From: Emily Gregoire
To: Morissette, Rachel
Subject: RE: questions for GRN 000950
Date: Wednesday, December 9, 2020 5:22:16 PM
Attachments: image001.png
2020-12-9 GRN 000950 Chr. Hansen response to FDA.zip

Dear Rachel,

Please see attached response and supporting material. Let me know if you have any questions.

Kind Regards / Venlig hilsen

Emily Gregoire

From: Emily Gregoire
Sent: Thursday, December 3, 2020 7:47 AM
To: Morissette, Rachel <Rachel.Morissette@fda.hhs.gov>
Subject: RE: questions for GRN 000950

Thank you Rachel, much appreciated.

Kind Regards / Venlig hilsen

Emily Gregoire

From: Morissette, Rachel <Rachel.Morissette@fda.hhs.gov>
Sent: Thursday, December 3, 2020 7:12 AM
To: Emily Gregoire <USEMGR@chr-hansen.com>
Subject: RE: questions for GRN 000950

Thanks. Sorry, I didn't see this email before I responded. Go ahead and submit everything at the same time so it's cleaner for the administrative record. Next Friday by COB is fine.

Best,

Rachel

Rachel Morissette, Ph.D.
Regulatory Review Scientist

Division of Food Ingredients
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
U.S. Food and Drug Administration
rachel.morissette@fda.hhs.gov

U.S. FOOD & DRUG ADMINISTRATION

From: Emily Gregoire <USEMGR@chr-hansen.com>
Sent: Wednesday, December 2, 2020 8:17 PM
To: Morissette, Rachel <Rachel.Morissette@fda.hhs.gov>
Subject: RE: questions for GRN 000950

Dear Rachel,

I have attached what I was able to complete. Please consider my request for extension to respond to the remaining questions.

Kind Regards / Venlig hilsen

Emily Gregoire

From: Emily Gregoire
Sent: Wednesday, December 2, 2020 3:43 PM
To: Morissette, Rachel <Rachel.Morissette@fda.hhs.gov>
Subject: RE: questions for GRN 000950
Importance: High

Dear Rachel,

At this time, I would like to request an extension to answer questions 5, 6, and 7 related to dietary exposure. We are being assisted by a consultant on these questions in order to correctly capture the correct data, and are not able to meet the desired deadline.

I can have the rest of the responses to you by the end of today, or return a completed response to you next week. Is it possible to have 5 more business days to respond?

My apologies for this request coming at the last minute.

Kind Regards / Venlig hilsen

Emily Gregoire

From: Morissette, Rachel <Rachel.Morissette@fda.hhs.gov>
Sent: Tuesday, November 17, 2020 10:57 AM
To: Emily Gregoire <USEMGR@chr-hansen.com>
Subject: RE: questions for GRN 000950

10 business days from today would be December 2, 2020.

Rachel

Rachel Morissette, Ph.D.
Regulatory Review Scientist

Division of Food Ingredients
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
U.S. Food and Drug Administration
rachel.morissette@fda.hhs.gov

FDA U.S. FOOD & DRUG ADMINISTRATION

From: Emily Gregoire <USEMGR@chr-hansen.com>
Sent: Tuesday, November 17, 2020 10:03 AM
To: Morissette, Rachel <Rachel.Morissette@fda.hhs.gov>
Subject: RE: questions for GRN 000950

Dear Rachel,

With the upcoming Thanksgiving Holiday is it possible for you to give an exact date?

Kind Regards / Venlig hilsen

Emily Gregoire

From: Morissette, Rachel <Rachel.Morissette@fda.hhs.gov>
Sent: Tuesday, November 17, 2020 8:27 AM
To: Emily Gregoire <USEMGR@chr-hansen.com>
Subject: questions for GRN 000950

Dear Ms. Gregoire,

Please see attached our questions for GRN 000950. Let me know if you have any questions at this time.

Best regards,

Rachel

Rachel Morissette, Ph.D.
Regulatory Review Scientist

Division of Food Ingredients
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
U.S. Food and Drug Administration
rachel.morissette@fda.hhs.gov

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Rachel Morissette, Ph.D.

Regulatory Review Scientist
FDA Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
Division of Food Ingredients

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Milwaukee, WI 53214 - 4298

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info@chr-hansen.com

December 9, 2020
USEMGR

Chr. Hansen Response to FDA's questions regarding GRN 000950

Dear Dr. Morissette,

We are happy to provide you with the answers to the majority questions regarding GRN 000950 received on Nov. 17, 2020.

We have marked any confidential material as such. Please reach out with any further questions.

Yours sincerely,

Emily Gregoire
Global Regulatory Affairs Partner

usemgr@chr-hansen.com
Mobile: 414-553-7198

Regulatory:

1. **A GRAS conclusion is for the use of a substance, not the substance itself. We note that in Part 1 of the notice (Signed Statements and Certification) in sections 1.1 Basis of GRAS Conclusion, 1.4 Statutory Basis for GRAS Determination, and 1.5 Premarket Approval Status, and the first paragraph of Part 6 Narrative, the notice states that *Bifidobacterium longum*subsp. *infantis* DSM 33361 is GRAS, not its use. Please provide revised text for those sections indicating that the use of *Bifidobacterium longum* subsp. *infantis* DSM 33361 is GRAS.**

Please see attached "Revised Part 1 for GRN 000950"

Chemistry

2. **Please provide specifications for the identification and assay of *Bifidobacterium longum subsp. infantis* DSM 33361, along with at least three nonconsecutive batch analyses to demonstrate that *Bifidobacterium longum* subsp. *infantis* DSM 33361 can be manufactured to meet the specifications.**

(b) (4) [redacted]

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3. **Please provide any specified limits for contaminants, such as heavy metals (e.g. lead), and residual components of the manufacturing process that may be of concern, along with at least three nonconsecutive batch analyses.**

Specified limits for contaminants

Environmental contaminants are controlled on the raw material level.
However one batch of the freeze-dried material has been analyzed for heavy metals as carry-over from process.

Parameter	limit	Results batch 3441814
Lead (Pb)	0,01 mg/kg	<3 µg/kg
Cadmium (Cd)	0,01 mg/kg	<0,01 mg/kg
Mercury (Hg)	0,01 mg/kg	<0,005 mg/kg
Arsenic (As)	0,1 mg/kg*	<0,1 mg/kg

Analysis reference to EN 13805:2014, EN ISO 17294m:2016. Method: ICP-MS. Performed by external laboratorium.
*based on rice destined for baby food ref. 2015/2006 amendment

4. **Please confirm that all analytical methods used to test for each specification parameter are validated for that purpose. If using standard methods of analysis, please provide complete and appropriate citations.**

Environmental Contaminants: Analysis reference to EN 13805:2014, EN ISO 17294m:2016. Method: ICP-MS. Performed by external laboratory

5. **We note that the estimated dietary exposure described in the notice for the intended use in conventional foods is based on assumptions that an average, healthy individual consumes approximately 20 servings of food/day and that approximately 10 of those servings would contain 1 x 10^{10} cfu/serving of the notified substance. Please provide a citation for the source of the estimated 20 servings of food/day and discuss the basis for the 10 servings/day assumption.**

Reference:

Millen, A. E., Midthune, D., Thompson, F. E., Kipnis, V., & Subar, A. F. (2006). The National Cancer Institute Diet History Questionnaire: Validation of Pyramid. *American Journal of Epidemiology*, 279-288.

By calculating intake assuming that half of all servings of food per day (10 servings) contain *Bifidobacterium longum* subsp. *infantis* DSM 33361, we are using a "worst-case-scenaria" approach, as it is highly unlikely that half of the conventional food consumed in a day would contain *B. infantis*.

