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February 11, 2020

Food and Drug Administration
Center for Food Safety & Applied Nutrition
Office of Food Additive Safety (HFS-255)
5001 Campus Drive
College Park, MD 20740-3835

Attention: Dr. Rachel Morissette
Re: Supplement to GRN 735– Modification of *2'-Fucosyllactose* to Update the Manufacturing Process and Specifications

Dear Dr. Morissette:

GRAS Associates, LLC, acting as the Agent for FrieslandCampina Domo B.V. ("FrieslandCampina", The Netherlands), is submitting for FDA review Form 3667 and an enclosed CD, free of viruses, containing a Supplement to GRN 735 for *2'-Fucosyllactose.* FrieslandCampina reviewed the composite safety information of the subject and has determined that 2'-fucosyllactose, produced using alternative raw materials in the manufacturing process, as well as under updated specifications, is GRAS under the intended conditions of use as an ingredient in infant formulas and conventional foods at levels ranging from 0.24 to 4.0 grams 2'-fucosyllactose per serving. The attached documentation contains the specific information that addresses the safe human food uses for the subject notified substance as discussed in the GRAS guidance document.

If additional information or clarification is needed as you and your colleagues proceed with the review, please feel free to contact me via telephone or email. We look forward to your feedback.

Sincerely,

Katrina Emmel, Ph.D.
Senior Scientist/Project Manager/Associate at GRAS Associates
Agent for FrieslandCampina
GRAS Associates, LLC
11810 Grand Park Ave
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North Bethesda, MD 20852
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Enclosure: Supplement to GRN 735 on behalf of FrieslandCampina – *2'-Fucosyllactose*

GRAS ASSOCIATES, LLC

Supplement to GRAS Notification 735

Purified 2'-Fucosyllactose

on behalf of

FrieslandCampina Domo B.V.

Amersfoort, The Netherlands

February 12, 2020

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DESCRIPTION OF PROPOSED SUPPLEMENT

FrieslandCampina Domo B.V. (hereinafter "FrieslandCampina") has determined that our Purified 2'-Fucosyllactose preparation (hereinafter "Purified 2'-FL") is Generally Recognized as Safe (GRAS) in accordance with Section 201(s) of the Federal Food, Drug, and Cosmetic (FD&C) Act and has successfully notified this conclusion to FDA in GRAS Notification (GRN) 735.[1] The purpose of this Supplement to GRN 735 is to evaluate the safety of changes in manufacture and specifications of Purified 2'-FL.

In addition to the manufacturing process, chemical properties, consumption and safety-related information provided in GRN 735, FrieslandCampina evaluated data pertaining to the changes in manufacture and product specifications along with other related documentation described in this dossier. The updated manufacturing process includes replacement of three raw materials used in GRN 735: (a) the use of a glucose syrup (99% glucose) as an alternative to dextrose monohydrate; (b) cobalt sulfate heptahydrate as an alternative to cobalt chloride hexahydrate; and (c) manganese sulfate monohydrate as an alternative to manganese chloride tetrahydrate.

Regarding specifications, the finished product minimum content of 2'-fucosyllactose (2'-FL) has been lowered from 90% to 88% on a dry matter basis, the maximum water content has been increased from 5% to 9%, and the maximum aflatoxin M1 concentration has been lowered from 0.2 μg per kg to 0.025 μg per kg. In addition, a search of the scientific and regulatory literature was conducted through October 23, 2019. Those references that were deemed pertinent to this Supplement are listed in Part 7. The composite safety information, in concert with dietary exposure information, ultimately provides the specific scientific foundation for the GRAS conclusion.

At FrieslandCampina's request, GRAS Associates, LLC ("GA") convened an Expert Panel to complete an independent safety evaluation of this Supplement to GRN 735. The purpose of the evaluation is to evaluate the scientific basis for FrieslandCampina's conclusion that Purified 2'-FL, when manufactured as described in Part 2 and meeting the specifications described therein, is GRAS under the intended conditions of use. In addition, FrieslandCampina has asked GA to act as Agent for the submission of this GRAS Supplement to GRN 735.

[1] GRN 735 for Purified 2'-Fucosyllactose (2'-FL) Food Usage Conditions for General Recognition of Safety, submitted to FDA by GRAS Associates, LLC on behalf of Glycosyn, LLC and FrieslandCampina Domo B.V. and dated September 29, 2017, was filed and subsequently received a "no questions" letter from FDA on April 6, 2018 (FDA, 2018a).

PART 1. SIGNED STATEMENTS AND CERTIFICATION

A. Claim of Exclusion from the Requirement for Premarket Approval Pursuant to 21 CFR 170 Subpart E

FrieslandCampina has previously determined that our Purified 2'-Fucosyllactose preparation (Purified 2'-FL) and designated food uses are Generally Recognized as Safe (GRAS) in accordance with Section 201(s) of the Federal Food, Drug, and Cosmetic (FD&C) Act as reported in GRN 735. This supplement includes details on slight modifications to the manufacturing process and specifications for Purified 2'-FL, but maintains the same food uses and use levels. The GRAS determination is based primarily on scientific procedures as described in this Supplement to GRN 735. The evaluation accurately reflects the intended conditions of food use for Purified 2'-FL and is the subject of this Supplement to GRN 735.

Signed:

Agent for FrieslandCampina

William J. Rowe Date: 2/12/2020
President
GRAS Associates, LLC
11810 Grand Park Avenue
Suite 500
North Bethesda, MD 20852

B. Name and Address of Responsible Party

FrieslandCampina Domo B.V.
Stationsplein 4,3818 LE Amersfoort
P.O. Box 1551, 3800 BN Amersfoort
The Netherlands

As the Responsible Party, FrieslandCampina accepts responsibility for the GRAS conclusion that has been made for our Purified 2'-FL, as described in the subject safety evaluation; consequently, our Purified 2'-FL, having purity no less than 88% 2'-fucosyllactose and which meets the conditions described herein, is not subject to premarket approval requirements for food ingredients.

C. Common Name and Identity of Subject Substance

The common name of the ingredient to be used on food labels is 2'-fucosyllactose.

D. Conditions of Intended Use in Food

Purified 2'-FL is intended for use as an ingredient in infant formulas and conventional foods at the use levels described in GRN 735.

E. Basis for GRAS Conclusion

Pursuant to 21 CFR170.30(a) and (b)[2], FrieslandCampina's Purified 2'-FL preparation (> 88% 2'-fucosyllactose) has been concluded to be GRAS on the basis of scientific procedures as discussed below.

Purified 2'-FL is not subject to premarket approval requirements of the FD&C Act based on FrieslandCampina's conclusion that the substance is GRAS under the conditions of intended food use.

FrieslandCampina certifies, to the best of our knowledge, that this GRAS review is a complete, representative, and balanced assessment that includes all relevant information available—both favorable and unfavorable—that is pertinent to the evaluation of safety and GRAS status of the subject Purified 2'-FL preparation. The preparation of this safety evaluation also included an updated comprehensive literature search through October 23, 2019.

F. Availability of Information

The data and information that serve as the basis for this GRAS Supplement will be maintained at the offices of FrieslandCampina Domo B.V., Stationsplein 4,3818 LE Amersfoort, P.O. Box 1551, 3800 BN Amersfoort, The Netherlands, and will be made available during customary business hours.

FrieslandCampina certifies that no data or information contained herein are exempt from disclosure under the Freedom of Information Act (FOIA). No non-public, safety-related data were used by the Expert Panel to reach a GRAS conclusion.

[2] Available at: https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfCFR/CFRSearch.cfm?fr=170.30 (Accessed 11/19/19)

PART 2. IDENTITY, METHOD OF MANUFACTURE, SPECIFICATIONS, AND PHYSICAL OR TECHNICAL EFFECT

The chemical identity, manufacturing process, and specifications for Purified 2'-FL have changed as described in Sections A, B, and C, below. No changes have been made to the physical or technical effect or uses and use levels which were described in GRN 735.

A. Alternative Chemical Identity of the Ingredient

"Purified 2'-Fucosyllactose" is the common or usual name of the preparation that contains > 88% 2'-FL, which is manufactured according to the process described in Part B below, and is the subject of this GRAS Supplement to GRN 735. In GRN 735, "Purified 2'-Fucosyllactose" is defined as the preparation which contains > 90% 2'-FL that is manufactured according to the process detailed therein.

B. Alternative Manufacturing Process for Purified 2'-FL

As stated in GRN 735, Purified 2'-FL is produced through the enzymatic transfer of fucose to lactose in an α-1,2-linkage. The 2'-FL production process consists of two stages: fermentation and purification. The reaction is catalyzed by fucosyltransferase present in an engineered host strain of *Escherichia coli* K12 bacteria. No changes have been made to the organism used to produce the ingredient or the purification process. No other changes have been made to the fermentation process, except for the use of the three raw materials indicated below:

(a) a glucose syrup high in glucose (99%) as an alternative to dextrose monohydrate;

(b) cobalt sulfate heptahydrate as an alternative to cobalt chloride hexahydrate; and

(c) manganese sulfate monohydrate as an alternative to manganese chloride tetrahydrate

The three alternative raw materials, glucose syrup, cobalt sulfate heptahydrate, and manganese sulfate monohydrate, are suitable food-grade or high purity materials, and are used in accordance with applicable US Federal Regulations, as detailed in Table 1.

Certificates of Analysis (CoA) and/or specifications for the raw materials are provided in Appendix 1. No changes have been made to the processing aids used to manufacture Purified 2'-FL; therefore, all resins and polymers remain suitable for use in food manufacturing, and are compliant with applicable US Federal Regulations, as defined in 21 CFR 173.25,[3] 21 CFR 177.2440,[4] and 21 CFR 173.340.[5]

[3] Available at: https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=173.25 (Accessed 11/19/19)
[4] Available at: https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=177.2440 (Accessed 11/19/19)
[5] Available at: https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=173.340 (Accessed 11/19/19)

Table 1. Alternative Raw Materials used to Manufacture Purified 2'-FL

Name	CAS No.	Function	Grade	Appendix Location
Glucose syrup[a]	8029-43-4	Energy and carbon source, precursor 2'-FL	FCC	Appendix 1.1
Cobalt (II) sulfate heptahydrate[b]	10026-24-1	Fermentation media ingredient	98+% purity[d]	Appendix 1.2
Manganese (II) sulfate monohydrate[c]	10034-96-5	Fermentation media ingredient	FCC	Appendix 1.3

FCC – Food Chemicals Codex

[a] Alternative to glucose monohydrate

[b] Alternative to cobalt (II) chloride hexahydrate

[c] Alternative to manganese chloride tetrahydrate

[d] There are no FCC, European Union, or Codex Alimentarius specifications for this substance.

C. Product Specifications for Purified 2'-FL Preparation Produced Using the Alternative Manufacturing Process

The product specifications for Purified 2'-FL manufactured using the alternative manufacturing process, described in Section 2.B. above, have been modified from those stated in GRN 735 to permit a higher maximum water content (9%), lower minimum 2'-FL content (88%), and lower maximum aflatoxin M1 content (0.025 μg per kg). The specifications for Purified 2'-FL prepared with the alternative manufacturing process are compared with the specifications provided in GRN 735 in Table 2. Results of analyses performed by FrieslandCampina demonstrate that 5 non-consecutive production batches of Purified 2'-FL manufactured according to Section 2.B. above meet the designated specifications, as shown in Table 2.

CoAs for the five representative lots of Purified 2'-FL manufactured by the alternative process are provided in Appendix 2, along with methodologies used to measure each parameter. A report detailing the quantitative polymerase chain reaction (qPCR) method to evaluate 2'-FL for the absence of residual genetic material from the *E. coli* production strain is located in GRN 735. Chromatograms for five representative lots of Purified 2'-FL manufactured according to the alternative process are provided in Appendix 3. The collection of these reports demonstrates that the substance is well characterized and meets the established purity criteria.

Table 2. Specifications for FrieslandCampina's Purified 2'-FL Preparations

Physical & Chemical Parameters	Specification For Purified 2'-FL per GRN 735	Specification for Purified 2'-FL per this Supplement to GRN 735	Purified 2'-FL Produced by the Alternative Manufacturing Process				
			Lot# 815358-4	Lot# 815383-5	Lot# 815418-7	Lot# 815440-7	Lot# 815463-4
Appearance Form	Homogeneous powder	Homogeneous powder	complies	complies	complies	complies	complies
Appearance Color	White	White	complies	complies	complies	complies	complies
Assay (% dm)	Min. 90	Min. 88	96.9	94.0	92.9	95.7	93.9
pH (10% solution)	3.0-7.5	3.0-7.5	4.36	4.59	4.71	3.89	4.28
Water (%)	Max. 5	Max. 9	3.61	4.02	3.72	3.86	3.91
Sulfated Ash (%)	Max. 0.2	Max. 0.2	0.05	0.03	0.02	<0.01	<0.003
Residual Proteins (%)	Max. 0.01	Max. 0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Aluminum (mg/kg)	Max. 4.8	Max. 4.8	0.48	0.62	0.54	0.51	0.31
Lead (mg/kg)	Max. 0.05	Max. 0.05	<0.02	<0.02	<0.02	<0.02	<0.02
Arsenic (mg/kg)	Max. 0.1	Max. 0.1	<0.01	<0.01	<0.01	<0.01	<0.01
Cadmium (mg/kg)	Max. 0.01	Max. 0.01	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
Mercury (mg/kg)	Max. 0.05	Max. 0.05	<0.006	<0.006	<0.006	<0.006	<0.006
Lactose (%)	Max. 3	Max. 3	1.5	0.9	1.1	0.8	0.8
Allo-lactose (%)	Max. 2	Max. 2	1.0	0.8	1.2	1.2	0.9
Glucose (%)	Max. 2	Max. 2	0.2	0.2	0.2	0.2	0.3
Galactose (%)	Max. 2	Max. 2	<0.1	<0.1	<0.1	<0.1	<0.1
Fucose (%)	Max. 2	Max. 2	0.4	0.3	0.2	0.2	0.3
Nitrite (mg/kg)	Max. 1	Max. 1	<0.1	<0.1	<0.1	<0.1	<0.1
Nitrate (mg/kg)	Max. 50	Max. 50	9.0	2.3	2.3	7.5	8.9
Scorched particles	Max. disc A	Max. disc A	A	A	A	A	A
Aerobic mesophilic total count (cfu/g)	Max. 3,000	Max. 3,000	<100	<100	<100	<100	100
Yeast (cfu/g)	Max. 10	Max. 10	<10	<10	<10	<10	<10
Mold (cfu/g)	Max. 10	Max. 10	<10	<10	<10	<10	<10

Physical & Chemical Parameters	Specification For Purified 2'-FL per GRN 735	Specification for Purified 2'-FL per this Supplement to GRN 735	Purified 2'-FL Produced by the Alternative Manufacturing Process				
			Lot# 815358-4	Lot# 815383-5	Lot# 815418-7	Lot# 815440-7	Lot# 815463-4
***Salmonella* (in 25 g)**	absent	absent	absent	absent	absent	absent	absent
Enterobacteriaceae (in 15 g)	absent	absent	absent	absent	absent	absent	absent
***Cronobacter (Enterobacter) sakazakii* (in 25 g)**	absent	absent	absent	absent	absent	absent	absent
***Bacillus cereus* (presumptive) (cfu/g)**	Max. 100	Max. 100	<10	<10	<10	<10	<10
***E. coli* (in 10 g)**	absent	absent	absent	absent	absent	absent	absent
***Staphylococcus aureus* (in 1 g)**	absent	absent	absent	absent	absent	absent	absent
Sulphite reducing clostridia spores (cfu/g)	Max. 30	Max. 30	<10	<10	<10	<10	<10
***Clostridium perfringens* (in 1 g)**	absent	absent	absent	absent	absent	absent	absent
Residual Endotoxins (EU/mg)	Max. 10	Max. 10	0.01	0.003	0.003	0.01	<0.001
Aflatoxin M_1 (µg/kg)	Max. 0.2	Max. 0.025	<0.01	<0.01	<0.01	<0.01	<0.01
GMO detection	negative	negative	negative	negative	negative	negative	negative

cfu – colony forming units; dm – dry matter; EU – endotoxin units; GMO – genetically modified organism

D. Stability

FrieslandCampina has not performed a stability study on Purified 2'-FL produced by the alternate method of manufacture. Results of the accelerated storage stability study reported in GRN 735 show that the Purified 2'-FL produced according to the method described GRN 735 is stable for up to 6 months, when stored at 40°C at a relative humidity of 75%. Results of the shelf-storage stability study reported in GRN 735 (with pull dates of 0, 3, 6, 12, 24, and 36 months) show that the Purified 2'-FL produced according to the method described GRN 735 is stable for up to 6 months when stored at 25°C at a relative humidity of 60% (with results for subsequent pull dates pending).

