillus fermentum* CECT5716
- *Lactobacillus reuteri* DSM 17938
- *Bifidobacterium (B.) lactis* BB-12[1]
- bifidobacteria of the species *B. breve*, *B. bifidum*, *B. infantis* and *B. longum* (strain names unknown).

For the following assessment, a comprehensive literature search was conducted in the databases *PubMed* and *Web of Science*, whereby only German and English-language publications were considered (last search: July 2020). Intervention studies on the safety and/or efficacy of the above-mentioned microorganisms in healthy, full-term infants (not premature babies!) were primarily taken into account. In the studies, the bacterial strains were administered in the form of fortified infant or follow-on formula as well as in other forms (e.g. as drops, tablets or powder).

[1] *Bifidobacterium animalis ssp. lactis* BB-12 is currently used exclusively in formulae for premature babies and for newborns with a bodyweight ranging from 1800 g to approx. 4000 g after discharge from hospital. Since the latter cannot clearly be classified as preterm formulae from a nutritional point of view and are very similar in composition to infant formula, *B. lactis* BB-12 was included in the assessment.

Furthermore, guidelines on the safety assessment of probiotics in food (EFSA, 2005; EFSA, 2007; FAO and WHO, 2006) and on the assessment of probiotics in infant formula (Braegger et al., 2011; IOM, 2004; Thomas et al., 2010) were taken into consideration.

Definition of terms:

Probiotics are defined, living, non-pathogenic microorganisms which, when ingested in sufficient quantities, provide positive health effects.

Probiotic foods are foods that contain probiotics in an amount that produces the probiotic effects after consuming the food.

Infant formula is a food intended for consumption by infants during the first few months of life and that, on its own, will meet the nutritional requirements of these infants until appropriate complementary foods are introduced. Infant formula is also suitable after the introduction of an appropriate complementary feeding (from the 5th to 7th month of life) as a liquid component of an increasingly diversified diet for infants.

Follow-on formula refers to foods intended for consumption by infants in the course of the introduction of an appropriate complementary feeding and which constitutes the major liquid portion of an increasingly diversified diet for these infants.

In the following Opinion, the terms infant formula and follow-on formula are used, depending on the formula used in the particular studies described. Since the terms mentioned were not clearly differentiated in all of the studies and in practice there is no need to switch from infant formula to follow-on formula in the course of the first year of life, the term “infant formula” is used in all cases in which a distinction was not possible or necessary.

BfR risk profile:
Infant and follow-on formula with added probiotic bacteria*: No. 040/2020

A	**Affected persons**	Healthy infants				
B	**Probability of a health impairment upon administration of infant and follow-on formula with addition of the “probiotic” strains of bacteria assessed here**	Practically impossible	**Unlikely**	Possible	Probable	Certain
C	**Severity of the health impairment from exposure through infant formula**	**No impairment**	Mild impairment [reversible]	Moderate impairment [reversible/irreversible]	Severe impairment [reversible/irreversible]	
D	**Validity of available data**	High: The most important data are available and are internally consistent	Medium: Some important data are missing or contradictory	**Low: A large volume of important data is missing or inconsistent**		
E	**Controllability by the consumer [1]**	**Control not necessary**	Controllable with precautionary measures	Controllable by avoidance	Not controllable	

Dark blue fields indicate the properties of the risks assessed in this Opinion (further information on this can be found in the text of the Opinion).

Explanations

The risk profile is intended to visualise the risk outlined in the BfR Opinion. It is not intended to be used to compare risks. The risk profile should only be read in conjunction with the corresponding Opinion.

Row E - Controllability by the consumer
[1] – The information in the row "controllability by the consumer" is not a recommendation by the BfR, but is of a descriptive nature.

* A health benefit of the (strains of) bacteria assessed here has not been proven.

2 Results

The species *Lactobacillus fermentum*, *Lactobacillus reuteri*, *Bifidobacterium animalis*, *Bifidobacterium breve*, *Bifidobacterium longum* and *Bifidobacterium bifidum* have been classified as generally safe by the European Food Safety Authority (EFSA) due to their long and safe use in the food sector. These six species were therefore awarded QPS status (QPS = qualified presumption of safety).

Lactic acid bacteria, in particular species from the genera *Lactobacillus* and *Bifidobacterium*, are routinely used in food production. Their use as production cultures in food (e.g. in fermented milk products, cheese, etc.) for over 100 years attests to the safety of these microorganisms in fermented products. On the other hand, experience with the use of these bacteria in infant formulae is lacking, and relatively few studies are available in which infants were fed infant formulae containing probiotic bacteria.

Human studies indicate that there are large differences between individuals regarding the composition of the stool microbiota. Factors affecting bacterial colonisation in early infancy include gestational age, mode of delivery, diet (breast milk or infant formula), use of antibiotics, as well as family, geographic and cultural influences. The introduction of bacteria designated as probiotic causes only temporary, insignificant changes in the overall structure and diversity of the stool microbiota.

The following can be concluded about the **safety** of the (strains of) bacteria assessed here and used in infant and follow-on formulae on the German market:
The BfR is aware of three studies in which an infant formula with *Lactobacillus fermentum* CECT5716 as an ingredient was used to feed healthy infants. Negative effects were neither observed after five or eleven months of feeding in the first year of life nor in toddler age in a follow-up observation of one of the two studies.

Regarding the safety of *Lactobacillus reuteri* DSM 17938, little scientific data from studies with healthy infants is available. However, the results available do not indicate any adverse effects.

Also, based on the available study results, there are no indications of adverse effects from the use of *Bifidobacterium lactis* BB-12 in infant formula in healthy infants.

No reliable conclusions can be drawn regarding another mixture of *Bifidobacterium breve*, *Bifidobacterium bifidum*, *Bifidobacterium infantis* and *Bifidobacterium longum* used in infant and follow-on formulae on the German market, as it is not known which strains of these bacterial species are used in the formulae. A German intervention study using infant formula containing *Bifidobacterium breve* BR3, *Bifidobacterium bifidum* BF3, *Bifidobacterium longum ssp. infantis* BT1 and *Bifidobacterium longum* BG7, did not show any negative effects on the growth and development of the infants examined. However, the BfR is not aware of any other studies in which the safety of a mixture of these four bifidobacteria strains was studied for use in infant formula. It is also questionable whether the composition of the formula used in the study and the mixture of bifidobacteria contained in it corresponds to that found in infant formula marketed in Germany.

In general, from the BfR's point of view, the question arises as to whether/how immunocompromised infants, who have an increased risk of negative health effects through the intake of probiotic microorganisms, can be identified and, if necessary, excluded from the consumption of infant formulae with added microorganisms. It is recommended to combine the marketing of infant and follow-on formulae with probiotics with a market surveillance program to record any undesirable effects that may occur.

Regarding possible **positive effects** or benefits of the evaluated bacterial strains, the following can be concluded:

Based on available study data, there is insufficient scientific evidence of positive effects or health benefits in regard to growth, development and/or the frequency and severity of infectious diseases or other health-related effects for any of the bacterial species or strains assessed, when used as ingredient in infant formula in healthy infants. Scientific data suggest that *L. reuteri* DSM 17938 in oily suspension appears to be effective in the treatment of colic in breastfed infants. However, no health benefit of routine feeding of infant and follow-on formulae with *L. reuteri* DSM 17938 can be derived from this.

In summary, the infant and follow-on formulae on the market that have been supplemented with the (strains of) bacteria assessed here are not considered to be more suitable for feeding **healthy infants** than conventional infant and follow-on formulae. Furthermore, there is insufficient data available to assess the suitability of infant formula based on protein hydrolysates (with the additional designation “HA”) supplemented with “probiotic” (strains of) bacteria.

3 Rationale

The species *Lactobacillus (L.) fermentum*, *L. reuteri* and *Bifidobacterium (B.) animalis*, *B. breve*, *B. longum* and *B. bifidum* have been classified as generally safe by the EFSA due to their long and safe use in the food sector (EFSA, 2008) and were already part of EFSA's first list of proposals for QPS status (QPS = qualified presumption of safety) (EFSA, 2007). Lactic acid bacteria, in particular species of the genera *Lactobacillus* and *Bifidobacterium*, have been in use in the production of food for decades. Their use as production cultures in food (e.g. in fermented milk products and cheese) for over 100 years attests to the safety of these microorganisms.

