Part 3: Dietary Exposure

3.1 Intended Uses and Food Categories of GRAS Organism

Bifidobacterium longum subsp. infantis DSM 33361 intended to be consumed by the general population as well as term infants. Intended applications include but are not limited to the following: milk and dairy products such as yogurt and other fermented milk products; dairy alternatives (plant-based (oat, soy, almond, coconut, pea, etc.) fermented milk and yogurt products); beverages such as juice and protein shakes; shelf-stable products such as bars (granola, protein, meal replacement bars), confectionery (gummy candy, hard candy, soft chew candy, chewing gum, coatings), cereals (RTE and hot), and non-exempt infant formula (including cow-milk, soy, and protein hydrolysate based formulas). The addition level may be as high as $2.8x10^{10}$ CFU/serving to account for loss of viability throughout the shelf of the product for conventional foods, and $1x10^{10}$ cfu/g for infant formula.

3.2 Estimated Daily Intake

The initial addition level of *Bifidobacterium longum subsp. infantis* DSM 33361 may be as high as $2.8x10^{10}$ cfu/serving. It is expected to be present at a concentration of $1x10^{8}$ to $1x10^{11}$ cfu/serving at the time of consumption. The maximum ingestion of *Bifidobacterium longum subsp. infantis* DSM 33361 through conventional foods is likely to be less than $2.8x10^{10}$ cfu/day based on the assumption that the average consumption of a healthy individual is approximately 20 servings of all combined food per day. Intake of $1x10^{11}$ cfu/ day would be achieved by those who consume 10 servings of food containing $1x10^{10}$ cfu/serving of *Bifidobacterium longum subsp. infantis* DSM 33361 per day.

Bifidobacterium longum subsp. infantis DSM 33361 is also intended for use as a bacterial ingredient in non-exempt infant formula (including cow-milk, soy, and protein hydrolysate-based formulas) at levels up to $1x10^{10}$ cfu/g of powdered formula. According to the CDC (2018), a newborn will eat a 2oz serving of formula approximately 12 times in a day (24 hour period). Assuming that formula is the sole source of nutrition and is reconstituted at 14.1 g/100 mL (average reconstitution rate) the addition of $1x10^{10}$ cfu/g of *Bifidobacterium longum subsp. infantis* DSM 33361 in infant formula will result in a daily intake of $1x10^{12}$ cfu/day for a newborn baby.

Part 4: Self-Limiting Levels of Use

Bifidobacterium longum subsp. *infantis* does not have any self-limiting intake levels under the conditions of use described in this GRAS notification, other than it is restricted to applications that can sustain living *Bifidobacterium longum subsp. infantis* DSM 33361 for the intended level throughout the shelf life of the product.

Part 5: Experience Based on Common Use in Food Before 1958

The GRAS conclusion for the intended use of *Bifidobacterium longum* subsp. *infantis* DSM 33361 is based on scientific procedures and not based on common use in food before 1958.

Part 6: Narrative

In the following sections, the data and information providing the basis for our conclusion that the ingredient *Bifidobacterium longum* subsp. *infantis* DSM 33361 is GRAS through scientific procedures is presented. The information provided below and elsewhere in this document is generally available and has been properly cited. Chr. Hansen has rigorously applied the decision tree recommended by Pariza *et al.* and the risk assessment conducted by EFSA as per the QPS approach for the determination of the safety of *Bifidobacterium longum subsp. infantis DSM 33361* . Additionally, Chr. Hansen has conducted a thorough search of the scientific literature through October 2019 on the safety of *B. infantis.*