Additional results (12 and 24 months) for the shelf-storage stability study are now available and are shown in Table 3. The results that were not reported in GRN 735 are shown in bolded text. As indicated by the results presented below, Purified 2'-FL is stable for at least 24 months; however, the amount of moisture is higher than the 5% limit stated in the specifications. This substantiates the shift to a broader moisture specification (Max. 9% instead of 5%) and a lower purity specification (Min. 88% instead of 90%), as moisture in the sample is > 5% after 24 months.

Table 3. Purified 2'-Fucosyllactose Shelf-Storage Stability Data For Product Produced According to Method Described in GRN 735

Parameter	Specifications per GRN 735	t = 0	t = 3 months	t = 6 months	t = 12 months	t = 24 months	t = 36 months
Assay	Min. 90%	96.3%	98.2%	94.8%	**96.7%**	**91.0%**	NA
Moisture	Max. 5%	3.3%	3.7%	4.0%	**4.3%**	**6.3%**	NA
Ash	Max. 0.2%	0.11%	<0.01%	0.01%	**0.02%**	**0.04%**	NA
Lactose	Max. 3%	0.6%	0.6%	1.3%	**0.6%**	**1.3%**	NA
Allo-lactose	Max. 2%	0.1%	1.1%	1.8%	**0.6%**	**0.8%**	NA
Glucose	Max. 2%	0.1%	0.1%	0.3%	**0.2%**	**0.2%**	NA
Mesophilic aerobic cell count	Max. 3,000 cfu/g	< 10 cfu/g	<10 cfu/g	<10 cfu/g	**<10 cfu/g**	**<10 cfu/g**	NA
Enterobacteriaceae	Absent in 10 g	Neg.	Neg.	Neg.	**Neg.**	**Neg.**	NA
Salmonella	Absent in 25 g	Neg.	Neg.	Neg.	**Neg.**	**Neg.**	NA
Cronobacter spp.	Absent in 25 g	Neg.	Neg.	Neg.	**Neg.**	**Neg.**	NA
Appearance	White homogeneous powder	Pass	Pass	Pass	**Pass**	**Pass**	NA

NA – not available

It should be noted that the stability study is currently ongoing; according to the timetable, the remaining measurements are scheduled to be performed at t=36 months (December 6th, 2019). The methodologies used to assess the parameters outlined in Table 3 are the same

methodologies used to analyze the composition of the Purified 2'-FL as described elsewhere in this dossier (Appendix 2).

According to measurements of sorption isotherms of the product, 9% moisture on a wet basis (9.9% moisture on a dry basis) represents water activity below 0.5 (Figure 1). As noted by Roos (2002), and also confirmed by FrieslandCampina's microbiology expert, water activity of 0.6 is the generally accepted boundary for growth/no growth of any micro-organism (Figure 2).

Figure 1. Water Activity for FrieslandCampina's Purified 2'-FL Preparation as a Function of Water Content on a Dry Basis

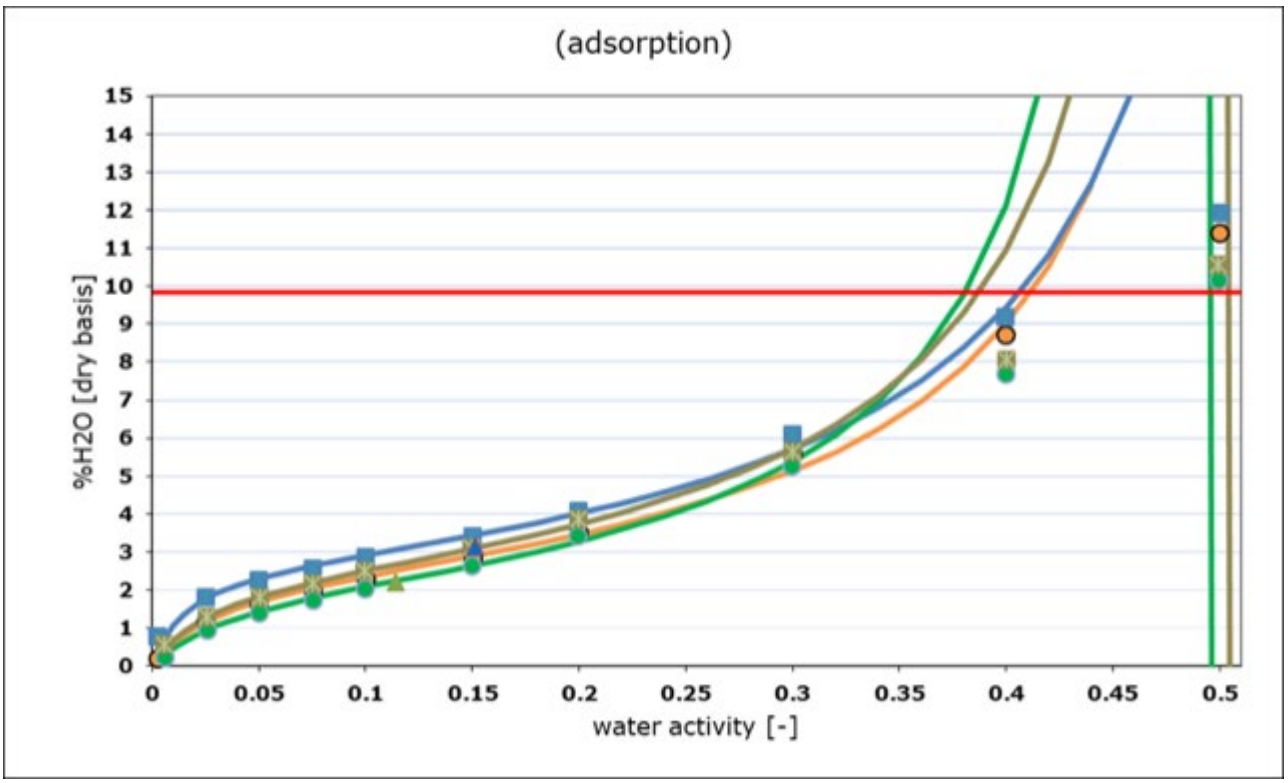
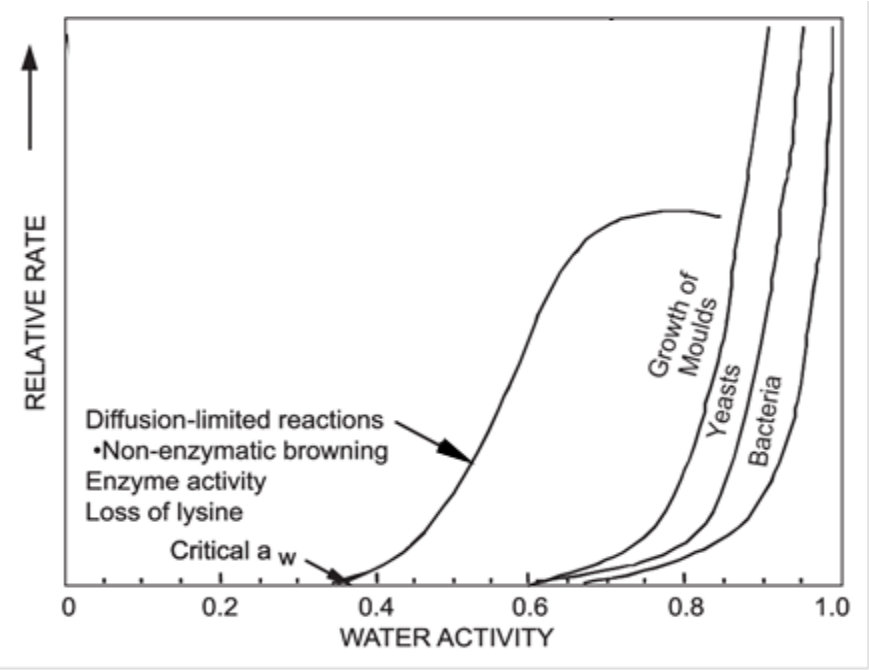


Figure 2. Microbial Growth of Dairy Products Containing Amorphous Lactose as a Function of Water Activity[a]



[a] Adapted from Roos (2002)

FrieslandCampina contends that stability testing results obtained with the Purified 2'-FL produced according to the method detailed in GRN 735 also applies to the Purified 2'-FL produced according to the alternative method described in this Supplement to GRN 735 because the only differences between the two preparations are in the concentrations of Purified 2'-FL and moisture content. The changes to the specifications are necessary to support room temperature storage of the substance for up to three years.

PART 3. DIETARY EXPOSURE

There are no proposed changes to uses or use levels or to permissible levels of contaminants. Therefore, the dietary exposure to the ingredient or any potential contaminants will not change. Please refer to GRN 735 for a detailed dietary exposure assessment.

PART 4. SELF-LIMITING LEVELS OF USE

As stated in GRN 735, there are no known self-limiting levels of use.

PART 5. EXPERIENCE BASED ON COMMON USE IN FOOD AND REGULATORY HISTORY

A. Other Information on Dietary Exposure

As mentioned in GRN 735, there is a history of use of 2'-FL because it is present in human breast milk. The statutory basis for the conclusion of GRAS status of Purified 2'-FL is based on scientific procedures, rather than common use in food before 1958.

According to data obtained by FrieslandCampina, infant milk formula products containing 2'-FL are currently available in 45 countries. In August 2019, the Market Plan stated that fucosyllactose accounted for a market share of almost 48% of the global human milk oligosaccharides market, with an estimated value of over US$9 Million at the end of 2017. Value is projected to increase at a Compound Annual Growth Rate (CAGR) of 14.4% from 2017-2027 (Xploremr, 2019).

B. Summary of Regulatory History of 2'-FL

The regulatory status of 2'-FL in the US and Europe up to September 2017 was reviewed in GRN 735 and is not reiterated here. The purpose of this section is to update the regulatory history to the present date.

1. U.S. Regulatory History

A search of FDA's GRAS Notice Inventory website[6] using the search terms "fucosyllactose" identified four new GRAS Notice submissions since the last search of the website was conducted for GRN 735: GRN 749 received a "no questions" letter from FDA; GRNs 815 and 852 are pending FDA response; and FDA ceased to evaluate GRN 859 at the notifier's request. These recently filed GRNs are summarized in Table 4.

Table 4. Summary of 2'-Fucosyllactose GRAS Notices in FDA GRAS Inventory

Substance	GRN No. / Closure Date	Intended Use and Use Rate	Company/ Reference
2'-fucosyllactose	GRN 735 04/06/18	Use as an ingredient in 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, whole and skim; milk products; processed fruits and fruit juices; sweet sauces, toppings, and syrups; non-exempt infant and follow-on	Glycosyn LLC and Friesland Campina Domo B.V. (2018) FDA (2018a)

[6] GRAS Notice Inventory website available at: https://www.accessdata.fda.gov/scripts/fdcc/?set=GRASNotices (accessed for search on 10/22/2019)

Substance	GRN No. / Closure Date	Intended Use and Use Rate	Company/ Reference
		formula; and baby foods at levels ranging from 0.24 to 4 g/serving	
2'-O-fucosyllactose	GRN 749 04/23/18	Use as an ingredient in term infant formula, toddler formulas, baby foods and beverages for young children at levels ranging from 0.24 to 2.04 g/serving	DuPont Nutrition & Health (2018) FDA (2018b)
2'-fucosyllactose and difucosyllactose	GRN 815 Pending	Use as an ingredient in beverage and beverage bases, infant formula and toddler foods, grain products and pastas, milk (whole and skim), and milk products at levels ranging from 1.2 to 40 g/kg	Glycom A/S (2018)
2'-fucosyllactose	GRN 852 Pending	Use as an ingredient in beverages and beverage bases; breakfast cereals; dairy product analogues; frozen dairy desserts and mixes; gelatins, puddings, and fillings; grain products and pastas; jams and jellies; milk, whole and skim; milk products; processed fruits and fruit juices; sweet sauces, toppings, and syrups; non-exempt infant and follow-on formula; and baby foods at levels ranging from 0.24 to 1.2 g/serving.	BASF SE (2019)
2'-fucosyllactose	GRN 859 Cease to evaluate request from notifier, 9/6/19	Use an ingredient in whey, milk, and soy-based, non-exempt infant formulas at a level of 2.4 g/L of formula as consumed; infant and toddler foods at levels ranging from 0.24-1.2 g/serving; and 0 in beverage 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 syrup at levels ranging from 0.28-1.2 g/serving [Note: the 0 is not a typographical error]	Advanced Protein Technologies Corp. (2019)

GRN – GRAS Notification; No. – number; g – gram; kg – kilogram

2. European Regulatory History

In December 2017, the European Union approved the use of 2'-FL in a number of different foods (including infant formula) (European Commission, 2017). Permitted foods and inclusion levels are shown in Table 5. Specifications for 2'-FL produced synthetically or from microbial sources were included the 2017 authorization. The specifications for 2'-FL produced from microbial sources were revised in 2019 (European Commission, 2019). The 2017 specification for synthetic 2'-FL and the 2019 specifications for 2'-FL produced from microbial sources are shown in Table 6. As reported herein, the new specifications for FrieslandCampina's Purified 2'-FL meet all specifications for 2'-FL produced from microbial sources (genetically modified strains of *E. coli* K12 or BL21) established by the European Commission.