The BfR is of the opinion that, in accordance with Article 3 of the Commission Delegated Regulation (EU) 2016/127, infant or follow-on formulae containing ingredients such as “probiotic” microorganisms should only be placed on the market if their suitability has been proven via systematic assessment of the available data in relation to the expected benefits and safety considerations.

The BfR considers that probiotic effects are only to be regarded as scientifically proven if they a) have been demonstrated in randomised, double-blind and placebo-controlled studies with defined, well-characterised bacterial strains in the target group (healthy infants) and b) the results of those studies can be transferred to the product under evaluation.
It must be taken into account that there are large inter-individual as well as intra-individual differences in the effects of individual bacterial strains on the colonisation of the intestinal mucosa and faeces as well as the possible associated health effects (e.g. Butel et al., 2018; Suez et al., 2018; Zmora et al., 2018). Safety and efficacy assessments of microorganisms should therefore be undertaken on a strain-specific basis and consider target group-specific characteristics, whereby factors such as age, lifestyle, diet and health status, as well as the use of antibiotics have to be included. Knowledge gained about individual strains cannot be

transferred to other strains (Braegger et al., 2011). There is also evidence that the changes in the overall structure and diversity of the stool microbiota in infants resulting from the intake of “probiotic” bacteria are not significant and only temporary (Bazanella et al., 2017; Laursen et al., 2017; Roos et al., 2013).

The safety and expected benefits of the (strains of) bacteria used in infant and follow-on formulae on the German market are assessed below.

3.1 Lactobacillus fermentum CECT5716

3.1.1 Hazard identification

Lactobacillus (L.) fermentum CECT5716 was originally isolated from human milk (Martín et al., 2003). The strain shows a high survival rate under gastrointestinal tract-like conditions in *in vitro* studies and has a strong ability to adhere to HT-29 and Caco-2 cells (human cell lines of intestinal origin). It produces lactic acid and hydrogen peroxide, which are thought to be part of the non-specific antimicrobial defence due to their pH-reducing and oxidative properties (Martín et al., 2005). In addition, the strain shows immunomodulatory, antibacterial and anti-inflammatory properties *in vivo* and *in vitro*, which is why it was classified as probiotic (Diaz-Ropero et al., 2007; Lara-Villoslada et al., 2007; Mañé et al., 2009; Olivares et al., 2006).

L. fermentum CECT5716’s toxic potential and its sensitivity to antibiotics was investigated in mice. Doses 10,000 times higher than that ingested by humans (based on kg bodyweight) did not show any pathogenic properties (Lara-Villoslada et al., 2009).

Based on data on *L. fermentum* CECT5716 submitted to the US Food and Drug Administration (FDA) by a manufacturer, the FDA has approved the use of this bacterial strain in infant formula for healthy infants after one month of life as generally safe [Generally Recognised As Safe (GRAS)][2].

3.1.2 Risk assessment

To date, only few human studies have been conducted with *L. fermentum* CECT5716, including three intervention studies with healthy infants in the first and/or second half-year of life (Gil-Campos et al., 2012; Maldonado et al., 2012; Maldonado et al., 2019). As some of the subjects from Gil-Campos et al. (2012) were reexamined at the age of three, the first results on medium-term effects of *L. fermentum* CECT5716 are also available (Maldonado-Lobón et al., 2015). Another intervention study, which included healthy infants from six months of age, primarily aimed to investigate the health benefit of *L. fermentum* CECT5716-fortified follow-on formula (Maldonado et al., 2012).

The study by Gil-Campos et al. (2012) included 137 infants aged one month. Before study begin, about a third of the infants had been breastfed. The intervention took place over five months with infant formula containing either *L. fermentum* CECT5716 (10^7 colony-forming units (CFU)/g) and 0.3 g/100 mL galactooligosaccharides (GOS) or only 0.3 g/100 mL GOS. The infants were examined clinically at the start of the study and after two, four and six months. Stool samples were also taken after four and six months. Weight gain was measured as the primary endpoint after four months. Secondary parameters included increases in

[2] GRAS Notice GRN No. 531 from 20 March 2015: *Lactobacillus fermentum* CECT5716 for use in powdered milk-based infant formula at 10^7 colony forming units per gram of powdered formula. (http://www.accessdata.fda.gov/scripts/fdcc/?set=GRASNotices&id=531; last accessed: 20 July 2020)

length and head circumference of the children as well as the frequency of gastrointestinal infections, feeding behaviour and undesirable effects associated with feeding. In addition, the species and number of faecal bacteria and the concentration of short-chain fatty acids and immunoglobulin (Ig) A in the faeces were determined. The drop-out rate was 15% in the intervention group and 7% in the control group.

According to the authors, the formula was well tolerated. On average, 600 ml of it was consumed per day. Examinations after four and six months did not show any significant differences in growth or in most of the other parameters measured (stool frequency, colour, texture, flatulence, vomiting, duration of sleep and sleep behaviour). No adverse effects were reported in connection with the study formula. In both groups, comparable concentrations of *Lactobacillus spp.*, *Bifidobacterium spp.*, *Clostridium spp.* and *Bacteroides spp.* as well as short-chain fatty acids and IgA were measured in stool (Gil-Campos et al., 2012).

In a follow-up study to the study by Gil-Campos et al. (2012), a total of 91 children - 46 from the former control group and 45 from the former intervention group - were contacted again at the age of three to determine growth and frequency of infections (retrospectively for the past three years) of the children, as well as various stool parameters (at three years of age): No significant differences were found between children in the two former study arms, neither in terms of growth nor in the other recorded parameters (Maldonado-Lobón et al., 2015).

Maldonado et al. (2012) recruited 215 six month-old infants for another placebo-controlled intervention study. Up to the end of the first year of life, the infants received a follow-on formula containing either *L. fermentum* CECT5716 (2×10^8 CFU/g) and GOS (0.4 g/100 ml) or only GOS (0.4 g/ml) in addition to complementary feeding. The primary aim of this study was to investigate the benefit of a follow-on formula supplemented with *L. fermentum* CECT5716 in infants in the second half-year of their life. Of the infants originally included, 188 completed the study; i.e. the drop-out rate was 22.5%. Again, no differences in the growth of the infants were observed and good tolerability of the formula was reported. Since the infants received larger amounts of complementary foods in addition to the study formula with increasing age, the actual exposure to the study formula or the microorganisms it contained is unclear; however, it was more likely to be lower than when only feeding study formula in the first half-year of life.

Finally, Maldonado et al. (2019) conducted another intervention study in which they tested 236 healthy infants from the first month of life up to the age of twelve months either with standard infant and follow-on formula (control group) or with comparable infant formula supplemented with *L. fermentum* CECT5716 (Lf group) or *B. breve* CECT7263 (Bb group) - each in concentrations of 10^7 CFU/g. The children were examined at the start of the study as well as after two, four, six, nine and twelve months. The drop-out rate in this study was 19%, with drop-outs roughly evenly distributed across the three arms. The primary endpoint was mean weight gain up to four months of age. In addition, secondary endpoints were recorded as in the study by Gil-Campos et al. (2012). No significant differences were observed between the three study arms at any time. According to the authors no undesirable effects occurred in connection with the study formula (Maldonado et al., 2019). However, it cannot be ruled out that the higher incidence of reflux, constipation and colic, which led to discontinuation of the study in 10.8% of the infants in the Lf group (compared to 1.3% in the Bb group and 6.5% in the control group), was causally related to the study formula.

In summary, the intervention studies undertaken in healthy infants in the first or second half-year of life and the results of a follow-up examination in infants at the age of three indicate that feeding infant formula supplemented with *L. fermentum* CECT5716 for five or eleven

months is neither associated with growth retardation nor other short or medium-term negative health effects.