6. **The dietary exposure estimate for the notified substance for infants is based on the intended use in non-exempt infant formula and an estimated consumption of approximately 24 fl. oz/day of formula for newborn infants. Please discuss the dietary exposure for infants 0 to 12 months of age and estimate the age group with the highest exposure to *Bifidobacterium longum* subsp. *infantis* DSM 33361. We recommend using consumption data for infant formula from the National Health and Nutrition Examination Survey (NHANES), for example, as summarized in Grimes, et al., Beverage Consumption among U.S. Children Aged 0–24 Months: National Health and Nutrition Examination Survey (NHANES). Nutrients 2017, 9, 264; doi:10.3390/nu9030264.**

The infant formula intake data described in Grimes, et al. confirms that infants age 0-6 months consume the highest amount of formula, 834 g reconstituted formula per day. Using the Grimes daily intake as well as the average reconstitution rate of 14.1 g powdered infant formula per 100mL water, we can use the following calculations to predict the maximum daily intake:

$$\left(\frac{1 \times 10^{10}\ CFU\ B.infantis}{1g\ powdered\ formula}\right)\left(\frac{14.1\ g\ powdered\ formula}{100\ mL}\right)\left(\frac{834\ g\ reconstituted\ formula}{1\ day}\right)$$

$$= \frac{1.18 \times 10^{12} CFU\ B.infantis}{Day}$$

There is no significant difference between daily intake of infants consuming the highest amount of formula per day (age 0-6 months) using the NHANES data presented in the Grimes paper versus the calculations done in the original dossier using CDC infant feeding guidelines.

7. **Please discuss the potential cumulative dietary exposure to *Bifidobacterium longum* subsp. *infantis* DSM 33361 in infants consuming both conventional foods and infant formula that contain the notified substance.**

There is no potential for cumulative exposure as *B. infantis* DSM 33361, as well as all safe lactic acid bacteria, are transient in the gut. Additionally, *B. infantis* DSM 33361 will not proliferate in the foods for which it is intended to be added to. As shown in Grimes et al., as non-formula beverage intake increases, formula intake decreases. This also indicates that the amount of *B. infantis* DSM 33361 consumed will not significantly increase as the infant ages.

CHR. HANSEN

Improving food & health

Microbiology

8. **Please provide a statement that *Bifidobacterium longum* subsp. *infantis* is a non-toxigenic microorganism.**

Please see attached "BB-02_General Safety Statement_November 2020"

9. **Please provide a more detailed manufacturing protocol describing the individual steps used, e.g., sterilization of fermentation medium, whether this is a batch-, fed-batch-, or continuous-fermentation method, etc.**

Bifidobacterium longum subsp. *infantis* DSM 33361 is batched produced by inoculating the microorganism into sterilized growth substrate. The flow chart on page 16 of the GRAS notice shows a flow chart in which you can see that the media is sterilized prior to fermentation by UHT treatment.

Anaerobic conditions are maintained during the fermentation; pH and temperature are controlled. When the microbiological growth stops, fermentation is stopped by cooling. The microorganisms are then harvested and concentrated by centrifugation and a cryoprotectant is added. They are then frozen into pellets and then lyophilized (freeze dried) into granules. The individual steps of production are described in the GRAS dossier on pages 16-17.

10. **Description of final product formulation (freeze-dried bulk) prior to grinding and blending.**

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(b) (4)		

11. **Please describe the manufacturing step during which milk allergens could be introduced.**

(b) (4)

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12. The notice only includes a specification for "total aerobic microbial count (cfu/g)," which is an incomplete list of microorganisms that could be found in infant formula. a) Please provide a complete list of microorganisms that you test for, along with the protocols used. Please be sure to include the following:

(a)

- ***Enterobacteriaceae or Escherichia coli at an appropriate test sensitivity e.g., cfu/10 g.***
- ***Staphylococcus aureus at an appropriate test sensitivity e.g., cfu/10g .***
- ***Salmonella at an appropriate test sensitivity e.g., cfu/25 g.***
- ***Cronobacter sakazakii at an appropriate test sensitivity e.g., cfu/10 g.***
- ***Molds and yeasts at an appropriate test sensitivity e.g., cfu/g.***

Specification	Criteria	Reference	Frequency of analysis
Total Aerobic Microbial Count	≤ 2000 CFU/g	Ph.Eur. 2.6.12 (modified)	Every batch
Total Yeast and Moulds Count	≤ 100 CFU/g	Ph.Eur. 2.6.12 (modified)	Every batch
Staphylococcus aureus	< 10 CFU/g *	Ph.Eur. 2.6.13 (modified)	Every batch
Salmonella spp.	Absent/10x10g	ISO 6579 (modified)	Every batch
Enterobacteriaceae	Absent/10x10g	ISO 21528 (modified)	Every batch
Cronobacter spp.	Absent/10x10g	ISO 22964 (modified)	Every batch
Bacillus cereus	< 100 CFU/g	ISO 7932 (modified)	Every batch

* Not detected in 0.1g

(b) **Additionally, please provide at least three nonconsecutive batch analyses to demonstrate that *Bifidobacterium longum* subsp. *infantis* DSM 33361 can be manufactured to meet the specifications.**

The GRAS Notice for *Bifidobacterium longum subsp. infantis DSM 33361* is for the safe use of the bulk freeze-dried material. As such, full microbial contaminant testing is not conducted until the bulk product is further processed. The above table shows one example of the specifications for a finished microbial ingredient produced using the freeze-dried bulk, to be sold to the infant formula producer. Testing and CoA's are managed by the co-packer manufacturing the finished microbial ingredient. Additionally, it is the responsibility of the infant formula producer to test for microbial contaminants in their finished product.

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Toxicology

13. On page 23 of the notice, you state that a thorough search of the scientific literature was conducted through October 2019, while the cover letter of your notice is dated May 26, 2020. Please provide the results of an updated literature search through at least May 2020 for studies relevant to the safety of *Bifidobacterium longum* subsp. *infantis* DSM 33361. As part of this discussion, please include search terms, time frames, and databases utilized for the search.

A search in the period July 2019 through May 2020 was performed with the following search terms:

Database	Search term	Period	hits	relevant hits
NCBI PubMED	Bifidobacterium longum infection	July 2019 through May 2020	19	19
NCBI PubMED	Bifidobacterium longum case report	July 2019 through May 2020	1	1
NCBI PubMED	Bifidobacterium longum safety	July 2019 through May 2020	15	13
NCBI PubMED	Bifidobacterium infantis infection	July 2019 through May 2020	5	5
NCBI PubMED	Bifidobacterium infantis case report	July 2019 through May 2020	0	0
NCBI PubMED	Bifidobacterium infantis safety	July 2019 through May 2020	9	9

With the search term '*Bifidobacterium longum* infection' 19 hits were obtained. An assessment of all the hits did not reveal any safety concern. The search term '*Bifidobacterium longum* case report' returned one hits. The study described the first case of bacteremia in a premature infant caused by

Bifidobacterium longum after using the product Florababy Pro® since 2016. They mention that an isolated strain from the infected infant is similar based on molecular typing to *B. longum*, but the method is not described. Although the methods is not described it cannot be ruled out that the *B. longum* from the product used caused the infection. The product also contained a *B. longum* subsp. *infantis* strain and no infection with this strain was observed in the period the product has been used (Pillai et al, 2020). Finally the search term '*Bifidobacterium longum* safety' returned 15 hits. When assessing the hits two were not relevant. Of the relevant hits three were reviews or meta studies, one was an *in vitro* study and the rest were clinical trials dealing with efficacy and safety of *Bifidobacterium longum* subspecies *infantis* or *B. longum* either alone or in combination with other strains. None of the studies reported and adverse effects or safety concerns. The additional searched using the search term 'infantis' did not return any hits of safety concern. This is in line with the latest literature searches done by EFSA as part of the regular update of the list of QPS species (EFSA BIOHAZ Panel 2020a, EFSA BIOHAZ Panel 2020b). The searches run from April 2019 to March 2020 did only find one article relevant for the evaluation (Pruccoli et al., Jun 2019). The paper described a

case of bacteraemia in a 5-month child with a diagnosis of heart disease. Although the composition of the probiotics that the child received was checked and revealed the presence of *Bifidobacterium longum*, the bacterial isolation from the patients referred only to a positive blood culture for Bifidobacterium spp. without further identification and specifications. Based on the available evidence as described above, the QPS status of Bifidobacterium spp. is not changed.
Overall we conclude that infections with *Bifidobacterium longum* subspecies *infantis* are rare.