Table 5. Conditions for Use of 2'-Fucosyllactose as a Novel Food in the European Union[a]

Specific Food Category	Maximum Level	Additional Specific Labelling Requirements
Unflavored pasteurized and sterilized (including UHT) milk-based products	1.2 g/L	1. The designation of the novel food on the labelling of the foodstuffs containing it shall be '2'-fucosyllactose'. 2. The labelling of food supplements containing 2'-fucosyllactose shall bear a statement that the supplements should not be used if other foods with added 2'-fucosyllactose are consumed the same day. 3. The labelling of food supplements containing 2'-fucosyllactose intended for young children shall bear a statement that the supplements should not be used if breast milk or other foods with added 2'-fucosyllactose are consumed the same day.
Unflavored fermented milk-based products	1.2 g/L beverages	
	19.2 g/kg products other than beverages	
Flavored fermented milk-based products including heat-treated products	1.2 g/L beverages	
	19.2 g/kg products other than beverages	
Dairy analogues, including beverage whiteners	1.2 g/L beverages	
	12 g/kg for products other than beverages	
	400 g/kg for whitener	
Cereal bars	12 g/kg	
Table-top sweeteners	200 g/kg	
Infant formula as defined in European Union Regulation No. 609/2013	1.2 g/L alone or in combination with up to 0.6 g/L of lacto-*N*-neotetraose at a ratio of 2:1 in the final product ready for use, marketed as such or reconstituted as instructed by the manufacturer	
Follow-on formula as defined in European Union Regulation No. 609/2013	1.2 g/L alone or in combination with up to 0.6 g/L of lacto-*N*-neotetraose at a ratio of 2:1 in the final product ready for use, marketed as such or reconstituted as instructed by the manufacturer	
Processed cereal-based food and baby food for infants and young children as defined European Union Regulation No. 609/2013	12 g/kg for products other than beverages	
	1.2 g/L for liquid food ready for use, marketed as such or reconstituted as instructed by the manufacturer	
Milk-based drinks and similar products intended for young children	1.2 g/L for milk-based drinks and similar products added alone or in combination with up to 0.6 g/L	

Specific Food Category	Maximum Level	Additional Specific Labelling Requirements
	lacto-*N*-neotetraose, at a ratio of 2:1 in the final product ready for use, marketed as such or reconstituted as instructed by the manufacturer	
Foods for special medical purposes as defined in European Union Regulation No. 609/2013	In accordance with the particular nutritional requirements of the persons for whom the products are intended	
Total diet replacement for weight control as defined in European Union Regulation No. 609/2013	4.8 g/L for drinks	
	40 g/kg for bars	
Bread and pasta products bearing statements on the absence or reduced presence of gluten in accordance with the requirements of Commission Implementing European Union Regulation No. 828/2014 60 g/kg	60 g/kg	
Flavored drinks	1.2 g/L	
Coffee, tea (excluding black tea), herbal and fruit infusions, chicory; tea, herbal and fruit infusions and chicory extracts; tea, plant, fruit and cereal preparations for infusions, as well as mixes and instant mixes of these products	9.6 g/L - the maximum level refers to the products ready to use	
Food supplements as defined in Directive 2002/46/EC, excluding food supplements for infants	3.0 g/day for general population	
	1.2 g/day for young children	

[a] Adapted from European Commission (2017)

Table 6. Specifications for Novel Food Use of 2'-Fucosyllactose in the European Union[ab]

Substance	Specification
2'-Fucosyllactose (synthetic)	**Definition:** Chemical name: α-L-Fucopyranosyl-(1→2)-β-D-galactopyranosyl-(1→4)-D-glucopyranose Chemical formula: $C_{18}H_{32}O_{15}$ CAS No: 41263-94-9 Molecular weight: 488.44 g/mole **Description**: 2'-Fucosyllactose is a white to off-white powder that is produced by a chemical synthesis process and is isolated by crystallization. **Purity:** 2'-Fucosyllactose: ≥ 95 % D-Lactose: ≤ 1.0 w/w % L-Fucose: ≤ 1.0 w/w % Difucosyl-D-lactose isomers: ≤ 1.0 w/w % 2'-Fucosyl-D-lactulose: ≤ 0.6 w/w % pH (20 °C, 5 % solution): 3.2-7.0 Water (%): ≤ 9.0 % Ash, sulphated: ≤ 0.2 % Acetic acid: ≤ 0.3 % Residual solvents (methanol, 2-propanol, methyl acetate, acetone): ≤ 50.0 mg/kg singly, ≤ 200.0 mg/kg in combination) Residual proteins: ≤ 0.01 % **Heavy Metals:** Palladium: ≤ 0.1 mg/kg Nickel: ≤ 3.0 mg/kg **Microbiological criteria:** Aerobic mesophilic bacteria total count: ≤ 500 cfu/g Yeasts and Molds: ≤ 10 cfu/g Residual endotoxins: ≤ 10 EU/mg
2'-Fucosyllactose (microbial source)	**Definition:** Chemical name: α-L-Fucopyranosyl-(1→2)-β-D-galactopyranosyl-(1→4)-D-glucopyranose Chemical formula: $C_{18}H_{32}O_{15}$

Substance	Specification	
	CAS No: 41263-94-9 Molecular weight: 488.44 g/mole	
	Source: Genetically modified strain of *Escherichia coli* K12	**Source:** Genetically modified strain of *Escherichia coli* BL21
	Description: 2'-Fucosyllactose is a white to off-white crystalline powder that is produced by a microbial process. **Purity:** 2'-Fucosyllactose: ≥ 83 % D-Lactose: ≤ 10.0 % L-Fucose: ≤ 2.0 % Difucosyl-D-lactose: ≤ 5.0 % 2'-Fucosyl-D-lactulose: ≤ 1.5 % Sum of saccharides (2'-Fucosyllactose, D-Lactose, L-Fucose, Difucosyl-D-lactose, 2'-Fucosyl-D-lactulose): ≥90 % pH (20 °C, 5 % solution): 3.0-7.5 Water: ≤ 9.0 % Ash, sulphated: ≤ 2.0 % Acetic acid: ≤ 1.0 % Residual proteins: ≤ 0.01 % **Microbiological criteria:** Aerobic mesophilic bacteria total count: ≤ 3,000 cfu/g Yeasts: ≤ 100 cfu/g Molds: ≤ 100 cfu/g Endotoxins: ≤ 10 EU/mg	**Description**: 2'-Fucosyllactose is a white to off white powder and the liquid concentrate (45 % ± 5 % w/v) aqueous solution is a colorless to slight yellow clear aqueous solution. 2'-Fucosyllactose is produced by a microbiological process. **Purity**: 2'-Fucosyllactose: ≥ 90 % Lactose: ≤ 5.0 % Fucose: ≤ 3.0 % 3-Fucosyllactose: ≤ 5.0 % Fucosylgalactose: ≤ 3.0 % Difucosyllactose: ≤ 5.0 % Glucose: ≤ 3.0 % Galactose: ≤ 3.0 % Water: ≤ 9.0 % (powder) Ash, sulphated: ≤ 0.5 % (powder and liquid) Residual proteins: ≤ 0.01 % (powder and liquid) **Heavy Metals:** Lead: ≤ 0.02 mg/kg (powder and liquid) Arsenic: ≤ 0.2 mg/kg (powder and liquid) Cadmium: ≤ 0.1 mg/kg (powder and liquid) Mercury: ≤ 0.5 mg/kg (powder and liquid) **Microbiological criteria:** Total plate count: ≤ 10^4 cfu/g (powder), ≤ 5,000 cfu/g (liquid)

Substance	Specification	
		Yeasts and Molds: ≤ 100 cfu/g (powder); ≤ 50 cfu/g (liquid) Enterobacteriaceae/Coliforms: absence in 11 g (powder and liquid) *Salmonella*: negative/100 g (powder), negative/200 ml (liquid) *Cronobacter*: negative/100 g (powder), negative/200 ml (liquid) Endotoxins: ≤ 100 EU/g (powder), ≤ 100 EU/ml (liquid) Aflatoxin M_1: ≤ 0.025 µg/kg (powder and liquid)

[a] Specifications for 2'-Fucosyllactose (synthetic) adapted from European Commission (2017)

[b] Specifications for 2'-Fucosyllactose (microbial source) adapted from European Commission (2019)

cfu – colony forming units; D – dextro; L – levo; w/w – weight/weight

PART 6. NARRATIVE

GRN 735 stated the requirements for a GRAS determination under this heading and these requirements have not changed since GRN 735 was filed by FDA. Therefore, these requirements are not reiterated here. The purpose of this section is to provide information that has been published since GRN 735 was submitted, with specific regard to the safety of 2'-FL, to support GRAS status. As noted below, the new information reviewed herein does not impact the GRAS status of Purified 2'-FL.

A. Safety Considerations Including Updated Scientific Literature Review of 2'-FL

As mentioned previously, a GRAS Notice for Purified 2'-FL was submitted by GRAS Associates, LLC on behalf of Glycosyn, LLC and FrieslandCampina Domo B.V. on September 29, 2017, filed by FDA as GRN 735, and subsequently received a "no questions" letter from FDA on April 6, 2018. The key safety information in GRN 735 included multiple published toxicology and clinical studies on various 2'-FL preparations and corroborative information from unpublished toxicology studies on Purified 2'-FL, which was supported by a history of safe consumption. The aggregate evidence from experimental studies was used to demonstrate the safety of Purified 2'-FL for human food consumption.

An updated review of the scientific literature was performed covering the time period between July 13, 2017 (the date of the literature search for GRN 735) through the present to ascertain whether or not any new safety information has been published or any adverse effects have been reported due to ingestion of 2'-FL. The literature search strategy was based on CAS No. 41263-94-9 and the common name "2'-Fucosyllactose," and used the TOXLINE, ToxPlanet, and PubMed databases. The PubMed search yielded 9 relevant articles. No relevant articles were identified within the TOXLINE and ToxPlanet databases, with the exception of GRN 749, which was also located through a search of the GRAS Notice Inventory website (see Part 5 above). TOXLINE and PubMed were used in the literature search for GRN 735, as well as RTECS and NAPRALERTSM. The latter two sites were not searched for this GRAS Supplement because they did not yield any information for GRN 735.

Because 2'-FL is manufactured using fucosyltransferase produced by *E. coli,* information related to the safety of the organism used by FrieslandCampina, *E. coli* K12 strain E997 (E638/pG217), was also sought. In this regard, the identification and taxonomic description of the bacterial strain, as well as precedents for its safe use in the context of human foods and drugs, were reviewed. No information on this particular strain was obtained from the search; however, a PubMed search yielded two relevant articles for *E. coli* K12.

Information from the relevant publications is summarized in the following sections. The updated literature search reveals a growing body of evidence that 2'-FL is safe for human consumption.

1. Information Pertaining to the Safety of *E. coli* K12

The literature search identified two new studies on the safety of *E. coli* K12, both of which were conducted *in vitro.*

Bhat et al. (2019) examined the ability of *E. coli* K12 ATCC 14948 to disrupt intestinal epithelial barrier function using Caco-2 cells. Caco-2 cells exposed to this *E. coli* strain showed a statistically significant ($P<0.01$), time-dependent decrease in transepithelial electrical resistance (TEER) and concomitantly increased phenol red flux across cell monolayer in contrast to control cells that were not exposed to the *E. coli*. Caco-2 cells exposed to the *E. coli* K12 also exhibited suppressed levels of mRNA for the tight junction proteins Zona Occludens (ZO-1) Claudin-1, Occludin, and Cingulin-1 ($p<0.05$) and higher levels of mRNA for polymeric immunoglobulin receptor (PIgR) and human-beta defensin 2 (hbd-2) ($p<0.05$), two proteins that protect against pathogen adherence and invasion. Immunofluorescent and electron micrographs revealed the disrupted distribution and localization of specific tight junction proteins (ZO-1 and Claudin-1) and actin filaments in Caco-2 cells exposed to the *E. coli* K12 that ultimately resulted in deformed cellular morphology. As only one *E. coli* K12 strain was tested in this study, it is unknown whether other strains would cause similar findings in this system. As mentioned in GRN 735, Purified 2'-FL is produced from a genetically modified *E. coli* strain GI724, which is in the W3110 lineage of *E.coli* K12. ATCC 14948 originates from W3100 (ATCC, 2019). There is no evidence in the literature that *E.coli* MG1655 or W3110 disrupt intestinal epithelial barrier function. Further, as shown in the 90-day oral study in rats that was conducted using Purified 2'-FL, there is no effect of Purified 2'-FL on the histopathology of the intestine (van Berlo et al., 2018). Thus, the finding that contact with *E. coli* K12 ATCC14948 disrupts the junctions of Caco-2 cells *in vitro* has no bearing on the safety of Purified 2'-FL.

Fejes et al. (2018) examined the effect of non-pathogenic (K12) and pathogenic (O18:K1) *E.coli* strains on platelet activation, RNA expression patterns, and fibrinogen binding capacity. Platelets in contact with *E. coli* K12 (but not *E. coli* O18:K1) exhibited increased surface expression of the activation markers P-selectin and CD63, PAC-1 antibody and bound fibrinogen on the surface. Incubation of platelets with *E. coli* K12 caused an enrichment of RNAs with the following functional characteristics: involved in splicing (Cluster 4, 15 RNAs), cell-cell adhesion (Cluster 5 with 7 RNAs), related to Golgi apparatus (Cluster 2, 11 members) and ubiquitin related processes (Cluster 1 with 13 and Cluster 3 with 5 RNAs) (cluster enrichment score >1). The overall effect of these changes on platelet function was not assessed. Because the investigators did not examine whether the changes elicited by *E. coli* K12 were due to contact with live bacteria or substances secreted from the bacteria, it is unclear whether these findings are pertinent for FrieslandCampina's Purified 2'-FL (which contains no *E. coli* according to specifications). Further, as shown in the 90-day oral study in rats that was conducted using Purified 2'-FL, there is no effect of Purified 2'-FL on prothrombin time (van Berlo et al., 2018). Thus, the finding that contact with *E. coli* K12 ATCC14948 causes molecular changes to platelets that are consistent with activation has no bearing on the safety of Purified 2'-FL.