For reliable conclusions on the safety of routine use of *L. fermentum* CECT5716 in infant and follow-on formulae, further controlled studies with sufficiently long study duration, number of subjects and follow-up time, in accordance with the criteria established by international organisations for the safety assessment of infant formula, are necessary.

3.1.3 Benefit assessment

To assess the health benefits of *L. fermentum* CECT5716 for healthy infants, the already mentioned intervention studies by Gil-Campos et al. (2012), Maldonado et al. (2012), Maldonado-Lobón et al. (2015) and Maldonado et al. (2019) can be used, whereby Maldonado et al. (2012) was the only one that was conducted with the aim of investigating the benefit of a follow-on formula supplemented with *L. fermentum* CECT5716 in infants in the second half-year of their life.

Primary endpoints in the study by Maldonado et al. (2012) included the frequency of infections of the gastrointestinal tract, respiratory tract, middle ear, urinary tract, and other less common infections. The secondary endpoints recorded were the development of weight, length and head circumference of the infants as well as the occurrence of fever, administration of antibiotics, but also the concentrations of short-chain fatty acids, IgA and the composition of the stool microbiota as well as repeated occurrence of respiratory infections (defined as three times or more).

As described above, 215 infants aged six months were included in the study and were given a follow-on formula supplemented with *L. fermentum* CECT5716 (2×10^8 CFU/g) and GOS (0.4 g/100ml) or only GOS (0.4 g/ml) in addition to complementary foods until the end of their first year of life. The drop-out rate was 22.5%.

A significantly lower incidence of upper respiratory and gastrointestinal infections was observed in the intervention group. No differences between the intervention and control groups were found for the secondary parameters. At the end of the intervention period, the probiotic group had significantly higher concentrations of lactic acid and bifidobacteria in the stool; however, the *L. fermentum* CECT5716 bacterial strain used was not quantified.

Similar, in the previously described studies by Gil-Campos et al. (2012) and Maldonado et al. (2019) significantly lower frequencies of gastrointestinal infections were observed in the *L. fermentum* study arms in the first half-year of life. In addition, Maldonado et al. (2019) observed that infants whose mothers smoked during pregnancy were less likely to have upper respiratory tract infections if they were fed infant formula with *L. fermentum* CECT5716 instead of a conventional one. Upper respiratory tract infections were also less common in infants delivered by caesarean section when they received infant formula supplemented with *L. fermentum* CECT5716. These findings suggest that the mode of delivery (vaginal or caesarean) or other maternal and child factors could influence the effects of *L. fermentum* CECT5716 (Maldonado et al., 2019). Since this study primarily aimed to investigate the effects of *L. fermentum* CECT5716 on the growth of infants, the number of subjects and thus the power of the study was too small to detect a possible protective effect of *L. fermentum* CECT5716 on the development of infections in infancy.

In the studies by Gil-Campos et al. (2012) and Maldonado et al. (2012) the mode of delivery (vaginal or caesarean) was not taken into account. In addition, no reliable statements can be drawn from these two studies regarding the benefits of infant formula supplemented with *L.*

fermentum CECT5716. However, the studies indicate a protective effect against gastrointestinal infections and infections of the upper respiratory tract in infants. The significance of the results is limited by the relatively high drop-out rates. Also, in Gil-Campos et al. (2012) about 50% of infants in both study arms were breastfed during the intervention period and in Maldonado et al. (2012) around 70% of the infants had been breastfed with varying intensity and duration before the start of the intervention. This could also be one of the reasons that Gil-Campos et al. (2012) found no significant differences in the species and number of microorganisms or in the concentrations of short-chain fatty acids and IgA in the stool.

A search of the database US National Library of Medicine for clinical trial registration[3] found that another placebo-controlled, multi-centre study was completed in 2019, in which infants were treated for colic using *B. breve* CECT7263 (2×10^8 CFU/day) or a mixture of *B. breve* CECT7263 (1×10^8 CFU/day) and *L. fermentum* CECT5716 (1×10^8 CFU/day) - each in powder form - in comparison to a drug commonly used for flatulence and bloating. The results of this study have not been published yet, and can therefore not be evaluated.

In summary, based on the currently available study results, there are indications, but insufficient evidence, of a health benefit of infant or follow-on formula with *L. fermentum* CECT5716 for the feeding of healthy infants. Data on the suitability of infant formula with *L. fermentum* CECT5716 for feeding infants with flatulence, constipation, colic or increased belching and spitting up are not available.

To be able to draw reliable conclusions on the benefits of this bacterial strain as a component of infant or follow-on formula, the results of further controlled studies must be awaited.

3.2 Lactobacillus reuteri DSM 17938

3.2.1 Hazard identification

L. reuteri DSM 17938 was generated by removing two genes responsible for antibiotic resistance from *L. reuteri* ATCC 55730 which was originally isolated from human milk. Therefore, in the literature it is sometimes viewed as a daughter strain of *L. reuteri* ATCC 55730. In an *in vitro* study Rosander et al. (2008) showed that *L. reuteri* DSM 17938 and *L. reuteri* ATCC 55730 behaved similarly in the presence of acids and bile, and with respect to mucosal binding. In addition, in a placebo-controlled human study with 16 adult test subjects they observed that *L. reuteri* DSM 17938 also had similar properties to *L. reuteri* ATCC 55730 *in vivo* (Rosander et al., 2008).

Despite this and other data from a few, uncontrolled human studies with adult test subjects, in which a temporary colonisation of the intestine with *L. reuteri* DSM 17938 was shown after oral intake of 10^9 CFU/day of this bacterial strain for seven to 21 days (Dommels et al., 2009; Smith et al., 2011), there are still different opinions as to whether *L. reuteri* DSM 17938 and *L. reuteri* ATCC 55730 can be considered equivalent. In this context, Urbańska and Szajewska (2014) pointed out that the uncertainty about the bioequivalence of the two strains is fuelled by the fact that the manufacturing process can influence the properties of probiotic bacteria, which for example was shown for *L. rhamnosus* GG by Grześkowiak et al. (2011).

In addition to the considerations on the equivalence of the two bacterial strains, it should be noted that, to the knowledge of the BfR, of the studies, in which a total of around 300 infants were subject to supplementation (placebo-controlled) with 10^8-10^{11} CFU *L. reuteri* ATCC 55730 per day over 5 to 28 days or (in one study) over 12 months), only one study (Weizman

[3] https://clinicaltrials.gov/ct2/show/NCT03467334?cond=lactobacillus+fermentum&draw=2&rank=3 (last accessed: 27 July 2020)

and Alsheikh, 2006) was conducted with the primary aim to investigate the safety of *L. reuteri* ATCC 55730 in infants. All other studies aimed to gain knowledge about the efficacy of this bacterial strain for the therapy of - mostly gastrointestinal - diseases. Negative effects were not observed (Abrahamsson et al., 2007; Abrahamsson et al., 2013; Connolly et al., 2005 (partial collective from Abrahamsson et al., 2007); Forsberg et al., 2013; Roos et al., 2013; Weizman and Alsheikh, 2006).

3.2.2 Risk assessment

Regarding the safety of infant formula with *L. reuteri* DSM 17938 as an ingredient, results from three intervention studies in healthy infants are available (Cekola et al., 2015; Lee et al., 2015; Papagaroufalis et al., 2014):

Cekola et al. (2015) recruited 84 infants in the first 14 days after birth who were fed a study formula containing *L. reuteri* DSM 17938 (10^6 CFU/g or 10^8 CFU/day) for about four months; 79 other infants received a comparable infant formula without probiotics (control group). The drop-out rate was 29%. No differences were observed between the two study arms in terms of tolerability of the formula, growth of the infants and in the stool parameters investigated. Also, the number of infants with adverse effects was equally high in both groups; however, regarding the recorded effects that were classified as being likely associated with the study formula, there were 13 of them in the intervention group, but only six in the control group.