14. On pages 29 and 30 of the notice, you state that safety studies on a *Bifidobacterium longum* subsp. *infantis* strain described in GRN 000758 are applicable to the current GRAS notice and are incorporated therein. For each safety study that Chr. Hansen intends to incorporate into the current notice, please provide a brief discussion of the incorporated study, as well as the findings and results relevant to *Bifidobacterium longum* subsp. *infantis* DSM 33361. Please also indicate the page number in GRN 000758 where that information can be found.

Please strike the following paragraph (pg. 29-30) from the GRAS submission as it is not necessary to include GRN 758 studies to prove safety.

"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 5x107 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)".

References:

Anish Pillai, Jason Tan, Vanessa Paquette, Julia Panczuk 2020. Does probiotic bacteremia in premature infants impact clinically relevant outcomes? A case report and updated review of literature. Clinical Nutrition ESPEN, Volume 39, Pages 255-259, ISSN 2405-4577, https://doi.org/10.1016/j.clnesp.2020.05.020.

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), 2020a. Statement on the update of the list of QPS-recommended biological agents intentionally added to food or feed as notified to EFSA 11: suitability of taxonomic units notified to EFSA until September 2019. EFSA Journal 2020;18(2):5965, 57 pp. https://doi.org/10.2903/j.efsa.2020.5965

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), 2020b. Statement on the update of the list of QPS-recommended biological agents intentionally added to food or feed as notified to EFSA 12: suitability of taxonomic units notified to EFSA until March 2020. EFSA Journal 2020;18(7):6174, 42 pp. https://doi.org/10.2903/j.efsa.2020.6174

Grimes, et al., Beverage Consumption among U.S. Children Aged 0–24 Months: National Health and Nutrition Examination Survey (NHANES). Nutrients 2017, 9, 264; doi:10.3390/nu9030264

Pruccoli G, Silvestro E, Pace Napoleone C, Aidala E, Garazzino S, Scolfaro C. Are probiotics safe? Bifidobacterium bacteremia in a child with severe heart failure. Infez Med. 2019 Jun 1;27(2):175-178. PMID: 31205041.

Graphic presentation for Materials

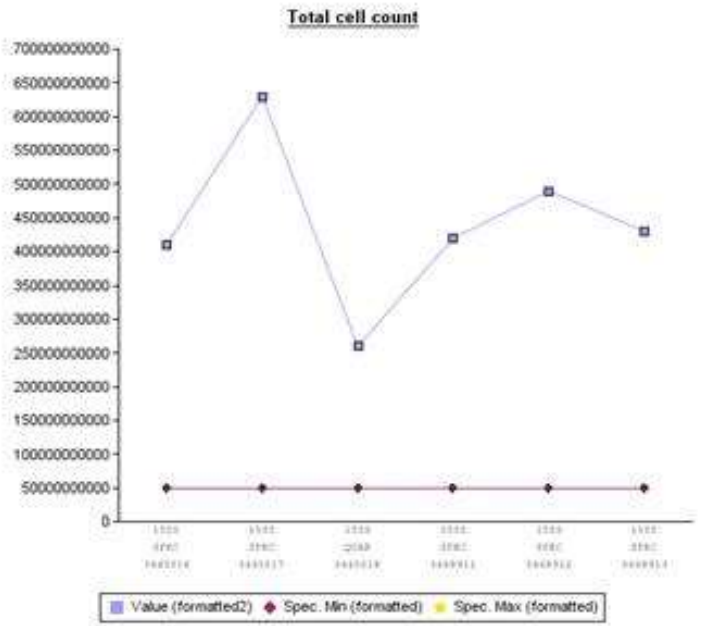
User Name: USKXG
Refreshed: 05/Dec/2020

718388	FD BlFin10012 HA-IF-pre	V. 1500	Roskilde	Profile 8+1 time. New culture implemented according to CCF: 2018-0523-DKMAPJ. 21.08.2018 LCn//Archived, end point must be updated and minimum drying time must be implemented. 21.12.2018 LCn

G-MRSCS-02	Total cell count

Prod. date	Batch	Equipment	S.P.	Version	An	Approval	Test	Value	Min	Max	Uom
11/Jan/2019	3445016	Ray kabinet	ROS-720A	1500	1	SPEC	1	4.10E+11	5.0e+10		cfu/g
11/Jan/2019	3445017	Ray kabinet	ROS-720A	1500	2	SPEC	2	6.30E+11	5.0e+10		cfu/g
11/Jan/2019	3445018	Ray kabinet	ROS-720A	1500	1	QUAR	1	2.60E+11	5.0e+10		cfu/g
31/Jan/2019	3448911	Ray kabinet	ROS-720A	1500	1	SPEC	1	4.20E+11	5.0e+10		cfu/g
31/Jan/2019	3448912	Ray kabinet	ROS-720A	1500	1	SPEC	1	4.90E+11	5.0e+10		cfu/g
31/Jan/2019	3448913	Ray kabinet	ROS-720A	1500	1	SPEC	1	4.30E+11	5.0e+10		cfu/g
	No batches					Average:		Std:	Target:	Cpki/ Cpki log	Cpks/ Cpks log
	6					4.40e+11		1.20e+11		2.47	

LCL	7.90e+10
UCL	8.01e+11
2 STD	1.99e+11
2 STD	6.81e+11



Graphic presentation for Materials

User Name: USKXG

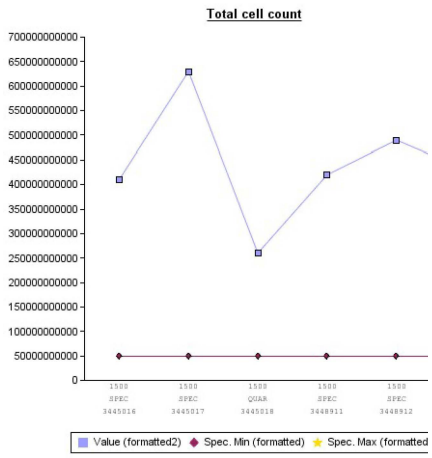
Refreshed: 08/Dec/2020

FD BIFin10012 HA-IF-pre	V. 1500	Roskilde	Profile 8+1 time. New culture implemented according to CCF: 2018-0523-DKMAPJ. 21.08.2018 LCn//Archived, end p be updated and minimum drying time must be implemented. 21.12.2018 LCn

Total cell count

Batch	Equipment	S.P.	Version	Ani	Approval	Test	Value	Min	Max	Uom
3445016	Ray kabinet I	ROS-720A	1500	1	SPEC	1	4.10E+11	5.0e+10		cfu/g
3445017	Ray kabinet I	ROS-720A	1500	2	SPEC	2	6.30E+11	5.0e+10		cfu/g
3445018	Ray kabinet I	ROS-720A	1500	1	QUAR	1	2.60E+11	5.0e+10		cfu/g
3448911	Ray kabinet I	ROS-720A	1500	1	SPEC	1	4.20E+11	5.0e+10		cfu/g
3448912	Ray kabinet I	ROS-720A	1500	1	SPEC	1	4.90E+11	5.0e+10		cfu/g
3448913	Ray kabinet I	ROS-720A	1500	1	SPEC	1	4.30E+11	5.0e+10		cfu/g
No batches					**Average:**		**Std:**	**Target:**	**Cpki/ Cpki log**	**Cpks/ Cpks log**
6					**4.40e+11**		**1.20e+11**		**2.47**	

7.90e+10
8.01e+11
1.99e+11
6.81e+11



General Safety Statement

November, 2020
Valid two years from date of issue

Bifidobacterium infantis (BB-02™)