6.1 History of Consumption of GRAS Organism

Strains of *Bifidobacterium*, *Lactobacillus* and *Saccharomyces* have a long history of safe and effective use. The existing consumption of fermented milks for centuries and the growing knowledge about Bifidobacteria taxonomy and physiology supports the safe use of Bifidobacteria. In a review article, Picard et al. (2005) reported that Bifidobacteria are naturally present as the dominant colonic microbiota. These bacteria represent up to 80% of the cultivable fecal bacteria in infants and 25% in adults. As a lactic-acid producing bacteria in foods, these bacteria are considered to have little or no pathogenic potential (Picard et al. 2005). In the human gut, the presence of a variety of bacterial species has been reported by several researchers. The available literature suggests both individual and regional differences in gut microflora. However, a number of Bifidobacterium species are commonly present in the gastrointestinal tract of both infants and adults. As the age increases, the number of fecal Bifidobacteria decreases with reported concentrations of 10^{10} to 10^{11} in infants and 10^{5} to 10^{8} cfu/g feces in adults (Naidu et al., 1999). In another report the number of Bifidobacteria in the colon of adults is reported as 10^{8} to 10^{11} cfu/g but this number decreases with age (Orrhage and Nord, 2000). Typical Bifidobacterium species in infants were reported as *B. bifidum, B. infantis, B. breve*, and *B. parvulorum*, while *B. bifidum* and *B. longum* could often be found in both infants and adults (Reuter, 2001). There are many species of Bifidobacteria and *B. infantis* is one of the most abundant species isolated from feces of exclusively breast-fed infants (LoCascio et al., 2010).

Among the probiotic microorganisms, *Lactobacillus* spp. and *Bifidobacterium* spp. have been utilized globally in fermented food products and commercially-produced food supplements. The identification and description of Bifidobacteria first appeared during the early 20th Century (Poupard et al., 1973). All known species of Bifidobacteria are reported to be non-motile, non-sporulating, anaerobic, Gram-positive, catalase-negative (except *B. indicum and B. asteroids*) and saccharoclastic (Poupard et al., 1973; Holt et al., 1994). All species of Bifidobacteria are grouped in six different ecological niches: the human intestine, oral cavity, food, the animal gastrointestinal tract, the insect intestine and sewage (Ventura et al., 2004). Bifidobacteria

were first isolated from the feces of breast-fed infants in 1899 by Tissier and since then Bifidobacteria have been isolated from a range of different ecological niches (O'Callaghan and van Sinderen, 2016). Identification of these bacteria at species levels is based on phenotypic and biochemical features that include cell morphology, sugar fermentation profiles and electrophoretic mobility of enzymes. These features constitute the first taxonomical keys used in any bacterial classification. More recent tools, such as DNA-DNA reassociation studies provides approximately 70% or greater DNA-DNA relatedness, while the accurate identification of many bacterial species can be accomplished by reference to rRNA gene sequences (mainly the 16s rRNA gene). All species of the genus *Bifidobacterium* form a coherent phylogenetic unit with over 93% identity among the 16s rDNA sequences found within the members of this genus (Ventura et al., 2004). Members of this genus produce lactic acid and acetic acid from glucose and are normal residents of the normal flora of the human intestine.

In summary, the historical uses of *Bifidobacterium* and available current information on marketing of products containing *B. infantis,* including the subject of present GRAS, supports the safety of *Bifidobacterium longum subsp. infantis DSM 33361* .

6.2 *Bifidobacterium* and Safety

Similar to other orally consumed product, there exists potential for microorganisms or probiotics to enter the bloodstream through cuts in the mouth, gastric lesions, or during surgical procedures. In an extensive review, Borriello et al., (2003) reported that the risk of infection with Bifidobacteria is similar to that of infection with commensal strains, and that consumption of such products presents a negligible risk to consumers, including immunocompromised subjects. Septicemia caused by *Bifidobacterium* spp. is exceedingly rare. These genera have been used in a variety of food products for centuries and are regularly consumed by humans on a daily basis. *Bifidobacterium* spp. have been safely used in yogurts for more than half a century (Salminen et al., 1998). Additionally, these bacteria are components of the normal flora of the human gastrointestinal tract (Ahrne et al. 1998). Borrielo et al. (2003) noted that Lactobacilli and Bifidobacteria account for approximately 0.05 to 0.4% of cases of infective endocarditis or bacteremia. Cannon et al. (2005) reported that of the over 200 reported cases of bacterial infection in the literature, nearly all were found in a patient with an underlying, often severe, pathology.

