From:	Gavin P Thompson
To:	Hice, Stephanie
Cc:	Julia Parkot; Anne Oehne (Anne.Oehme@jennewein-biotech.de); Stephen ORourke; Cassie Huang
Subject:	RE: GRN 000929 - Questions for Notifier - Responses
Date:	Monday, October 19, 2020 5:46:32 PM
Attachments:	GRN000929-Response_to_Questions-2020-10-19.pdf

Dear Dr. Hice,

On behalf of the notifier, Jennewein Biotechnologie, GmbH, we herein provide substantive responses regarding the GRN review team's questions and requests concerning GRN 000929.

If you have any difficulty accessing the response document (attached to this email), please let know and we will make a secure Internet access point where the document can be downloaded.

We trust that this information fully addresses the questions and requests; however, if you have additional questions, comments, or requests at any time during your review of GRN 000929, please contact us so that we may provide clarification or other response in a timely manner.

Regards,
Gavin

Gavin P Thompson, PhD
Principal Consultant
Product Safety & Stewardship
Ramboll Environment & Health
+1 (703) 589 8023 mobile
gthompson@ramboll.com

_____

2111 East Highland Avenue, Suite 402
Phoenix, AZ 85016 USA
Profile: https://ramboll.com/contact/environ/gthompson

From: Gavin P Thompson
Sent: October 17, 2020 6:50 PM
To: Hice, Stephanie <Stephanie.Hice@fda.hhs.gov>
Subject: RE: GRN 000929 - Questions for Notifier

Dear Dr. Hice,

Please be advised that on behalf of the notifier, we will be submitting a letter of responses to your requests and questions via email on Monday afternoon, October 19, 2020.

Regards,
Gavin

Gavin P Thompson, PhD
Principal Consultant
Product Safety & Stewardship

Ramboll Environment & Health
+1 (703) 589 8023 mobile
gthompson@ramboll.com

_____

2111 East Highland Avenue, Suite 402
Phoenix, AZ 85016 USA
Profile: https://ramboll.com/contact/environ/gthompson

From: Hice, Stephanie <Stephanie.Hice@fda.hhs.gov>
Sent: October 6, 2020 7:44 AM
To: Gavin P Thompson <GThompson@ramboll.com>
Subject: GRN 000929 - Questions for Notifier

Dear Dr. Thompson,

During our review of GRAS Notice No. 000929, we noted further questions that need to be addressed and are attached to this email.

We respectfully request a response within **10 business days**. If you are unable to complete the response within that time frame, please contact me to discuss further options. Please do not include any confidential information in your responses.

If you have questions or need further clarification, please feel free to contact me. Thank you in advance for your attention to our comments.

Sincerely,
Stephanie Hice

Stephanie Hice, PhD
Staff Fellow (Biologist)
Division of Food Ingredients
Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
U.S. Food and Drug Administration
stephanie.hice@fda.hhs.gov

October 6, 2020

Dear Dr. Thompson,

After reviewing Jennewein Biotechnologie GmbH (Jennewein)'s GRAS Notice, GRN 000929 for the intended use of 2′-fucosyllactose (2′-FL), below we noted further questions and comments that need to be addressed, as well as our recommendation that we strongly suggest you consider. We respectfully request a response within 10 business days. If you are unable to complete the response within that time frame, please contact me to discuss further options. Please do not include any confidential information in your responses.

Outstanding Comment:

We have reviewed the scope of GRN 000929, and do not believe that the narrative provided sufficient publicly available safety data and information that could support the intended use of 2′-FL in exempt infant formula for pre-term infants. As such, our questions regarding the intended use for pre-term infants would warrant an extensive discussion on the notifier's part, beyond the scope of what is typically provided in amendments. We note that we specifically advised the notifier at the December 7, 2015 GRAS pre-submission meeting that a robust discussion of the relevant developmental vulnerabilities of pre-term infants is needed to justify the use of 2′-FL. Thus, we suggest that the notifier remove the intended use of 2′-FL in exempt infant formula for pre-term infants to ensure the successful completion of this review in a timely manner. Should the notifier have any questions, they are welcome to request a teleconference to discuss this further. A separate list of outstanding questions will be provided to the notifier regarding the intended use of 2′-FL in exempt infant formula for pre-term infants.

We note that the remaining Regulatory, Chemistry, Microbiology and Toxicology questions below apply should the notifier agree to remove the intended use of 2′-FL in exempt infant formula for pre-term infants.

Regulatory:

1. Hypoallergenic exempt infant formulas can include those that are amino acid-based or extensively hydrolyzed protein-based. For the administrative record, please state whether use of 2′-FL as an ingredient in hypoallergenic exempt infant formulas includes amino acid-based or extensively hydrolyzed protein-based infant formula.
2. The notice does not mention the type of formula the notifier intends to add the 2′-FL to. Please state the intended source of the protein base (e.g., cow milk) of infant formula.
3. Please state whether the intended use of 2′-FL includes use in human milk fortifiers (HMF). If the notifier plans to use 2′-FL in HMF, the estimated daily intake (EDI) should consider the 2′-FL content of HMF in addition to 2′-FL in maternal or donor human milk.

4. Please note that, while the United States does not have a definition for "toddler formula", the Agency recognizes it as formula intended for infants 12+ months of age. However, if it is intended for infants under 12 months of age (for example, 9-18 months) then these products must follow the infant formula regulations as the intended population includes infants less than 12 months of age. If the notifier's use of the term "toddler formula" applies to infant formula for older infants (e.g., 9-12 months), we would request clarification of this use for the administrative record.

5. For the administrative record, please state whether the entirety of GRN 000571 and its supplement are incorporated by reference.

6. On page 9 of the notice and page 4 of Appendix A, the notifier refers to 2′-FL as a "nutrient". Because this ingredient is intended for use in infant formula, the definition of a "nutrient" is defined in 21 CFR Part 106.3. In our view, 2′-FL does not meet the definition of a "nutrient" as defined in 21 CFR Part 106.3. For the administrative record, please make a statement that corrects this reference.

7. In section 2.9 of the notice, the notifier includes discussion on "beneficial bacteria" (page 9). We note that the Agency's evaluation of GRAS notices focuses exclusively on the safety of the ingredient in food and not about purported beneficial effects of the substance. For the administrative record, the notifier should clarify that their GRAS conclusion is based strictly on data and information pertaining to safety, without regards to any potential beneficial effects of 2′-FL.

8. The notifier states that the intended use of 2′-FL is GRAS based on scientific procedures (21 CFR 170.30(b)); however, the notifier also includes a discussion in Part 5, Experience Based on Common Use in Foods (pages 13-16). Please note that the information provided in Part 5 does not meet the regulatory definition of "Common Use in Foods" as defined by 21 CFR Part 170.245. We note that the provided discussion should be incorporated into Part 6, Narrative, as defined by 21 CFR Part 170.250. For the administrative record, please make a statement that corrects this reference.

9. On page 19 of the notice, the notifier references Appendix X, however, Appendix X was not included in the notice. For the administrative record, please clarify this discrepancy.

10. In Appendix A, the notifier states that the Expert Panel, "… independently and collectively, critically evaluated the available information presented in the documents prepared and presented by Ramboll and other materials deemed appropriate and necessary for this review" (page 1). For the administrative record, please clarify if the Expert Panel reviewed materials not included in GRN 000929.

Chemistry:

11. The notifier incorporates information and data from GRN 000571 and its supplement by reference, including the results of batch analyses. For the administrative record, please provide the results from a minimum of three (preferably five) current non-consecutive batch analyses to demonstrate that 2′-FL meets the established specifications (see also

point 12 below). In addition, please confirm that all analytical methods used to test for each specification parameter are validated for that purpose.

12. The notifier states that 2′-FL is manufactured using the same process as described in GRN 000571 and its supplement. In GRN 000571 (Appendix J), the notifier lists cobalt (II) chloride hexahydrate as a component of the fermentation medium used in the manufacture of 2′-FL. Per 21 CFR 189.120, food containing added cobaltous salts, including cobalt (II) chloride, is deemed adulterated. Please discuss the potential presence of cobalt in the final product and provide analytical data from three (preferably five) non-consecutive batches to demonstrate that cobalt is not present in the final product.

13. In addition to the estimates of dietary exposure to 2′-FL from hypoallergenic infant and toddler formulas, we request that the notifier address cumulative dietary exposure to 2′-FL that also includes 2′-FL from infant and toddler foods.

14. As currently written, Section 2.3 of the notice (page 5) includes reference to previous notices and a statement that "… a summary of the manufacturing process of Jennewein 2′-FL using *E.coli* BL21 (DE3) strains as a processing aid is included below". The actual method of fermentation and subsequent purification is not described. This information is relevant for the population of milk-allergic infants. Provide a summary of the manufacturing process that includes relevant purification steps to ensure specifications can be met.