BB-02™ is a trademark of Chr. Hansen A/S.
Scientific Ref.: *Bifidobacterium longum* subsp. *infantis* Mattarelli et al. 2008

According to Bergey's Manual of Systematics of Archaea and Bacteria, Section on *Bifidobacterium* (Biavati and Mattarelli, 2015) the genus *Bifidobacterium* is in general considered non-pathogenic and from long-term experience, it is concluded that *Bifidobacterium* spp. are safe with the possible exception of the species *B. dentium*. Hemolysis is not seen among species of *Bifidobacterium* except for a weak hemolysis observed in *B. scardovii*.
The species *Bifidobacterium longum* has been the subject of several safety assessment papers and have in all cases been found to be a safe species with no cause for concern regarding adverse effects or production of virulence factors or toxins (Saarela *et al.*, 2002; Ouwehand *et al.*, 2004; Meile *et al.*, 2008; Smilowitz *et al.*, 2017; Kim *et al.*, 2018). The study by Ouwehand *et al.* (2004) showed that strains of *B. longum* did not produce any virulence factors or cause hemolysis. Infections by *Bifidobacterium* sp. are described, but they are extremely rare and in patients with underlying conditions (Saarela *et al.*, 2002; Esaiassen *et al.*, 2017, Wilson and Ong, 2017).
The species *Bifidobacterium longum* has been evaluated by the EFSA Panel on Biological Hazards (BIOHAZ) and found to be suited for the QPS (Qualified Presumption of Safety) status since the start in 2007 (EFSA, 2007; EFSA BIOHAZ Panel, 2020).

Four strains of *B. longum* have obtained GRAS status in food and/or milk based powdered infant formula (GRN000268, GRN000758, GRN000813 and GRN000877).

The BB-02™ strain has a long history of safe use and clinical studies with administration of the BB-02™ in combination with other probiotic strains to preterm infants found the probiotic strains to be safe e.g. Jacobs *et al.* (2013). This is in line with the strain being non-hemolytic and non-cytotoxic. Altogether, all evidence supports that the BB-02™ strain is well tolerated and safe.

Biosafety & Molecular Assays
R&D Microbial Platform

Yvonne Agersø (PhD)
Strain Safety Specialist

Electronically generated, therefore not signed

Chr. Hansen A/S -10-12 Bøge Allé – DK-2970 Hørsholm, Denmark - Phone: +45 45 74 74 74 - Fax: +45 45 74 88 88 - www.chr-hansen.com

References

Biavati, B. and Mattarelli, P. (2015) Bifidobacterium. In, *Bergey's Manual of Systematics of Archaea and Bacteria.*, pp. 1–57.

European Food Safety Authority (EFSA) (2007) Introduction of a Qualified Presumption of Safety (QPS) approach for assessment of selected microorganisms referred to EFSA. *EFSA J.* **587**: 1–16.

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), Koutsoumanis K, Allende A, et al. (2020). Statement on the update of the list of QPS-recommended biological agents intentionally added to food or feed as notified to EFSA 12: suitability of taxonomic units notified to EFSA until March 2020. EFSA Journal **18**:6174.

Esaiassen, E., Hjerde, E., and Cavanagh, P. (2017) Bifidobacterium Bacteremia: Clinical Characteristics and a Genomic Approach To Assess Pathogenicity. *J. Clin. Microbiol.* **55**: 2234–2248.

Jacobs, S.E., Tobin, J.M., Opie, G.F., Donath, S., Tabrizi, S.N., Pirotta, M., et al. (2013) Probiotic Effects on Late-onset Sepsis in Very Preterm Infants: A Randomized Controlled Trial. *Pediatrics* **132**: 1055–1062.

Kim MJ, Ku S, Kim SY *et al.* (2018) Safety Evaluations of *Bifidobacterium bifidum* BGN4 and *Bifidobacterium longum* BORI. Int J Mol Sci. 9;19:1422.

Meile, L., Le Blay, G., and Thierry, A. (2008) Safety assessment of dairy microorganisms: Propionibacterium and Bifidobacterium. *Int. J. Food Microbiol.* **126**: 316–320.

Ouwehand, A.C., Saxelin, M., and Salminen, S. (2004) Assessment of potential risk factors and related properties of clinical, faecal and dairy Bifidobacterium isolates. *Biosci. Microflora* **23**: 37–42.

Saarela, M., Mättö, J., and Mattila-Sandholm, T. (2002) Safety Aspects of Lactobacillus and Bifidobacterium Species Originating from Human Oro-gastrointestinal Tract or from Probiotic Products. *Microb. Ecol. Health Dis.* **14**: 234–241.

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Wilson HL and Ong CW (2017). Bifidobacterium longum vertebrodiscitis in a patient with cirrhosis and prostate cancer. Anaerobe **47**, 47–50.

Chr. Hansen A/S -10-12 Bøge Allé – DK-2970 Hørsholm, Denmark - Phone: +45 45 74 74 74 - Fax: +45 45 74 88 88 - www.chr-hansen.com

Statement

Human Health, Health & Nutrition Business Unit
January, 2020
Valid two years from date of issue

To whom it may concern

Fermentation media ingredients for Human Health cultures

Thank you for your inquiry into Chr. Hansen's products.

Chr. Hansen is pleased to inform you that the ingredients applied to the fermentation media and the yield of biomass is considered Chr. Hansen proprietary information.

In the fermentation process, Chr. Hansen uses various sources of carbohydrates, amino acids, fatty acids, vitamins and minerals for growing the biomass. The ingredient amounts vary from culture to culture due to specific nutrient requirements of the different species and/or strains. The main dry matter components used for fermentation of Human Health cultures are:

- Carbohydrates - primary source of energy, which is metabolized to lactate and for some species also acetate.
- Yeast extract - primary source of amino acids, vitamins and other nutrients to grow the biomass.
- Skimmed milk powder - prime source of carbohydrates, amino acids and other nutrients to grow the biomass (only added to some cultures).
- Micronutrients applied in minor quantities such as minerals, vitamins and fatty acids as growth factors.

All ingredients are approved, sourced and controlled from Chr. Hansen manufacturing sites in Denmark authorized by the Danish Food Administration and Danish Medicines Agency and Chr. Hansen has been inspected by US FDA. Furthermore, we have a system, organization and practice in place to monitor food alerts globally.

Chr. Hansen sources raw materials globally and controls and keep record of the geographical origin of all raw materials used.

The origin is specific for a given raw material and may change over time, depending on changes in supply.

Please do not hesitate to contact your local Chr. Hansen representative in case of further questions.

Yours sincerely
Chr. Hansen A/S

Lotte Harlou
QA Human Health Management

Chr. Hansen A/S -10-12 Bøge Allé - DK-2970 Hørsholm, Denmark - Phone: +45 45 74 74 74 - Fax: +45 45 74 88 88 - www.chr-hansen.com

1.1 Basis of GRAS Conclusion

In accordance with the 21 CFR 170 Subpart E, regulations for Generally Recognized as Safe (GRAS) notifications, Chr. Hansen, Inc. has concluded, through scientific procedures, that *Bifidobacterium longum subsp. infantis* DSM 33361 is Generally Recognized As Safe (GRAS) and is not subject to the premarket approval requirements for use as a bacterial ingredient in conventional foods including (but not limited to) dairy products and other fermented milk products, fermented plant-based products, beverages, shelf stable products, confectionery, and breakfast cereals. *Bifidobacterium longum subsp. infantis* DSM 33361 is also intended as an ingredient in 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.

1.4 Statutory Basis for GRAS Determination

Pursuant to the GRAS rule [81 Fed. Reg. 159 (17 August 2016)], Chr. Hansen has concluded that *Bifidobacterium longum subsp. infantis* DSM 33361 is GRAS as a microbial ingredient, through scientific procedures, in accordance with 21 CFR 170.30 (b).