Cohen et al. (2016) reported that until 2015, only 15 cases of *Bifidobacterium* bacteremias in adults had been reported in the literature, and these were predominantly among patients with underlying gastrointestinal disease and/or impaired immunity. In a large cohort study focusing on bloodstream infections caused by probiotic bacteria in 3,500 hematopoietic transplant recipients, no cases of Bifidobacterium bacteremia were found. The publicly available information lacks the evidence to suggest that consumption of foods containing Bifidobacteria increase the risk of opportunistic infection among immunocompromised patients. Additionally, available information from human clinical studies conducted to investigate safety of bacterial

ingredients in small groups of specific immunocompromised patients (HIV infection) support the safety of these ingredients consumed by such groups (Borriello et al., 2003). In general, *Bifidobacterium* strains are considered to be non-pathogenic to humans (Carr et al., 2002). The available evidence indicates that *Bifidobacterium* spp. lack invasive properties, i.e., these bacteria will not cross the epithelial boundary of the intestine and reach deep tissue, and that they are not mucinolytic (Zhou et al., 2000a, 2000b; 2001).

The available extensive literature of clinical studies in humans on *Lactobacillus* and *Bifidobacterium* supports the safe use of lactic acid producing group of microorganisms. These studies on the effects of *Bifidobacterium* are primarily focused on the efficacy, not on safety *per se*. However, the reasonable conclusion can be drawn from these studies that *Bifidobacterium* is safe for consumption. Ouwehand et al. (2004) investigated the presence of known virulence factors in clinical blood isolates, dairy and fecal isolates of Bifidobacteria. No significant differences were noted between clinical and fecal isolates. These findings confirm the general opinion that Bifidobacteria are safe. As *Bifidobacterium* has not been shown to lead to systemic infections in humans, such beneficial effects in the gastrointestinal tract support the safety of the organism. In a review article, Saarela et al. (2002) stated that the long history of safe use of lactobacilli and Bifidobacteria remains the best proof of their safety.

6.3 Clinical Trials Using *Bifidobacterium infantis*

The clinical database of studies with *B. infantis* includes multiple clinical trials in infants and adults, of which several have been identified as double-blind, placebo-controlled trials. As double-blind, placebo-controlled clinical trials are least likely to result in bias and will capture the adverse effects, the clinical studies of *B. infantis* provide an opportunity to assess the safety and tolerability in a fairly diverse population. The objective of majority of clinical trials in adults and infants was to study the efficacy of *B. infantis*, however clinical observations also included adverse effects. The findings from these studies did not reveal any significant adverse events of *B. infantis* treatment. Thus, the available evidence from the composite clinical trials supports the safety of *B. infantis* at the levels tested.

6.3.1 Clinical Trial Summaries

In a randomized, double-blind, placebo-controlled study, Langkamp-Henken et al. (2015) examined the effect of three bacteria on the proportion of healthy days over a 6-week period in academically stressed undergraduate students (n=581) who received $3x10^9$ cfu/day of *L. helveticus* R0052, *B. infantis* R0033, *B. bifidum* R0071 or placebo. 145 healthy students received one capsule of *B.* infantis ($3x10^9$ cfu). One participant receiving *B. infantis* withdrew after one day because of abdominal pain. After approximately 2 weeks of supplementation, two participants discontinued the supplement (placebo and *B. infantis*) due to diarrhea but completed the remainder of the study-related activities. No other adverse effects of *B. infantis*

were reported. The findings from this clinical study showed that consumption of these three strains, including B. infantis, was well tolerated.

In an exploratory, randomized, double-blind, placebo-controlled study, Smecuol et al. (2013) investigated the effects of *B. infantis* natren in active celiac disease. In this study, 22 adult patients with 2 positives celiac disease-specific tests were randomized to receive 2 capsules before meals for 3 weeks of either *B. infantis* $2x10^9$ cfu/capsule; 2 capsules 3 times per day 15 minutes before meals (n = 12) or placebo (n = 10). The subjects also consumed at least 12 g of gluten/day. The administration of *B. infantis* ($1.2x10^{10}$ cfu/day) for 3 weeks was reported to be safe.