Another study by Lee at al. (2015) included 140 infants in the first 14 days after birth. Up to the age of six months they were fed either an infant formula supplemented with just *L. reuteri* DSM 17938 (n = 68) or a combination of *L. reuteri* DSM 17938 and a mixture of fructooligosaccharides (FOS) and GOS (n = 72). Both study formulae contained *L. reuteri* in concentrations that resulted in a total intake of 10^8 CFU per day. The primary aim of the study was to examine weight development up to the age of four months. In addition to bodyweight, further anthropometric values (length and head circumference), food tolerance as well as possible undesirable effects and disease frequencies were recorded at regular intervals. In addition, D- and L-lactate in the urine and the bacterial composition of the faeces were analysed at the age of two months. According to the authors, differences in weight gain, anthropometric parameters or food tolerability were not observed between the two study arms. The excretion of D- and L-lactate was also comparable in both groups (D-lactate: on average 3 and 4 mmol/mol creatinine; L-lactate: on average 58 and 66 mmol/mol creatinine). In the *L. reuteri* + GOS/FOS group, there were 14 cases of severe adverse effects; in the group without GOS/FOS there were seven. A total of five cases of bacterial pneumonia and isolated cases of respiratory and urinary tract infections were registered in both *L. reuteri* groups. It is critical to note that the study did not include a control group without probiotic exposure. Therefore, no reliable conclusions on the safety of an *L. reuteri* DSM 17938-containing infant formula can be drawn from this study.

In 2014, Papagaroufalis et al. carried out a randomised, placebo-controlled intervention study in which 88 infants received *L. reuteri* DSM 17938-containing infant formula (1.2×10^6 CFU per ml formula) or a standard infant formula for 28 days within 72 hours after birth (stratified according to mode of birth and gender). After seven, 14 and 28 days, D- and L-lactate concentrations in the urine as well as anthropometric parameters were measured and information from the parents on stool frequency and consistency was recorded. In addition, the bacterial concentrations in the stool were analysed after 14 and 112 days. Follow-up observations were made after 112 and 168 days. Up to day 112, food intake and tolerance, stool frequency and consistency, as well as sleep behaviour and any undesirable health effects were recorded by the parents in a diary. In both groups, eight and nine out of 44 infants each

dropped out before day 28, thus, 71 infants - 36 in the intervention group and 35 in the control group - were included in the analysis. Lactic acid bacteria were detected in stool in 60% of the verum group (compared to 30% of the control group); however, the proportion of children containing *L. reuteri* in stool was not stated by the authors. Overall, the intervention group had significantly higher concentrations of bifidobacteria, lactic acid bacteria and *L. reuteri*. No differences were observed in the children's growth or sleeping and crying behaviour. There were also no differences in stool frequency, but in stool consistency (more often soft stools in the verum group) and in the frequency of spitting up (less often in the verum group). According to the authors, the D- and L-lactate concentrations in both groups were comparable after 14 days.

Two further intervention studies were undertaken with the aim of determining the safety of *L. reuteri* DSM 17938 in infants with colic (N = 20) (Fatheree et al., 2017) and in children between two and five years of age with a high prevalence of diarrhoeal diseases (N = 60) (Kosek et al., 2019):

In both studies, *L. reuteri* DSM 17938 was administered in oily suspension at doses of 10^8 CFU/day for either 5 or 42 days. There were no significant differences between the intervention and control groups with regard to the laboratory parameters examined and the occurrence of undesirable effects. Also the occurrence of diseases, crying and restless times caused by colic (Fatheree et al., 2017) as well as diarrhoea, fever and other symptoms (Kosek et al., 2019) were similar between groups.

A number of additional intervention studies with *L. reuteri* DSM 17938 were conducted with the aim of alleviating the symptoms of colic in infants (see 3.2.3). Again, no negative effects on growth or health of the infants were observed. In most of these studies, however, 10^8 CFU *L. reuteri* DSM 17938 were only taken over a period of three weeks. In addition, the microorganisms were not administered as part of an infant formula, but in form of an oily suspension and the infants were otherwise exclusively breastfed.

In summary, the available study results provide indications that *L. reuteri* DSM 17938 is well tolerated by infants in the doses used in the oily suspensions for a period of up to six weeks or as part of infant formula over the course of four months. In the studies to date, no negative effects on the growth of healthy infants have been observed. In some cases, however, adverse effects and in some instances even serious illnesses occurred. A causal relationship between them and the use of the bacterial strain can neither be proven nor completely be ruled out. It should be noted that no long-term studies or follow-up examinations of the studies were carried out.

Overall, the available data for assessing the safety of infant formula with *L. reuteri* DSM 17938 as an ingredient are limited. For a reliable assessment of the (long-term) safety of routine use of *L. reuteri* DSM 17938 in infant formula for healthy infants further controlled studies with a sufficiently long study duration and follow-up period are necessary.

3.2.3 Benefit assessment

3.2.3.1 Prevention of diarrhoeal diseases

Gutiérrez-Castrellón et al. (2014) investigated the effect of *L. reuteri* DSM 17938 on the incidence and duration of diarrhoeal diseases in infants and young children cared for in day-care centres in Mexico. A total of 336 children between the ages of six and 36 months were recruited in four day-care centres. They received five drops of an oily suspension with *L. reuteri* (1×10^8 CFU/day) (n = 168) or a placebo (n = 168) per day. The intervention lasted twelve

weeks. The data from all 336 children were then evaluated. In the *L. reuteri* DSM 17938 group, a significantly lower frequency and duration of diarrhoeal diseases and respiratory infections as well as fewer days absent from the care facility, less frequent visits to the doctor and less administrations of antibiotics were registered. No differences in weight, height or stool frequency were observed. It should be noted that the age range of the children (six to 36 months) was very broad and also included small children. In addition, no information was given on the randomisation procedure. Since the study was carried out in Mexico and *L. reuteri* DSM 17938 was administered in the form of an oily suspension, the results cannot be transferred to infant formula supplemented with *L. reuteri* DSM 17938.

In another intervention study by Garofoli et al. (2014), 40 healthy, breastfed infants were recruited in the first three days after birth and treated with *L. reuteri* DSM 17938 in an oily suspension (10^8 CFU in five drops) (n = 20) or placebo (n = 20) for 28 days to examine the effect on the occurrence of gastrointestinal infections. The parents were asked to record daily crying times in minutes, stool frequency and consistency, occurrence of reflux symptoms and adverse effects. At the end of the intervention period, all infants were examined, various growth parameters were measured and the occurrence of gastrointestinal diseases was recorded. Saliva samples were also taken to determine the concentration of secretory (s)IgA. There were no differences between the intervention and control groups in growth, crying times, stool frequency and consistency and sIgA concentrations. However, three infants in the control group had been treated with a drug for gastrointestinal disorders. Furthermore, symptoms of reflux were observed significantly less frequent in the verum group over the period of the intervention. Since the study groups differed significantly in terms of gender distribution (proportion of male infants in the verum and control group: 20 versus 70%), the authors also examined whether the occurrence of reflux symptoms could have been influenced by the gender of the children. A gender-specific analysis showed that male infants were indeed more frequently affected by reflux than female ones. Although this difference was not significant, the data suggests that, contrary to the authors' claim, the gender of the children might have had an impact on the treatment effect. Adverse effects on the growth and health of the children were not observed. As *L. reuteri* was only administered for a short time and in form of drops, the results cannot be transferred to the routine use of *L. reuteri*-containing infant formula.

L. reuteri DSM 17938 drops in different concentrations (4×10^8 or 10^9 CFU/day) were found to be ineffective in preventing hospital-acquired diarrhoea in hospitalised infants and young children (Urbańska et al., 2016).

In summary, no sufficient evidence for a health benefit from the use of *L. reuteri* DSM 17938-containing infant formula for the **prevention** of diarrhoeal diseases in infants can be derived on the basis of the available study data.