1.5 Premarket Approval Status

It is the opinion of Chr. Hansen that *Bifidobacterium longum subsp. infantis* DSM 33361 is not subject to premarket approval requirements of the Federal Food, Drug, and Cosmetics Act based on our conclusion that *Bifidobacterium longum subsp. infantis* DSM 33361 is GRAS as a microbial ingredient under the intended use conditions.

It is also our opinion that other qualified and competent scientists reviewing the same publicly available toxicological and safety information would reach the same conclusion. Therefore, we have also concluded that *Bifidobacterium longum subsp. infantis* DSM 33361, when used as a microbial ingredient as described in this dossier, is GRAS based on scientific procedures.

From: Arie Carpenter
To: Morissette, Rachel; Kate Urbain
Cc: Emily Gregoire
Subject: RE: follow-up questions for GRN 950 to be addressed
Date: Wednesday, January 13, 2021 3:26:02 PM
Attachments: image001.png
2021-13-1 GRN 950 response.pdf
Amendment 1.pdf
Amendment 2.pdf

Hi Dr. Morissette,

Thank you so much for allowing us an extension to get your questions answered.

You will find answers to your questions from December 21st, 2020 to Emily Gregoire attached.

If you have any additional questions, please let me know.

Thanks so much,

Arie Carpenter
Sr. Regulatory Affairs Specialist, Food Cultures and Enzymes
Cell: 414-544-2317 Desk: 414-777-7526
usarbr@chr-hansen.com | www.chr-hansen.com

From: Morissette, Rachel <Rachel.Morissette@fda.hhs.gov>
Sent: Thursday, January 7, 2021 8:18 AM
To: Kate Urbain <USKAUR@chr-hansen.com>
Cc: Arie Carpenter <USARBR@chr-hansen.com>; Highbarger, Lane A <Lane.Highbarger@fda.hhs.gov>
Subject: RE: follow-up questions for GRN 950 to be addressed

Hi Kate and Arie,

Thank you for the call this morning. If you have any further clarification questions about the additional questions we sent on December 21, 2020, please let me know.

Best regards,

Rachel

Rachel Morissette, Ph.D.
Regulatory Review Scientist

Division of Food Ingredients
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition

U.S. Food and Drug Administration
rachel.morissette@fda.hhs.gov

U.S. FOOD & DRUG ADMINISTRATION

From: Kate Urbain <USKAUR@chr-hansen.com>
Sent: Wednesday, January 6, 2021 2:41 PM
To: Morissette, Rachel <Rachel.Morissette@fda.hhs.gov>
Cc: Arie Carpenter <USARBR@chr-hansen.com>
Subject: RE: follow-up questions for GRN 950 to be addressed

Hi Dr. Morissette,

We can do tomorrow morning at 9am EST, please include my colleague Arie Carpenter (copied) on the invite. We understand your feedback, and we don't believe we need more than a few days to answer the questions satisfactorily. Would the agency object to an extension of five business days? We understand the potential inconvenience and are happy to work with the agency to minimize the disruption. We can also discuss on the call.

Best Regards,

Kate Urbain
Head of Regulatory Affairs North America – Compliance
Chr. Hansen, Inc. - 9015 W Maple St. West Allis, WI 53214 – USA
Mobile: +1-414-520-3441
Office: +1-414-607-5819
uskaur@chr-hansen.com

https://www.chr-hansen.com/en/about-us/purpose-and-strategy

From: Morissette, Rachel <Rachel.Morissette@fda.hhs.gov>
Sent: Wednesday, January 6, 2021 1:37 PM

To: Kate Urbain <USKAUR@chr-hansen.com>
Cc: Arie Carpenter <USARBR@chr-hansen.com>
Subject: RE: follow-up questions for GRN 950 to be addressed

Hi Kate,

Thanks for getting back to me. Sure, we are available for a phone call tomorrow from 9-11 am EST or 12:30-2 pm EST and Friday from 9-10 am EST or 11-3 pm EST. If you need me to check into next week, just let me know. Regarding your second question, we can allow an extension. However, I just want to point out that this is the second round of questions we've had and typically the GRAS process is not meant to be iterative. So if these questions cannot be addressed to our satisfaction, we will have to recommend asking us to cease our evaluation and you can resubmit a clean notice at a later date without prejudice should you choose to.

Best regards,

Rachel

Rachel Morissette, Ph.D.
Regulatory Review Scientist

Division of Food Ingredients
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
U.S. Food and Drug Administration
rachel.morissette@fda.hhs.gov

From: Kate Urbain <USKAUR@chr-hansen.com>
Sent: Wednesday, January 6, 2021 2:29 PM
To: Morissette, Rachel <Rachel.Morissette@fda.hhs.gov>
Cc: Arie Carpenter <USARBR@chr-hansen.com>
Subject: FW: follow-up questions for GRN 950 to be addressed
Importance: High

Dear Dr. Morisette,

I need to notify you that Emily is out of the office on medical leave. I will be taking over the correspondence for GRN950 and any other dossiers Emily was point of contact for. We would like to respectfully propose two items as listed below.

A. Schedule a short call to better understand a few of the questions you have below. (I don't think it will take even 30 minutes but want to make sure we are on the same page in our interpretation since I am coming into this in the middle).

B. Respectfully request an extension to allow other people on our team time to familiarize with the dossier and question history before responding in order to ensure the most thorough response.

Best Regards,

Kate Urbain
Head of Regulatory Affairs North America – Compliance
Chr. Hansen, Inc. - 9015 W Maple St. West Allis, WI 53214 – USA
Mobile: +1-414-520-3441
Office: +1-414-607-5819
uskaur@chr-hansen.com

https://www.chr-hansen.com/en/about-us/purpose-and-strategy

From: Emily Gregoire <USEMGR@chr-hansen.com>
Sent: Tuesday, January 5, 2021 4:00 PM
To: Kate Urbain <USKAUR@chr-hansen.com>
Subject: FW: follow-up questions for GRN 950 to be addressed

Kind Regards / Venlig hilsen

Emily Gregoire

From: Morissette, Rachel <Rachel.Morissette@fda.hhs.gov>
Sent: Monday, December 21, 2020 8:08 AM
To: Emily Gregoire <USEMGR@chr-hansen.com>
Subject: follow-up questions for GRN 950 to be addressed

Dear Ms. Gregoire,

Thank you for sending responses to our questions. We note the following issues that need to be

resolved before we can move forward with the review of your GRAS notice.

1. There are several places throughout the responses that are stamped confidential. As you are aware, data and information pertaining to safety cannot be confidential in a GRAS notice. Therefore, you have three options to consider.
    a. Keep the responses as is and provide copies where the confidential stamps are removed in both your response document and anywhere in the attachments.
    b. Revise your responses to our questions so that all confidential markings are removed, all confidential information is removed, and your responses are based on publicly releasable information only.
    c. Request that we cease to evaluate your GRAS notice.

2. You did not provide an appropriate response to question 1. The revised text that you provided in the attachment is simply the same text that was found in the original GRAS notice. Also, the incorrect text in Part 6 of the narrative was not addressed in the attachment you provided. Please revise your response to question 1.

3. Microbiological testing of the ingredient is part of our safety evaluation; without batch analyses we do not consider the safety aspect of the GRAS conclusion to have been met. Please provide at least 3 non-consecutive batch analyses for all specifications as requested in question 12b.

4. In your amendment to the notice, you provided specified limits for lead, cadmium, arsenic, and mercury and the results of one batch analysis of *Bifidobacterium longum* subsp. *infantis* DSM 33361. Please clarify if your product is routinely analyzed for the presence of heavy metals. Also, we request the results at least 3 non-consecutive batch analyses to demonstrate that your ingredient can be consistently manufactured to meet the specifications.

Due to the holidays, we are requesting your response by COB January 8. Let me know if you have any questions. Please note that I will be on leave starting December 23rd and will return to the office January 4, 2021.

