JHeimbach LLC

April 30, 2020

Susan J. Carlson, Ph.D., Director
Division of Biotechnology and GRAS Notice Review (HFS-255)
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740

Dear Dr. Carlson:

Pursuant to 21 CFR Part 170, Subpart E, DuPont Nutrition and Biosciences, through me as its agent, hereby provide notice of a claim that the addition of 3-fucosyllactose produced by genetically engineered *Escherichia coli* K12 MG1655 to nonexempt term infant formula, formula intended for young children 12 months of age and older, and other foods and beverages that are consumed by toddlers under 3 years of age and by the general US population aged 3 years and above is exempt from the premarket approval requirement of the Federal Food, Drug, and Cosmetic Act because DuPont Nutrition and Biosciences has determined that the intended use is generally recognized as safe (GRAS) based on scientific procedures.

As required, one copy of the GRAS monograph and one signed copy of the conclusion from the members of the GRAS Panel are provided. Additionally, I have enclosed a virus-free CD-ROM with the GRAS monograph and the signed statement of the GRAS Panel.

If you have any questions regarding this notification, please feel free to contact me at 804-742-5543 or jh@jheimbach.com.

Sincerely,

[redacted]

James T. Heimbach, Ph.D., F.A.C.N.
President

Encl.

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COMPREHENSIVE GRAS ASSESSMENT

for the proposed use of

3-FUCOSYLLACTOSE

in

Nonexempt term infant formula, formula intended for young children 12 months of age and older, and other foods and beverages consumed by toddlers under 3 years of age and by the general US population aged 3 years and above

April 2020

DuPont Nutrition & Biosciences

Edited by
JHeimbach LLC

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PART 1. SIGNED STATEMENTS AND CERTIFICATIONS

1.1. GRAS Notice Submission

In accordance with 21 CFR 170.255, Danisco USA Inc. (dba DuPont Nutrition & Biosciences [DuPont]) submits this GRAS notice through its agent James T. Heimbach, president of JHeimbach LLC, for 3-fucosyllactose (3FL) produced by a genetically engineered *Escherichia coli* K12 MG1655 production strain.

1.2. Name and Address of Notifier

DuPont Nutrition & Biosciences
DuPont Experimental Station - E320
200 Powder Mill Road
Wilmington, DE 19803

Notifier Contact
Angela Lim
Global Regulatory Strategy Lead (HMOs & Food Protection)
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Angela.Lim@DuPont.com

Agent Contact
James T. Heimbach, Ph.D., F.A.C.N.
President
JHeimbach LLC
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Tel: 804-742-5543
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1.3. Name of the Notified Substance

3-fucosyllactose (3FL)

1.4. Intended Conditions of Use of the Notified Substance

3FL is intended be used as a food ingredient in nonexempt term infant formulas, formula intended for young children 12 months of age and older, and other foods and beverages that are consumed by toddlers under 3 years of age and by the general US population aged 3 years and above. Proposed use levels range from 0.2 g/serving (2.0 g/L) in infant formula and formula intended for young children 12 months of age and older to levels ranging from 0.14 to 4.0 g/serving (1.2 to 40 g/kg) in other foods and beverages.

1.5. Statutory Basis for Conclusion of GRAS status

DuPont has concluded that the notified substance, 3-fucosyllactose (3FL), as described herein is generally recognized as safe (GRAS) under the conditions of its intended use. This GRAS conclusion was reached through scientific procedures and in concert with the views of a panel of experts who

are qualified by scientific training and experience to evaluate the safety of substances added to foods, in accordance with 21 CFR 170.30(a) and (b).

1.6. Claim of Exclusion from the Requirement for Premarket Approval

Based upon DuPont's GRAS conclusion as stated in Part 1, Section 1.5 above, it is DuPont's view that 3FL is not subject to the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act.

1.7. Availability of Data and Information

DuPont agrees to make available the data and information that are the basis for DuPont's 3FL GRAS conclusion available for review and copying at FDA's request during customary business hours at the office listed in Part 1, Section 1.2 above. A complete copy of the data and information will be provided to FDA upon request.

1.8. Disclosure under the Freedom of Information Act

This GRAS notice does not contain data and information that are exempt from disclosure under the Freedom of lnformation Act (FOIA), USC 552.