2. Toxicology Studies on 2'-FL

Three toxicology studies on 2'-FL were identified from the current literature search, one of which [van Berlo et al. (2018)] reported the results of the unpublished toxicology studies conducted by Triskelion Laboratories on the Purified 2'-FL formulation that was the subject of GRN 735 (which were included as Appendices 9-12 in GRN 735). Because the results of these studies were unpublished when GRN 735 was submitted, the results were considered to be corroborative of the published safety evidence for GRAS status of Purified 2'-FL. The study designs and findings reported for the genetic toxicity studies in the van Berlo et al. (2018) publication are identical to those reported in GRN 735, with the exception of the viability of cells reported for the 2,000 μg per mL concentration in the continuous treatment micronucleus test [100% in van Berlo et al. (2018) and 93% in Appendix 12 of GRN 735]; therefore, they are not presented here.

The results for body weight, food, and water consumption for the 90-day toxicity study are reported differently in the van Berlo et al. (2018) publication than were reported in GRN 735; they were averaged over the study period rather than reported over intervals. The results of the van Berlo et al. (2018) study for these parameters are reported in Table 7. As shown, there was no effect of Purified 2'-FL on body weight, food, or water consumption at the concentrations tested (3, 6, or 10% in the diet) compared to controls.

Table 7. Mean Body Weight, Food, and Water Consumption and 2'-FL Intake for Rats Over the 13-Week Exposure Period

Parameter	2'-FL concentration in diet (%)			
	0	3	6	10
Males				
Body weight (g)	261 ± 26.1	264 ± 24.5	266 ± 28.2	251 ± 30.2
Food consumption (g/rat/day)	18.6 ± 2.5	19 ± 2.4	18.7 ± 2.3	18 ± 2.3
Water consumption (g/rat/day)	21.2 ± 4.7	22.1 ± 4.9	21.5 ± 4.6	22.9 ± 5.1
2'-FL intake (g/kg bw/day)	0 ± 0	2.17 ± 0.21	4.27 ± 0.48	7.25 ± 0.89
Females				
Body weight (g)	171 ± 11.4	173 ± 14.3	164 ± 21.3	169 ± 11.3
Food consumption (g/rat/day)	14.1 ± 1.3	14.1 ± 1.3	14.2 ± 1.2	13.1 ± 1.1
Water consumption (g/rat/day)	19.3 ± 3.3	18.2 ± 3.6	18.5 ± 3.4	19 ± 3.1
2'-FL intake (g/kg bw/day)	0 ± 0	2.45 ± 0.20	5.22 ± 0.71	7.76 ± 0.51

bw – body weight; g – gram; kg – kilogram

The test material intake at each of the concentrations tested is identical to that reported in GRN 735. There are two other differences between the results of the 90-day toxicity study reported in the van Berlo et al. (2018) publication and GRN 735: (1) the units for absolute organ weights are erroneously reported as g per kg bw in van Berlo et al. (2018) and should be g (as reported in GRN 735) and 2): the value for absolute weight of the spleen in females receiving 6% in the diet is reported as 0.04026 (g per kg bw) in van Berlo et al. (2018) and 0.4026 g in GRN 735. The value

reported in the van Berlo et al. (2018) study appears to be an error in transposition and does not affect the conclusion that the no observed adverse effect level (NOAEL) was 10% in the diet (7.25 g per kg bw per day for males and 7.76 g per kg bw per day for females).

The results of the van Berlo et al. (2018) study support the safety of the Purified 2'-FL formulation at the usage rate stipulated in GRN 735. This study also supports the safety of the Purified 2'-FL preparation that is the subject of this supplement at same usage rate stipulated in GRN 735 because although minor differences exist between specifications, none are expected to have an adverse impact on safety.

Additional information about safety can be gleaned from two new studies conducted in neonatal rats. One of the studies examined the effect of administration of 2'-FL to neonatal male and female Lewis rats from Days 2-16 of life (Azagra-Boronat et al., 2019b). The neonatal rats and their respective dams were randomly distributed into two groups (3 litters of 8 pups per group, with a similar number of each sex in each litter). In the 2-FL group, pups received 0.2 g of 2'-FL per 100 g bw (2 g per kg bw or 4.5 µL per g per day); and in the control group pups received 4.5 µL per g per day mineral water (vehicle) by oral gavage. The naso–anal and tail lengths were measured to determine the body/tail ratio. Body weight, fecal weight, and stool consistency were monitored daily. On Days 8 and 16 of life, half of each litter (four randomly selected pups/dam) were euthanized to obtain tissue samples. The weight of spleen, thymus, liver, small intestine, and large intestine were recorded, and the length of the small and large intestines was measured. Mesenteric lymph nodes were obtained to study the proportion of specific immune cell populations. Plasma samples were also collected for immunoglobulin measurement and gut samples for cytokine release. Morphometry and gene expression of the intestine, mesenteric lymph node cell composition, fecal microbiota composition, cecal short-chain fatty acids content, and urinary metabolic profile were also assessed. Animals given 2′-FL had a greater body-to-tail-length ratio at both Days 8 and 16 and higher body weights than control animals at Day 16 ($p < 0.05$). There was no effect of 2'-FL on organ weight, with the exception of a relatively lower colonic weight on Day 16 ($p < 0.05$). No treatment-related effects on stool consistency were observed. Villus heights and areas were increased on Day 8 ($p < 0.08$), which is considered to be trophic and not adverse. Effects of 2'-FL on some of the immunoglobulins, cytokines, fecal microbiota, short chain fatty acids, and urinary metabolites that were measured were also observed, none of which were determined by the authors to be adverse. The results of the study show that 2 g of 2'-FL per kg bw can be safely consumed by weanling rats during the first two weeks of life.

Azagra-Boronat et al. (2019a) performed an additional study with 2'-FL in neonatal Wistar rats to examine its effects on rotavirus (RV) diarrhea. Upon natural delivery, litters from 15 dams were randomly assigned to the experimental groups and culled to 8 pups per lactating dam, with a similar number of females and males in each litter. Pups were randomly distributed into five groups (3 litters per group):

1. reference (water control);
2. RV SA11;
3. RV SA11 plus 0.8 g per 100 g bw of a mixture of short chain galactooligosaccharides (scGOS) and long chain fructooligosaccharides (lcFOS) in a 9:1 ratio (RV+scGOS/lcFOS);
4. RV SA11 plus 0.2 g per 100 mL 2'-FL (RV+2'-FL); or
5. RV SA22 plus both 0.8 g per 100 g bw scGOS/lcFOS and 0.2 g per 100 mL 2'-FL (RV+scGOS/lcFOS/2'-FL).

Each material was given at a volume of 4.5 μL per g by oral gavage from Days 2-8 of life, except for RV SA11, which was given by oral gavage on Day 5. Fecal sampling was performed once daily (from Days 4 to 8 of life) and severity and incidence of diarrhea was assessed. Feces from one animal per litter collected on Day 8 was analyzed for fecal microbiota by 16S rRNA sequencing. At Day 8 of life, half of each litter (4 random pups, 3 litters per group, n = 12) were euthanized to obtain samples of the small intestine for analysis of gene expression. The fate of the other treated animals was not mentioned. As this study was designed as an efficacy study against a pathogen, little information about safety of 2'-FL can be obtained from it. Nonetheless, the results showed that treatment with 2'-FL did not have an adverse effect on any of the variables that were measured in the study.

3. Human Clinical Studies

The results of clinical studies that were located by the new literature search for 2'-FL are summarized in Table 8. For the purpose of this document, we have focused on any discussion of potential adverse effects associated with 2'-FL intake.

Larsson et al. (2019) performed a prospective, observational, cohort study in 30 breastfed infants (13 high weight gain and 17 normal weight gain) to examine the relationship between concentrations of specific oligosaccharides (including 2'-FL) in breast milk and anthropometric endpoints at 5 and 9 months of age. The investigators found no difference between the 2'-FL content of breast milk in the high weight or normal weight gain groups at 5 or 9 months. Content of 2′-FL in breast milk was positively associated with weight velocity from 0 to 5 months (p=0.015) and fat mass index (FMI) at 5 months (p=0.024), but not with body mass index (BMI), or height-for-age Z-score (HAZ). There was no adjustment for potential cofounders that could affect anthropometric measurements of infants (i.e., anthropometric characteristics of parents, solid food intake, or concentrations of nutrients in breast milk). Maternal BMI at 5 months was positively associated with 2′-FL, suggesting that, at a minimum, the results should have been adjusted for maternal BMI. Although the results of the study suggest that there is a positive relationship between 2'-FL and body weight gain and FMI at 5 months, they do not definitively demonstrate that 2'-FL is responsible for these findings or that the findings have an adverse effect on the health of infants.

By contrast, the double-blind, controlled, randomized study performed by Storm et al. (2019) showed no difference between body weights of infants on a partially hydrolyzed whey-based infant formula supplemented with 0.25 g per L 2'-FL and infants provided control formula for six weeks. Results of this study also show that formula containing 0.25 g per L 2'-FL is well tolerated by healthy full-term infants. A study performed by Nowak-Wegrzyn et al. (2019) demonstrates that extensively hydrolyzed whey-based infant formula containing 1.0 g per L 2'-FL is well tolerated by infants with cow's milk protein allergy.

Table 8. Summary of New Clinical Trials for 2'-FL

Study Setup and Details	Human Study Results, Significance, Safety	Reference
Study Design: prospective, observational, cohort **Study Length**: 4 months **Subjects**: n= 13 high weight gain (HW) breastfed infants with at least + 1.0 SD increment in weight-for-age z-score (WAZ) during the first 5–6 months post-partum and 17 normal weight gain (NW) breastfed infants with an increment in WAZ during the first 5–6 months post-partum within normal range, defined as <0.67 SD. **Dose, Delivery, and Frequency**: Not relevant	**Outcome Measurements:** Weight, length, body composition, fat free mass, fat mass, fat mass percentage of infants, 24 h milk intake at 5 months; Maternal pre-pregnancy body mass index (BMI), gestational weight gain, weight, and height; concentrations of oligosaccharides (OS)in breast milk at 5 and 9 months; differences in OS content between the HW and NW groups at 5 and 9 months; Associations between OS composition and anthropometry at 5 months and weight velocities from birth to 5 months in HW and NW groups combined, excluding Non-secretors. No adjustment for potential confounders. **Results and Significance**: In the HW and NW groups 8/11and 15/17 infants received milk from secretor mothers, respectively. In secretor mothers, four OS were significantly different between the HW and NW group at 5 months [difucosyl-lactose, lacto-N-neotetraose (LNnT), difucosyl-lacto-N-hexaose, and OS-bound fucose] and two remained significant at 9 months (LNnT and OS-bound fucose). Total OS and total OS-bound fucose at 5 months were positively associated with fat mass index (FMI) and weight velocity from 0 to 5 months (all $p < 0.025$). 2'-FL was positively associated with weight velocity from 0 to 5 months ($p=0.015$) and FMI at 5 months ($p=0.024$), but not with BMI or height-for-age Z-score (HAZ). In contrast, LNnT was lower in the HW group ($p = 0.012$) and negatively associated with HAZ ($p = 0.008$), weight velocity from 0 to 5 months ($p = 0.009$), and FMI ($p = 0.033$). Maternal BMI at 5 months was negatively associated with 6'-sialyllactose and sialyl-lacto-N-tetraose and positively with 2'-FL, total OS, and total OS-bound fucose (all $p \leq 0.03$). **Safety Measurements/Adverse Events Reported:** Not reported	Larsson et al. (2019)
Study Design: double-blind, controlled, randomized **Study Length**: 6 weeks **Subjects**: n=78 (38 test, 40 control) healthy full-term formula-fed infants (14 ± 5 days old) **Dose, Delivery, and Frequency**: 100% whey, partially hydrolyzed infant formula with	**Outcome Measurements**: Infant Gastrointestinal Symptom Questionnaire (IGSQ) and anthropometric measurements. IGSQ is a validated 13-item questionnaire that assesses an infant's gastrointestinal (GI)-related signs and symptoms as observed by caregivers/parents over the previous week in 5 domains: stooling, spitting up/vomiting, flatulence, crying, and fussing. The possible range in scores is 13 to 65, where a score of 13 indicates no GI distress and a score of 65 represents extreme GI distress. Adverse events (AEs) were collected throughout the study and were assessed by the site investigator or designee for duration, intensity, frequency, and relationship to test product. **Results and Significance**: In the Test group, 1 subject was lost to follow-up, 1 caregiver wished to withdraw, 3 withdrew due to AEs, and 3 were noncompliant with feeding only study formula. In the Control group, 1 subject was lost to follow-up, 1 caregiver wished to withdraw, 3 withdrew due to AEs, and 2 were	Storm et al. (2019)

Study Setup and Details	Human Study Results, Significance, Safety	Reference
the probiotic *Bifidobacterium animalis* ssp *lactis* strain Bb12 ± 0.25 g/L 2'-FL	noncompliant. Therefore, 30 subjects from the Test group and 33 subjects from the Control group were included in the analysis. IGSQ scores were similar between groups (Test 20.9 ± 4.8, Control 20.7 ± 4.3, p = 0.82). Average formula consumption, body weight, length, stool frequency or consistency, crying or fussing duration, vomiting frequency, proportion of babies spitting up and numbers of infants with difficult to pass stools did not differ between groups. Among the babies whose caregivers reported spit-up, significantly more were reported to have spit-up >5 times per day in the Test group than the Control group. More stools were reported to be difficult to pass in the Control group (33 [21%] Control and 4 [3%] Test, p= 0.04). **Safety Measurements/Adverse Events Reported**: No serious AEs were reported. Seventy-two AEs occurred in the study (36 in 17 Test subjects, 36 in 19 Control subjects). With the exception of more infants spitting up > 5 times per day (see above), no AEs occurred at a higher rate in the Test group compared to the Control group. No safety concerns noted with either of the study formulas.	
Study Design: double-blind, controlled, randomized, crossover food challenge, followed by open label home study **Study Length**: Food challenge (1 day); Home study (7 days) **Subjects**: Infants with cow's milk protein allergy (2–57 months old, n=64 for challenge study and n=61 for home study) **Dose, Delivery, and Frequency**: 100% whey, extensively hydrolyzed infant formula + 2'-FL (1.0 g/L) and lacto-N-neotetraose (0.5 g/L) or hypoallergenic control formula for challenge study (100 mL min. in divided doses over approx.3 hr), min. 240 mL test formula/day for home study	**Outcome Measurements: Challenge study:** Any allergic signs or symptoms (cutaneous, gastrointestinal, respiratory, or cardiovascular) **Home study**: Daily formula intake, stool frequency, color, consistency, and odor; frequency of flatulence, spitting-up and/or vomiting, any potential allergic symptoms, adverse or serious adverse events. **Results and Significance: Challenge study**: A 12-month-old girl reacted to both formulae with widespread urticaria and an erythematous rash, but no other systemic clinical features, after ingesting a total of 165 mL of the test and 85 mL of the control formulae. The reactions settled after treatment with an antihistamine. 63 out of 64 subjects (98.4%) tolerated the test formula, and 61 out of 62 subjects (98.4%) tolerated the control formula **Home study:** Fifty-five (90.2%) subjects consumed a min. of 240 mL of the test formula/day. Two subjects reported GI symptoms. One subject vomited on Day 1 but completed the home study without further problems. Another patient developed diarrhea on the last day, which was attributed to gastroenteritis. The episode resolved after 4 days. Otherwise, no significant GI symptoms (flatulence, abnormal stool frequency/consistency, increased spitting-up or vomiting) were reported. No reactions warranted early discontinuation. No serious adverse events occurred during the entire study.	Nowak-Wegrzyn et al. (2019)

Approx – Approximately; BMI – body mass index; 2'-FL – 2'-fucosyllactose; FMI – fat mass index; GI – gastrointestinal; HAZ – height-for-age Z-score; HW – high weight gain; IGSQ – Infant Gastrointestinal Symptom Questionnaire; LW – low weight gain; Min – minimum; OS – oligosaccharides; SD – standard deviation; WAZ – weight-for-age z-score

4. Reviews

Four review articles were located by the current literature search, which did not include any new information that would affect the conclusion of GRAS status for 2'-FL. The review articles

discussed results of studies that are presented in GRN 735, plus three additional unpublished studies.