Best regards,

Rachel

Rachel Morissette, Ph.D.
Regulatory Review Scientist

Division of Food Ingredients
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
U.S. Food and Drug Administration
rachel.morissette@fda.hhs.gov

FDA U.S. FOOD & DRUG ADMINISTRATION

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Rachel Morissette, Ph.D.

Regulatory Review Scientist
FDA Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
Division of Food Ingredients

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January 13, 2021
USARBR

Chr. Hansen Response to FDA's questions regarding GRN 000950

Dear Dr. Morissette,

We are happy to provide you with the answers to your questions regarding GRN 000950 received in e-mail on December 21, 2020.

Please see answers below.

Yours sincerely,

Arie Carpenter
Sr. Regulatory Affairs Specialist

usarbr@chr-hansen.com
Mobile: 414-544-2317

1. **There are several places throughout the response that are stamped confidential. As you are aware, data and information pertaining to safety cannot be confidential in a GRAS notice.**

   We would like to keep the responses as is with the confidential stamp removed for questions 2 and 11 and then revise the response for question 10 to read as follows:

   Bifidobacterium longum subsp. *infantis*, cysteine chloride, trisodium citrate dihydrate, sucrose, maltodextrin

   These changes have all been made in 2020-12-9 GRN 000950 (attached as Amendment 1)

2. **You did not provide an appropriate response to question 1. The revised text that you provided in the attachment is simply the same text that was found in the original GRAS notice. Also, the incorrect text in Part 6 of the narrative was not addressed in the attachment you provided. Please revise your response to question 1.**

   We apologize for the misunderstanding.

   Please see attached and amended Revised Part 1 for GRN 000950 named Amendment 2.

3. **Microbiological testing of the ingredient is part of our safety evaluation; without batch analyses we do not consider the safety aspect of the GRAS conclusion to have been met. Please provide at least 3 non-consecutive batch analyses for all specifications as requested in question 12b.**

   Below you will find the micro analysis for 4 non-consecutive batches of FD *Bifidobacterium longum* subsp. *infantis.* All results, are reported as cfu/g unless otherwise stated.

Batch	Batch 1	Batch 2	Batch 3	Batch 4
Total Aerobic Micro Count	<250	<250	<250	<250
Yeast and Mould	<25	<25	<25	<25
Staphylococcus aureus	<10	<10	<10	<10
Salmonella spp.	Absent/25g	Absent/25g	Absent/25g	Absent/25g
Enterobacteriaceae	Absent/10g	Absent/10g	Absent/10g	Absent/10g
Cronobacter spp.	Absent/10g	Absent/10g	Absent/10g	Absent/10g
Bacillus cereus	<10	<10	<10	<10

4. **In your amendment to the notice, you provided specified limits for lead, cadmium, arsenic, and mercury and the results of one batch analysis of *Bifidobacterium longum* subsp. *infantis* DSM 33361. Please clarify if your product is routinely analyzed for the presence of heavy metals. Also, we request the results at least 3 non-consecutive batch analyses to demonstrate that your ingredient can be consistently manufactured to meet the specifications.**

Heavy metal contamination is not a potential hazard in our process or products. The only potential source of heavy metals comes from raw materials used during the fermentation process and cryoprotectants added post fermentation. To mitigate risk of contamination through these materials, risk assessments are conducted during implementation of new raw materials and cryoprotectants. Supplier approval includes a statement of compliance to the legislation in force on compliance to heavy metal limits. Potable water is used in our process, which complies with heavy metal limits for drinking water. Seed materials (pre-inoculation material-PIM and direct inoculation material-DIM) are grown with those same standards. PIM and DIM are inoculated into the fermentations at extremely low levels which suggests that their contribution to heavy metal contamination of a production batch is exceedingly small.

Because heavy metals are not a reasonable hazard in Chr. Hansen's production of microbial cultures, heavy metal contaminant testing is not a release criteria and therefore does not appear on certificates of analysis. Heavy metal testing is, however, conducted on a monitoring basis.

As an act of caution, we monitor for heavy metals by selecting representative products annually. When it is produced, at least one batch of *Bifidobacterium longum subsp. infantis* DSM 33361 is analyzed annually as a representative sample. Similar microbial products are analyzed at the same time to ensure a good representation of raw materials and cryoprotectants used in the production process, since this is the point at which heavy metals theoretically could be introduced.

Testing is performed by an external laboratory that complies with the requirements and is accredited according to ISO 17025 standard. The external laboratory uses internationally recognized and accredited reference methods suitable for testing trace elements in foodstuffs.

The raw materials that go into the production of *Bifidobacterium longum subsp. infantis* DSM 33361 are represented in the cultures seen in Table 1 along with the results of heavy metal testing. The cultures tested were nonconsecutive. In all cases, heavy metal results were under limits set forth.

It should also be noted that we provided incorrect information in the previous response. In the absence of numeric limits set for heavy metals by the FDA, we utilize limits set by the EU in EC no 1881/2006, last consolidated October 14, 2020. The limits are intended to be for the final food product. The product category closest to our intended use and with the lowest limit was used. Originally for lead, 0.010 mg/kg was reported. This is the limit set for infant formula marketed as liquid. Since the product for which GRN 950 is written is freeze dried and going into powdered infant formula, we test to the limit for infant formula

marketed as powder, which is 0.050 mg/kg. The limits set forth by EC no 1881/2006 can be found in Table 2.

Table 1: Heavy metal results for 3 non-consecutive batches of product going into infant formula

Lot ID	Arsenic (As)	Lead (Pb)	Cadmium (Cd)	Mercury (Hg)
Limit	**0.1 mg/kg**	**0.05 mg/kg**	**0.01 mg/kg**	**0.01 mg/kg**
3441814	<0.1 mg/kg	<3 µg/kg	<0.01 mg/kg	<0.005 mg/kg
3448913	<0.1 mg/kg	<0.05 mg/kg	<0.01 mg/kg	0.0079 mg/kg
3468358	<0.1 mg/kg	<0.05 mg/kg	<0.01 mg/kg	<0.005 mg/kg

Table 2: EC 1881/2006 heavy metal limits for infant formula or most closely related food products

Arsenic	Rice destined for the production of food for infants and young children	0.10 mg/kg
Lead	Infant formulae and follow-on formulae marketed as powder	0.050 mg/kg
Cadmium	powdered formulae manufac- tured from cows' milk proteins or protein hydrolysates	0.010 mg/kg
Mercury	Food supplements	0.10 mg/kg

Rachel Morissette, Ph.D.

Regulatory Review Scientist
FDA Center for Food Safety and Applied Nutrition
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December 9, 2020
USEMGR

Chr. Hansen Response to FDA's questions regarding GRN 000950

Dear Dr. Morissette,

We are happy to provide you with the answers to the majority questions regarding GRN 000950 received on Nov. 17, 2020.

We have marked any confidential material as such. Please reach out with any further questions.

Yours sincerely,

Emily Gregoire
Global Regulatory Affairs Partner

usemgr@chr-hansen.com
Mobile: 414-553-7198

Regulatory:

1. **A GRAS conclusion is for the use of a substance, not the substance itself. We note that in Part 1 of the notice (Signed Statements and Certification) in sections 1.1 Basis of GRAS Conclusion, 1.4 Statutory Basis for GRAS Determination, and 1.5 Premarket Approval Status, and the first paragraph of Part 6 Narrative, the notice states that *Bifidobacterium longum*subsp. *infantis* DSM 33361 is GRAS, not its use. Please provide revised text for those sections indicating that the use of *Bifidobacterium longum* subsp. *infantis* DSM 33361 is GRAS.**

Please see attached "Revised Part 1 for GRN 000950

Chemistry

2. **Please provide specifications for the identification and assay of *Bifidobacterium longum subsp. infantis* DSM 33361, along with at least three nonconsecutive batch analyses to demonstrate that *Bifidobacterium longum* subsp. *infantis* DSM 33361 can be manufactured to meet the specifications.**

Complete strain identification is carried out on our master CHCC* reference material (please see Figure 2, pg. 19 of GRAS Notice). This is a part of our quality management system. In addition to continually monitor and ensure strain identity in our production we do quality control pulsed field gel electrophoresis at regular intervals. More specifically, every time inoculate material** is produced for our products, DNA fingerprinting is done on the PIM/DIM** batch against the master reference material from the CHCC* strain bank. This secures that we always have the correct strain in our production and products remain always the same.