1.9. Certification Statement

To the best of our knowledge, this dossier/notice presents a complete, representative, and balanced submission that includes unfavorable information, as well as favorable information, known to us and pertinent to the evaluation of the safety and GRAS status of the intended use of 3FL.

1.10. FSIS Statement

Not applicable.

1.11. Name, Position, and Signature of Notifier

James T. Heimbach, Ph.D., F.A.C.N.
President
JHeimbach LLC
Agent to DuPont Nutrition & Biosciences

PART 2. IDENTITY, METHOD OF MANUFACTURE, SPECIFICATIONS AND EFFECT

2.1. Substance Identity

Common name: 3-Fucosyllactose

Abbreviated names: 3FL, 3-FL

Alternative names: 3-O-Fucosyllactose

IUPAC name[1]: 6-Deoxy-α-L-galactopyranosyl-(1->3)-β-D-galactopyranosyl-(1->4)-D-glucopyranose

CAS number: 41312-47-4

Chemical formula: $C_{18}H_{32}O_{15}$

Molecular weight: 488.4 g/mol

Structural formula:

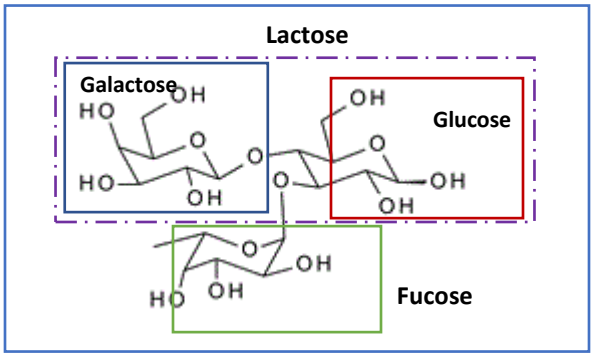


Figure 1: Structural Formula of 3FL

2.2. Description

3FL is a milk oligosaccharide composed of a lactose core of D-glucose and D-galactose units linked via an α(1-3) bond to L-fucose. It is a white to ivory-colored powder that is soluble in water.

Originally identified and isolated from human breast milk in the mid-1950s (Kunz, 2012), 3FL belongs to a group of complex carbohydrates described as human milk oligosaccharides (HMOs). HMOs are the third largest component in breast milk, totaling on average 12.9 g/L in mature milk and 20.9 g/L at 4 days post-partum (Andreas et al., 2015).

The 3FL under discussion in this monograph is produced by fermentation using a modified *E. coli* K12 host production strain. Nuclear magnetic resonance spectroscopy (^{1}H NMR) was used to compare three batches of *E. coli*-derived 3FL to 3FL isolated from human milk[2]. The ^{1}H NMR spectra (Figure 2) show that all major well-resolved signals in the spectra of the 3FL samples are identical,

[1] CSID:141824, http://www.chemspider.com/Chemical-Structure.141824.html (accessed 21:55, Sep 18, 2019)

[2] Sourced from IsoSep AB, Lot No.:1042-092, purity >95%

indicating that there is no significant difference between 3FL derived from *E. coli* K12 MG1655 sINB008971 and 3FL isolated from human milk. Additionally, the recorded ^{1}H NMR spectra of the samples are identical to spectral data reported in the published literature (van Leeuwen et al., 2014).