In 2018, Reverri and coworkers published a review of clinical studies performed on infants receiving formula supplemented with 2'-FL (Reverri et al., 2018). Clinical studies involving 610 healthy infants were reviewed, two of which examined subpopulations of infants in the Marriage et al. (2015) study. Information from two of the studies [Marriage et al. (2015) and Goehring et al. (2016)] was reported in GRN 735 and is not discussed here. Results of a prospective randomized, multi-center, double-blinded, controlled tolerance study in 131 healthy term infants who were fed formula supplemented with 0.2 g 2'-FL per L and 2.0 g short-chain fructooligosaccharides (scFOS) per L were included in the review (Kajzer, 2016). The authors of the review concluded that "formula with 2'-FL and scFOS was safe and well tolerated in infants, as evidenced by stool consistency, formula intake, percent feedings with spit-up/vomit, and reported AEs like those of the infants who were fed formula without oligosaccharides or those of the BF [breastfed] infants." Reverri et al. (2018) also reviewed an unpublished prospective, multi-center, single-arm study by Abbott Nutrition (no reference number reported) in 59 healthy, but fussy infants who were fed a low lactose formula containing partially hydrolyzed whey-based formula with 0.2 g 2'-FL per L and 1.8 g scFOS per L. Reverri et al. (2018) also conducted a post-hoc analysis of respiratory AEs from 205 infants that participated in the Marriage et al. (2015) study and found no association between consumption of formula supplemented with 2'-FL and increased incidences of respiratory AEs.

Sprenger et al. (2019) reviewed available clinical studies performed on 2'-FL [all of which were mentioned in GRN 735 with the exception of the Kajzer (2016) and unpublished Abbott Nutrition studies mentioned above] and concluded that "clinical intervention trials with specific HMOs [human milk oligosaccharides] demonstrate their growth safety and digestive tolerance." Vandenplas et al. (2018) also reviewed available studies (all of which were mentioned in GRN 735 or this section to this point) and concluded that "no adverse effects have been reported for 2'-FL" and "2'-FL is a safe supplementation of infant formula." In addition, a review by Hegar et al. (2019) included studies already mentioned, plus an unpublished study in an unstated number of infants performed by Janas et al. (2015). Hegar et al. (2019) concluded that "there have been no adverse effects reported till date for 2′-FL. Clinical studies have demonstrated that infants fed on a formula supplemented with 2′-FL exhibit a normal growth pattern, normal defecation, and no adverse effects. Therefore, it can be concluded that 2′-FL is a safe supplementation for infant formula."

5. Summary

None of the updated literature summarized above triggers any safety concerns for the intended uses of FrieslandCampina's Purified 2'-FL preparations, described in GRN 735 and herein, in food. FrieslandCampina's alternative manufacturing process uses the same raw materials evaluated in GRN 735, with the exception of three alternative sources of glucose, cobalt, and/or manganese. The alternative manufacturing process produces material that meets the same specifications as the Purified 2'-FL described in GRN 735, with minor changes in purity and water content. The

specifications of the Purified 2'-FL manufactured with the alternative method described in this Supplement are sufficiently similar to the European Union specifications for 2'-FL produced from genetically modified strains of *E. coli* K12 and BL21 and raise no safety concerns. Furthermore, FrieslandCampina has reviewed this safety information and has concluded that our Purified 2'-FL manufactured using the alternative manufacturing process is GRAS for the proposed uses in foods as previously described in GRN 735.

B. Expert Panel Findings on Safety of Purified 2'-Fucosyllactose (2'-FL)

An evaluation of the safety and GRAS status of the alternative manufacturing process for the Purified 2'-FL preparation has been conducted by an Expert Panel convened by GRAS Associates; the Panel consisted of Robert Kapp, Ph.D., Fellow Academy of Toxicological Sciences (ATS), Fellow Royal Society of Biology (FRSB) & European Registered Toxicologist (ERT); Kara Lewis, Ph.D.; and Katrina Emmel, Ph.D., as Panel Chair. The Expert Panel reviewed this Supplement, GRN 735, and the publicly available information available to them. The individuals who served as Expert Panelists are qualified to evaluate the safety of foods and food ingredients by merit of scientific training and experience.

The GRAS Expert Panel report is provided in Appendix 4.

C. Common Knowledge Element for GRAS Determinations

The first common knowledge element for a GRAS determination requires that data and information relied upon to establish safety must be generally available; this is most commonly established by utilizing studies published in peer-reviewed scientific journals. The second common knowledge element for a GRAS determination requires that there be a basis to conclude that consensus exists among qualified scientists about the safety of the substance for its intended use.

1. Generally Available Information

The common use of 2'-FL in food on a global basis with the associated absence of harm is based on published information of all types, including GRNs, European Union regulations, and nonclinical and clinical studies. The majority of the studies reviewed for GRN 735 (and the Supplement herein) have been published in peer-reviewed journals that are readily available. Published information about 2'-FL preparations produced from different manufacturing processes support the safety of the Purified 2'-FL formulation produced according to this GRAS Supplement at the usage rate stipulated in GRN 735.

The composite information thereby fulfills the general availability common knowledge element for GRAS determinations.

2. Scientific Consensus

The second common knowledge element for a GRAS determination requires that there must be a basis to conclude that consensus exists among qualified scientists about the safety of the substance for its intended use.

The most compelling documentation of consensus for the safety of Purified 2'-FL is described in GRN 735, and information in this Supplement supports GRN 735. In 2017, 2'-FL was approved by the European Union as a novel food. The conditions of use and acceptable specifications for 2'-FL for use as a novel food do not differ substantially from the Purified 2'-FL described in this GRAS Supplement. Further, the *in vitro* and toxicity studies conducted on Purified 2'-FL that were unpublished at the time of GRN 735 have been published and support a NOAEL of 10% in the diet of rats (7.25 g per kg bw per day for males and 7.76 g per kg bw per day for females). Results of new clinical studies in infants have no effect on the previous conclusion in GRN 735 that use of up to 1.2 g 2'-FL per L in infant formula is safe.

Based upon these data, FrieslandCampina has determined that a wide consensus exists in the scientific community to support a GRAS conclusion for the Purified 2'-FL preparation described in this GRAS Supplement as evidenced by the totality of published information supporting safety at the estimated levels of intake.

D. Conclusion

In consideration of the aggregate safety information available on 2'-FL, as well as the report from the designated Expert Panel provided in Appendix 4, FrieslandCampina concludes that the Purified 2'-FL preparation prepared under the alternative manufacturing process and as defined in this supplement to GRN 735, and produced under Current Good Manufacturing Practices (CGMP) is safe for use in term infant formulas and conventional foods as described within GRN 735, and is generally recognized as safe (GRAS) within the meaning of the Food, Drug, and Cosmetic Act.

This declaration has been made in accordance with FDA's standard for food ingredient safety, i.e., reasonable certainty of no harm under the intended conditions of use.

PART 7. LIST OF SUPPORTING DATA AND INFORMATION

A. List of Acronyms and References

1. List of Acronyms

AEs	Adverse Events
Approx	Approximately
ATS	Academy of Toxicological Sciences
BMI	Body mass index
bw	Body weight
CAGR	Compound Annual Growth Rate
CAS	Chemical Abstracts Service
CFR	Code of Federal Regulations
cfu	Colony Forming Unit
CGMP	Current Good Manufacturing Practice
CoA	Certificate of Analysis
D	Dextro
dm	Dry Matter
E. coli	Escherichia coli
ERT	European Registered Toxicologist
EU	Endotoxin Units
FCC	Food Chemicals Codex
FD&C	Federal Food Drug and Cosmetics Act
FMI	Fat mass index
FOIA	Freedom of Information Act
FRSB	Fellow Royal Society of Biology
g	Gram
GA	GRAS Associates
GI	Gastrointestinal
GMO	Genetically Modified Organism
GRAS	Generally Recognized as Safe
GRN	GRAS Notice
h	Hour
HAZ	Height-for-age Z-score
hbd-2	Human-beta defensin 2
HW	High weight gain
IGSQ	Infant Gastrointestinal Symptom Questionnaire
kg	Kilogram
L	Levo
lcFOS	Long chain fructooligosaccharides
LNnT	lacto-N-neotetraose
Max	Maximum
mg	Milligram
Min	Minimum
mL	Milliliter
NA	Not Available
Neg	Negative
No.	Number

NOAEL	No observed adverse effect level
NW	Normal weight gain
OS	Oligosaccharides
PIgR	polymeric immunoglobulin receptor
qPCR	quantitative polymerase chain reaction
rRNA	Ribosomal ribonucleic acid
RV	Rotavirus
scFOS	Short-chain fructooligosaccharides
scGOS	Short chain galactooligosaccharides
SD	Standard deviation
spp.	Species
TEER	Transepithelial electrical resistance
ug	Microgram
US	United States
w/w	Weight by weight
w/w	weight/weight
WAZ	Weight-for-age z-score
ZO-1	Zona Occludens

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B. Appendices

Appendix 1 Specifications for Alternative Raw Materials and Production Processing Aids

Appendix 1.1 Glucose syrup

Appendix 1.2 Cobalt (II) sulfate heptahydrate

Appendix 1.3 Manganese (II) sulfate monohydrate

Appendix 1.1 Glucose syrup

ROQUETTE

SPECIFICATIONS **Ref: J41-102A10**

PAGE 1/2

SIROP DE GLUCOSE 70/100

DEFINITION :

High dextrose GLUCOSE SYRUP
obtained from starch.

CAS n° : 8029-43-4
EINECS : 232-436-4

SPECIFICATIONS :

* PHYSICO-CHEMICAL VALUES

APPEARANCE		Colourless to yellowish syrupy liquid.
TASTE		Sweet
ODOUR		Neutral
REFRACTOMETRIC READING AT 20°C (BRIX)	Refractometric reading	68.7 - 69.7
REFRACTIVE INDEX	Refractometric reading	1.4623 - 1.4649
DRY SUBSTANCE	calculation/R.reading	70.1 - 71.2 %
GLUCOSE	H.P.L.C	99.2 %/D.S. min.
SULPHATED ASH	NF EN 5809	0.1 % max.
pH IN SOLUTION	At 50 Refract.reading	3.0 - 5.5
SO2	NF EN 1185	10 ppm max.

* MICROBIOLOGICAL VALUES

- TOTAL COUNT	Internal method	1000/g max.
- YEASTS	Internal method	50/g max.
- MOULDS	Internal method	50/g max.
- E.COLI	Internal method	Absent in 1 g
- SALMONELLAE	Internal method	Absent in 10 g

MCL,MMC : ROQUETTE Methods

QUALITY ASSURANCE / HUMAN FOOD ++++ August 16, 2018

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ROQUETTE

SPECIFICATIONS **Ref: J41-102A10**

SIROP DE GLUCOSE 70/100

PAGE 2/2

TYPICAL VALUES :

VISCOSITIES - 40°C	50 mPa.s approx
SPECIFIC GRAVITY At 50° C	1.33 approx.
ENERGY VALUE calculated, on 100g commercial product	1198 kJ (282 kcal)

CONFORMITY :

- E.U. Council Directive: 73/437/CEE (JO CE L.356 of 27/12/73).
- CODEX STAN 9 - 1981.
- US code of Federal Regulations 21 CFR, 1994 § 168.120.
- FOOD CHEMICALS CODEX, current edition.

STORAGE :

Standard packaging: bulk road tanker.

It is recommended that this syrup be stored at a temperature near 50 °C to avoid crystallization.

MCL,MMC : ROQUETTE Methods

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Appendix 1.2 Cobalt (II) sulfate heptahydrate

ACROS ORGANICS

Version	0
Molecular weight	281.09
Molecular formula	Co O4 S . 7 H2 O
CAS No	10026-24-1
Linear formula	CoSO4.7H2O
Flash point (°C)	

Certificate of Analysis

This is to certify that units of the lot number below were tested and found to comply with the specifications of the grade listed. Certain data have been supplied by third parties. Acros Organics expressly disclaims all warranties,expressed or implied, including the implied warranties of merchantability and fitness for a particular purpose. Products are for research use or further manufacturing. Not for direct administration to human or animals. It is the responsiblity of the purchaser, formulator or those performing further manufacturing to determine suitibility based upon the intended use of the end product. Products are tested to meet the analytical requirements of the noted grade. The following information is the actual analytical results obtained.