Assays are done for each PIM/DIM batch and recorded in an electronic database. Reports may be pulled to extract desired data. Please see attached "718388 Total cell count batch records".

3. **Please provide any specified limits for contaminants, such as heavy metals (e.g. lead), and residual components of the manufacturing process that may be of concern, along with at least three nonconsecutive batch analyses.**

Specified limits for contaminants

Environmental contaminants are controlled on the raw material level.
However one batch of the freeze-dried material has been analyzed for heavy metals as carry-over from process.

Parameter	limit	Results batch 3441814
Lead (Pb)	0,01 mg/kg	<3 µg/kg
Cadmium (Cd)	0,01 mg/kg	<0,01 mg/kg
Mercury (Hg)	0,01 mg/kg	<0,005 mg/kg
Arsenic (As)	0,1 mg/kg*	<0,1 mg/kg

Analysis reference to EN 13805:2014, EN ISO 17294m:2016. Method: ICP-MS. Performed by external laboratorium.
*based on rice destined for baby food ref. 2015/2006 amendment

4. **Please confirm that all analytical methods used to test for each specification parameter are validated for that purpose. If using standard methods of analysis, please provide complete and appropriate citations.**

Environmental Contaminants: Analysis reference to EN 13805:2014, EN ISO 17294m:2016. Method: ICP-MS. Performed by external laboratory

5. **We note that the estimated dietary exposure described in the notice for the intended use in conventional foods is based on assumptions that an average, healthy individual consumes approximately 20 servings of food/day and that approximately 10 of those servings would contain 1 x 10^{10} cfu/serving of the notified substance. Please provide a citation for the source of the estimated 20 servings of food/day and discuss the basis for the 10 servings/day assumption.**

Reference:

Millen, A. E., Midthune, D., Thompson, F. E., Kipnis, V., & Subar, A. F. (2006). The National Cancer Institute Diet History Questionnaire: Validation of Pyramid. *American Journal of Epidemiology*, 279-288.

By calculating intake assuming that half of all servings of food per day (10 servings) contain *Bifidobacterium longum* subsp. *infantis* DSM 33361, we are using a “worst-case-scenaria” approach, as it is highly unlikely that half of the conventional food consumed in a day would contain *B. infantis*.

6. **The dietary exposure estimate for the notified substance for infants is based on the intended use in non-exempt infant formula and an estimated consumption of approximately 24 fl. oz/day of formula for newborn infants. Please discuss the dietary exposure for infants 0 to 12 months of age and estimate the age group with the highest exposure to *Bifidobacterium longum* subsp. *infantis* DSM 33361. We recommend using consumption data for infant formula from the National Health and Nutrition Examination Survey (NHANES), for example, as summarized in Grimes, et al., Beverage Consumption among U.S. Children Aged 0–24 Months: National Health and Nutrition Examination Survey (NHANES). Nutrients 2017, 9, 264; doi:10.3390/nu9030264.**

The infant formula intake data described in Grimes, et al. confirms that infants age 0-6 months consume the highest amount of formula, 834 g reconstituted formula per day. Using the Grimes daily intake as well as the average reconstitution rate of 14.1 g powdered infant formula per 100mL water, we can use the following calculations to predict the maximum daily intake:

$$\left(\frac{1 \times 10^{10}\ CFU\ B.infantis}{1g\ powdered\ formula}\right)\left(\frac{14.1\ g\ powdered\ formula}{100\ mL}\right)\left(\frac{834\ g\ reconstituted\ formula}{1\ day}\right)$$

$$= \frac{1.18 \times 10^{12} CFU\ B.infantis}{Day}$$

There is no significant difference between daily intake of infants consuming the highest amount of formula per day (age 0-6 months) using the NHANES data presented in the Grimes paper versus the calculations done in the original dossier using CDC infant feeding guidelines.

7. **Please discuss the potential cumulative dietary exposure to *Bifidobacterium longum* subsp. *infantis* DSM 33361 in infants consuming both conventional foods and infant formula that contain the notified substance.**

There is no potential for cumulative exposure as *B. infantis* DSM 33361, as well as all safe lactic acid bacteria, are transient in the gut. Additionally, *B. infantis* DSM 33361 will not proliferate in the foods for which it is intended to be added to. As shown in Grimes et al., as non-formula beverage intake increases, formula intake decreases. This also indicates that the amount of *B. infantis* DSM 33361 consumed will not significantly increase as the infant ages.

Microbiology

8. **Please provide a statement that *Bifidobacterium longum* subsp. *infantis* is a non-toxigenic microorganism.**

Please see attached "BB-02_General Safety Statement_November 2020"

9. **Please provide a more detailed manufacturing protocol describing the individual steps used, e.g., sterilization of fermentation medium, whether this is a batch-, fed-batch-, or continuous-fermentation method, etc.**

Bifidobacterium longum subsp. *infantis* DSM 33361 is batched produced by inoculating the microorganism into sterilized growth substrate. The flow chart on page 16 of the GRAS notice shows a flow chart in which you can see that the media is sterilized prior to fermentation by UHT treatment.

Anaerobic conditions are maintained during the fermentation; pH and temperature are controlled. When the microbiological growth stops, fermentation is stopped by cooling. The microorganisms are then harvested and concentrated by centrifugation and a cryoprotectant is added. They are then frozen into pellets and then lyophilized (freeze dried) into granules. The individual steps of production are described in the GRAS dossier on pages 16-17.

10. **Description of final product formulation (freeze-dried bulk) prior to grinding and blending.**

The final product formulation for the freeze-dried bulk includes the following ingredients:

Bifidobacterium longum subsp. *infantis*, cysteine chloride, trisodium citrate dihydrate, sucrose, maltodextrin

11. **Please describe the manufacturing step during which milk allergens could be introduced.**

Skimmed milk powder may be used in the fermentation media for propagation of some of our strains. However, as each strain requires a proprietary blend of fermentation media ingredients, not all batches will include skimmed milk powder. Please see "Fermentation Media – HH Statement" attached.

12. The notice only includes a specification for "total aerobic microbial count (cfu/g)," which is an incomplete list of microorganisms that could be found in infant formula. a) Please provide a complete list of microorganisms that you test for, along with the protocols used. Please be sure to include the following:

(a)

- ***Enterobacteriaceae or Escherichia coli at an appropriate test sensitivity e.g., cfu/10 g.***
- ***Staphylococcus aureus at an appropriate test sensitivity e.g., cfu/10g .***
- ***Salmonella at an appropriate test sensitivity e.g., cfu/25 g.***
- ***Cronobacter sakazakii at an appropriate test sensitivity e.g., cfu/10 g.***
- ***Molds and yeasts at an appropriate test sensitivity e.g., cfu/g.***

Specification	Criteria	Reference	Frequency of analysis
Total Aerobic Microbial Count	≤ 2000 CFU/g	Ph.Eur. 2.6.12 (modified)	Every batch
Total Yeast and Moulds Count	≤ 100 CFU/g	Ph.Eur. 2.6.12 (modified)	Every batch
Staphylococcus aureus	< 10 CFU/g *	Ph.Eur. 2.6.13 (modified)	Every batch
Salmonella spp.	Absent/10x10g	ISO 6579 (modified)	Every batch
Enterobacteriaceae	Absent/10x10g	ISO 21528 (modified)	Every batch
Cronobacter spp.	Absent/10x10g	ISO 22964 (modified)	Every batch
Bacillus cereus	< 100 CFU/g	ISO 7932 (modified)	Every batch

* Not detected in 0.1g

(b) **Additionally, please provide at least three nonconsecutive batch analyses to demonstrate that *Bifidobacterium longum* subsp. *infantis* DSM 33361 can be manufactured to meet the specifications.**

The GRAS Notice for *Bifidobacterium longum subsp. infantis DSM 33361* is for the safe use of the bulk freeze-dried material. As such, full microbial contaminant testing is not conducted until the bulk product is further processed. The above table shows one example of the specifications for a finished microbial ingredient produced using the freeze-dried bulk, to be sold to the infant formula producer. Testing and CoA's are managed by the co-packer manufacturing the finished microbial ingredient. Additionally, it is the responsibility of the infant formula producer to test for microbial contaminants in their finished product.