3.2.3.2 Prevention of colic, reflux and constipation

Indrio et al. (2014) investigated the possibility of using *L. reuteri* DSM 17938 for the prevention of colic, reflux and constipation in healthy infants. They recruited 554 infants within the first week after birth and after randomisation (stratified according to gender and gestational age) treated them with either *L. reuteri* DSM 17938 in oily suspension (10^8 CFU per day) or an otherwise identical placebo over 90 days. After one and after three months, the data from 468 infants (15% drop-out) were evaluated. The results suggest a positive effect of *L. reuteri* DSM 17938 - administered in oily suspension - on the gastrointestinal functions of the infants: after one month, the verum group exhibited significantly less crying time and a significantly higher stool frequency, and after three months also fewer reflux symptoms. It should be noted critically that the gestational age and the average birth weight of the infants in the

probiotic group were significantly higher than in the control group and that more infants in the probiotic group were born vaginally and breastfed. It is unclear whether these differences could have distorted the study results. Regardless of this, no reliable conclusions can be drawn regarding the health effects of *L. reuteri* DSM 17938 used as an ingredient in infant formula.

The above mentioned study by Cekola et al. (2015) also recorded parameters on the mood of the infants (satisfaction and crying behaviour) and sleep behaviour (duration of sleep), from which conclusions about the occurrence of colic might potentially be drawn. As no differences were found in these parameters, a preventive effect of *L. reuteri* DSM 17938-containing infant formula on the occurrence of infant colic could not be derived from this study either (Cekola et al., 2015).

Finally, Savino et al. (2015) undertook a study with 105 infants who received either *L. reuteri* DSM 17938 drops in combination with vitamin D (n = 55) or only vitamin D (n = 58) from their parents at home from the first 14 days after birth up to the age of three months. In both groups, 90% of the infants were exclusively breastfed at the start of the study. At the age of three months results showed that the *L. reuteri* DSM 17938 group had received less colic preparations and that mothers in this group had called their doctor less often about colic incidences than in the vitamin D group. In addition, the number of infants who were still exclusively breastfed was higher in the *L. reuteri* group than in the vitamin D group. The results of this methodologically flawed study (e.g., the assignment to one of the two groups was neither blinded for the parents nor for the attending physician) provide indications, but no reliable evidence of a benefit of *L. reuteri* DSM 17938 for the prevention of colic in breastfed infants.

In summary, there is insufficient scientific evidence that *L. reuteri* DSM 17938-containing infant formula reduces the risk of colic, reflux and constipation in healthy infants.

3.2.3.3 Treatment of colic, reflux, constipation or diarrhoeal diseases

a) Colic

In a controlled intervention study, Savino et al. (2010) investigated whether the supplementation of *L. reuteri* DSM 17938 reduced the symptoms and crying behaviour in otherwise healthy infants with colic (measured by the frequency of crying: three days per week or more and the duration of crying: three hours per day or longer). For this purpose, 50 exclusively breastfed infants aged ten to 60 days were supplemented with 10^8 CFU of *L. reuteri* DSM 17938 per day (in a mixture with sunflower oil) for 21 days. After 21 days, a significantly reduced duration of crying and a 50% reduction in the frequency of crying were identified in the intervention group. The treatment also resulted in a significant increase in the concentration of *lactobacilli* with detection of *L. reuteri* DSM 17938 in twelve of 13 faecal samples and a decrease in the *E. coli* concentration in the intervention group. No significant differences were found in the children's growth (weight, length, head circumference) by the end of the study. There were also no differences in stool frequency or in the frequency of constipation and spitting up, and no adverse effects were observed in connection with supplementation (Savino et al., 2010).

Szajewska et al. (2013), Chau et al. (2015) and Mi et al. (2015) also reported positive effects of *L. reuteri* DSM 17938 **in droplet form** from placebo-controlled intervention studies with 80, 52 and 42 exclusively or predominantly breastfed healthy infants (1×10^8 CFU/day) after use for 21 days. However, due to methodological deficiencies (small study groups, unclear or no blinding, subjective and non-comparable endpoints: reduction in crying times per day or

over the entire period of the intervention) the results of these three studies offer insufficient evidence that *L. reuteri* DSM 17938 can be used successfully for the treatment of colic.

Another study investigating the effectiveness of *L. reuteri* DSM 17938 in droplet form for treating colic was undertaken by Sung et al. (2014). The authors recruited 167 infants with symptoms of colic (according to Wessel criteria) within the first three months of life and treated them either with five drops of *L. reuteri* in an oily suspension (0.2×10^8 CFU per drop) or an otherwise identical placebo over a period of one month. The behaviour of the infants (crying phases and phases of restlessness) was documented daily by the parents and evaluated after 7, 14, 21 and 28 days; a follow-up took place after six months. In addition, the bacterial composition and calprotectin in the faeces were analysed after 28 days. *L. reuteri* was detected in only 45% of the infants in the probiotic group. Looking at the other endpoints, such as daily crying times, number of crying events per day and sleep duration, it was found that the group treated with *L. reuteri* had significantly longer periods of crying and restlessness than the control group after 28 days. Correspondingly, the sleep phases in the infants treated with probiotics were significantly shorter. Overall, there was a decrease in crying and restless times for all children over time; however, the decrease was more pronounced in the placebo group than in the intervention group. At the age of six months, the crying and restless times of the two groups no longer differed significantly. A stratified analysis of the data showed that the crying behaviour of breastfed infants was comparable in both groups, while those treated with *L. reuteri* DSM 17938 exhibited significantly longer crying times in non-breastfed infants (on average for infants up to six months: 78 minutes longer and for infants over six months: 88 minutes longer) than those in the placebo group. No other adverse effects were observed when using *L. reuteri* DSM 17938 drops. The study was sufficiently blinded and, according to the authors, a validated diary was used to document crying times. Therefore, the study overall could be considered methodologically more reliable than those previously published on this topic. It is noteworthy, however, that at the start (and presumably also in the further course) of the study 34% of the placebo group, but only 22% of the verum group, received a diet without cow's milk protein. In addition, the verum group showed longer crying times right from the beginning, which could have distorted the result.

Finally, Turco et al. (2020) investigated the effectiveness of a partially hydrolysed infant formula with reduced lactose content and *L. reuteri* DSM 17938 as an ingredient in comparison to a standard infant formula for the treatment of infant colic. The study included 241 (124/117) otherwise healthy infants with colic less than four months of age. The intervention was undertaken over a period of four weeks. The mean crying times measured on day 28 (primary endpoint) were significantly shorter in the group that had received the standard food than in the intervention group. No significant negative effects were reported.

Overall, the available study results do not provide sufficient evidence that a *L. reuteri* DSM 17938-containing (partially hydrolysed) infant formula can be used successfully for the treatment of infant colic. Although there are indications that *L. reuteri* DSM 17938 in an oily suspension in drop form appears to be effective in treating colic in breastfed infants, there is no proof of a benefit in this regard in the case of non-breastfed infants based on the study data available.

b) Reflux

Indrio et al. (2011) investigated the effect of *L. reuteri* (1×10^8 CFU/day) administered as drops to three to twelve months old non-breastfed infants (n = 42) who suffered from reflux. Study parameters included the symptoms of reflux (documented by the parents) and gastric emptying time (measured with ultrasound). Eight of the infants included (five in the verum

group and three in the control group) terminated the study prematurely. After 30 days, the remaining 34 infants in the verum group vomited significantly less frequently and had significantly accelerated gastric emptying. Adverse effects were not documented. Given the small number of subjects and a drop-out rate of 19%, these results provide indications, but no evidence, of a therapeutic benefit of *L. reuteri* drops in infants with reflux symptoms.