Catalog Number	21310	**Quality Test / Release Date**	1 June 2018
Lot Number	A0397174	**Suggested Retest Date**	June 2023
Description	Cobalt(II) sulfate heptahydrate,99+%,extra pure		
Country of Origin	FINLAND		
Declaration of Origin	synthetic		

Origin Comment	

Result Name	Specifications	Test Value
Appearance (Color)	Red-brown	Red-brown
Appearance (Form)	Adhering crystalline powder or crystals	Adhering crystalline powder and crystals
Titration Complexometric	>=99.0 %	99.1 %
Nickel (Ni)	=<500 ppm	=<5 ppm
Iron (Fe)	=<50 ppm	2 ppm
Lead (Pb)	=<50 ppm	=<5 ppm

L. Van den Broek, QA Manager Issued: 5 March 2019

Acros Organics
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1 Reagent Lane, Fair Lawn, NJ 07410,USA Fax 201-796-1329

Appendix 1.3 Manganese (II) sulfate monohydrate

Specification

1.05999.1000 Manganese(II) sulfate monohydrate spray dried suitable for use as excipient EMPROVE® exp Ph Eur,USP,FCC

	Spec. Values	
Assay		
complexometric, $MnSO_4 \cdot H_2O$	98.0 - 102.0	%
complexometric, calc. on the ignited basis	99.0 - 101.0	%
Identity	passes test	
Appearance of solution	passes test	
Chloride (Cl)	≤ 0.005	%
Heavy metals (as Pb)	≤ 0.002	%
As (Arsenic)	≤ 0.0003	%
Ca (Calcium)	≤ 0.01	%
Fe (Iron)	≤ 0.001	%
Pb (Lead)	≤ 0.0004	%
Se (Selenium)	≤ 0.003	%
Zn (Zinc)	≤ 0.005	%
Substances not precipitated by ammonium sulfide (as SO_4)	≤ 0.5	%
Residual solvents (Ph. Eur./USP/ICH)	excluded by manufacturing process	
Loss on ignition (500 °C)	10.5 - 12.0	%

Residues of metal catalysts or metal reagents acc. to EMEA/C HMP/SWP/4446/2000 are not likely to be present.

conforms to Ph Eur, USP, FCC

Dr. Andreas Lang

responsible laboratory manager quality control

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Appendix 2 Certificates of Analysis for Multiple Batches of Purified 2'-FL Produced According to this Supplement

Appendix 2.1 Lot 815358-4

Appendix 2.2 Lot 815383-5

Appendix 2.3 Lot 815418-7

Appendix 2.4 Lot 815440-7

Appendix 2.5 Lot 815463-4

Appendix 2.1 Lot 815358-4

DOMO®

Certificate of analysis

Product : 2'-Fucosyllactose
Batch number : 815358-4
Date of production : 7/2/2019
Best Before : 7/2/2022

Description : Human milk oligosaccharide

Typical analysis : Dry matter (solids) 96%, moisture 4%. On dry matter: 2'-fucosyllactose 94%, lactose 1%, allo-lactose 1%, glucose 1%, fucose 1%

Sensorial: : White homogeneous powder, neutral to slightly sweet, no off flavor

Chemical/physical	Specification	Result	Method of analysis
Total moisture	max. 9%	3.61%	ISO 760 (modified), Karl Fischer
2'-Fucosyllactose	min. 88% on dm	96.9%	FC-method using HPAEC-PAD
Lactose	max. 3%	1.5%	FC-method using HPAEC-PAD
Allo-Lactose	max. 2%	1.0%	FC-method using HPAEC-PAD
Glucose	max. 2%	0.2%	FC-method using HPAEC-PAD
Galactose	max. 2%	<0.1%	FC-method using HPAEC-PAD
Fucose	max. 2%	0.4%	FC-method using HPAEC-PAD
Protein	max. 0.01%	<0.01%	Bradford
Sulphated ash	max. 0.2%	0.05%	NEN 6810 (modified)
Nitrite	max. 1 mg/kg	<0.1 mg/kg	ISO 14673-2/IDF 189-2
Nitrate	max. 50 mg/kg	9.0 mg/kg	ISO 14673-2/IDF 189-2
Scorched particles	max. disc A	A	FC-method equivalent to ADPI 916/ISO 5739/IDF 107
pH (10%)	3.0 - 7.5	4.36	FC-method using NEN 3775
Aluminum	max. 4.8 mg/kg	0.48 mg/kg	FC-method using ISO 17294
Arsenic	max. 0.1 mg/kg	<0.01 mg/kg	FC-method using ISO 17294
Cadmium	max. 0.01 mg/kg	<0.005 mg/kg	FC-method using ISO 17294
Mercury	max. 0.05 mg/kg	<0.006 mg/kg	FC-method using ISO 17294
Lead	max. 0.05 mg/kg	<0.02 mg/kg	FC-method using ISO 17294
Aflatoxin M1	max. 0.2 µg/kg	<0.01 µg/kg	ISO 14501/IDF 171

Microbiological	Specification	Result	Method of analysis
Aerobic mesophilic count	max. 3000 cfu/g	<100 cfu/g	FC-method equivalent to ISO 4833
Enterobacteriaceae	absent in 10 g	Absent in 10g	FC-method, BPW 18h 37°C, SD, VRBG 18-24h 37°C
E. coli	absent in 10 g	Absent in 10g	FC-method, LMX 25h, Coli ID 24h
Yeasts	max. 10 cfu/g	<10 cfu/g	FC-method equivalent to ISO 6611
Moulds	max. 10 cfu/g	<10 cfu/g	FC-method equivalent to ISO 6611
Presumptive Bacillus cereus	max. 100 cfu/g	<10 cfu/g	FC-method equivalent to ISO 7932
Staphylococcus aureus	absent in 1 g	Absent in 1 g	FC-method, GB/C 42h 37°C, PCR
Sulphite reducing clostridia spores	max. 30 cfu/g	<10 cfu/g	FC-method using IJFM 27 (1995) 185-200 Weenk
Clostridium perfringens	absent in 1 g	Absent in 1 g	FC-method, RPM 20h 46°C, confirmation
Salmonella	absent in 25 g	Absent in 25 g	FC-method equivalent to ISO 6579
Cronobacter spp.	absent in 25 g	Absent in 25 g	FC-method equivalent to ISO/TS 22964
Endotoxin	max. 10 EU/mg	0.01 EU/mg	Eur. Ph. 2.6.14 and USP <85>
GMO-detection	negative	Negative	qPCR

Wageningen, 01/06/2019

Jan Bastiaans

Function: R&D Manager

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Appendix 2.2 Lot 815383-5

DOMO®

Certificate of analysis

Product	: 2'-Fucosyllactose
Batch number	: 815383-5
Date of production	: 26/2/2019
Best Before	: 26/2/2022
Description	: Human milk oligosaccharide
Typical analysis	: Dry matter (solids) 96%, moisture 4%. On dry matter: 2'-fucosyllactose 94%, lactose 1%, allo-lactose 1%, glucose 1% fucose 1%
Sensorial:	: White homogeneous powder, neutral to slightly sweet, no off flavor

Chemical/physical	**Specification**	**Result**	**Method of analysis**
Total moisture	max. 9%	4.02%	ISO 760 (modified), Karl Fischer
2'-Fucosyllactose	min. 88% on dm	94.0%	FC-method using HPAEC-PAD
Lactose	max. 3%	0.9%	FC-method using HPAEC-PAD
Allo-Lactose	max. 2%	0.8%	FC-method using HPAEC-PAD
Glucose	max. 2%	0.2%	FC-method using HPAEC-PAD
Galactose	max. 2%	<0.1%	FC-method using HPAEC-PAD
Fucose	max. 2%	0.3%	FC-method using HPAEC-PAD
Protein	max. 0.01%	<0.01%	Bradford
Sulphated ash	max. 0.2%	0.03%	NEN 6810 (modified)
Nitrite	max. 1 mg/kg	<0.1 mg/kg	ISO 14673-2/IDF 189-2
Nitrate	max. 50 mg/kg	2.3 mg/kg	ISO 14673-2/IDF 189-2
Scorched particles	max. disc A	A	FC-method equivalent to ADPI 916/ISO 5739/IDF 107
pH (10%)	3.0 - 7.5	4.59	FC-method using NEN 3775
Aluminum	max. 4.8 mg/kg	0.62 mg/kg	FC-method using ISO 17294
Arsenic	max. 0.1 mg/kg	<0.01 mg/kg	FC-method using ISO 17294
Cadmium	max. 0.01 mg/kg	<0.005 mg/kg	FC-method using ISO 17294
Mercury	max. 0.05 mg/kg	<0.006 mg/kg	FC-method using ISO 17294
Lead	max. 0.05 mg/kg	<0.02 mg/kg	FC-method using ISO 17294
Aflatoxin M1	max. 0.2 µg/kg	<0.01 µg/kg	ISO 14501/IDF 171

Microbiological	**Specification**	**Result**	**Method of analysis**
Aerobic mesophilic count	max. 3000 cfu/g	<100 cfu/g	FC-method equivalent to ISO 4833
Enterobacteriaceae	absent in 10 g	Absent in 10g	FC-method, BPW 18h 37°C, SD, VRBG 18-24h 37°C
E. coli	absent in 10 g	Absent in 10g	FC-method, LMX 25h, Coli ID 24h
Yeasts	max. 10 cfu/g	<10 cfu/g	FC-method equivalent to ISO 6611
Moulds	max. 10 cfu/g	<10 cfu/g	FC-method equivalent to ISO 6611
Presumptive Bacillus cereus	max. 100 cfu/g	<10 cfu/g	FC-method equivalent to ISO 7932
Staphylococcus aureus	absent in 1 g	Absent in 1 g	FC-method, G&C 42h 37°C, PCR
Sulphite reducing clostridia spores	max. 30 cfu/g	<10 cfu/g	FC-method using IJFM 27 (1995) 185-200 Weenk
Clostridium perfringens	absent in 1 g	Absent in 1 g	FC-method, RPM 20h 46°C, confirmation
Salmonella	absent in 25 g	Absent in 25 g	FC-method equivalent to ISO 6579
Cronobacter spp.	absent in 25 g	Absent in 25 g	FC-method equivalent to ISO/TS 22964
Endotoxin	max. 10 EU/mg	0.003 EU/mg	Eur. Ph. 2.6.14 and USP <85>
GMO-detection	negative	Negative	qPCR

Wageningen, 01/05/2019

Jan Bastiaans

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Appendix 2.3 Lot 815418-7

DOMO®

Certificate of analysis

Product	: 2'-Fucosyllactose
Batch number	: 815418-7
Date of production	: 27/3/2019
Best Before	: 27/3/2022
Description	: Human milk oligosaccharide
Typical analysis	: Dry matter (solids) 96%, moisture 4%. On dry matter: 2'-fucosyllactose 94%, lactose 1%, allo-lactose 1%, glucose 1% fucose 1%
Sensorial:	: White homogeneous powder, neutral to slightly sweet, no off flavor

Chemical/physical	**Specification**	**Result**	**Method of analysis**
Total moisture	max. 9%	3.72%	ISO 760 (modified), Karl Fischer
2'-Fucosyllactose	min. 88% on dm	92.9%	FC-method using HPAEC-PAD
Lactose	max. 3%	1.1%	FC-method using HPAEC-PAD
Allo-Lactose	max. 2%	1.2%	FC-method using HPAEC-PAD
Glucose	max. 2%	0.2%	FC-method using HPAEC-PAD
Galactose	max. 2%	<0.1%	FC-method using HPAEC-PAD
Fucose	max. 2%	0.2%	FC-method using HPAEC-PAD
Protein	max. 0.01%	<0.01%	Bradford
Sulphated ash	max. 0.2%	0.02%	NEN 6810 (modified)
Nitrite	max. 1 mg/kg	<0.1 mg/kg	ISO 14673-2/IDF 189-2
Nitrate	max. 50 mg/kg	2.3 mg/kg	ISO 14673-2/IDF 189-2
Scorched particles	max. disc A	A	FC-method equivalent to ADPI 916/ISO 5739/IDF 107
pH (10%)	3.0 - 7.5	4.71	FC-method using NEN 3775
Aluminum	max. 4.8 mg/kg	0.54 mg/kg	FC-method using ISO 17294
Arsenic	max. 0.1 mg/kg	<0.01 mg/kg	FC-method using ISO 17294
Cadmium	max. 0.01 mg/kg	<0.005 mg/kg	FC-method using ISO 17294
Mercury	max. 0.05 mg/kg	<0.006 mg/kg	FC-method using ISO 17294
Lead	max. 0.05 mg/kg	<0.02 mg/kg	FC-method using ISO 17294
Aflatoxin M1	max. 0.2 µg/kg	<0.01 µg/kg	ISO 14501/IDF 171

Microbiological	**Specification**	**Result**	**Method of analysis**
Aerobic mesophilic count	max. 3000 cfu/g	<100 cfu/g	FC-method equivalent to ISO 4833
Enterobacteriaceae	absent in 10 g	Absent in 10g	FC-method, BPW 18h 37°C, SD, VRBG 18-24h 37°C
E. coli	absent in 10 g	Absent in 10g	FC-method, LMX 25h, Coli ID 24h
Yeasts	max. 10 cfu/g	<10 cfu/g	FC-method equivalent to ISO 6611
Moulds	max. 10 cfu/g	<10 cfu/g	FC-method equivalent to ISO 6611
Presumptive Bacillus cereus	max. 100 cfu/g	<10 cfu/g	FC-method equivalent to ISO 7932
Staphylococcus aureus	absent in 1 g	Absent in 1 g	FC-method, G&C 42h 37°C, PCR
Sulphite reducing clostridia spores	max. 30 cfu/g	<10 cfu/g	FC-method using IJFM 27 (1995) 185-200 Weenk
Clostridium perfringens	absent in 1 g	Absent in 1 g	FC-method, RPM 20h 46°C, confirmation
Salmonella	absent in 25 g	Absent in 25 g	FC-method equivalent to ISO 6579
Cronobacter spp.	absent in 25 g	Absent in 25 g	FC-method equivalent to ISO/TS 22964
Endotoxin	max. 10 EU/mg	0.003 EU/mg	Eur. Ph. 2.6.14 and USP <85>
GMO-detection	negative	Negative	qPCR

Wageningen, 01/06/2019

[redacted]

Jan Bastiaans

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Appendix 2.4 Lot 815440-7

DOMO®

Certificate of analysis

Product : 2'-Fucosyllactose
Batch number : 815440-7
Date of production : 17/4/2019
Best Before : 17/4/2022

Description : Human milk oligosaccharide

Typical analysis : Dry matter (solids) 96%, moisture 4%. On dry matter: 2'-fucosyllactose 94%, lactose 1%, allo-lactose 1%, glucose 1%, fucose 1%

Sensorial: : White homogeneous powder, neutral to slightly sweet, no off flavor

Chemical/physical	Specification	Result	Method of analysis
Total moisture	max. 9%	3.86%	ISO 760 (modified), Karl Fischer
2'-Fucosyllactose	min. 88% on dm	95.7%	FC-method using HPAEC-PAD
Lactose	max. 3%	0.8%	FC-method using HPAEC-PAD
Allo-Lactose	max. 2%	1.2%	FC-method using HPAEC-PAD
Glucose	max. 2%	0.2%	FC-method using HPAEC-PAD
Galactose	max. 2%	<0.1%	FC-method using HPAEC-PAD
Fucose	max. 2%	0.2%	FC-method using HPAEC-PAD
Protein	max. 0.01%	<0.01%	Bradford
Sulphated ash	max. 0.2%	<0.01%	NEN 6810 (modified)
Nitrite	max. 1 mg/kg	<0.1 mg/kg	ISO 14673-2/IDF 189-2
Nitrate	max. 50 mg/kg	7.5 mg/kg	ISO 14673-2/IDF 189-2
Scorched particles	max. disc A	A	FC-method equivalent to ADPI 916/ISO 5739/IDF 107
pH (10%)	3.0 - 7.5	3.89	FC-method using NEN 3775
Aluminum	max. 4.8 mg/kg	0.51 mg/kg	FC-method using ISO 17294
Arsenic	max. 0.1 mg/kg	<0.01 mg/kg	FC-method using ISO 17294
Cadmium	max. 0.01 mg/kg	<0.005 mg/kg	FC-method using ISO 17294
Mercury	max. 0.05 mg/kg	<0.006 mg/kg	FC-method using ISO 17294
Lead	max. 0.05 mg/kg	<0.02 mg/kg	FC-method using ISO 17294
Aflatoxin M1	max. 0.2 µg/kg	<0.01 µg/kg	ISO 14501/IDF 171