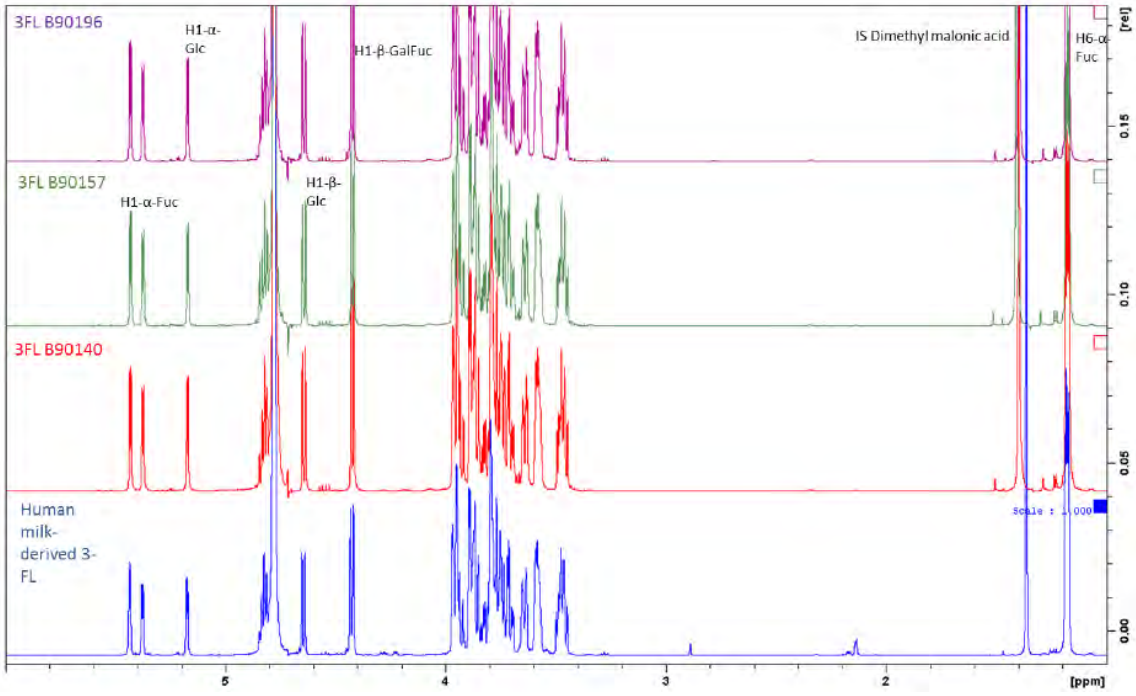


Figure 2: Comparison of 1H NMR Spectra of 3FL Batches to Human Milk-Derived 3FL

The 3FL was further characterized using hydrophilic interaction liquid chromatography (HILIC) with refractive index detection (HILIC-RID) and with mass spectrometric detection (HILIC-MS) along with the relevant reference standards. The data showed that, in addition to the 3FL molecule, lactose, galactose/glucose and other carbohydrates are present as minor components and/or as residuals from the downstream processing step. The residual components were identified as mono-, di- and trimeric carbohydrates with a polyol nature.

The engineered *E. coli* K12 MG1655 production strain is removed during the downstream processing step and is not present in the 3FL product. The absence of the production host and any residual rDNA is confirmed by test as shown below.

2.3. Method of Manufacture

2.3.1. Process Description

DuPont's 3FL production process can be divided into two main stages: fermentation and post-fermentation processing.

The fermentation stage involves inoculating a small batch of sterilized fermentation media with a seed culture of the genetically engineered *E. coli* K12 MG1655 production strain. Once the

inoculated cells reach an optimal cell concentration, the seeded batch is used to seed increasing volumes of sterilized fermentation media to reach commercial production size batches.

The lactose-sucrose based fermentation medium is supplemented with other nutrients such as trace minerals, vitamins, and amino acids. These include food-grade ammonium chloride, ammonium sulfate, potassium phosphate, sodium chloride, citric acid monohydrate, magnesium sulfate, lactose, sucrose, thiamine, zinc chloride, copper chloride, manganese chloride, calcium chloride, iron (II) chloride, glycine, glutamine, methionine, and betaine. Food-grade processing aids such as antifoam and pH control agents may also be used in the process. All ingredients and processing aids are permitted for direct addition to foods as GRAS ingredients and/or food additives.

The fermentation stage is maintained under controlled temperature and pH conditions to optimize growth of the production strain and its expression of 3FL. The resulting fermentate contains various carbohydrates, cell biomass, residual fermentation media, by-products of the fermentation process, and other impurities.

The post-fermentation processing stage (outlined in Figure 3) serves to purify and selectively concentrate the 3FL component by removing the cell biomass, residual fermentation media, by-products of the fermentation process, and other impurities, to achieve the targeted product purity.