In a non-randomized, open-label, controlled before-and-after study, Ma et al. (2018) investigated the effects of *B. infantis* M-63. In this study, of the 53 participants, 20 with IBS were given *B. infantis* M-63 ($1x10^9$ cfu/sachet/day) for three months and 33 served as controls. No adverse effects of *B. infantis* were reported.

Giannetti et al. (2017) investigated the effects of a probiotic mixture of *B. infantis* M-63, *B. breve* M-16V, and *B. longum* BB536. The findings from this study show that a mixture containing *B. infantis* M-63 and two other strains (*B. breve* M-16V and *B. longum* BB536) to be safe.

In a review article, Giannetti and Staiano (2016) reported that in children with IBS, consuming a mixture of *B. infantis* M-63®, *B. breve* M-16V® and *B. longum* BB536® is safe.

In summary, the findings from above described clinical studies, including double-blind placebo-controlled, studies suggest *B. infantis* is unlikely to cause adverse effects.

6.3.4 Clinical Trials in Infants

In a randomized, double-blind, placebo-controlled trial, Bin-Nun *et al.* (2005) compared the effect of a combination (*B. longum* subsp. *infantis* DSM 33361, *S. thermophilus* TH-4 and *B. animalis* subsp. *lactis* BB-12®) of $1x10^9$ cfu /day with placebo. The authors did not find any adverse events of the intake of this combination which included the *B. infantis* strain (DSM 33361) that is the subject of this GRAS notification.

In a prospective multicenter, double-blinded, placebo-controlled, randomized trial (also known as ProPrems trial), Jacobs et al. (2013) compared daily administration of a combination (*B. longum* subsp. *infantis* BB-02, *S. thermophilus* TH-4 and *B. animalis* subsp. *lactis* BB-12, containing $1x10^9$ total organisms) with placebo (maltodextrin) in infants born before 32 completed weeks' gestation weighing <1500 g. The *B. infantis* used in this study is the subject of this GRAS notice (*Bifidobacterium longum subsp. infantis* DSM 33361). The investigators also stated that consumption of these bacterial ingredients appears to be safe.

In a double-blinded, randomized, multicenter, controlled clinical trial, Escribano et al. (2018) investigated the effectiveness of an infant formula supplemented with *B. longum* subsp. *infantis* CECT7210 (*B. infantis* IM1) in reducing diarrhea incidence in healthy term infants. In this study, formula-fed infants (<3 months) received either an infant formula supplemented with 10^7 cfu/g of *B. infantis* IM1, or not (Control) over 12 weeks. There were no significant differences in the presence of adverse events between study groups nor in the absolute number score of the severity of the adverse event.

In a randomized, double-blind, placebo-controlled trial, Manzano *et al*. (2017) investigated the safety and tolerance of three bacterial strains (*B. longum* subsp. *infantis* R0033, *B. bifidum* R0071 and *L. helveticus* R0052) in healthy infants aged 3 to 12 months. The investigators concluded that the use of *B. infantis* R0033, *L. helveticus* R0052 and *B. bifidum* R0071 in infancy is safe, and well tolerated.

In a Phase I clinical trial, Smilowitz et al. (2017) investigated the safety and tolerability of supplementing breastfed infants with *B. infantis* (EVC001). There was no difference in the number or type of reported adverse events between supplemented and non-supplemented infants. The investigators concluded that *B. infantis* supplement was safely consumed and well-tolerated.

In a sub-study of the above mentioned ProPrems trial, Plummer et al. (2018) investigated the effect of the bacterial strain B. infantis DSM 33361 (subject of this notification) on the gut microbiota in a cohort of very preterm infants. The study details of ProPrems are described above (Jacobs et al., 2013). No adverse effects of treatment were reported.