15. Given the subpopulation of milk-allergic infants that would consume the amino-acid based or extensively-hydrolyzed formulas listed in the description of intended use of 2′-FL, we request that the notifier address the potential source of milk protein and removal of these components by the method of purification of 2′-FL. Specifically, we request that the notifier:

    a. Describe food grade media components, identifying potential sources of milk protein or other allergenic proteins.

    b. Provide statements regarding regulatory status of food contact materials (i.e., filtration materials, cation and anion exchange chromatography, simulated moving bed chromatography, electrodialysis (page 24 of GRN 000571)) used in the method of manufacture, including citations to a relevant regulation and/or effective food contact notification for those uses.

    c. Provide a brief discussion of the steps incorporated during purification to ensure removal of milk protein. We note the protein specification (≤100 μg/g) is higher than limits of detection in the other assays described in Section 2.8 of GRN 000929 (pages 8-9).

Microbiology:

16. The notifier states that the production strain used in GRN 000571 is *Escherichia coli* BL21(DE3) strain "#1540", which contains a heat-inducible *lac*Z Ω gene fragment and a *lacZ*α gene fragment under the control of the *E. coli* BL21(DE3) *pgb*A promoter, resulting in production of high amounts of β-galactosidase when the temperature is shifted from 30°C to 42°C, facilitating excess lactose degradation.

    In the supplement to GRN 000571, the notifier states that the production strain used is *E. coli* BL21(DE3) strain "#1242", which lacks the aforementioned gene fragments and does not have the ability to produce β-galactosidase. As a result, food grade lactase is added at the end of production to remove excess lactose.

    As noted in GRN 000571, the notifier states that *E. coli* BL21(DE3) is used to derive the two productions organisms used in GRN 000571 and its supplement. For the administrative record, please clarify which strain is used as the production strain in the production of 2′-FL in GRN 000929.

17. Please state whether the production strain has been deposited in a recognized culture collection and provide the non-trade name designation. If the strain is not deposited, describe how the source was verified and identified.

18. As each GRAS notice stands on its own, for the administrative record, please provide detailed description of the production strain including phenotypic (e.g., pathogenicity, toxigenicity and antibiotic resistance) and genotypic characteristics (e.g., introduced and excised genes). Please include a summary and reference to GRN 000571 and its supplement regarding how the production strain was derived from *E. coli* BL21(DE3).

19. Please describe the virulence profile of the production strain, and state whether the production strain is expected to result in any safety concerns.

20. Please state whether the production strain is capable of DNA transfer to other organisms.

21. The notifier provides specifications for *Salmonella* serovars and *Cronobacter sakazakii* listed as negative by test in 100 grams. In GRNs 000921-000923 and in GRN 000925, which correspond to other human milk oligosaccharides produced by Jennewein Biotechnologie GmbH intended for use in infant formula, Jennewein provides specifications for *Salmonella* serovars and *Cronobacter sakazakii* listed as negative by test in 25 grams and 10 grams, respectively. For the administrative record, please clarify this discrepancy.

22. Please state whether the fermentation process follows cGMPs and is conducted in a contained, sterile environment.

Toxicology:

23. On page 14 of the notice, the notifier states, "In Google Scholar, only the first few pages of search results were screened for relevance". Please provide a complete literature review of relevant publications associated with the safety of 2′-FL.

24. The notifier provides a clinical study conducted in infants and young children with cow's milk protein allergy, which assessed the hypoallergenicity and safety of an extensively hydrolyzed formula supplemented with 1.0 g/L 2′-FL and 0.5 g/L LNnT against a control (Nowak-Wegrzyn et al. (2019). This study used lower level of 2′-FL (1.0 g/L compared to the proposed 2.0 g/L) and/or in combination with LNnT. Please provide a thorough discussion on why 2′-FL would not have an impact on the health of infants requiring hypoallergenic infant formulas.

Sincerely,

Stephanie A. Hice, Ph.D.
Staff Fellow (Biologist)
FDA Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
Division of Food Ingredients

October 19, 2020

Stephanie A. Hice, PhD
Staff Fellow (Biologist)
FDA Center for Food Safety and Applied Nutrition
Office of Food Additive Safety Division of Food Ingredients
5001 Campus Drive
College Park, MD 20740
T: +1 301-348-1740
E: Stephanie.Hice@fda.hhs.gov

RE: GRN 000929: Responses to questions

Dear Dr. Hice,

On behalf of the notifier, Jennewein Biotechnologie GmbH, we herein provide substantive answers and responses to the questions and requests from the GRN technical review team regarding GRN 000929.

Regarding FDA's outstanding comment: The notifier confirms that it hereby removes preterm infant formula (IF) use from GRN 000929. The Notifier will request a pre-notification consultation (PNC) to discuss a separate GRN for preterm IF use of 2'-FL.

Regarding FDA's questions concerning regulatory, chemistry, microbiology, and toxicology are shown in italics and the notifier's responses are immediately below each question respectively.

Regulatory:

Q-1. Hypoallergenic exempt infant formulas can include those that are amino acid-based or extensively hydrolyzed protein-based. For the administrative record, please state whether use of 2′-FL as an ingredient in hypoallergenic exempt infant formulas includes amino acid-based or extensively hydrolyzed protein-based infant formula.

> A-1. The notifier intends to use 2'-FL as an ingredient in term infant formula including both 1) extensively hydrolyzed protein-based hypoallergenic exempt infant formulas, and 2) amino acid-based hypoallergenic exempt infant formulas.

Q-2. The notice does not mention the type of formula the notifier intends to add the 2′-FL to. Please state the intended source of the protein base (e.g., cow milk) of infant formula.

A-2. The notifier confirms that the source of extensively hydrolyzed protein is from cow's milk.

Q-3. Please state whether the intended use of 2′-FL includes use in human milk fortifiers (HMF). If the notifier plans to use 2′-FL in HMF, the estimated daily intake (EDI) should consider the 2′-FL content of HMF in addition to 2′-FL in maternal or donor human milk.

A-3. The notifier states that this use of 2'-FL in HMF is not included in GRN 000929.

Q-4. Please note that, while the United States does not have a definition for "toddler formula", the Agency recognizes it as formula intended for infants 12+ months of age. However, if it is intended for infants under 12 months of age (for example, 9-18 months) then these products must follow the infant formula regulations as the intended population includes infants less than 12 months of age. If the notifier's use of the term "toddler formula" applies to infant formula for older infants (e.g., 9-12 months), we would request clarification of this use for the administrative record.

A-4. By use of the term "toddler formulas", the notifier means formulas intended for toddlers 12 months or greater and less than 36 months. Due to NHANES age grouping, the EDIs for toddler formulas are based on the age group 12 to 35 months. In GRN 000929 Section 1.4 "Intended Conditions of Uses of the Notified Substance" (p. 1), the notifier specifies that "toddler formulas for hypoallergenic use are formulas considered suitable for children from 12 through 35 months of age."

Q-5. For the administrative record, please state whether the entirety of GRN 000571 and its supplement are incorporated by reference.

A-5. The notifier states that the entirety of GRN 000571 and Supplement 1 to GRN 000571 are incorporated by reference into this GRN 000929. The notifier originally submitted these proposed uses of 2'-FL as a supplement to GRN 000571 in September 2019. Subsequently, in December 2019, FDA advised the notifier to submit these uses of 2'-FL as a separate GRN, not as a Supplement. The notifier submitted a separate GRN for these uses of 2'-FL in December 2019. Per FDA's request in March 2020, the GRN was revised to remove repeated information from GRN 000571 and Supplement 1 to GRN 000571. This revised GRN for these uses of 2'-FL was submitted to FDA in April 2020 and filed by FDA as GRN000929 on June 19, 2020.

Q-6. On page 9 of the notice and page 4 of Appendix A, the notifier refers to 2′-FL as a

"nutrient". Because this ingredient is intended for use in infant formula, the definition of a "nutrient" is defined in 21 CFR Part 106.3. In our view, 2′-FL does not meet the definition of a "nutrient" as defined in 21 CFR Part 106.3. For the administrative record, please make a statement that corrects this reference.

A-6. Although HMOs such as 2'-FL are naturally-occurring sources of carbohydrate to infants from mother's milk, the notifier clarifies and corrects that 2'-FL is not a nutrient as defined in 21 CFR Part 106.3.

Q-7. In section 2.9 of the notice, the notifier includes discussion on "beneficial bacteria" (page 9). We note that the Agency's evaluation of GRAS notices focuses exclusively on the safety of the ingredient in food and not about purported beneficial effects of the substance. For the administrative record, the notifier should clarify that their GRAS conclusion is based strictly on data and information pertaining to safety, without regards to any potential beneficial effects of 2′-FL.

A-7. The notifier confirms that the GRAS assessment and determination are solely based on the safety data presented in GRN 000929 and references incorporated as cited and discussed.

Q-8. The notifier states that the intended use of 2′-FL is GRAS based on scientific procedures (21 CFR 170.30(b)); however, the notifier also includes a discussion in Part 5, Experience Based on Common Use in Foods (pages 13-16). Please note that the information provided in Part 5 does not meet the regulatory definition of "Common Use in Foods" as defined by 21 CFR Part 170.245. We note that the provided discussion should be incorporated into Part 6, Narrative, as defined by 21 CFR Part 170.250. For the administrative record, please make a statement that corrects this reference.

A-8. The notifier confirms this GRAS assessment and determination were based solely on scientific procedures.