Indrio et al. (2017) examined the effect of *L. reuteri* DSM 17938-containing infant formula on gastric emptying and the frequency of reflux in infants with functional regurgitation[4]. The study included 80 infants (40/40) with a mean age of 60 days. Over a period of four weeks, the infants were fed either a *L. reuteri* DSM 17938-containing (2.8×10^6 CFU/g powder) infant formula based on partially hydrolysed whey protein and starch or a commercial infant formula based on 70% whey protein and 30% casein. At the end of the intervention period, significantly faster gastric emptying and less frequent regurgitation were observed in the verum group. Furthermore, the two groups did not differ in drinking quantities or the anthropometric parameters measured. Also, no negative effects were observed in connection with the intervention (Indrio et al., 2017). The study exhibits a number of methodological deficits and is not suitable to prove the effectiveness of an infant formula with *L. reuteri* DSM 17938 for the prevention or dietary treatment of regurgitation in infants due to the many differences in the formulae used (in addition to probiotics, the study formula was thickened with starch and contained partially hydrolysed protein). This is particularly true since a positive influence on regurgitation symptoms in infants is also discussed in connection with the other food characteristics (protein hydrolysate, added starch, ratio of whey protein to casein) (Leung and Hon, 2019; Salvatore et al., 2018; Tolia et al., 1992). In addition, given the short intervention period of four weeks, no conclusions can be drawn on the long-term effects of *L. reuteri* DSM 17938-containing infant formula on the basis of this study.

c) Constipation

Coccorullo, et al. (2010) investigated the effect of *L. reuteri* in droplet form (1.2×10^9 CFU/day) on stool frequency and consistency as well as on occurrence of crying phases in 44 non-breastfed infants older than six months and suffering from chronic constipation. The intervention was placebo-controlled over eight weeks. After two, four and eight weeks, the stool frequency in the verum group - according to the parents' statements - was significantly higher. However, the two study arms showed no differences in stool consistency or in the frequency of crying phases. Adverse effects were not documented (Coccorullo et al., 2010).

Sung et al. (2013), Anabrees et al. (2013) and Urbańska and Szajewska (2014) as well as Harb et al. (2016), Schreck Bird et al. (2017), Sung et al. (2018) and Xu et al. (2015) concluded in (systematic) reviews that although there was evidence that *L. reuteri* may have a positive effect on functional gastrointestinal disorders such as reflux, colic or constipation and abdominal pain, the evidence is not sufficient to recommend a routine use of *L. reuteri* DSM 17938 for the treatment of these disorders on scientific grounds.

d) Acute diarrhoeal diseases

Two intervention studies were identified in which *L. reuteri* DSM 17938 was used to treat acute diarrhoea in young children (Maragkoudaki et al., 2018; Szymanski and Szajewska, 2019). In both studies, an oral rehydration solution (ORS) supplemented with *L. reuteri* (2×10^8 CFU/day) alone or in combination with zinc, was used in well-nourished and otherwise healthy children (age: 1.3 to 2.4 or <5 years) with acute diarrhoea. The ORS supplemented

[4] Regurgitation = passaging of stomach contents into the oesophagus, also known as gastroesophageal reflux (GER). This is a physiological process that occurs more frequently in infants than in older children or adults. GER is considered pathological if the reflux episodes occur too frequently or last too long. If a pathological GER is detected in a pH-metry, it does not have any disease value per se and is not in need of therapy. Only gastroesophageal reflux disease (GERD) requires treatment.

with *L. reuteri* DSM 17938 was well tolerated by the children, but showed no positive effect on the duration of the illness compared to conventional ORS, although Szymanski and Szajewska (2019) reported slightly shorter hospital stays of children in the verum group (p = 0.048).

A systematic review on the use of probiotics in acute diarrhoeal diseases revealed – based on the included studies, which according to the authors were of poor methodological quality – very weak evidence of a positive effect of *L. reuteri* DSM 17938 for the treatment of diarrhoea in infancy and early childhood (Szajewska et al., 2014a; Szajewska et al., 2014b).

In summary, the available data do not provide sufficient evidence of positive effects of *L. reuteri* DSM 17938 for the treatment of colic, reflux, constipation or diarrhoeal diseases in infants. There is evidence that *L. reuteri* DSM 17938 in oily suspension appears to be effective for treating colic in breastfed infants. However, no health benefit can be derived from routinely feeding healthy infants with *L. reuteri* DSM 17938-containing infant formula.

3.3 Bifidobacteria

3.3.1 Hazard identification

Additions of *Bifidobacterium (B.) lactis* BB-12 and a mixture of the bifidobacteria *B. breve*, *B. bifidum*, *B. infantis* and *B. longum* were identified in infant and follow-on formulae offered on the German market. For the latter, information on the strains used is not available.

Bifidobacteria are natural components of the intestinal flora of adults and one of the genera that first colonise the gut of infants. They belong to the intestinal bacteria that, inter alia, stimulate the maturation of gut-associated lymphoid tissue (GALT), regulate the permeability of the intestinal mucosa, inhibit the growth of pathogenic bacteria, attenuate inflammatory processes, ferment undigested food components and inhibit the production of pro-inflammatory cytokines (Isolauri et al., 2001), which is why they are considered probiotic. In the technical rules for biological agents (TRBA 466) of the Federal Office for Occupational Safety and Health, bifidobacteria were classified in safety class 1 as non-pathogenic for humans.

Only very few local or severe systemic diseases in humans have been reported in connection with bifidobacteria. A total of 21 cases of human bacteraemia caused by *Bifidobacterium spp.* have been identified in the literature, seven of which occurred in infants (Weber et al., 2015).

Antibiotic resistance studies have shown that *B. animalis ssp. lactis* has acquired tetracycline resistance (Kastner et al. 2006; Masco et al. 2006). However, the expected health risk for children was considered to be low, partly because tetracycline is not normally used in infants and young children, and laboratory tests have shown that the resistance gene is not transferred to other bacteria in the gut (Gibson et al., 2009).

3.3.1.1 Bifidobacterium animalis ssp. lactis BB-12

Bifidobacterium animalis ssp. lactis BB-12 (former name: *Bifidobacterium bifidum* BB-12 or also: *Bifidobacterium lactis* BB-12; hereinafter *B. lactis* BB-12) is considered to be sufficiently characterised for use in food (EFSA, 2011). *B. lactis* BB-12 was granted GRAS status by the FDA in 2003 for use in foods, including infant formulae for infants four months of age and older[5]. It should be pointed out that GRAS status is granted by the FDA solely on the basis of

[5] GRN No. 49 of 19 March 2002 and 28 November 2005: Substance: *Bifidobacterium lactis strain* Bb12 and *Streptococcus thermophilus strain* Th4; Intended Use: Ingredients in milk-based infant formula that is intended for consumption by infants four

the documents submitted by the manufacturers and that the FDA itself does not conduct any safety assessments for the bacterial strains.

3.3.1.2 B. breve, *B. animalis*, *B. longum* and *B. bifidum*

EFSA has classified *B. breve*, *B. animalis*, *B. longum* and *B. bifidum* as QPS (Qualified Presumption of Safety) (EFSA, 2013). The FDA has issued GRAS notices for *B. breve* M-16V (GRN 455[6]) for use in infant formulae for special purposes and for *B. longum* BB536 (GRN 268[7]) for use in foods, including infant formulae for infants of nine months of age and older. It should also be noted here that GRAS status is granted by the FDA solely on the basis of the documentation submitted by the manufacturers and that the FDA itself does not perform any safety assessments for the bacterial strains.

Although bifidobacteria can generally be regarded as safe, the BfR is of the opinion that specific investigations of the behaviour towards antibiotics should be carried out for each strain - in accordance with the specifications of EFSA (2005).
For the microorganisms to be assessed here, i.e. *B. breve*, *B. bifidum*, *B. infantis* and *B. longum*, no assessment can be made of possible antibiotic resistance and general safety for infants, as it is not known which bacterial strains are used in the infant formulae offered on the German market.

3.3.2 Risk assessment of *Bifidobacterium animalis* ssp. *lactis* BB-12

The tolerability and safety of infant formula with *B. lactis* BB-12 - alone or in combination with other bacterial strains - was investigated in three controlled studies with healthy infants (Weizman and Alsheikh, 2006; Saavedra et al., 2004; Vlieger et al., 2009). In these studies, after placebo-controlled feeding of infant formula containing *B. lactis* BB-12 in doses of 10^6 and 10^7 CFU per g of formula (in powder form) to healthy, term infants over a period of one to a maximum of six months, no differences were observed in growth or other parameters such as feeding behaviour (meal frequency and amounts consumed) as well as stool frequency and characteristics (e.g. consistency, colour, pH value, type and number of bacteria in the faeces). Furthermore, no significant differences in the frequency and severity of adverse effects such as vomiting, spitting up and diarrhoea were observed and, according to the authors, no other adverse effects associated with feeding occurred.