Ishizeki et al. (2013) investigated the effects of Bifidobacteria on the intestinal microbiota in low-birth-weight infants, and the transition of each strain of administered Bifidobacteria. In this study, a single strain of *B. breve* M-16V ($5x10^8$; one-species group) or a mixture of three species composed of *B. breve* M-16V, *B. infantis* M-63 and *B. longum* BB536 ($5x10^8$) of each strain; three-species group) were administered daily for 6 weeks. No adverse effects were reported.

In a multicenter randomized controlled double-blinded clinical trial, Al-Hosni et al. (2012) investigated the effects of bacterial ingredient-supplemented feeding (including a strain of *B. infantis*) in extremely low-birth-weight infants. There were no bacterial consumption-related adverse events reported.

In summary, available information from several well controlled clinical studies, including studies with subject of present GRAS, in infants suggest that *B. infantis* is well tolerated.

6.4 Animal Toxicity and Other Studies of *Bifidobacterium infantis*

The results from acute and subchronic toxicity studies in rats demonstrate that under conditions of the tests, a specific strain of *B. infantis* presented no toxicological concerns at the highest doses tested. Based on the subchronic toxicity study of *B. infantis* in rats, no observed

adverse effect levels (NOAELs) was established to be the highest dose tested. The dose tested was $7.6x10^{10}$ cfu *B. infantis*/kg bw/day. Results from this study with *B. infantis* provide corroborative data to support the available evidence that *Bifidobacterium longum subsp. infantis* DSM 33361 is safe for human consumption.

Abe et al. (2009) evaluated safety of two Bifidobacterial strains, *B. breve* M-16V and *B. infantis* M-63, following single dose and 90-day repeated dose oral toxicity tests using rats. These studies were also described in the GRAS notice (GRN 268) submitted by Morinaga (2008) that received no question letter from FDA. In the single dose oral toxicity test using $1.4x10^{12}$ cfu/kg of *B. breve* M-16V or $3.2x10^{11}$ cfu/kg of *B. infantis* M-63, there were no death and no abnormalities. In the 90-day repeated dose oral toxicity test using $2.3x10^{11}$ CFU/kg bw/day of *B. breve* M-16V or $7.6x10^{10}$ CFU/kg bw/day of *B. infantis* M-63, no death and no abnormalities in body weight, food consumption, water consumption, urinalysis, hematology, blood biochemistry, organ weights, and histophathological findings were observed. The findings related to *B. infantis* in the acute and subchronic study are further discussed below.

In the single dose oral toxicity study, *B. infantis* M-63 powder was administered to a group of 5 male and 5 female Sprague Dawley rats once via a stomach tube at a dosage of 4000 mg/kg-bw powder, or approximately $3.2x10^{11}$ cfu/kg bw (Abe et al., 2009; Morinaga, 2008). All animals survived until scheduled euthanasia. General conditions and behavior were not adversely affected by the test substance in any of the animals. The body weights of all animals reportedly increased at a normal rate. No abnormalities were found in any of the animals at necropsy. According to the researcher, no histopathological examinations were conducted due to the absence of abnormalities at necropsy. The investigator reported that acute gastric administration of 4000 mg/kg bw of *B. infantis* powder was well tolerated. The LD_{50} for this study was determined to be greater than 4000 mg/kg bw powder, the highest dose tested, which is equivalent to approximately $3.2x10^{11}$ cfu *B. infantis*/kg bw.

In the subchronic study, *B. infantis* M-63 powder was administered to Sprague- Dawley rats (10/sex/group) by oral gavage at powder dose levels of either 0 or 1000 mg/kg-bw/day for 91 days (Abe et al., 2009; Morinaga, 2008). The number of viable bacteria in the powder was recorded as being $7.6x10^{10}$/g, equivalent to $7.6x10^{10}$ cfu/kg bw/day. Clinical conditions and behavior not affected by test substance. As compared to control, no changes in body weight or food consumption observed compared to control group. No treatment-related abnormalities found in urinalysis, ophthalmoscopic, hematological, blood chemistry or histopathological examinations. A decrease in absolute and relative weights of seminal vesicles in males and relative weights of spleen in females; changes were not considered to be related to treatment. The investigators concluded that oral intake of 1000 mg/kg bw/day of *B. infantis* M-63 powder ($7.6x10^{10}$ cfu/kg bw/day) for 91 days occurred without signs of toxicity. The NOAEL for this study was estimated to be above 1000 mg/kg bw/day, the highest dose tested. Based on these findings, Abe et al. (2009) determined the acceptable daily intake (ADI) of *B. infantis* M-63.