The notifier considers this information regarding background intakes of 2'-FL to be part of the dietary intake discussion and proposes to move the information to GRN 000929 Part 3 Dietary Exposure (in accordance with 21 CFR Part 170.235). Therefore, to conform to FDA's request and comply with 21 CFR Part 170 Subpart E, the notifier hereby requests:

(1) the information in GRN 000929 Part 5.1 – Naturally-Occurring 2'-FL: History of Exposure and Use – be re-ordered, re-numbered and re-named Part 3.1.1 – Background Intake Level: Naturally-Occurring 2'-FL; and

(2) the information in GRN 000929 Part 5.2 – Manufactured 2'-FL: Existing Exposure and Use – be re-ordered, re-numbered and re-named Part 3.1.2 – Background

Intake Level: Manufactured 2'-FL.

Q-9. On page 19 of the notice, the notifier references Appendix X, however, Appendix X was not included in the notice. For the administrative record, please clarify this discrepancy.

A-9. The Notifier clarifies and corrects that the Appendix referenced on page 19 of GRN 000929 is Appendix E – Previously Reviewed Safety Studies in GRN 000571 and Supplement 1 to GRN 000571.

Q-10. In Appendix A, the notifier states that the Expert Panel, "… independently and collectively, critically evaluated the available information presented in the documents prepared and presented by Ramboll and other materials deemed appropriate and necessary for this review" (page 1). For the administrative record, please clarify if the Expert Panel reviewed materials not included in GRN 000929.

A-10. All information considered by the Expert Panel is referenced in the Panel's report. All citations to information that the panel considered are included in GRN 000929 and/or referenced therein including GRN 000571 and Supplement 1 to GRN 000571.

Chemistry:

Q-11. The notifier incorporates information and data from GRN 000571 and its supplement by reference, including the results of batch analyses. For the administrative record, please provide the results from a minimum of three (preferably five) current non-consecutive batch analyses to demonstrate that 2′-FL meets the established specifications (see also point 12 below). In addition, please confirm that all analytical methods used to test for each specification parameter are validated for that purpose.

A-11. Results from five (5) current non-consecutive batch analyses are presented herein, in Table A-11 in Attachment 1. To ensure a consistent food-grade product, each batch of 2'-FL manufactured by Jennewein Biotechnologie is evaluated against the same product specifications that were established in GRN 000571 (Table 1 in GRN 000571). These product specifications control the amount of 2'-FL, carbohydrate by-products, DNA and endotoxin residues derived from the production strain, heavy metals, and selected microbiological parameters. Each parameter is measured using the same, fit-for-purpose, compendial and/or internally validated methods that were used and determined to be GRAS in GRN 000571. Data from five batches of the finished ingredient show that the manufacturing process continues to reproducibly produce a product that meets the specifications that were established in GRN 000571.

Currently no batch data for 2'-FL concentrate are available. Thus, the notifier requests that FDA cease evaluation of 2'-FL concentrate as part of GRN 000929 and only continue evaluation for 2'-FL powder.

Q-12. The notifier states that 2′-FL is manufactured using the same process as described in GRN 000571 and its supplement. In GRN 000571 (Appendix J), the notifier lists cobalt (II) chloride hexahydrate as a component of the fermentation medium used in the manufacture of 2′-FL. Per 21 CFR 189.120, food containing added cobaltous salts, including cobalt (II) chloride, is deemed adulterated. Please discuss the potential presence of cobalt in the final product and provide analytical data from three (preferably five) non-consecutive batches to demonstrate that cobalt is not present in the final product.

A-12. The notifier confirms that cobalt (II) chloride is no longer used as a process aid in the fermentation media (trace element solution) for the production of Jennewein 2'-FL. The notifier has analyzed previous batches of 2'-FL for cobalt (II) chloride. The residual analyses for cobalt (II) chloride for five non-consecutive batches are presented below in Table A-12. Thus, all Jennewein Biotechnologie trace element solutions and consequently all fermentation media are produced without cobalt.

Table A-12. Residual analyses for cobalt (II) chloride in production batches of Jennewein 2'-FL

Method	LOQ	Batch Number				
		16130039	16116049	16151039	26108010	26120020
		July 2019	April 2019	December 2019	February 2020	May 2020
PV-347 ICP-MS	0.04 mg/kg	< 0.04	< 0.04	< 0.04	< 0.04	< 0.04

Determined by the Institut für Produktqualität GmbH, which is a DIN EN ISO/IEC 17025-accredited laboratory.
Abbreviations: ICP-MS, inductively coupled plasma mass spectrometry; LOQ, limit of quantitation

As stated in the response to Q-11, currently no batch data for 2'-FL concentrate are available. Therefore, the notifier requests that FDA cease evaluation of 2'-FL concentrate as part of GRN 000929 and only continue evaluation for 2'-FL powder.

Q-13. In addition to the estimates of dietary exposure to 2′-FL from hypoallergenic infant and toddler formulas, we request that the notifier address cumulative dietary exposure to 2′- FL that also includes 2′-FL from infant and toddler foods.

A-13. As discussed in GRN 000929 Part 3.5 – Estimated Intakes of 2'-FL from the Proposed Uses, the use level of 2 g 2'-FL per liter formula is in the range of concentrations of 2'-FL normally found in human milk. Thus, the overall intake of 2'-FL by combination breast milk/formula-fed infants and toddlers likely would be comparable to the intake of 2'-FL by infants solely breastfeeding. Based on NHANES

2015-16, many infants consume both formula and other foods. A smaller proportion of toddlers consume both formula and other foods, presumably because most toddlers are consuming solid foods as the majority of their diet.

Table A-13-1. Proportions of Infants and Toddlers Eating both Formula and Other Foods

Population[a]	N [b]	N (eating both)	Percent users[c]
Infants, 0-5 mo	90	89	36.9%
Infants, 6-11 mo	122	111	82.6%
Toddlers, 12-35 mo	201	13	7.2%

[a] Breastfeeding infants and children were excluded from the sample population.

[b] Number of people consuming food category during the study period.

[c] Weighted percent.

Abbreviations: d = day; g = grams; L = liters; mo = months.

2'-FL is GRAS in other foods that infants and toddlers may consume (GRNs 546, 650, 735, 749, 853, 897). The uses described in these GRNs include milk-based beverages, milk products (e.g. whole milk, skim milk, yogurt), dairy product analogs, cereals, processed fruits and fruit juices, jams and jellies, baby foods (e.g. baby crackers and cookies) and other uses. The use levels also vary by GRN and the food uses described, ranging from 0.8 to 80 g/L or g/kg (unit is dependent on whether it is a beverage or a solid food). Based on a survey of proposed food uses of 2'-FL in milk-based beverages and specific cereals, a use level of 10 g 2'-FL per L beverage or per kg food is used to estimate the cumulative dietary exposure to 2'-FL that also includes 2'-FL from infant and toddler foods. Using NHANES 2015-2016 food categories consumed by toddlers wherein the addition of 2'-FL is GRAS, the cumulative estimated daily intakes of 2'-FL is presented in Table A-13-2.

Table A-13-2. Cumulative Estimated Daily Intake of Jennewein 2'-FL from Infant Formula, Toddler Formula and Other Foods Containing 2'-FL

Population	n[b]	Percent users[c]	Intakes of Certain Foods[d]		2'-FL Intake[e]	
			kg food/d		g 2'-FL/d	
			Mean	90th Percentile	Mean	90th Percentile
Infants, 0-5 mo	90	100.0%	0.94	1.25	2.00	2.60
Infants, 6-11 mo	122	100.0%	0.91	1.25	2.92	4.62
Toddlers, 12-35 mo	201	100.0%	0.72	1.13	6.96	11.32

[a] Breastfeeding infants and children were excluded from the sample population.

[b] Number of people consuming food category during the study period.

[c] Weighted percent of n[b].

[d] Intake of the foods that may contain 2'-FL as an ingredient: infant formula, milk, milk products, milk-based beverages, dairy product analogs, cereal or granola bars, baby crackers and cookies, processed fruit and fruit juices, jams and jellies, fitness water and thirst quenches, sports and isotonic, fluid replacement/electrolyte solution. These daily average data are based on a 2-day survey; some participants who had day 1 but not day 2 data are included using a single day of consumption.

[e] 2'-FL intake based on use level of 2 g 2'-FL per L formula and 10 g 2'-FL per kg food and other beverages

Abbreviations: bw = body weight; d = day; g = grams; kg = kilograms; L = liters; mg = milligrams; mo = months

Note: Data from NHANES 2015-16.

Q-14. As currently written, Section 2.3 of the notice (page 5) includes reference to previous notices and a statement that "… a summary of the manufacturing process of Jennewein 2′-FL using E. coli BL21 (DE3) strains as a processing aid is included below". The actual method of fermentation and subsequent purification is not described. This information is relevant for the population of milk-allergic infants. Provide a summary of the manufacturing process that includes relevant purification steps to ensure specifications can be met.