The initial post-fermentation processing involves removing the modified *E. coli* K12 cell biomass, endotoxins, large molecules (e.g. proteins from the fermentate), and antifoam; this is achieved using microfiltration and/or ultrafiltration. The cell-free fermentate may be treated with the lactase enzyme beta-galactosidase (EC 3.2.1.23) to hydrolyze residual lactose prior to the nanofiltration step, which removes water, inorganic salts and small carbohydrate molecules. The resulting concentrated fermentate is passed through ion exchange columns and overactive carbon where small molecules (e.g. organic acids, inorganic salts) formed/used in the fermentation stage and other compounds that may cause off-color and off-flavor are separated out. Potential microbial contaminants are then eliminated via sterile filtration and the resulting output is concentrated by evaporation/nanofiltration to further remove excess water and facilitate crystallization of the 3FL product. Depending upon customer preference, the resulting crystals may be commercialized as is or be dissolved in water and spray-dried. All processing aids used in the post fermentation processing stage, including but not limited to beta-galactosidase, ion exchange resins, filter aids, and regenerating solutions, are food-grade aids permitted for use in food processing.

To accommodate production at different manufacturing sites, equivalent technologies/unit operations may be used to accomplish the key purification, concentration, and finishing steps outlined. All manufacturing sites operate in accordance with current good manufacturing practice (cGMP) and/or Global Food Safety Initiative (GFSI) certification requirements and comply with the requirements of the Food Safety Modernization Act.