Given that bifidobacteria have cariogenic potential due to their acid-producing properties, Taipale et al. (2012) conducted an intervention study to investigate the effects of *B. lactis* BB-12 on oral colonisation with *Streptococcus mutans* in infants aged one to two months. The study included 94 infants who were given placebo-controlled tablets containing either xylitol alone or in combination with *B. lactis* BB-12 (5×10^9 CFU). Of the infants treated with xylitol preparations - with or without *B. lactis* BB-12 - (N = 75; 37/38), 67 (32/35) were followed up until the age of two years. At eight months and two years of age, the effect on *Streptococcus*

months and older, at levels not to exceed good manufacturing practice (https://www.accessdata.fda.gov/scripts/fdcc/?set=GRASNotices&id=49&sort=GRN_No&order=DESC&startrow=1&type=column&search=GRN%20No%2E%C2%A4DECIMAL%C2%A449; last accessed 07 July 2020).

[6] GRN No. 455 of 23 January 2013: Substance: *Bifidobacterium breve* M-16V; Intended Use: As an ingredient in exempt term powdered amino acid-based formulas, at levels providing 10^8 colony forming units per gram of infant formula powder (https://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=455; last accessed: 18 August 2020).

[7] GRN No. 268 of 08 July 2009: Substance: *Bifidobacterium longum strain* BB536; Intended Use: Ingredient in breads/baked goods, cereals, dairy products/dairy-based foods and dairy substitutes, fruit products, candy, chewing gum, cocoa powder, condiment sauces, flavoured beverage syrups, fruit flavoured powder beverage mixes, gelatine desserts, gravies, margarine, peanut and other nut butter/spreads, snack foods, weaning foods at a maximum level of $1x10^{10}$ colony forming units (cfu) per serving and in milk based powdered infant formula at a level of $1x10^{10}$ cfu per gram of infant formula powder that is intended for consumption for term infants aged 9 months and older *B. longum strain* BB536 (https://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=268; last accessed: 07 July 2020).

mutans colonisation was measured. No permanent oral colonisation with *B. lactis* BB-12 and no significant differences in the colonisation with *Streptococcus mutans* were identified, from which the authors concluded that *B. lactis* BB-12 had no negative effects on dental health. It must be said that the number of test subjects was small and that only some of the test subjects followed the prescribed intake of *B. lactis* BB-12 over the entire period of two years; the preparations were only taken for an average of 15 months. Adverse effects associated with the intake of *B. lactis* BB-12 were not observed (Taipale et al., 2013; Taipale et al., 2016).

A number of further intervention studies were carried out with the primary aim of investigating any possible beneficial health effects of *B. lactis* BB-12-fortified infant formula (see 3.3.3). No adverse effects were observed in these studies either.

In summary, based on the available study results, there is no evidence of adverse effects of *B. lactis* BB-12 or infant formula containing *B. lactis* BB-12 up to the concentrations used in the studies in healthy infants.

The available studies were of varying methodological quality and were predominantly conducted with the primary aim of demonstrating positive effects of *B. lactis* BB-12. In some cases, only small study groups were examined, with short intervention and follow-up periods. The criteria established by international organisations for the safety assessment of infant formula to which new, non-essential substances such as bacterial strains have been added were only partially met in the studies.

3.3.3 Benefit assessment of *Bifidobacterium animalis ssp. lactis* BB-12

a) Effects on the intestinal flora and the immune system

A placebo-controlled intervention study by Bakker-Zierikzee et al. (2005) investigated the effects of normal infant formula in comparison to infant formula supplemented with GOS/FOS (90:10) or *B. lactis* BB-12 (6×10^{10} CFU per litre of formula) on the intestinal flora. After 16 weeks of intervention, the group fed with *B. lactis* BB-12-containing formula showed no differences in the faecal concentration of bifidobacteria, short-chain fatty acids, lactic acid and pH of the stool compared to the control group. The concentrations of sIgA (secretory immunglobuline A) measured in the stool of the *B. lactis* BB-12 group also did not differ from those in the control group (Bakker-Zierikzee et al., 2006).

Rautava et al. (2006) investigated the effect of *B. lactis* BB-12 on the development of infectious diseases in 81 infants under two months of age. The infants were fed placebo-controlled formula with *B. lactis* BB-12 and *L. rhamnosus* (1×10^{10} CFU per g of formula) until the age of twelve months. Concentrations of sIgA and other immunologically relevant factors (TGF-β2, sCD4) were examined in the serum. No significant differences were found between the intervention and control groups in this study either.

In another study by Holscher et al. (2012), the effect of *B. lactis* BB-12-containing infant formula on the concentrations of sIgA in the serum was investigated in 172 infants. After two and six weeks, vaginally delivered infants who had been fed the intervention formula tended to show a greater increase in sIgA concentrations compared to the control group. However, in infants delivered by caesarean section no effect of the intervention on sIgA levels was observed.

b) Effects on the growth of healthy infants

The effects of *B. lactis* BB-12 added to infant formula on infant growth have been investigated in several controlled intervention studies, which were included in a systematic review

by Szajewska et al. (2010) as well as Szajewska and Chmielewska (2013)[8]. In total, the systematic review analysed seven intervention studies including healthy, full-term infants, who had been fed *B. lactis* BB-12-containing infant formula for one to a maximum of seven months. The doses of *B. lactis* BB-12 used ranged from 10^6 to 3.6×10^9 CFU per g of formula. The compositions of the infant formulae used in the studies were otherwise very heterogeneous, especially with regard to their protein content (1.8 to 2.2 g protein/100 kcal) and their degree of hydrolysis. Furthermore, the studies were of varying quality with regard to the randomisation and blinding procedures, and sometimes had high drop-out rates with relatively small numbers of subjects. For the growth parameters investigated - increase in weight, length and head circumference as well as body mass index (BMI) - no significant differences were found between intervention and control groups, neither in the individual studies nor in the overall results (Szajewska and Chmielewska, 2013).

Among the studies considered in another analysis by Steenhout et al. (2009), some were carried out in South Africa with infants whose mothers were HIV-positive and who showed a tendency towards higher weight gains when fed *B. lactis* BB-12-containing infant formula (Steenhout et al., 2009). The results of these studies suggest that feeding *B. lactis* BB-12-containing infant formula may have a beneficial effect on infant weight gain under certain conditions. However, the results cannot be transferred to healthy infants in industrialised countries.

In an intervention study by Mitra et al. (2014), the results of which are only available to the BfR in form of a conference abstract, 92 infants were fed infant formula containing either *L. reuteri* DSM 17938 or *B. lactis* BB-12 or no microorganisms. A reference group of breastfed infants was used for comparison. In the second half-year of life, the three groups fed infant formula had significantly higher anthropometric values (z-scores of weight, length and head circumference) than the breastfed infants. However, there were no significant differences between the three intervention arms.
From the BfR's point of view, the differences in growth observed in this study between breastfed and non-breastfed infants are less related to the addition of either *L. reuteri* DSM 17938 or *B. lactis* BB-12 than to the feeding of infant formula versus breast milk. This assumption is supported by findings from other studies in which body composition and anthropometric measures differed significantly between breastfed and non-breastfed infants (Dewey, 1998; Dewey et al., 1995). Furthermore, it should be noted here that the study by Mitra et al. (2014) was carried out in India; thus it is questionable whether the results can be transferred to the situation in Germany.

c) Effects on diarrhoeal diseases, other infections and allergies

In an intervention study by Chouraqui et al. (2004) no differences in the frequency and severity of acute diarrhoea, but in the number of days with diarrhoea per child, were observed in 90 infants who were initially about four months old and fed, in a placebo-controlled manner, infant formula containing *B. lactis* BB-12 for around 140 days.

Rautava et al. (2009) observed significantly fewer cases of acute otitis media and a significantly lower use of antibiotics as well as a lower recurrence rate of respiratory infections in a total of 72 infants fed infant formula with *Lactobacillus GG* and *B. lactis* BB-12 over a period of seven months. The effects observed cannot be clearly attributed to *B. lactis* BB-12, as the infant formula used contained two different bacterial strains.