A-14. In according with instructions from FDA in March 2020, the notifier removed a recapitulation of the manufacturing process of 2'-FL previously described in GRN 000571 and Supplement 1 to GRN 000571. In response to your request, we provide the following expanded description:

The manufacturing process of 2'-FL uses genetically engineered *E. coli* BL21 (DE3) as a processing aid to produce the 2'-FL. To initiate the synthesis of 2'-FL by the *E. coli* BL21 (DE3) microbial cells via fermentation, the cells were genetically modified by introducing genes necessary to achieve the import of lactose and enhancing of GDP-fucose production. The *E. coli* BL21 (DE3) cells are inoculated into a chemically-defined, salt-based, minimal fermentation medium containing lactose. Batch fermentation is performed in a chemically-defined, salt-based, minimal medium that excludes inhibitors or antibiotics, with glycerol as the only carbon source and lactose

as the substrate. The major constituents of the fermentation medium are: glycerol, lactose, ammonium dihydrogen phosphate ($NH_4H_2PO_4$), dipotassium phosphate (K_2HPO_4), citric acid, potassium hydroxide (KOH), and magnesium sulfate heptahydrate ($MgSO_4 \cdot 7H_2O$). The fermentation process, which does not use antibiotics or inhibitors, leads to the production of 2'-FL from lactose by the *E. coli* BL21 (DE3) cells. The synthesis of 2'-FL is followed by its export into the medium, and the subsequent degradation of excess lactose if necessary. Supplement 1 to GRN 000571 describes a slight modification to the *E. coli* BL21 (DE3) cells used in the manufacturing process, wherein the *E. coli* BL21 (DE3) cells do not have the ability to degrade excess lactose and a food-grade commercial lactase is added if excess lactose is present in the media. The culture supernatant containing 2'-FL is then isolated from the medium and the microbial biomass removed via 10 kDa cross-flow filtration producing a sterile 2'-FL product.

The design of the production strains and the purification process of 2'-FL allows for fermentation without the use of antibiotics, inhibitors or otherwise toxic additives. The purification process completely removes the biomass from the media. A sequence of filtration and chromatographic steps yields 2'-FL of food grade quality that is consistent and compliant with the specifications. Proteins are removed from the 2'-FL solution by the ultrafiltration step using a 10 kiloDalton (kDa) filter immediately after fermentation, as well as by the final ultrafiltration process using a 3/5 kDa filter during filling of the 2'-fucosyllactose concentrate at the Critical Control Point (CCP) – removal of endotoxins and of micro-impurities (e.g. traces of DNA and proteins).

Q-15. Given the subpopulation of milk-allergic infants that would consume the amino-acid based or extensively-hydrolyzed formulas listed in the description of intended use of 2′- FL, we request that the notifier address the potential source of milk protein and removal of these components by the method of purification of 2′-FL. Specifically, we request that the notifier:

a) Describe food grade media components, identifying potential sources of milk protein or other allergenic proteins.

A-15a. As described in GRN000571 Part 2.2 – Production and Manufacturing Process of Jennewein 2'-FL – "only food or pharmaceutical grade chemicals, solvents and processing aids (e.g., ion exchange resins, activated carbon and filtration membranes) are used in the manufacture of Jennewein 2'-FL." Furthermore, all production batches meet the established specifications for food-grade materials (see Attachment 1 for specifications and batch data for current batches). 2'-FL does not contain residual components or impurities from the manufacturing process with allergenic potential and there is no evidence to suggest that it may cause adverse effects in sensitive populations. Residual components in 2'-FL are also present in human milk (lactose, LDFT, 3-FL, fucose, glucose and galactose) or otherwise naturally present in the human body (fucosylgalactose) and were present in the material used in the Notifier's toxicological studies.

As described in Supplement 1 to GRN 000571 Part 4.3 and 4.4, the potential source of allergens from the lactose from cow's milk has been evaluated and the production process is designed to specifically address excess lactose. The analytical program is designed to confirm that it is removed. As discussed in Supplement 1 to GRN 000571, Part 4.4, also demonstrated in the batch data and specifications, Jennewein 2'-FL is consistently devoid of proteins (not detected), bacteria or bacterial endotoxins, residual recombinant DNA, antibiotics, and chemical sensitizers including metals, and meet the specifications. Therefore, the allergenic potential of Jennewein 2'-FL is extremely low and no sensitive populations have been identified or are anticipated.

b) *Provide statements regarding regulatory status of food contact materials (i.e., filtration materials, cation and anion exchange chromatography, simulated moving bed chromatography, electrodialysis (page 24 of GRN 000571)) used in the method of manufacture, including citations to a relevant regulation and/or effective food contact notification for those uses.*

A-15b. As presented in GRN 000571, information regarding the food contact materials is provided in Appendix E. The notifier provides a table of the food contact materials and the relevant regulatory citations for food contact clearance.

Table A-15-1: Processing aids and their regulatory status

Compound or Material	Function	Regulatory Status
Styrene-divinylbenzene-copolymer	ion exchange; removal of small molecules	21 CFR § 173.25(a)
Polyethersulfone	ultrafiltration; removal of impurities	21 CFR § 177.2440
polysulfone membrane	ultrafiltration; removal of impurities	21 CFR § 177.1655
Filter: Polyethersulfone, asymmetric; polypropylene; and silicone	filtration	21 CFR § 177.2440, 21 CFR § 175.105, 21 CFR § 175.300, 21 § CFR 175.1520, 21 CFR § 177.2600
Lactose	substrate	21 CFR § 168.122, 21 CFR § 184.1979a
Glycerol	carbon source	21 CFR § 182.1320
Dipotassium phosphate	fermentation ingredient (K source)	21 CFR § 182.6285
Citric acid monohydrate	fermentation ingredient (pH adjuster)	21 CFR § 184.1033
Calcium chloride dihydrate	fermentation ingredient (Ca source)	21 CFR § 184.1193
Magnesium sulphate heptahydrate	fermentation ingredient (Mg source)	21 CFR § 184.1443

Table A-15-1: Processing aids and their regulatory status		
Compound or Material	Function	Regulatory Status
Mono ammonium phosphate	fermentation ingredient (nitrogen source)	21 CFR § 184.1141a
Potassium hydroxide	fermentation ingredient (pH adjuster)	21 CFR § 184.1631
Alkoxylated fatty acid esters on vegetable base	anti-foaming agent	21 CFR § 173.340
Activated carbon	purification	FD&C Act Section 201(s)
Abbreviations: 21 CFR - United States Code of Federal Regulations Title 21; FD&C - United States Federal Food, Drug & Cosmetic		

c) *Provide a brief discussion of the steps incorporated during purification to ensure removal of milk protein. We note the protein specification (≤100 µg/g) is higher than limits of detection in the other assays described in Section 2.8 of GRN 000929 (pages 8-9).*

A-15c. The notifier asserts that the lactose utilized in the production of 2'-FL is theoretically free of milk protein. To further ensure this milk protein-free status, as the last step in the last step of the manufacturing process a 3/5 kDa filter removes any milk proteins.

Described in Appendix C of GRN 000929, the Size Exclusion Chromatography test (HPLC with a size exclusion chromatography column) method is more sensitive and demonstrates no detected proteins. This method is not practical for batch quality control analyses.

Microbiology:

Q-16. The notifier states that the production strain used in GRN 000571 is Escherichia coli BL21(DE3) strain "#1540", which contains a heat-inducible lacZ Ω gene fragment and a lacZα gene fragment under the control of the E. coli BL21(DE3) pgbA promoter, resulting in production of high amounts of β-galactosidase when the temperature is shifted from 30°C to 42°C, facilitating excess lactose degradation.

In the supplement to GRN 000571, the notifier states that the production strain used is E. coli BL21(DE3) strain "#1242", which lacks the aforementioned gene fragments and does not have the ability to produce β-galactosidase. As a result, food grade lactase is added at the end of production to remove excess lactose.

As noted in GRN 000571, the notifier states that E. coli BL21(DE3) is used to derive the two productions organisms used in GRN 000571 and its supplement. For the administrative record, please clarify which strain is used as the production strain in the production of 2'-FL in GRN 000929.

A-16. Clarification of strain used: *E. coli* BL21 (DE3) #1242 strain is used in the production process. (This strain is also now referred to as JBT-2FL ΔlacZ.)

Q-17. Please state whether the production strain has been deposited in a recognized culture collection and provide the non-trade name designation. If the strain is not deposited, describe how the source was verified and identified.

A-17. The notifier confirms that, yes, the *E. coli* BL21 (DE3) #1242 strain has been deposited in the DSMZ - German Collection of Microorganisms and Cell Cultures GmbH with the deposition number DSM 33609.

Q-18. As each GRAS notice stands on its own, for the administrative record, please provide detailed description of the production strain including phenotypic (e.g., pathogenicity, toxigenicity and antibiotic resistance) and genotypic characteristics (e.g., introduced and excised genes). Please include a summary and reference to GRN 000571 and its supplement regarding how the production strain was derived from E. coli BL21 (DE3).

A-18. The information requested by FDA is provided in Appendix K of GRN 00571 and Part 2.3 – Modification to the production process: strain #1242 of Supplement 1 of GRN 000571 which have been incorporated into GRN 00929 by reference. Based on pre-notification consultation from FDA in December 2019 and a with FDA in March 2020 following the initial submission of a GRN for these uses in December 2019 advising re-submission of a pared-down GRN, the notifier did not recapitulate information from GRN 000571 and Supplement 1 to GRN 000571 that remains unchanged.