Using a specific safety factor of 100 fold, the ADI for *B. infantis* would be $4.56x10^{10}$ cfu/day for a 60 kg individual.

6.5 Recognition of Safety by an Authoritative Group of Qualified Experts

Bifidobacterium infantis species is described in the list of microorganisms with a documented history of safe use in food, assembled by International Dairy Federation (IDF) in collaboration with the European Food and Feed Cultures Association (EFFCA) (Bourdichon et al., 2012). The European Food Safety Authorities (EFSA) has granted Qualified Presumption of Safety (QPS) status for *B. infantis* (EFSA, 2017). In European countries, a strain belonging to a species listed on QPS and meeting the established criteria can freely be added to foods. The QPS concept was developed in 2007 to provide a harmonized generic pre-evaluation to support safety risk assessments of microorganisms intentionally introduced into the food and feed chain. The identity, body of knowledge, safety concerns and antimicrobial resistance of valid taxonomic units were assessed. The QPS status is given if the taxonomic group does not raise safety concerns or, if safety concerns exist, can be defined and excluded. The list of QPS recommended biological agents is updated every three years, with the latest version being released in December 2018.

Another document that is also used in Europe as reference regarding food culture safety besides the QPS list from EFSA is the Inventory of Microorganisms with Technological Beneficial Use from the International Dairy Federation (IDF). The IDF represents the global dairy sector and ensures that the best scientific expertise is used to support high quality milk and nutritious, safe, and sustainable dairy products (Laulund et al., 2017). In collaboration with the European Food and Feed Cultures Association (EFFCA), the International Dairy Federation (IDF) has assembled a list of microorganisms with a documented history of safe use in food (Salminen et al. 2002). This inventory includes the species *B. infantis*.

In Canada, Natural and Nonprescription Health Products Directorate (NNHPD) of Health Canada has listed *Bifidobacterium longum* ssp. *infantis* as eligible to be used for the general support of gastrointestinal health as well as it is also listed to show that its use in foods is allowed without a pre-marketing authorization. The Food Directorate of Health Canada published a list of species eligible to generic health claims as well in 2009, allowing freely its use, which in other terms reflects an established safe history of use. Similarly, the Australian Therapeutic Goods Administration (TGA) includes *B. infantis* on the "List of approved substances that can be used as Active ingredients in "Listed" Medicines". Additionally, *B. infantis* is permitted for use in other countries such as South Africa, India, China, etc.

In 2018, FDA reviewed a GRAS notice (GRN 758) submitted by Lallemand (2018) on use of *L. helveticus* R0052, *B. longum* subsp. *infantis* R0033, and *B. bifidum* R0071, both individually and in combination, as an ingredient in non-exempt powdered infant formulas for term infants at $5x10^7$ cfu/g of powder in infant formulas. As this formula also contains one of the *B. longum*

subsp. *infantis* strains, the safety studies on this strain described in GRN 758 are also applicable to the present GRAS and are incorporated in the present GRAS by reference.

In GRN 758, The FDA reviewed the notification and responded that it had no question (FDA, 2018).

6.6 *Bifidobacterium longum subsp. infantis DSM* 33361 is safe.

Members of the genus *Bifidobacterium* are among the first microbes to colonize the human gastrointestinal tract and are believed to exert positive health benefits on their host. Due to their purported health-promoting properties, Bifidobacteria have been incorporated into many functional foods as active ingredients. The risk of infection with Bifidobacteria is similar to that of infection with commensal strains, and that consumption of such products presents a negligible risk to consumers, including immunocompromised subjects. Bifidobacteria are not regarded as pathogens, although few cases of infection are reported, and these were predominantly among patients with underlying gastrointestinal disease and/or impaired immunity. Documented cases of *Bifidobacterium* bacteremia are very rare, in comparison to the widespread use of *Bifidobacterium* strains in the environment, in food production, and in other applications. Consumption of live lactic acid bacteria such as Bifidobacteria included in lactic-acid-fermented foods has been a regular part of the food intake of humans for hundreds of years.