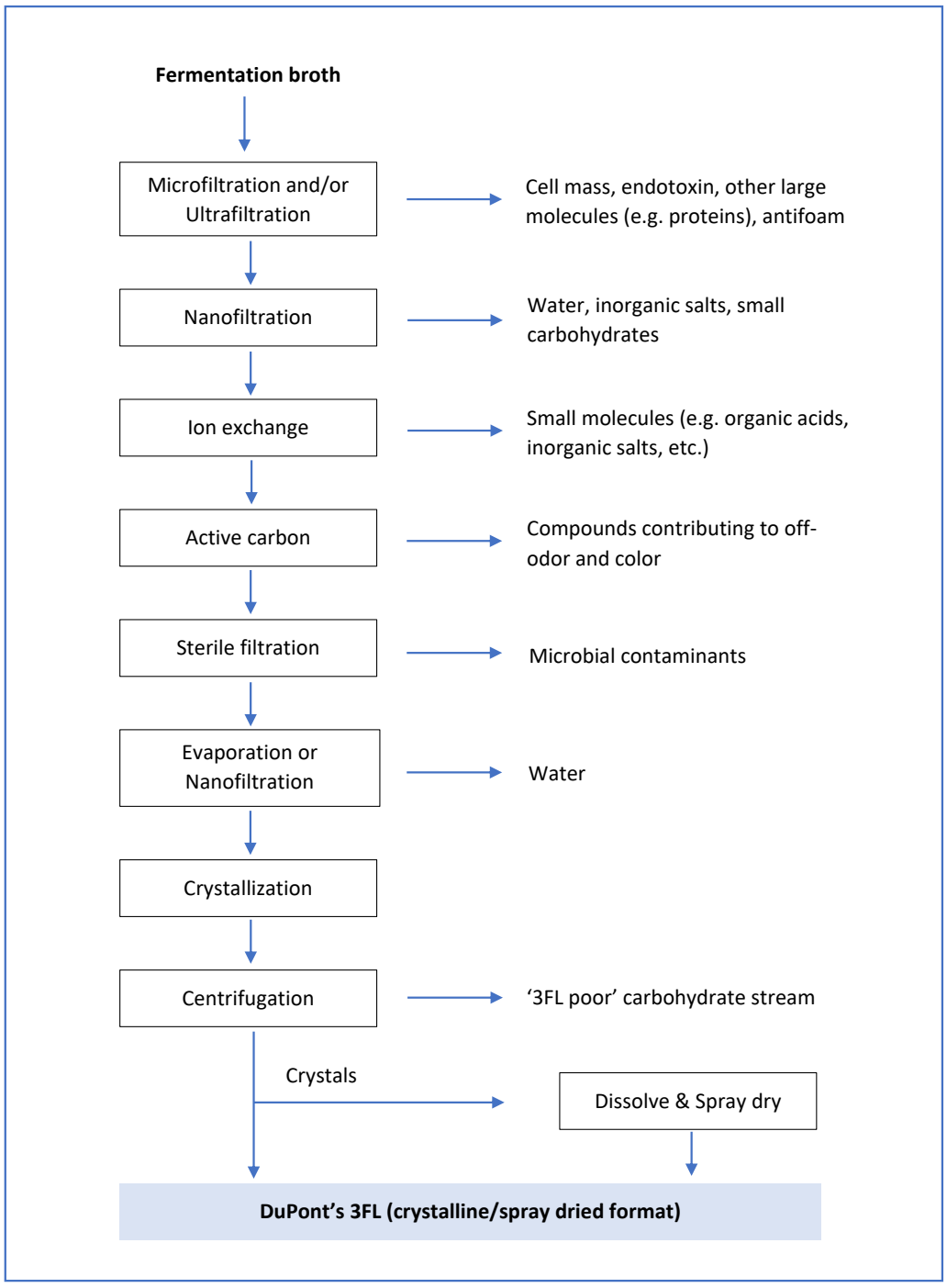


Figure 3: 3FL Process Flow Diagram (Post Fermentation)

2.3.2. Production Microorganism

Production strain: *E. coli* K12 MG1655 INB008971

The production strain, a genetically engineered *E. coli* K12 MG1655 strain, was constructed to produce high amounts of 3FL in large-scale industrial processes. This process makes use of endogenous production of GDP-fucose and the transfer of this nucleotide-activated sugar to the substrate lactose to result in 3FL.

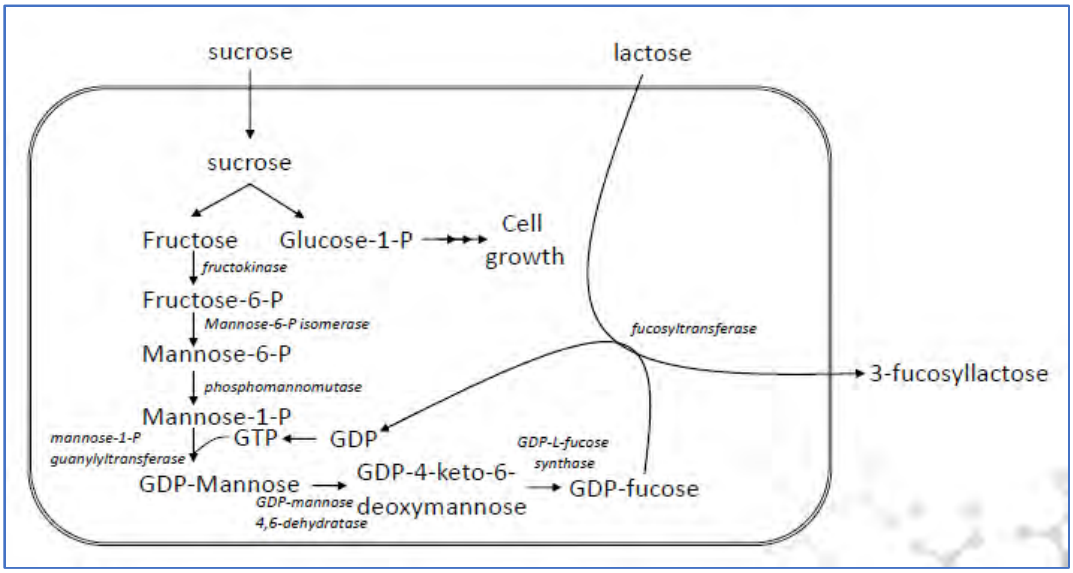


Figure 4: 3FL Biosynthesis Pathway in *E. coli* K12 MG1655

The strain was adapted to enable growth on sucrose, which is used to generate biomass as well as fructose. As shown in Figure 4, sucrose is used as a starting molecule in the production of GDP-fucose. To enable growth on sucrose and to generate fructose, two heterologous genes, EcCscB and BaSP, were inserted. EcCscB encodes the enzyme sucrose permease that facilitates intracellular uptake of sucrose while BaSP encodes sucrose phosphorylase that splits sucrose into glucose-1-phosphate and fructose. The glucose-1-phosphate is used by endogenous genes to generate biomass. The fructose is converted to fructose-6-phosphate by another enzyme, fructokinase, encoded by the heterologous gene ZmFrk, and subsequently to GDP-fucose.