As described in Appendix K of GRN 000571 and Supplement 1 to GRN 000571, the *E. coli* BL21 (DE3) production strains used as a processing aid during the fermentation process in the manufacture of 2'-FL are genetically engineered strains of the commensal bacterium *E. coli* BL21 (DE3). The phenotypic and genotypic characteristics are described in detail in Appendix K of GRN 000571. If the reviewer needs a copy of Appendix K of GRN 000571, the notifier can provide and attach a copy of Appendix K to GRN 000929.

Q-19. Please describe the virulence profile of the production strain, and state whether the production strain is expected to result in any safety concerns.

A-19. The notifier states that this information is presented in GRN 000571 and Appendix K of GRN 000571 and is summarized briefly here. The parental strain *E. coli* BL21 (DE3) is a commensal *E. coli* strain with no known toxicity. It is used for many years for the safe production of heterologous proteins for biopharmaceutical

applications and also used in the manufacture of at least one GRAS ingredient intended for use as a food ingredient (FDA GRN 485).

E. coli BL21 (DE3) shows absence of genes encoding invasion factors, adhesion molecules, and enterotoxins associated with virulence (Jeong et al. 2009). Because Jennewein engineered *E. coli* BL21 (DE3) #1242 (JBT-2FL ΔlacZ) with genes with known functions and that do not confer virulence using site-specific homologous recombination or transposition, *E. coli* BL21 (DE3) #1242 (JBT-2FL ΔlacZ) has the same virulence profile as E. coli BL21(DE3).

As described in GRN 000571, the risk of contamination of Jennewein 2'-FL with bacterial material like protein or DNA is very low because the manufacturing process is highly specific and designed to remove all components but 2'-FL. The use of metabolically engineered strain of *E. coli* BL21 (DE3) for the manufacture of 2'-FL is safe.

Q-20. Please state whether the production strain is capable of DNA transfer to other organisms.

A-20. The production strain is not capable of DNA transfer to other organisms.

Q-21. The notifier provides specifications for Salmonella serovars and Cronobacter sakazakii listed as negative by test in 100 grams. In GRNs 000921-000923 and in GRN 000925, which correspond to other human milk oligosaccharides produced by Jennewein Biotechnologie GmbH intended for use in infant formula, Jennewein provides specifications for Salmonella serovars and Cronobacter sakazakii listed as negative by test in 25 grams and 10 grams, respectively. For the administrative record, please clarify this discrepancy.

A-21. The specifications for *Salmonella* serovars and *Cronobacter sakazakii* for the 2'-FL that is the subject GRN 000929 were established and determined GRAS in 2015 in GRN 000571. Although these specifications have remained in place since then, Jennewein has learned that specifications for *Salmonella* serovars and *Cronobacter sakazakii* of absent in 25 g product and absent in 10 g of product, respectively, are sufficient to produce a safe infant formula ingredient. Based on these results, the specifications for *Salmonella* serovars and *Cronobacter sakazakii* being absent in 25 g product and absent in 10 g of product were established for the subjects of GRNs 000921-000923 and GRN 000925. However, because the *Salmonella* serovars and *Cronobacter sakazakii* specifications for the 2'-FL that is the subject of GRN 000929 are based on GRN 000571, to maintain consistency between this GRN and GRN 000571, the notifier decided to continue with the specifications that were established in GRN 000571.

Based on FDA's request, the notifier agrees to change the specification for the

subject of GRN 000929 from “absence of *Salmonella* serovars in 100 g” to “absence of *Salmonella* serovars in 25 g”, and from “absence of *Cronobacter sakazakii* in 100g” to “absence of *Cronobacter sakazakii* in 10g”.

Q-22. Please state whether the fermentation process follows cGMPs and is conducted in a contained, sterile environment.

A-22. As stated in GRN 000929 Part 6.5.1 – Summary of Safety Data for Exempt IF, 2’-FL is manufactured in accordance with current Good Manufacturing Practice (cGMP). The notifier states that the fermentation process follows cGMP in a contained, sterile environment, as described in GRN 000571 and Supplement 1 to GRN 000571.

Toxicology:

Q-23. On page 14 of the notice, the notifier states, “In Google Scholar, only the first few pages of search results were screened for relevance”. Please provide a complete literature review of relevant publications associated with the safety of 2′-FL.

A-23. As GRN000929 is a follow-on notice to GRN000571 presenting Jennewein 2’-FL as GRAS for different uses, the updated literature review of relevant publications in GRN 000929 includes summaries of publications included in the weight of evidence previously presented in GRN 000571 and Supplement 1 to GRN 000571. An updated literature search was conducted in the United States National Library of Medicine at the National Institutes of Health PubMed database to capture new safety literature in the time period after GRN 000571 and Supplement 1 to GRN 000571. The literature search was re-run on October 15, 2020 with an expanded timeframe to capture studies with safety or toxicological endpoints published since submittal of GRN 000929 to FDA in April 2020: (((fucosyllactose[All Fields] OR ("2'-fucosyllactose"[Supplementary Concept] OR "2'-fucosyllactose"[All Fields] OR "2' fucosyllactose"[All Fields])) OR ("2'-fucosyllactose"[Supplementary Concept] OR "2'-fucosyllactose"[All Fields] OR "2 fucosyllactose"[All Fields])) OR 2-FL[All Fields]) OR 2'-FL[All Fields] AND ("2019/06/25"[PDAT] : "2020/12/31"[PDAT])

Only toxicological studies evaluating 2’-FL as part of the test substance were included. All clinical studies that did not discuss safety, growth nor tolerance parameters of 2’-FL in the target population (i.e. infants and toddlers) were excluded. The search included 115 results; four publications were flagged as relevant and are summarized briefly below.

Toxicological Evaluations

The Notifier would like to add that Phipps et al. (2020) conducted toxicological safety

assessment of a biosynthesized mixture of lacto-*N*-fucopentaose I (LNFP-I) and 2'-FL intended for use in infant formula. The study authors conducted a bacterial reverse mutation test, an *in vitro* mammalian cell micronucleus test, and a 90-day oral gavage study in neonatal rats. LNFP-I/2'-FL was nongenotoxic *in vitro* and 5000 mg/kg bw/day LNFP-I/2'-FL, the highest dose tested in the neonatal rats, was the no-observed-adverse-effect level.

Bacterial reverse mutation tests, *in vitro* micronucleus tests, and a repeated-dose oral toxicity study in rats were conducted to evaluate the safety of a human milk oligosaccharide mixture containing the Notifier's 2'-FL ingredient (Parschat et al. 2020). The HMO mixture containing 2'-FL, 3-fucosyllactose, lacto-N-tetraose, 2'-sialyllactose, and 6'sialyllactose was not genotoxic and, in the repeated dose study, did not induce adverse effects.

Clinical Studies

Published since the submission of GRN 000929 in April 2020, Leung et al. (2020) describe a randomized, controlled, double-blind, parallel-group trial of 461 healthy Chinese children 1 to 2.5 years of age. Participants received either standard milk young child formula (YCF) or one of three YCFs containing bioactive proteins and/or 2'-FL and/or milk fat for six months. There were no significant between-group differences in upper respiratory tract infection and duration of gastrointestinal tract infections. Adverse events and anthropometric values were similar in all groups.

An open-label prospective study with 159 infants was conducted with a milk-based formula supplemented with 1 g/L of 2'-FL and 0.5 g/L of LNnT and contained *Lactobacillus reuteri* (Roman et al. 2020). The formula was given for 8 weeks to an exclusively formula-fed group (n=66) and also to a group that also breastfed (n=48). A third group was exclusively breastfeeding infants (n=45). Growth was comparable across groups. There was low gastrointestinal distress reported and low incidence of adverse events, comparable across all three groups.

Preterm / Hypoallergenic

As described in GRN 000929 Part 3.1.1 – Background Intake Level: Naturally Occurring 2'-FL, (formerly Part 5.1 – Naturally-Occurring 2'-FL: History of Exposure and Use – and Part 5.1.3 – 2'-FL in the Milk Produced by Mothers with Infants Born Preterm or with Low Birth Weight), a focused literature review was conducted to capture publications about 2'-FL related to infants born preterm or with low birth weight in the United States National Library of Medicine at the National Institutes of Health PubMed database using the search terms "2-FL", "2'-fucosyllactose", "2-fucosyllactose" combined with variations of "preterm" and "birth weight". Relevant studies were also identified from reviewing the references of potentially relevant publications. The Notifier clarifies and corrects that Google Scholar was used only in a supplementary, secondary capacity. Studies were excluded from the review if they did not evaluate 2'-FL and the target population of preterm infants or low birth weight or very low birth weight infants and/or their mothers.

Published since the April 2020 submission of GRN 000929 to FDA, the notifier notes that a study by Torres Roldan et al. (2020) provides further evidence that 2'-FL is naturally-occurring in preterm milk. Torres Roldan et al. (2020) conducted a retrospective cohort study of 153 mothers and their very-low-birthweight (<1500 g) infants, analyzing HMOs in 208 milk samples. 2'-FL was one of the most abundant oligosaccharides among those with the secretor phenotype (defined by the presence of α1-2-fucosylated HMOs), who made up 93% of the mothers.