The genome of *Bifidobacterium longum subsp. infantis* DSM 33361 was sequenced and genes were annotated and compared with databases of antibiotic resistance genes and virulence factors. No findings were suggestive of potential risk to consumers. The absence of genes encoding antibiotic resistance was confirmed by phenotypic testing for antibiotic resistance. Additional phenotypic testing demonstrated that the strain does not produce biogenic amines and this strain was found to produce 100% L-lactate, while D-lactate isomer is not produced.

All of the available evidence demonstrates clearly that there is no reason to suspect harm to individuals from the intended use of *Bifidobacterium longum subsp. infantis* DSM 33361.

6.7 Summary and Conclusion of GRAS Status

In summary, Chr. Hansen critically evaluated publicly available information and data on the safety of the intended use of *Bifidobacterium longum subsp. infantis* DSM 33361. Chr. Hansen used the Pariza et al. (2015) decision tree in its evaluation.

The basis for this GRAS conclusion is that the intended use of *Bifidobacterium longum subsp. infantis* DSM 33361 are GRAS includes the following:

1) The publicly available scientific literature documenting the safety of the use of this microorganism in a variety of foods as well as its use as a food ingredient;

2) *Bifidobacterium longum subsp. infantis* DSM 33361 is not genetically modified, is not able to produce biogenic amines, and does not carry any transferrable gene coding for antibiotic resistance;

3) Chr. Hansen's manufacturing and quality control programs ensure the safety and quality of the final *Bifidobacterium longum subsp. infantis* DSM 33361 product;

4) The expected daily intake of *Bifidobacterium longum subsp. infantis* DSM 33361 has been shown to be safe at proposed use levels and part of normal intestinal flora;

5) *Bifidobacterium infantis* has been evaluated and deemed safe and nonpathogenic by EFSA per the QPS approach and has been included in the IDF. *Bifidobacterium infantis* has been included as a microorganism in infant formula;

6) Chr. Hansen has used the Pariza et al. (2015) decision tree framework to evaluate and confirm safety of the organism for human consumption.

6.7.1 Pariza Decision Tree Analysis

As indicated above, in assessing the safety of *Bifidobacterium longum subsp. infantis* DSM 33361, Chr. Hansen has consulted and applied the Pariza et al. "Decision Tree for determining the Safety of Microbial Cultures to be Consumed by Humans or Animals" (2015). The decision tree is composed of thirteen questions which, when applied, provide a "comprehensive approach for determining the safety of microbial cultures that lack an established history of safe use for their intended new applications". This approach is described below:

Has the strain been characterized for the purpose of assigning an unambiguous genus and species name using currently accepted methodology?

YES (go to 2)

Has the strain genome been sequenced?

YES (go to 3)

Is the strain genome free of genetic elements encoding virulence factors and/or toxins associated with pathogenicity?

YES (go to 4)

Is the strain genome free of functional and transferable antibiotic resistance gene DNA?

YES (go to 5)

Does the strain produce antimicrobial substances?

NO (go to 6)

Has the strain been genetically modified using rDNA techniques?

NO (go to 8a)

8a. Was the strain isolated from a food that has a history of safe consumption for which the species, to which the strain belongs, is a substantial and characterizing component?

NO – the strain was isolated from the intestine of a healthy infant (go to 13a)

13a. For strains to be used in human food: Does the strain induce undesirable physiological effects in appropriately designed safety evaluation studies?

NO – (go to 14a)

14a. The strain is deemed to be safe for use in the manufacture of food, probiotics, and dietary supplements for human consumption.

Chr. Hansen concludes that the intended uses of *Bifidobacterium longum subsp. infantis* DSM 33361 are GRAS based on scientific procedures.

Part 7: List of Supporting Data, Information and References

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