To facilitate efficient intracellular 3FL production, over-expression of gene EcLacY ensures improved uptake of lactose for binding with the fucose moiety present in GDP-fucose. The GDP-fucose transfer step is enabled by the heterologous 3FT gene. Over-expression of endogenous EcMdfA encodes for a membrane protein which enables the transfer of intracellular 3FL into the extracellular environment, where it is subjected to downstream processing to yield the commercial 3FL product.

Genes encoding for unwanted proteins, including those for enzymes that interfere with the desired 3FL metabolic pathway, were deleted. These deletions help to make the strain more robust in industrial production settings, improve strain stability, and increase 3FL production.

E. coli K12 MG1655 INB008971 was characterized using whole genome sequencing and is deposited in DuPont's Global Culture Collection. The production strain is stable over 70 generations of fermentation. Stability of the strain was assessed using DNA sequencing data and by reviewing the consistency of repeated cell performance index values (i.e., 3FL titers in cultivated culture supernatant normalized to biomass production). DNA sequencing of the plasmid isolated from samples taken at the end of two fed batch fermentations showed that the plasmids are stable during the regular fermentation time.

Host strain: *E. coli* K12 MG1655

E. coli K12 MG1655 is derived from the well-known *E. coli* K12 strain via several classical mutagenesis steps and is available from both the American Type Culture Collection (ATCC) as ATCC 700926 and the Coli Genetic Stock Center (CGSC) as CGSC#7740. *E. coli* K12 MG1655 is classified by ATCC as a Biosafety Level 1 microorganism; the complete genome of this strain has been sequenced (GenBank Entry U00096.3).

E. coli K12 MG1655 also serves as the host strain in the production of DuPont's 2' fucosyllactose; GRAS notice GRN000749, incorporated by reference, included a discussion of the safety and characterization of *E. coli* K12 MG1655 on pp. 15-16.

Synthetic Donor Genes: DNA coding fragments with well characterized functions in the five strains listed were synthesized *in vitro* for insertion into the host strain. *In vitro* synthesis of inserted genes eliminated the potential introduction of undesirable genes from donor organisms.

Name	***Nature Identical Origin***	***Length (as bp)***	***Function***	***Location***
3FT	*Basilea psittacipulmonis*	990	α-1,3-Fucosyltransferase	Vector
BaSP	*Bifidobacterium adolescentis*	1515	Sucrose phosphorylase	Chromosome
ZmFrk	*Zymomonas mobilis*	906	Fructokinase	Chromosome
EcCscB	*Escherichia coli W*	1248	Sucrose transporter	Chromosome
EcLacY	*Escherichia coli K12* MG1655	1254	Lactose permease	Chromosome
EcMdfA	*Escherichia coli K12* MG1655	1233	MDR transporter	Chromosome

Production strain construction: The general method used to introduce modifications into the host genome is shown in Figure 5 and was based on the work of Datsenko & Wanner (2000) and Snoeck et al. (2019).