Q-24. The notifier provides a clinical study conducted in infants and young children with cow's milk protein allergy, which assessed the hypoallergenicity and safety of an extensively hydrolyzed formula supplemented with 1.0 g/L 2′-FL and 0.5 g/L LNnT against a control (Nowak-Wegrzyn et al. 2019). This study used lower level of 2′-FL (1.0 g/L compared to the proposed 2.0 g/L) and/or in combination with LNnT. Please provide a thorough discussion on why 2′-FL would not have an impact on the health of infants requiring hypoallergenic infant formulas.

A-24. Infants requiring hypoallergenic infant formulas have allergies against cow's milk protein or such allergies are feared by their parents. For this reason, hypoallergenic infant formulas contain extensively hydrolyzed protein or are based on amino acids instead of protein.

The molecule 2'-fucosyllactose is a carbohydrate, not a protein, and is secreted in varying levels in about 70% of human mothers (Kunz et al. 1999 as cited in GRN 000571). No allergies against human mother's milk itself or its natural components are described in literature. Studies of 2'-FL naturally-occurring in mother's milk discussed in Part 3.1.1 – Background Intake Level: Naturally-Occurring 2'-FL (formerly Part 5.1 – Naturally-Occurring 2'-FL: History of Exposure and Use) support the conclusion that 2'-FL itself is not allergenic at varying levels of 2'-FL.

The potential source of allergenic agents in the subject of GRN 000929 are proteins from the production process, especially cow's milk proteins that could originate from the substrate lactose. The size of the allergenic cow's milk proteins ranges from 14.2 to 160 kDa (Hochwallner et al., 2014). The notifier discusses the purification process and assertion of protein-free status in the responses to Q-14 and Q-15 in this response memorandum. The manufacturing process (including the ultrafiltration steps with pore sizes significantly smaller than the size of the cow's milk proteins, which are effectively separating off all potentially included cow's milk proteins) are described and discussed in GRN 000929, in GRN 000571 and Supplement 1 to GRN 000571. These ultrafiltration steps effectively remove all proteins from the process, as described in GRN 000929 Part 2.8 – Allergenic Potential and Absence of Protein, and as shown in GRN 000929 Appendix C – Protein Analyses. Thus, the subject of this GRN would not have any impact on the health of infants requiring hypoallergenic infant formulas.

We trust that our responses have addressed your questions and requests. As indicated in

several responses substantial additional information is provided in the predecessor safety dossiers, GRN 000571 and Supplement 1 to GRN 000571 incorporated by reference in GRN 000929. However, should you have any additional questions or comments at any time during your review, please contact us promptly, so that we may provide a substantive response in a timely manner.

Sincerely yours,

Gavin P Thompson, PhD
Principal Consultant
Ramboll Environment & Health
Phoenix, Arizona

CC: Julia Parkot, PhD, Jennewein Biotechnologie, GmbH

Attachment 1: Batch Data

Attachment 2: Reference Literature

References (Attachment 2)

Hochwallner, H., Schulmeister, U., Swoboda, I., Spitzauer, S., & Valenta, R. (2014). Cow's milk allergy: From allergens to new forms of diagnosis, therapy and prevention. *Methods*, *66*(1), 22-33.

Leung, T. F., Ulfman, L. H., Chong, M. K., Hon, K. L., Khouw, I. M., Chan, P. K., ... & Bovee-Oudenhoven, I. M. (2020). A randomized controlled trial of different young child formulas on upper respiratory and gastrointestinal tract infections in Chinese toddlers. *Pediatric Allergy and Immunology*, *31*(7), 745-754.

Parschat, K., Oehme, A., Leuschner, J., Jennewein, S., & Parkot, J. (2020). A safety evaluation of mixed human milk oligosaccharides in rats. *Food and Chemical Toxicology*, *136*, 111118.

Phipps, K. R., Lynch, B., Stannard, D. R., Gilby, B., Baldwin, N., Mikš, M. H., ... & Röhrig, C. H. Genotoxicity and neonatal subchronic toxicity assessment of a novel mixture of the human-identical milk oligosaccharides lacto-N-fucopentaose I and 2′-fucosyllactose. *Journal of Applied Toxicology*.

Román, E., JM, M. V., Alshweki, A., Cercamondi, C., Dahbane, S., & ML, V. G. (2020). Real-world study in infants fed an infant formula with two human milk oligosaccharides. *Nutricion Hospitalaria*.

Torres Roldan, V. D., Urtecho S, M., Gupta, J., Yonemitsu, C., Cárcamo, C. P., Bode, L., & Ochoa, T. J. (2020). Human milk oligosaccharides and their association with late-onset neonatal sepsis in Peruvian very-low-birth-weight infants. *The American Journal of Clinical Nutrition*.

ATTACHMENT 1:
2'-FL: Product Specifications and Batch Data

Table A-11. 2'-FL: Product Specifications and Batch Data							
Parameter	Analytical method	Specification	Batch number				
			16130039	16116049	16151039	26108010	26120020
			July 2019	April 2019	December 2019	February 2020	May 2020
Physical Parameters							
Appearance (Color)[4]	Visual	White to ivory-colored	Complies	Complies	Complies	Complies	Complies
Appearance (Form)[4]		Spray-dried powder	Complies	Complies	Complies	Complies	Complies
Chemical Parameters							
2'-Fucosyllactose	HPAEC-PAD	≥ 90 % (%DW)	92.2	98.4	95.5	97.8	94.9
Lactose		≤ 5 % (% Area)	1.1	< 0.5	2.5	< 0.5	0.5
3-Fucosyllactose		≤ 5 % (% Area)	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
Difucosyllactose		≤ 5 % (% Area)	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
Fucosylgalactose		≤ 3 % (% Area)	< 0.5	< 0.5	< 0.5	< 0.5	0.5
Glucose		≤ 3 % (% Area)	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
Galactose		≤ 3 % (% Area)	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
Fucose		≤ 3 % (% Area)	0.7	< 0.5	1.8	< 0.5	0.7
Protein content[4]	Nanoquant (modified Bradford)	≤ 100 µg/g	< 10	< 10	< 10	< 10	< 10
Ash[1]	ASU L 06.00-4	≤ 0.5 %	< 0.01	0.03	0.08	< 0.01	0.08
Moisture[4]	KF titration	≤ 9.0 %	5.8	5.8	6.3	6.6	5.2
Endotoxins[3]	Ph. Eur. 2.6.14	≤ 300 EU/g	14	< 5	< 5	< 5	< 5
Aflatoxin M1[1]	DIN EN ISO 14501	≤ 0.025 mg/kg	< 0.025	< 0.025	< 0.025	< 0.025	< 0.025
GMO residues[2]	PCR	Negative	Negative	Negative	Negative	Negative	Negative
Heavy Metals							
Arsenic[1]	ASU L 00.00-135 – ICP-MS	≤ 0.2 mg/kg	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
Cadmium[1]		≤ 0.1 mg/kg	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010
Lead[1]		≤ 0.02 mg/kg	< 0.010	< 0.010	0.020	< 0.010	< 0.010
Mercury[1]		≤ 0.5 mg/kg	< 0.005	0.007	< 0.005	< 0.005	< 0.005
Microbiology							
Standard Plate Count[1]	ISO 4833-2	≤ 10000 cfu/g	< 10	< 10	30	20	< 10
Yeast and Mold[1]	ISO 21527-2	≤ 100 cfu/g	< 20	< 20	< 20	< 20	< 20
Coliform	ISO 4832	Absent/11 g	Absent	Absent	Absent	Absent	Absent
Enterobacteriaceae[1]	ISO 21528-1	Absent/11 g	Absent	Absent	Absent	Absent	Absent

Table A-11. 2'-FL: Product Specifications and Batch Data							
Parameter	Analytical method	Specification	Batch number				
			16130039	16116049	16151039	26108010	26120020
			July 2019	April 2019	December 2019	February 2020	May 2020
Salmonella [1]	ISO 6579	Absent/100 g	Absent	Absent	Absent	Absent	Absent
Cronobacter sakazakii[1]	ISO/TS 22964	Absent/100 g	absent	absent	absent	absent	absent

Abbreviations: DW, dry weight; cfu, colony forming units; KF, Karl-Fischer; HPAEC-PAD, high performance anion exchange chromatography coupled with pulsed amperometric detection; PCR, polymerase chain reaction; ICP-MS, inductively coupled plasma mass spectrometry; EU, endotoxin unit; Ph Eur., European Pharmacopoeia.
[1]Determined by the Institut für Produktqualität GmbH, which is a DIN EN ISO/IEC 17025-accredited laboratory; ash limit of quantitation (LOQ) = 0.01 %. arsenic limit of detection (LOD) = 0.05 mg/kg; cadmium LOD = 0.01 mg/kg; mercury LOD = 0.005 mg/kg; lead LOD = 0.01 ppm; aflatoxin M1 LOQ = 0.025 µg/kg.
[2]Determined by GeneCon International GmbH, which is a DIN EN ISO/IEC 17025-accredited laboratory. Limit of detection = 0.01% of the finished product.
[3]Determined by Mikrobiologisches Labor. Dr. Michael Lohmeyer GmbH, which is a DIN EN ISO/IEC 17025-accredited laboratory; limit of quantitation = 5 EU/g.
[4]Determined by Jennewein Biotechnologie using internally validated methods. Protein LOQ = 10 µg/g; carbohydrate by-products with a percent area greater than 0.5% (limit of quantitation) are considered.