Toxicology

13. On page 23 of the notice, you state that a thorough search of the scientific literature was conducted through October 2019, while the cover letter of your notice is dated May 26, 2020. Please provide the results of an updated literature search through at least May 2020 for studies relevant to the safety of *Bifidobacterium longum* subsp. *infantis* DSM 33361. As part of this discussion, please include search terms, time frames, and databases utilized for the search.

A search in the period July 2019 through May 2020 was performed with the following search terms:

Database	Search term	Period	hits	relevant hits
NCBI PubMED	Bifidobacterium longum infection	July 2019 through May 2020	19	19
NCBI PubMED	Bifidobacterium longum case report	July 2019 through May 2020	1	1
NCBI PubMED	Bifidobacterium longum safety	July 2019 through May 2020	15	13
NCBI PubMED	Bifidobacterium infantis infection	July 2019 through May 2020	5	5
NCBI PubMED	Bifidobacterium infantis case report	July 2019 through May 2020	0	0
NCBI PubMED	Bifidobacterium infantis safety	July 2019 through May 2020	9	9

With the search term '*Bifidobacterium longum* infection' 19 hits were obtained. An assessment of all the hits did not reveal any safety concern. The search term '*Bifidobacterium longum* case report' returned one hits. The study described the first case of bacteremia in a premature infant caused by

Bifidobacterium longum after using the product Florababy Pro® since 2016. They mention that an isolated strain from the infected infant is similar based on molecular typing to *B. longum*, but the method is not described. Although the methods is not described it cannot be ruled out that the *B. longum* from the product used caused the infection. The product also contained a *B. longum* subsp. *infantis* strain and no infection with this strain was observed in the period the product has been used (Pillai et al, 2020). Finally the search term '*Bifidobacterium longum* safety' returned 15 hits. When assessing the hits two were not relevant. Of the relevant hits three were reviews or meta studies, one was an *in vitro* study and the rest were clinical trials dealing with efficacy and safety of *Bifidobacterium longum* subspecies *infantis* or *B. longum* either alone or in combination with other strains. None of the studies reported and adverse effects or safety concerns. The additional searched using the search term 'infantis' did not return any hits of safety concern. This is in line with the latest literature searches done by EFSA as part of the regular update of the list of QPS species (EFSA BIOHAZ Panel 2020a, EFSA BIOHAZ Panel 2020b). The searches run from April 2019 to March 2020 did only find one article relevant for the evaluation (Pruccoli et al., Jun 2019). The paper described a

case of bacteraemia in a 5-month child with a diagnosis of heart disease. Although the composition of the probiotics that the child received was checked and revealed the presence of *Bifidobacterium longum*, the bacterial isolation from the patients referred only to a positive blood culture for Bifidobacterium spp. without further identification and specifications. Based on the available evidence as described above, the QPS status of Bifidobacterium spp. is not changed.
Overall we conclude that infections with *Bifidobacterium longum* subspecies *infantis* are rare.

14. On pages 29 and 30 of the notice, you state that safety studies on a *Bifidobacterium longum* subsp. *infantis* strain described in GRN 000758 are applicable to the current GRAS notice and are incorporated therein. For each safety study that Chr. Hansen intends to incorporate into the current notice, please provide a brief discussion of the incorporated study, as well as the findings and results relevant to *Bifidobacterium longum* subsp. *infantis* DSM 33361. Please also indicate the page number in GRN 000758 where that information can be found.

Please strike the following paragraph (pg. 29-30) from the GRAS submission as it is not necessary to include GRN 758 studies to prove safety.

"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 5x107 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)".

References:

Anish Pillai, Jason Tan, Vanessa Paquette, Julia Panczuk 2020. Does probiotic bacteremia in premature infants impact clinically relevant outcomes? A case report and updated review of literature. Clinical Nutrition ESPEN, Volume 39, Pages 255-259, ISSN 2405-4577, https://doi.org/10.1016/j.clnesp.2020.05.020.

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), 2020a. Statement on the update of the list of QPS-recommended biological agents intentionally added to food or feed as notified to EFSA 11: suitability of taxonomic units notified to EFSA until September 2019. EFSA Journal 2020;18(2):5965, 57 pp. https://doi.org/10.2903/j.efsa.2020.5965

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), 2020b. Statement on the update of the list of QPS-recommended biological agents intentionally added to food or feed as notified to EFSA 12: suitability of taxonomic units notified to EFSA until March 2020. EFSA Journal 2020;18(7):6174, 42 pp. https://doi.org/10.2903/j.efsa.2020.6174

Grimes, et al., Beverage Consumption among U.S. Children Aged 0–24 Months: National Health and Nutrition Examination Survey (NHANES). Nutrients 2017, 9, 264; doi:10.3390/nu9030264

Pruccoli G, Silvestro E, Pace Napoleone C, Aidala E, Garazzino S, Scolfaro C. Are probiotics safe? Bifidobacterium bacteremia in a child with severe heart failure. Infez Med. 2019 Jun 1;27(2):175-178. PMID: 31205041.

1.1 Basis of GRAS Conclusion

In accordance with the 21 CFR 170 Subpart E, regulations for Generally Recognized as Safe (GRAS) notifications, Chr. Hansen, Inc. has concluded, through scientific procedures, that the use of *Bifidobacterium longum subsp. infantis* DSM 33361 as a bacterial ingredient in conventional foods including (but not limited to) dairy products and other fermented milk products, fermented plant-based products, beverages, shelf stable products, confectionery, and breakfast cereals as well as an ingredient in non-exempt infant formula (including cow-milk, soy, and protein hydrolysate based formulas, is Generally Recognized As Safe (GRAS) and is not subject to premarket approval. 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.

1.4 Statutory Basis for GRAS Determination

Pursuant to the GRAS rule [81 Fed. Reg. 159 (17 August 2016)], Chr. Hansen has concluded that the use of *Bifidobacterium longum subsp. infantis* DSM 33361 as a microbial ingredient is GRAS, through scientific procedures, in accordance with 21 CFR 170.30 (b).

1.5 Premarket Approval Status

It is the opinion of Chr. Hansen that *Bifidobacterium longum subsp. infantis* DSM 33361 is not subject to premarket approval requirements of the Federal Food, Drug, and Cosmetics Act based on our conclusion that the use of *Bifidobacterium longum subsp. infantis* DSM 33361 as a microbial ingredient under the intended use conditions is GRAS.

It is also our opinion that other qualified and competent scientists reviewing the same publicly available toxicological and safety information would reach the same conclusion. Therefore, we have also concluded that the use of *Bifidobacterium longum subsp. infantis* DSM 33361 when used as a microbial ingredient as described in this dossier, is GRAS based on scientific procedures.

Part 6: Narrative

In the following sections, the data and information providing the basis for our conclusion that the use of *Bifidobacterium longum subsp. infantis* DSM 33361 as a bacterial ingredient in conventional foods including (but not limited to) dairy products and other fermented milk products, fermented plant-based products, beverages, shelf stable products, confectionery, and breakfast cereals as well as an ingredient in non-exempt infant formula (including cow-milk, soy, and protein hydrolysate based formulas, is GRAS through scientific procedure. 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*.