Microbiological	Specification	Result	Method of analysis
Aerobic mesophilic count	max. 3000 cfu/g	<100 cfu/g	FC-method equivalent to ISO 4833
Enterobacteriaceae	absent in 10 g	Absent in 10g	FC-method, BPW 18h 37°C, SD, VRBG 18-24h 37°C
E. coli	absent in 10 g	Absent in 10g	FC-method, LMX 25h, Coli ID 24h
Yeasts	max. 10 cfu/g	<10 cfu/g	FC-method equivalent to ISO 6611
Moulds	max. 10 cfu/g	<10 cfu/g	FC-method equivalent to ISO 6611
Presumptive Bacillus cereus	max. 100 cfu/g	<10 cfu/g	FC-method equivalent to ISO 7932
Staphylococcus aureus	absent in 1 g	Absent in 1 g	FC-method, G&C 42h 37°C, PCR
Sulphite reducing clostridia spores	max. 30 cfu/g	<10 cfu/g	FC-method using IJFM 27 (1995) 185-200 Weenk
Clostridium perfringens	absent in 1 g	Absent in 1 g	FC-method, RPM 20h 46°C, confirmation
Salmonella	absent in 25 g	Absent in 25 g	FC-method equivalent to ISO 6579
Cronobacter spp.	absent in 25 g	Absent in 25 g	FC-method equivalent to ISO/TS 22964
Endotoxin	max. 10 EU/mg	0.01 EU/mg	Eur. Ph. 2.6.14 and USP <85>
GMO-detection	negative	Negative	qPCR

Wageningen, 01/06/2019

Jan Bastiaans

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Appendix 2.5 Lot 815463-4

DOMO®

Certificate of analysis

Product	: 2'-Fucosyllactose
Batch number	: 815463-4
Date of production	: 7/5/2019
Best Before	: 7/5/2022
Description	: Human milk oligosaccharide
Typical analysis	: Dry matter (solids) 96%, moisture 4%. On dry matter: 2'-fucosyllactose 94%, lactose 1%, allo-lactose 1%, glucose 1%, fucose 1%
Sensorial:	: White homogeneous powder, neutral to slightly sweet, no off flavor

Chemical/physical	**Specification**	**Result**	**Method of analysis**
Total moisture	max. 9%	3.91%	ISO 760 (modified), Karl Fischer
2'-Fucosyllactose	min. 88% on dm	93.9%	FC-method using HPAEC-PAD
Lactose	max. 3%	0.8%	FC-method using HPAEC-PAD
Allo-Lactose	max. 2%	0.9%	FC-method using HPAEC-PAD
Glucose	max. 2%	0.3%	FC-method using HPAEC-PAD
Galactose	max. 2%	<0.1%	FC-method using HPAEC-PAD
Fucose	max. 2%	0.3%	FC-method using HPAEC-PAD
Protein	max. 0.01%	<0.01%	Bradford
Sulphated ash	max. 0.2%	<0.003%	NEN 6810 (modified)
Nitrite	max. 1 mg/kg	<0.1 mg/kg	ISO 14673-2/IDF 189-2
Nitrate	max. 50 mg/kg	8.9 mg/kg	ISO 14673-2/IDF 189-2
Scorched particles	max. disc A	A	FC-method equivalent to ADPI 916/ISO 5739/IDF 107
pH (10%)	3.0 - 7.5	4.28	FC-method using NEN 3775
Aluminum	max. 4.8 mg/kg	0.31 mg/kg	FC-method using ISO 17294
Arsenic	max. 0.1 mg/kg	<0.01 mg/kg	FC-method using ISO 17294
Cadmium	max. 0.01 mg/kg	<0.005 mg/kg	FC-method using ISO 17294
Mercury	max. 0.05 mg/kg	<0.006 mg/kg	FC-method using ISO 17294
Lead	max. 0.05 mg/kg	<0.02 mg/kg	FC-method using ISO 17294
Aflatoxin M1	max. 0.2 µg/kg	<0.01 µg/kg	ISO 14501/IDF 171

Microbiological	**Specification**	**Result**	**Method of analysis**
Aerobic mesophilic count	max. 3000 cfu/g	100 cfu/g	FC-method equivalent to ISO 4833
Enterobacteriaceae	absent in 10 g	Absent in 10g	FC-method, BPW 18h 37°C, SD, VRBG 18-24h 37°C
E. coli	absent in 10 g	Absent in 10g	FC-method, LMX 25h, Coli ID 24h
Yeasts	max. 10 cfu/g	<10 cfu/g	FC-method equivalent to ISO 6611
Moulds	max. 10 cfu/g	<10 cfu/g	FC-method equivalent to ISO 6611
Presumptive Bacillus cereus	max. 100 cfu/g	<10 cfu/g	FC-method equivalent to ISO 7932
Staphylococcus aureus	absent in 1 g	Absent in 1 g	FC-method, G&C 42h 37°C, PCR
Sulphite reducing clostridia spores	max. 30 cfu/g	<10 cfu/g	FC-method using IJFM 27 (1995) 185-200 Weenk
Clostridium perfringens	absent in 1 g	Absent in 1 g	FC-method, RPM 20h 46°C, confirmation
Salmonella	absent in 25 g	Absent in 25 g	FC-method equivalent to ISO 6579
Cronobacter spp.	absent in 25 g	Absent in 25 g	FC-method equivalent to ISO/TS 22964
Endotoxin	max. 10 EU/mg	<0.001 EU/mg	Eur. Ph. 2.6.14 and USP <85>
GMO-detection	negative	Negative	qPCR

Wageningen, 22/07/2019

[redacted]

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Appendix 3 Representative Chromatograms for Five Production Batches of Purified 2'-FL Produced According to this Supplement

Chromatograms of HPAEC 2'-fucosyllactose method

Isocratic HPAEC of the 2'-fucosyllactose end product (ME-AV042FL Isocratic HPAEC)

In this document the Chromatograms of the production batches (Q1 2019) are presented, production batches 815358, 815383, 815418, 815440, 815463.

Identification and quantification of 2'-fucosyllactose is done with a standard, PMRS01, of which the 2'-fucosyllactose is identified and quantified with qNMR (see report Spectral Services, Köln, Germany)

Appendix 3.1 Lot 815358

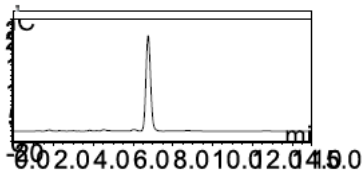
Appendix 3.2 Lot 815383

Appendix 3.3 Lot 815418

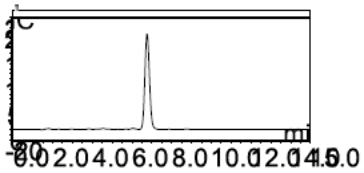
Appendix 3.4 Lot 815440

Appendix 3.5 Lot 815463

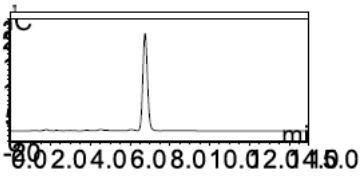
Appendix 3.1 Lot 815358



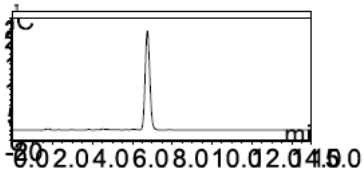
Appendix 3.2 Lot 815383



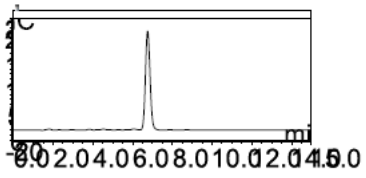
Appendix 3.3 Lot 815418



Appendix 3.4 Lot 815440



Appendix 3.5 Lot 815463



Appendix 4 GRAS Associates Expert Panel Report

The Generally Recognized as Safe (GRAS) Status of the Proposed Uses of Purified 2'-Fucosyllactose

November 21, 2019

Foreword

An independent panel of experts ("Expert Panel") was convened by GRAS Associates, LLC on behalf of FrieslandCampina Domo B.V., to evaluate the safety and Generally Recognized as Safe (GRAS) status of FrieslandCampina's Purified 2'-Fucosyllactose (2'-FL) when manufactured using the manufacturing process described in the document entitled "Supplement to GRAS Notification 735 Purified 2'-Fucosyllactose" and meeting the revised specifications described therein. The members of this Expert Panel† are qualified to serve in this capacity by qualification of scientific training and experience in the safety of food and food ingredients.

Discussion

A significant amount of safety information related to the consumption of 2'-fucosyllactose is generally available, and has been discussed in Part 6 of FrieslandCampina's Supplement dossier, and in further breadth in previous GRAS Notices (GRNs), including FrieslandCampina's GRN 735.

The Expert Panel has reviewed the chemistry of FrieslandCampina's Purified 2'-FL, the modified manufacturing process and specifications for producing Purified 2'-FL, and all available relevant published safety data in its evaluation of the GRAS status of Purified 2'-FL.

As a simple trisaccharide of L-fucose, D-galactose, and D-glucose, there is a high presumption that 2'-FL is safe for human consumption. The Expert Panel notes that the scientific literature establishes that, as with other human milk oligosaccharides (HMOs), 2'-FL is partially absorbed from the gastrointestinal (GI) tract. Unabsorbed 2'-FL is partially fermented by intestinal biota and exerts a prebiotic effect that promotes intestinal homeostasis.

The Expert Panel notes that FDA has issued "no questions" letters in response to 5 previous GRAS Notices on 2'-FL produced by various manufacturing processes as described in GRNs 546, 571, 650, 735, and 749. The Expert Panel further notes that the specifications for

† Dr. Emmel, Chair of the Expert Panel, is a chemist with substantial food safety experience in addressing steviol glycosides and other food ingredients. Dr. Kapp is a toxicologist with over 35 years of experience. He is a Fellow of the Academy of Toxicological Sciences, a Fellow of the Royal Society of Biology, and a European Registered Toxicologist. Dr. Lewis is a biologist with more than 10 years of experience preparing GRAS dossiers. All three panelists have extensive technical backgrounds in the evaluation of food ingredient safety and in participating in deliberations of GRAS Expert Panels.

FrieslandCampina's Purified 2'-FL have been modified from those presented in GRN 735 to allow for a higher maximum water content (9%), lower minimum 2'-FL content (88%), and lower maximum aflatoxin M1 content (0.025 μg per kg). The Expert Panel agrees that these revised purity specifications for Purified 2'-FL are adequate and comparable to those presented in previous GRNs that received "no questions" letters from FDA, as well as the specifications most recently established by the European Union for 2'-FL derived from microbial sources (European Commission, 2019).

The Expert Panel has carefully reviewed the alternative manufacturing process. FrieslandCampina states that no changes have been made to the *E. coli* K12 organism used to produce 2'-FL or the purification process. The Expert Panel notes that the three alternative raw materials (glucose syrup, cobalt sulfate heptahydrate, and manganese sulfate monohydrate) are suitable food-grade or high purity materials, and do not raise any safety concerns.

The majority of the safety studies conducted on 2'-FL have been discussed in detail in previous GRNs, including GRN 735, which was previously submitted for FrieslandCampina's Purified 2'-FL preparation manufactured using alternative raw materials and which received a "no questions" letter from FDA. The Expert Panel considered the following as evidence of safety for FrieslandCampina's Purified 2'-FL:

- No adverse effects attributed to 2'-FL were observed in a 90-day rat study using neonatal rats (from postnatal day 7 through postnatal day 98) at doses of up to 5 g per kg bw per day (Coulet et al., 2014).
- GRNs 571, 650, and 735, which received "no questions" letters from FDA, agreed with Coulet et al. (2014) that the no observed adverse effect level was 5,000 mg 2'-FL per bw per day.
- No adverse effects on growth and development, clinical pathology, or histopathology were seen in piglets fed a liquid diet of doses ranging up to 2,000 mg 2'-FL per L starting on postnatal day 2 for 3-weeks (Hanlon and Thorsrud, 2014). These doses were equivalent to up to 291.74 mg per kg bw per day in male piglets and 298.99 mg per kg mg per day in female piglets.
- No mutagenic activity was observed in a bacterial mutagenicity test, and no clastogenic or aneuploidy effect was seen in a mouse lymphoma assay (Coulet et al., 2014).
- The studies conducted on FrieslandCampina's 2'-FL (previously unpublished and discussed in GRN 735) are now published, and the results of the 90-day rat toxicity study (van Berlo et al., 2018) further support the use levels evaluated in GRN 735.
- An *in vivo* study by Azagra-Boronat et al. (2019) demonstrated that weanling rats can safely consume up to 2 g of 2'-FL per kg bw per day during the first two weeks of life.

- Clinical studies by Storm et al. (2019) and Nowak-Wegrzyn et al. (2019) demonstrated that formula supplemented with 0.25 g 2'-FL per L and 1.0 g 2'-FL per L, respectively, are well tolerated by infants.

Furthermore, the Expert Panel notes that no changes have been made to the proposed uses or use levels for Purified 2'-FL; therefore, the estimated dietary intake evaluation presented in GRN 735 is remains suitable and can be applied to the Purified 2'-FL manufactured using the alternative process detailed in FrieslandCampina's Supplement dossier.

In summary, a compelling case can be made that scientific consensus exists regarding the safety of FrieslandCampina's Purified 2'-FL in support of a GRAS conclusion under the conditions of its intended use, given the following conditions:

- FrieslandCampina's Purified 2'-FL continues to meet the designated specifications;
- The proposed uses and use levels for FrieslandCampina's Purified 2'-FL remain unchanged from those presented in GRN 735; and
- FrieslandCampina's Purified 2'-FL is produced in accordance with Current Good Manufacturing Practices (CGMPs).

Conclusion

The Expert Panel critically reviewed the data provided by FrieslandCampina for their alternative Purified 2'-FL preparation, as well as publicly available published information obtained from peer-reviewed journals and other safety assessments prepared by other Expert Panels and well-respected international regulatory bodies.

The Expert Panel unanimously concluded that the alternative manufacturing process and modified product specifications for Purified 2'-FL do not raise any safety concerns. Therefore, FrieslandCampina's Purified 2'-FL, manufactured as described in Part 2.B of the Supplement, and declared within the subject notification meets FDA's definition of safety in that there is "reasonable certainty of no harm under the intended conditions of use" as described herein and in GRN 735, and FrieslandCampina's Purified 2'-FL is generally recognized as safe (GRAS).

Robert W. Kapp, Jr., Ph.D.
Fellow ATS, FRSB, & ERT(UK)

Kara Lewis, Ph.D.