ATTACHMENT 2:
Reference Literature

References

Hochwallner, H., Schulmeister, U., Swoboda, I., Spitzauer, S., & Valenta, R. (2014). Cow's milk allergy: From allergens to new forms of diagnosis, therapy and prevention. *Methods*, *66*(1), 22-33.

Leung, T. F., Ulfman, L. H., Chong, M. K., Hon, K. L., Khouw, I. M., Chan, P. K., ... & Bovee-Oudenhoven, I. M. (2020). A randomized controlled trial of different young child formulas on upper respiratory and gastrointestinal tract infections in Chinese toddlers. *Pediatric Allergy and Immunology*, *31*(7), 745-754.

Parschat, K., Oehme, A., Leuschner, J., Jennewein, S., & Parkot, J. (2020). A safety evaluation of mixed human milk oligosaccharides in rats. *Food and Chemical Toxicology*, *136*, 111118.

Phipps, K. R., Lynch, B., Stannard, D. R., Gilby, B., Baldwin, N., Mikš, M. H., ... & Röhrig, C. H. Genotoxicity and neonatal subchronic toxicity assessment of a novel mixture of the human-identical milk oligosaccharides lacto-N-fucopentaose I and 2′-fucosyllactose. *Journal of Applied Toxicology*.

Román, E., JM, M. V., Alshweki, A., Cercamondi, C., Dahbane, S., & ML, V. G. (2020). Real-world study in infants fed an infant formula with two human milk oligosaccharides. *Nutricion Hospitalaria*.

Torres Roldan, V. D., Urtecho S, M., Gupta, J., Yonemitsu, C., Cárcamo, C. P., Bode, L., & Ochoa, T. J. (2020). Human milk oligosaccharides and their association with late-onset neonatal sepsis in Peruvian very-low-birth-weight infants. *The American Journal of Clinical Nutrition*.

68 reference pages have been removed in accordance with copyright laws. The removed references are:

Hochwallner, “Cow’s milk allergy: From allergens to new forms of diagnosis, therapy and prevention” Elsevier, Methods 66, 2013

Leung, “A randomized controlled trial of different young child formulas on upper respiratory and gastrointestinal tract infections in Chinese toddlers” Wiley 2020

Parschat, “A safety evaluation of mixed human milk oligosaccharides in rats”
Elsevier, Food and Chemical Toxicology 136 (2020)

Phipps, “Genotoxicity and neonatal subchronic toxicity assessment of a novel mixture of the human-identical milk oligosaccharides lacto-N-fucopentaose I and 2′-fucosyllactose”
Journal of Applied Toxicology,2020

Roman, “Real-world study in infants fed with an infant formula with two human milk oligosaccharides” Nutricion Hospitalaria, Trabajo Original

Torres, “Human milk oligosaccharides and their association with late-onset neonatal sepsis in Peruvian very-low-birth-weight infants” ASN, 2020

From:	Gavin P Thompson
To:	Hice, Stephanie
Cc:	Julia Parkot; Anne Oehne (Anne.Oehme@jennewein-biotech.de); Stephen ORourke; Cassie Huang
Subject:	RE: GRN 000929 - Questions for Notifier
Date:	Monday, December 7, 2020 11:59:27 PM
Attachments:	image001.png GRN000929-Responses_to_FDA_Questions.pdf

Dear Dr. Hice:

Regarding GRN 000929 and on behalf of the Notifier, Jennewein Biotechnologies, GmbH, we provide (attached hereto) responses to the FDA GRN technical review team's two questions presented to us on November 23, 2020.

Please contact us if you have any additional questions or comments at any time during your review of GRN 000929.

Regards,
Gavin

Gavin P Thompson, PhD
Principal Consultant
Product Safety & Stewardship
Ramboll US Consulting
+1 (602) 734 7704 direct
+1 (703) 589 8023 mobile
gthompson@ramboll.com

_____

2111 East Highland Avenue, Suite 402
Phoenix, AZ 85016 USA
Profile: https://ramboll.com/contact/environ/gthompson

From: Hice, Stephanie <Stephanie.Hice@fda.hhs.gov>
Sent: November 23, 2020 1:19 PM
To: Gavin P Thompson <GThompson@ramboll.com>
Subject: RE: GRN 000929 - Questions for Notifier
Importance: High

Dear Dr. Thompson,

During our review of GRAS Notice No. 000929, we noted further questions that need to be addressed and are attached to this email.

We respectfully request a response within **10 business days**. If you are unable to complete the response within that time frame, please contact me to discuss further options. Please do not include any confidential information in your response.

If you have questions or need further clarification, please feel free to contact me. Thank you in advance for your attention to our comments.

Sincerely,

Stephanie Hice

Stephanie Hice, PhD
Staff Fellow (Biologist)
Division of Food Ingredients
Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
U.S. Food and Drug Administration
stephanie.hice@fda.hhs.gov

RAMBOLL

ENVIRONMENT
& HEALTH

December 7, 2020

Stephanie A. Hice, PhD
Staff Fellow (Biologist)
FDA Center for Food Safety and Applied Nutrition
Office of Food Additive Safety Division of Food Ingredients
5001 Campus Drive
College Park, MD 20740
T: +1 301-348-1740
E: Stephanie.Hice@fda.hhs.gov

GRN 000929:
Notifier's Responses to FDA Questions (November 23, 2020)

Dear Dr. Hice,

Ramboll US Consulting

2111 East Highland Avenue
Suite 402
Phoenix, AZ 85016
USA

T +1 602 734 7704
F +1 602 734 7701
gthompson@ramboll.com

On behalf of the Notifier, Jennewein Biotechnologie GmbH, we herein provide substantive answers and responses to two questions from FDA's GRN technical review team regarding GRN 000929 provided to us on November 23, 2020.

Q-1. Chemistry:
The exposure estimate the notifier provide in the amendment (response to FDA's question 13) includes conservative assumptions about the use of 2′-fucosyllactose (2′-FL) in beverages but also does not explicitly include certain foods included among their proposed uses: breakfast cereals (for infants, toddlers, or all ages), ice cream and frozen yogurt, fruit fillings, syrups. Further, the notifier's estimate does not include certain foods included in other GRNs such as vegetable juice and carbonated beverages. We note that while the assumption of 10 g per kg of food is within proposed levels for various foods (although 12 g/kg is more commonly cited), the 10 g/L for all beverages results in an unrealistic overestimate of exposure, particularly for toddlers.

We request that the notifier either revise their exposure estimate using levels specific to each food category, possibly omitting foods not suited for hypoallergenic consumers, or incorporate (by reference or citation) the cumulative estimate of exposure provided in GRN 000735, addressing why background intakes from other uses of 2′-FL outside the scope of this notice but part of the current background diet in the US (e.g., vegetable juice, carbonated beverages, flavored waters), would not meaningfully increase the overall estimates of dietary exposure to 2′-FL for toddlers. Additionally, when citing this estimate the notifier may provide the caveat that some uses included in the calculation of cumulative exposure may not be suitable for milk-allergic individuals.

A-1. Notifier's Response:

The Notifier acknowledges that 2'-fucosyllactose (2'-FL) is added to many foods including infant formulas and toddler formulas. As recommended by FDA, the Notifier examined estimated intakes presented in GRN 735, which included in addition to infant formula, numerous other food uses for 2'-FL ranging from 0.8 to 60 g 2'-FL per kg food.[1] In GRN 735, the proposed levels of 2'-FL vary based on the proposed food uses; for example, 2'-FL is proposed to be added at 80 g 2'-FL per kg food in ready-to-eat breakfast cereals for adults and children, 60 g/kg in jellies and jams, fruit preserves and fruit butters, 1.2 g/L in flavored milks, and 10 g/L in yogurt and juice beverages identified as "baby" drinks.

The 2'-FL addition levels presented in GRN 929 are within the ranges presented in GRN 735. The uses presented in GRN 929 do not meaningfully increase the cumulative estimated daily intakes (CEDIs) of 2'-FL because the addition levels presented in GRN 929 are within similar ranges and food uses for the basis of the CEDI presented in GRN 735.

The CEDIs presented in GRN 735 are: 1.91 g 2'-FL per day (mean) and 3.00 g 2'-FL per day (90th percentile) among infants 0-5 months. The CEDIs reported on a mg/kg-bw/day basis for this age group are 315 mg/kg-bw/day (mean) and 532 mg/kg-bw/day (90th percentile). Among infants 6-11 months old, CEDIs are 2.28 g 2'-FL per day (mean) and 3.86 g/day (90th percentile). On a mg/kg-bw/day basis for this age group, the CEDIs are 259 mg/kg-bw/day (mean) and 447 mg/kg-bw/day (90th percentile). Among toddlers 12-35 months old, the CEDIs are 1.83 g/day (mean) and 2.97 g/day (90th percentile) and 148 mg/kg-bw/day (mean) and 243 mg/kg-bw/day (90th percentile).

The Notifier cites the EDIs presented in GRN 735, as those intakes are based on calculations of the various addition levels by food use category. The Notifier caveats that the EDIs in GRN 735 are an overestimate as the proposed uses include some foods that milk-allergic infants and toddlers would not consume such as frozen dairy desserts, dairy-based puddings, custards and mousses, milk and milk beverages, and yogurt.