[8] The meta-analysis by Szajewska and Chmielewska (2013) is an update of the earlier one by Szajewska et al. (2010).

In the intervention study by Taipale et al. (2011) already mentioned in section 3.3.2, *B. lactis* BB-12 was detected in the faeces of 62% of the infants supplemented with *B. lactis* BB-12 (5×10^9 CFU) at the age of eight months. No significant differences in the incidence of gastrointestinal infections, acute otitis media and the use of antibiotics, but a significantly lower rate of respiratory infections were observed. Since the primary aim of this study was to investigate the effects of *B. lactis* BB-12 on oral colonisation with *Streptococcus mutans*, no reliable conclusions on other health effects of *B. lactis* BB-12 can be drawn.

Weizman et al. (2005) investigated the effectiveness of infant formula supplemented with *B. lactis* BB-12 or *L. reuteri* ATCC 55730, each with 10^7 CFU per g of formula (powder), versus placebo in preventing infections in 201 (73/68/60) infants aged four to ten months. The infant formulae were fed for 12 weeks. Primary endpoints were frequency and duration of fever, respiratory diseases, diarrhoea and frequency of hospital visits, prescriptions of antibiotics, and days absent from childcare. In addition, feeding behaviour, growth parameters, behavioural changes, stool characteristics and any adverse effects were recorded. The children were examined at the beginning of the study and after four, eight and 12 weeks. The three study arms did not differ substantially at the start of the intervention. During the study period, fever and diarrhoea occurred less frequently in the *B. lactis* BB-12 group compared to the control group; the two study arms did not differ in the frequency of respiratory diseases and the other parameters.

Another controlled intervention study in day-care centres in Zagreb (Croatia) investigated whether the intake of *B. lactis* BB-12 reduced the occurrence of gastrointestinal and respiratory infections in children aged four to five years. 210 children (104/106) from three institutions were included in the study. After randomisation they received either the study product (10^9 CFU/day) or a placebo, each in powder form and stirred into milk, yoghurt or other beverages, over a period of 90 days. The parents kept a diary on compliance and the illnesses that occurred in the children as well as their duration and the resulting days of absence from the day-care centre. The evaluation of the data showed no difference between the intervention and control groups in the frequency and duration of respiratory or gastrointestinal infections and thus no benefit from *B. lactis* BB-12 for the prevention of these infections, which frequently occur in childhood (Hojsak et al., 2016).

Finally, Bocquet et al. (2013) conducted a multi-centre intervention study to assess the effects of infant and follow-on formula with either *B. lactis* CNCM I-3446 (identical to *B. lactis* BB-12) or *B. lactis* CNCM I-3446 and GOS/FOS to investigate the frequency of occurrence of infections. The study included 568 non-breastfed infants within the first month of life. The study formulae were fed until the end of the first year of life. Of 439 infants who remained in the study until the end (23% drop-out), 321 (156/165) data records, i.e. 56% of the originally recruited infants were included in the analysis. The authors stated protocol violations as the reason for the drop-out of 129 subjects. A per-protocol analysis of the data revealed no significant differences in the frequency of infectious diseases, growth, antibiotic use, as well as stool consistency and frequency between the intervention groups. In about half of the two study arms (49.5% of the *B. lactis* BB-12/GOS/FOS group and 54% of the *B. lactis* BB-12 group), unspecified gastrointestinal disorders were registered. It cannot be ruled out that these complaints were due to the intake of *B. lactis* BB-12. Given the high drop-out rate and the fact that there was no control group (without *B. lactis* BB-12 exposure) in this study, the study results are not suitable as evidence for the safe and effective use of *B. lactis* BB-12-containing infant formula.

To investigate the possibility of using *B. lactis* BB-12 for the treatment of allergies, Gore et al. (2012) conducted an intervention study in 208 three to six month old infants with allergic eczema. The infants were fed for three months extensively hydrolysed infant formula containing either *B. lactis* CNCM I-3446 (identical to *B. lactis* BB-12) or *L. paracasei* CNCM I-2, each at concentrations of 10^{10} CFU/day, or maltodextrin in the control group. The study aimed to investigate the benefits of the two bacterial strains in treating allergic eczema in addition to topical application of cortisone ointments. During the course of the intervention, there were significant improvements in eczema (determined using the SCORAD score) in all groups without distinction. Given that the improvements were also observed in the control group, the positive change cannot be attributed to the bacterial strains used. Also, no differences were found between the three study arms in other secondary parameters or in the progression of allergic diseases up to the age of one to three years. 31% of parents reported negative effects in their children such as vomiting, colic, green and soft stools and feed-refusal (Gore et al., 2012), which partly explain the high drop-out rate (17.5%). No positive health effect of *B. lactis* BB-12 can be derived from this study either. Based on the findings of this study, negative gastrointestinal effects caused by *B. lactis* cannot be completely ruled out.

In summary, the available study data do not allow any reliable conclusions on positive effects of feeding *B. lactis* BB-12-containing infant formula on immunological factors and/or growth as well as the development of infectious diseases in otherwise healthy infants. There is also insufficient scientific evidence for a health benefit of *B. lactis* BB-12 or *B. lactis* BB-12-containing (extensively or partially) hydrolysed infant formula.

3.3.4 Risk and benefit assessment of *B. breve*, *B. bifidum*, *B. infantis* and *B. longum*

The literature review for this Opinion identified an intervention study which used infant formula with a mixture of *B. breve*, *B. bifidum*, *B. infantis* and *B. longum* as study formula (Bazanella et al., 2017). The publication provides detailed information on the strain designations of the microorganisms used: *B. bifidum* BF3, *B. breve* BR3, *B. longum ssp. infantis* BT1 and *B. longum* BG7. The aim of this study was to investigate the effects of infant formula with the above mentioned bacterial strains on the intestinal flora of healthy infants in the first year of life and up to the age of two. The study included about 100 infants who were fed either the study formula or a comparable infant formula without added bacteria for six months. The concentration of bifidobacteria was a total of 10^7 CFU/g powder. In the first year of life, five stool samples from each child were examined and functional analyses carried out. In addition, the general state of health as well as the diet and growth of the children were determined or enquired via the parents. The data analysis revealed significant differences in the composition of the faecal flora between breastfed and non-breastfed infants as well as between vaginal and caesarean delivered infants. However, there were no significant differences between infants who were fed with or without the addition of bifidobacteria in the study formula. Also, no significant differences in growth, the use of antibiotics or other health parameters were observed between the two study arms (Bazanella et al., 2017).

The study results by Bazanella et al. (2017) suggest that infant formula with the bacterial strains *B. bifidum* BF3, *B. breve* BR3, *B. longum ssp. infantis* BT1 and *B. longum* BG7 at the concentrations used was well tolerated by healthy infants and that feeding this infant formula had no negative effects on the growth and development of healthy infants. There are no data from the study that would prove a health benefit of the infant formula used for healthy infants.

The BfR is not aware of any other studies in which infant formula containing a mixture of the bifidobacteria *B. breve*, *B. bifidum*, *B. infantis* and *B. longum* was used as study formula. It is also questionable whether the product used in the study by Bazanella et al. (2017) actually corresponds to one currently offered on the German market.

Furthermore, it is not possible to assess whether the results from other studies (Chouraqui et al., 2008; Hascoët et al., 2011; Kim et al., 2010; Puccio et al., 2007; Thibault et al., 2004), in which the efficacy of the bifido species *B. breve*, *B. bifidum* and *B. longum* was investigated, can be transferred to the infant formulae marketed in Germany.

Against this background, no reliable conclusions can be drawn on the safety and the expected benefits of infant formula containing these bacteria.

Overall, there is currently insufficient scientific evidence for positive effects of bifidobacteria, individually or in combination with other probiotics (synbiotics) or prebiotics, on growth or an improvement of clinical parameters in healthy infants (Braegger et al., 2011; EFSA, 2014; Gioia et al., 2014; Mugambi et al., 2012).

4 References

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