Katrina Emmel, Ph.D.
Panel Chair

END

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

GENERALLY RECOGNIZED AS SAFE *(GRAS)* NOTICE (Subpart E of Part 170)

Form Approved: OMB No. 0910-0342; Expiration Date: 09/30/2019
(See last page for OMB Statement)

FDA USE ONLY

GRN NUMBER	DATE OF RECEIPT
ESTIMATED DAILY INTAKE	INTENDED USE FOR INTERNET
NAME FOR INTERNET	
KEYWORDS	

Transmit completed form and attachments electronically via the Electronic Submission Gateway *(see Instructions)*; OR Transmit completed form and attachments in paper format or on physical media to: Office of Food Additive Safety *(HFS-200)*, Center for Food Safety and Applied Nutrition, Food and Drug Administration,5001 Campus Drive, College Park, MD 20740-3835.

SECTION A – INTRODUCTORY INFORMATION ABOUT THE SUBMISSION

1. Type of Submission *(Check one)*
☐ New ☐ Amendment to GRN No. _____ ☒ Supplement to GRN No. 735

2. ☒ All electronic files included in this submission have been checked and found to be virus free. *(Check box to verify)*

3 Most recent presubmission meeting *(if any)* with FDA on the subject substance *(yyyy/mm/dd)*: N/A

4 For Amendments or Supplements: Is your amendment or supplement submitted in response to a communication from FDA? *(Check one)* ☐ Yes ☒ No If yes, enter the date of communication *(yyyy/mm/dd)*: _____

SECTION B – INFORMATION ABOUT THE NOTIFIER

1a. Notifier

Name of Contact Person	Position or Title
Jan Bastiaans	R&D Manager

Organization *(if applicable)*: FrieslandCampina Domo B.V.

Mailing Address *(number and street)*: FrieslandCampina Innovation Centre, Bronland 20, 6708 WH

City	State or Province	Zip Code/Postal Code	Country
Wageningen			Netherlands

Telephone Number	Fax Number	E-Mail Address
+31 370711100	N/A	jan.bastiaans@frieslandcampina.com

1b. Agent or Attorney *(if applicable)*

Name of Contact Person	Position or Title
William J. Rowe	President

Organization *(if applicable)*: GRAS Associates

Mailing Address *(number and street)*: 11810 Grand Park Ave, Suite 500

City	State or Province	Zip Code/Postal Code	Country
North Bethesda	Maryland	20852	United States of America

Telephone Number	Fax Number	E-Mail Address
519-341-3367	888-531-3466	wrowe@nutrasource.ca

SECTION C – GENERAL ADMINISTRATIVE INFORMATION

1. Name of notified substance, using an appropriately descriptive term

2-Fucosyllactose; 2'-FL

2. Submission Format: *(Check appropriate box(es))*

☐ Electronic Submission Gateway

☒ Electronic files on physical media

☐ Paper

If applicable give number and type of physical media

3. For paper submissions only:

Number of volumes _____

Total number of pages _____

4. Does this submission incorporate any information in CFSAN's files? *(Check one)*

☒ Yes *(Proceed to Item 5)* ☐ No *(Proceed to Item 6)*

5. The submission incorporates information from a previous submission to FDA as indicated below *(Check all that apply)*

☒ a) GRAS Notice No. GRN 735

☐ b) GRAS Affirmation Petition No. GRP _____

☐ c) Food Additive Petition No. FAP _____

☐ d) Food Master File No. FMF _____

☐ e) Other or Additional *(describe or enter information as above)* _____

6. Statutory basis for conclusions of GRAS status *(Check one)*

☒ Scientific procedures *(21 CFR 170.30(a) and (b))* ☐ Experience based on common use in food *(21 CFR 170.30(a) and (c))*

7. Does the submission (including information that you are incorporating) contain information that you view as trade secret or as confidential commercial or financial information? (see 21 CFR 170.225(c)(8))

☐ Yes *(Proceed to Item 8*

☒ No *(Proceed to Section D)*

8. Have you designated information in your submission that you view as trade secret or as confidential commercial or financial information *(Check all that apply)*

☐ Yes, information is designated at the place where it occurs in the submission

☐ No

9. Have you attached a redacted copy of some or all of the submission? *(Check one)*

☐ Yes, a redacted copy of the complete submission

☐ Yes, a redacted copy of part(s) of the submission

☐ No

SECTION D – INTENDED USE

1. Describe the intended conditions of use of the notified substance, including the foods in which the substance will be used, the levels of use in such foods, and the purposes for which the substance will be used, including, when appropriate, a description of a subpopulation expected to consume the notified substance.

2'-Fucosyllactose is intended for use in a number of conventional foods as well as conventional infant formula for full term infants. No uses in pre-term infants are proposed at this time. Proposed use levels range from 0.24-4 g 2'-Fucosyllactose per serving.

2. Does the intended use of the notified substance include any use in product(s) subject to regulation by the Food Safety and Inspection Service (FSIS) of the U.S. Department of Agriculture?

(Check one)

☐ Yes ☒ No

3. If your submission contains trade secrets, do you authorize FDA to provide this information to the Food Safety and Inspection Service of the U.S. Department of Agriculture?

(Check one)

☒ Yes ☐ No , you ask us to exclude trade secrets from the information FDA will send to FSIS.

SECTION E – PARTS 2 -7 OF YOUR GRAS NOTICE

(check list to help ensure your submission is complete – PART 1 is addressed in other sections of this form)

☒ PART 2 of a GRAS notice: Identity, method of manufacture, specifications, and physical or technical effect (170.230).

☒ PART 3 of a GRAS notice: Dietary exposure (170.235).

☒ PART 4 of a GRAS notice: Self-limiting levels of use (170.240).

☒ PART 5 of a GRAS notice: Experience based on common use in foods before 1958 (170.245).

☒ PART 6 of a GRAS notice: Narrative (170.250).

☒ PART 7 of a GRAS notice: List of supporting data and information in your GRAS notice (170.255)

Other Information

Did you include any other information that you want FDA to consider in evaluating your GRAS notice?

☒ Yes ☐ No

Did you include this other information in the list of attachments?

☒ Yes ☐ No

SECTION F – SIGNATURE AND CERTIFICATION STATEMENTS

1. The undersigned is informing FDA that FrieslandCampina Domo B.V.
(name of notifier)

has concluded that the intended use*(s)* of 2-Fucosyllactose; 2'-FL
(name of notified substance)

described on this form, as discussed in the attached notice, is (are) not subject to the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act based on your conclusion that the substance is generally recognized as safe recognized as safe under the conditions of its intended use in accordance with § 170.30.

2. FrieslandCampina Domo B.V. agrees to make the data and information that are the basis for the conclusion of GRAS status available to FDA if FDA asks to see them; agrees to allow FDA to review and copy these data and information during customary business hours at the following location if FDA asks to do so; agrees to send these data and information to FDA if FDA asks to do so.
(name of notifier)

Stationsplein 4,3818 LE Amersfoort, P.O. Box 1551, 3800 BN Amersfoort, The Netherlands
(address of notifier or other location)

The notifying party certifies that this GRAS notice is a complete, representative, and balanced submission that includes unfavorable, as well as favorable information, pertinent to the evaluation of the safety and GRAS status of the use of the substance.The notifying party certifies that the information provided herein is accurate and complete to the best or his/her knowledge. Any knowing and willful misinterpretation is subject to criminal penalty pursuant to 18 U.S.C. 1001.

3. Signature of Responsible Official, Agent, or Attorney	Printed Name and Title	Date (mm/dd/yyyy)
[redacted]	Katrina Emmel on behalf of William J. Rowe, President	12/02/2019

SECTION G – LIST OF ATTACHMENTS

List your attached files or documents containing your submission, forms, amendments or supplements, and other pertinent information. Clearly identify the attachment with appropriate descriptive file names (or titles for paper documents), preferably as suggested in the guidance associated with this form. Number your attachments consecutively. When submitting paper documents, enter the inclusive page numbers of each portion of the document below.

Attachment Number	Attachment Name	Folder Location (select from menu) (Page Number(s) for paper Copy Only)
	Multiple Appendices 1-4	

William J. Rowe, Ph.D.
GRAS Associates, LLC
11810 Grand Park Ave Ste. 500
North Bethesda, MD 20852

Re: GRAS Notice No. GRN 000735

Dear Dr. Rowe:

The Food and Drug Administration (FDA, we) completed our evaluation of the supplement to GRN 000735 that you submitted on behalf of FrieslandCampina Domo B. V. (FrieslandCampina). We received the supplement on February 20, 2020. The supplement addresses changes in the method of manufacture and specifications for the subject of GRN 000735.

We previously responded to GRN 000735 on April 6, 2018. We stated that we had no questions at that time regarding Glycosyn and FrieslandCampina's conclusion that 2′-fucosyllactose (2′-FL) is GRAS for the intended use 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.[1] In the supplement dated February 12, 2020, FrieslandCampina informs us of its view that 2′-FL is GRAS, through scientific procedures, for the same uses described in GRN 000735.

In GRN 000735, Glycosyn and FrieslandCampina state that 2′-FL is enzymatically produced from lactose and glucose using a modified strain of *Escherichia coli* K-12 GI724 (E997), secreted into the fermentation medium, and obtained through a series of purification steps resulting in a spray-dried powder. In this supplement, FrieslandCampina states that no changes were made to the production organism, the fermentation process, or the purification steps; however, FrieslandCampina describes three changes to the components of the fermentation medium from GRN 000735. These changes include the use of glucose syrup in place of dextrose monohydrate, cobalt

[1] Glycosyn and FrieslandCampina stated that 2′-FL is not intended for use in products under the U.S. Department of Agriculture's jurisdiction.

sulfate heptahydrate in place of cobalt chloride hexahydrate, and manganese sulfate monohydrate in place of manganese chloride tetrahydrate. Additionally, FrieslandCampina discusses changes to the specifications from GRN 000735. These changes include a lower minimum content of 2′-FL in the finished product from ≥90% to ≥88% (on a dry matter basis), an increase in the maximum water content from ≤5% to ≤9%, and a lower limit for aflatoxin M1 from ≤0.2 µg/kg to ≤0.025 µg/kg. FrieslandCampina provides the results of five non-consecutive batch analyses to demonstrate that 2′-FL can be produced to meet these specifications.

FrieslandCampina states that it did not conduct a stability study with 2′-FL produced as described in this supplement. Rather, the supplement describes the results of stability studies that were reported in GRN 000735 and provides the more recent results of an on-going shelf-stability study showing that 2′-FL is stable for at least 24 months. The amount of moisture after 24 months exceeds the previously specified limit of 5% in GRN 000735, leading to the change in the specification described above.

FrieslandCampina conducted an updated literature search through October 2019 and discusses new published studies surrounding the safety of the production organism, as well as toxicological and human clinical studies with 2′-FL in support of safety. FrieslandCampina did not identify any data or information that would contradict its safety conclusion from GRN 000735.

FrieslandCampina includes the report of a panel of individuals (FrieslandCampina's GRAS panel). Based on its review, FrieslandCampina's GRAS panel concluded that 2′-FL is safe under the conditions of its intended use.

Based on the totality of the data and information described above, FrieslandCampina concludes that 2′-FL is GRAS for its intended use in food.

Standards of Identity

In the supplement, FrieslandCampina states its intention to use 2′-FL in several food categories, including foods for which standards of identity exist, located in Title 21 of the CFR. We note that an ingredient that is lawfully added to food products may be used in a standardized food only if it is permitted by the applicable standard of identity.

Potential Labeling Issues

Under section 403(a) of the Federal Food, Drug, & Cosmetic (FD&C) Act, a food is misbranded if its labeling is false or misleading in any way. Section 403(r) of the FD&C Act lays out the statutory framework for labeling claims characterizing a nutrient level in a food or the relationship of a nutrient to a disease or health-related condition (also referred to as nutrient content claims and health claims). If products containing 2′-FL bear any nutrient content or health claims on the label or in labeling, such claims are subject to the applicable requirements and are under the purview of the Office of Nutrition and Food Labeling (ONFL) in the Center for Food Safety and Applied Nutrition. The Office of Food Additive Safety (OFAS) did not consult with ONFL on this

issue or evaluate any information in terms of labeling claims. Questions related to food labeling should be directed to ONFL.

Allergen Labeling

The FD&C Act requires that the label of a food that is or contains an ingredient that contains a "major food allergen" declare the allergen's presence (section 403(w)). The FD&C Act defines a "major food allergen" as one of eight foods or food groups (i.e., milk, eggs, fish, Crustacean shellfish, tree nuts, peanuts, wheat, and soybeans) or a food ingredient that contains protein derived from one of those foods. 2′-FL derived from lactose may require labeling under the FD&C Act because it may contain protein derived from milk. Questions about petitions or notifications for exemptions from the food allergen labeling requirements should be directed to the Division of Food Ingredients in OFAS. Questions related to food labeling in general should be directed to the ONFL.

Intended Use in Infant Formula

Under section 412 of the FD&C Act, a manufacturer of a new infant formula must make a submission to FDA providing required assurances about the formula at least 90 days before the formula is marketed. Our response to FrieslandCampina's supplement does not alleviate the responsibility of any infant formula manufacturer that intends to market an infant formula containing 2′-FL to make the submission required by section 412. Infant formulas are the purview of ONFL.

Section 301(ll) of the FD&C Act

Section 301(ll) of the FD&C Act prohibits the introduction or delivery for introduction into interstate commerce of any food that contains a drug approved under section 505 of the FD&C Act, a biological product licensed under section 351 of the Public Health Service Act, or a drug or a biological product for which substantial clinical investigations have been instituted and their existence made public, unless one of the exemptions in section 301(ll)(1)-(4) applies. In our evaluation of FrieslandCampina's supplement concluding that 2′-FL is GRAS under its intended conditions of use, we did not consider whether section 301(ll) or any of its exemptions apply to foods containing 2′-FL. Accordingly, our response should not be construed to be a statement that foods containing 2′-FL, if introduced or delivered for introduction into interstate commerce, would not violate section 301(ll).

Conclusions

Based on the information that FrieslandCampina provided, as well as other information available to FDA, we have no questions at this time regarding FrieslandCampina's conclusion that 2′-FL is GRAS under its intended conditions of use. This letter is not an affirmation that 2′-FL is GRAS under 21 CFR 170.35. Unless noted above, our review did not address other provisions of the FD&C Act. Food ingredient manufacturers and food producers are responsible for ensuring that marketed products are safe and compliant with all applicable legal and regulatory requirements.

In accordance with 21 CFR 170.275(b)(2), the text of this letter responding to the supplement to GRN 000735 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

Susan J. Carlson -S

Digitally signed by Susan J. Carlson -S
Date: 2020.04.30 14:49:59 -04'00'

Susan Carlson, Ph.D.
Director
Division of Food Ingredients
Office of Food Additive Safety
Center for Food Safety
and Applied Nutrition