The Notifier examined toddler intakes of 2′-FL from uses presented in other GRNs that are outside the scope of GRN 735, but are part of the current background diet in the United States; according to NHANES survey data[2] these additional foods are not commonly consumed by toddlers. Therefore, considering these other uses of 2'-FL would not meaningfully increase the CEDI of 2′-FL for toddlers.

In response to FDA's October 6, 2020 questions, the Notifier refined its CEDIs (October 19, 2020) removing milk and dairy products from intake estimates to better reflect the CEDIs of 2'-FL by a population comprised of consumers of hypoallergenic infant and toddler formulas. The Notifier further refined its CEDIs to reflect varying addition levels for other food categories: 1.2 g/L dairy product analogs, 12 g/kg bars, 57 g/kg baby crackers baby crackers and cookies, 10 g/kg cereals, 60 g/kg jams and jellies, 0.8 g/L fitness water and thirst quenches, sports and isotonic, 2 g/L fluid replacement/electrolyte solutions, and 1.2

[1] Agency Response Letter GRAS Notice No. GRN 000735. FDA (April 6, 2018). FDA had "no questions at this time" regarding the GRAS conclusion based on scientific procedures for 2'-FL as an ingredient in milk- and soy-based, non-exempt infant formulas for term infants and in toddler formulas at a maximum level of 2.4 g/L of formula as consumed; infant and toddler foods at levels of 0.24-1.2 g/serving; and in the following food categories at levels of 0.28-1.2 g/serving: beverages and beverage bases; breakfast cereals; dairy product analogs; frozen dairy desserts and mixes; gelatins, puddings, and fillings; grain products and pastas; jams and jellies; milk and milk products; processed fruits and fruit juices; and sweet sauces, toppings, and syrups. Accessed November 30, 2020 at https://www.fda.gov/media/113453/download.

[2] National Health and Nutrition Examination Survey (NHANES) 2015-2016.

g/kg processed fruits and fruit juices. The revised CEDIs for infants 0-5 months are 1.9 g 2'-FL/day and 310 mg/kg-bw/day (mean) and 2.6 g/day and 403 mg/kg-bw/day (90th percentile). For older infants 6-11 months, the revised CEDIs are 2.0 g/day and 230 mg/kg-bw/day (mean) and 2.9 g/day and 320 mg/kg-bw/day (90th percentile). For toddlers 12-35 months old, the revised CEDIs are 0.7 g/day and 62 mg/kg-bw/day (mean) and 1.4 g/day and 130 mg/kg-bw/day (90th percentile). The ranges of 2'-FL intakes presented herein and in GRN 735 fall within the background range of 2'-FL intakes by infants and toddlers from naturally-occurring 2'-FL in mother's milk (as discussed further in the Notifier's response to Question 2 below).

Q-2. Toxicology:
We understand, based on the notifier's batch data and analyses, that their 2′-FL is devoid of proteins, thereby demonstrating and confirming the absence of potential allergenic proteins.

As a follow-up to the notifier's response to our question 24, FDA notes that the clinical study the notifier cited (Nowak-Wegrzyn et al., 2019) is the first and only hypoallergenicity trial of HMO-supplemented extensively hydrolyzed formula. This study used a concentration of 1.0 g/L 2′-FL in combination with 0.5 g/L LNnT. Furthermore, the notifier discussed a clinical study that assessed safety and tolerability of formula containing a combination of 2′-FL (1.0 - 1.2 g/L) and LNnT (0.5 – 0.6 g/L) in healthy, full-term infants (Pucio et al., 2017). Thus, neither of these human studies tested for tolerability of 2′-FL alone in infants and toddlers with potentially sensitive gastrointestinal systems at the proposed use level of 2.0 g/L.

Please confirm and provide a short rationale that infants and toddlers requiring consumption of hypoallergenic formulas would not be particularly sensitive to addition of 2.0 g/L 2′-FL.

A-2. Notifier's response:
As described in Sections 3 and 5 of GRN 929, 2'-FL is a naturally occurring human milk oligosaccharide (HMO) found in mother's milk. The levels of 2'-FL in milk vary from individual to individual. Most women produce milk containing 2'-FL and those that do not still produce fucosylated HMOs. Most infants, including infants with cow's milk allergy have a history of exposure to naturally produced 2'-FL via mother's milk.

2'-FL is naturally-occurring in milk along with other HMOs. There is a published clinical trial testing 2'-FL among milk-allergic infants (Nowak-Wegrzyn et al., 2019) and there is extensive literature reporting levels of 2'-FL in human milk, thus providing further evidence that 2'-FL is safe for infants and toddlers with cow's milk allergy. Though the clinical trial among individuals with cow's milk allergy tested 2'-FL at lower addition level than that proposed in GRN 929 (Nowak-Wegrzyn et al., 2019) the mean concentrations of 2'-FL in mother's milk are around 3 g/L, as reported in a systematic review of 21 studies of mothers in numerous countries (Thurl et al., 2017). Another large, multi-country study (410 healthy women in Ghana, Kenya, Peru, Spain, Sweden, Ethiopia, Gambia, and the United States) reported mean 2'-FL concentrations ranging between 0.7 and 3.4 g/L (McGuire et al., 2017). The background mean concentration of 2'-FL in mother's milk is consumed by infants (with or without an allergy to cow's milk) naturally. Thus, cow's milk allergic infants consuming mother's milk are exposed to 2'-FL at naturally occurring levels that are within the Notifier's proposed addition level of 2 g 2'-FL/L formula.

The clinical trial by Nowak-Wegrzyn et al., 2019 employed a combination of 2'-FL and LNnT to mimic the HMOs encountered naturally in mother's milk by incorporating in combination two of the most prevalent types of HMOs in human mother's milk: the fucosylated HMO 2'-

FL and the non-fucosylated neutral HMO LNnT. Although the Nowak-Wegrzyn et al. (2019) study was conducted with 1 g 2'-FL/L formula, the McGuire et al., 2017 survey of mother's milk shows that 2 g 2'-FL/L is well within the naturally occurring concentration to which the breast-fed cow's milk allergic infants are exposed.

Two additional studies of 2'-FL and cow's milk allergic infants are described below to support the Notifier's conclusion of the safety and tolerability of intake of 2'-FL by these infants:

Sjogren et al. (2007) reported similar levels of 2'-FL in the milk secreted by allergic mothers compared with milk secreted by non-allergic mothers as well as similar levels of 2'-FL in the milk consumed by infants with no allergy at 18 months compared with infants with confirmed allergy at 18 months. The allergies specifically discussed in the study included egg and/or milk allergy, asthma bronchiale, atopic eczema, and allergic rhinoconjunctivitis.

Seppo et al. (2016) studied mother's milk collected in a prospective birth cohort and reported that FUT2 Secretor status (2'-FL-producing mothers) did not significantly correlate with cow's milk allergy development within the first 18 months of life. The study reported that when stratifying infants by cow's milk allergy type, all infants with delayed-onset cow's milk allergy had mothers with FUT2 Secretor status (milk containing 2'-FL) whereas infants with immediate-type cow's milk allergy did not all have FUT2 Secretor mothers. The authors, however, also reported that 2'-FL concentrations in the milk of mothers of both groups were not statistically different (Seppo et al., 2016).

These studies and the range of background levels of 2'-FL in mother's milk of breast-fed cow's milk allergic infants support the Notifier's conclusion of the safety and tolerability of intake of 2'-FL at 2 g/L by these infants.

We trust that our responses have addressed your questions and requests. If you have any additional questions or comments at any time during your review of GRN 000929, please contact us promptly, so that we may provide a substantive response in a timely manner.

Sincerely yours,

Gavin P Thompson, PhD
Principal Consultant
Ramboll Environment & Health
Ramboll US Consulting

References

Sjögren, Y. M., Duchén, K., Lindh, F., Björkstén, B., & Sverremark-Ekström, E. (2007). Neutral oligosaccharides in colostrum in relation to maternal allergy and allergy development in children up to 18 months of age. *Pediatric allergy and immunology*, *18*(1), 20-26.

Seppo, A. E., Autran, C. A., Bode, L., & Järvinen, K. M. (2017). Human milk oligosaccharides and development of cow's milk allergy in infants. *Journal of Allergy and Clinical Immunology*, *139*(2), 708-711.

14 reference pages have been removed in accordance with copyright laws. The removed references are:

Neutral oligosaccharides in colostrum in relation to maternal allergy and allergy development in children up to 18 months of age.

Sjo¨ gren YM, Duche´n K, Lindh F, Bjo¨ rkste´n B, Sverremark-Ekstro¨mE. Neutral oligosaccharides in colostrum in relation to maternal allergy and allergy development in children up to 18 months of age. Pediatr Allergy Immunol 2007 18: 20–26.

Human milk oligosaccharides and development of cow’s milk allergy in infants

Seppo, A. E., Autran, C. A., Bode, L., & Järvinen, K. M. (2017). Human milk oligosaccharides and development of cow's milk allergy in infants. *Journal of Allergy and Clinical Immunology*, *139*(2), 708-711.