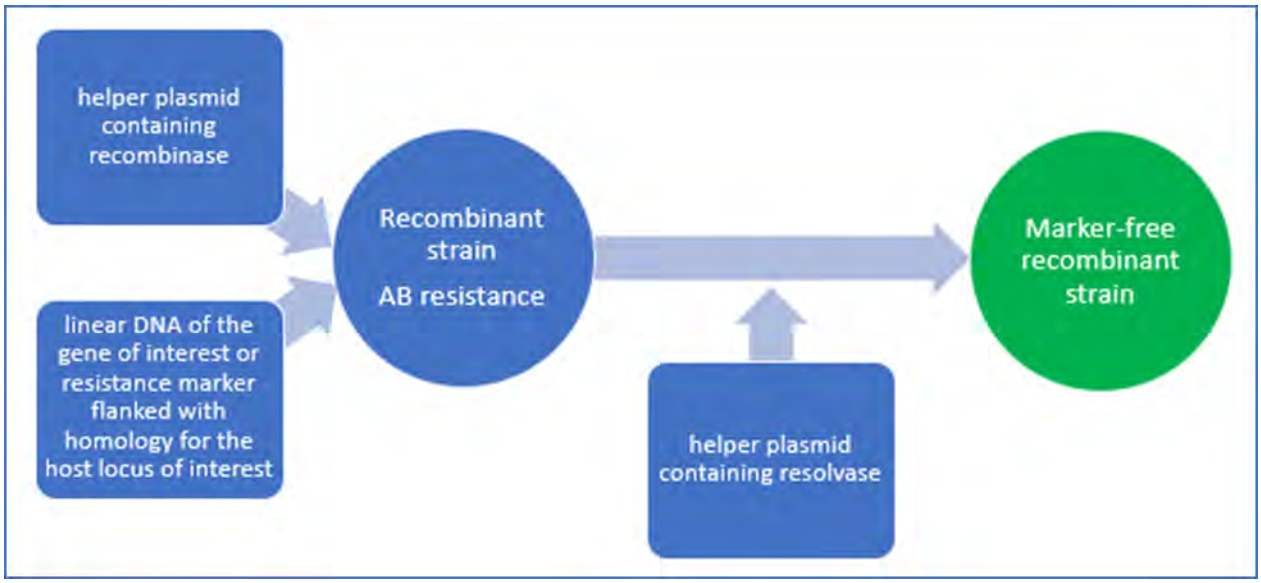


Figure 5: Schematic of General Method for Introducing Modifications into the Host Genome

To obtain a suitable production strain, *E. coli* K12 MG1655 was modified by engineering disruptions in genes that interfere with the metabolic pathway required to produce 3FL. The following deletions were made to remove the ability of the *E. coli* K12 MG1655 to produce the indicated proteins:

lacA (thiogalactoside acetyltransferase)
lacY (lactose permease)
lacZ (beta-D-galactosidase)
lon A (protease, enzyme with global regulatory function)
fsaA (fructose-6-phosphate aldolase 1)
agp (glucose-1-phosphatase/inositol phosphatase)
adhE (CoA-linked acetaldehyde dehydrogenase and iron-dependent alcohol dehydrogenase/ pyruvate-formate-lyase deactivase)
ldhA (fermentative NAD-dependent D-lactate dehydrogenase)
pfkB (6-phosphofructokinase II)
wcaJ (putative UDP-glucose lipid carrier transferase)
setB (sugar efflux transporter)
thyA (thymidylate synthase)
glgC (glucose-1-phosphate adenylyltransferase)
pfkA (6-phosphofructokinase I)
fsaB (fructose-6-phosphate aldolase 2)
iclR (regulator in central intermediary metabolism, glyoxylate bypass)
pgi (glucose-6-phosphate isomerase)
arcA (negative response regulator of genes in aerobic pathways, with sensors ArcB and CpxA)
yhcE (partial deletion), yhcG & yhcF (putative proteins)
between wza (putative polysaccharide export protein) and yegH (putative transport protein) - full deletion
between flhA (flagellar biosynthesis protein) and yecH (uncharacterized DUF-containing protein - full deletion

To facilitate the production of GDP-fucose to support 3FL production, single copies of the following five genes were inserted in the *E. coli* K12 MG1655 genome. Each gene consisted of codon use-adapted coding sequences plus artificial promoters and terminators used to drive expression of the inserted coding sequences; these are described in De Mey et al. (2007).

BaSP (*B. adolescentis* sucrose phosphorylase gene)
ZmFrk (*Z. mobilis* fructokinase gene)
EcCscB (*E. coli* W anion symport for sucrose gene)
EcMdfA (*E. coli* K12 MG1655 MDR transporter gene)
EcLacY (*E. coli* K12 MG1655 lactose permease)

The modified *E. coli* K12 MG1655 was transformed into the production strain *E. coli* K12 MG1655 INB008971 through introduction of a plasmid vector containing the *B. psittacipulmonis* α-1,3-fucosyltransferase gene (3FT). Synthetized *de novo*, the plasmid vector has a pINB003937 backbone with a pBR322 type ORI; it lacks any conjugation, mobilization, or transfer functions and does not contain an antibiotic resistance marker. In addition to the 3FT gene, it contains homologous *E. coli* thyA as a selectable marker. The 3FT gene is controlled by an artificial promoter.

Some small and widely dispersed genetic scars were left in the *E. coli* K12 MG1655 genome after constructing the gene knockouts and gene insertions. However, no trace remains of the helper plasmid or the antibiotic marker used in the construction of the helper plasmid. Removal of the helper plasmid was validated by PCR and replica plating on a plate containing the antibiotic corresponding to the marker present on the helper plasmid.

Production strain stability: *E. coli* K12 MG1655 INB008971 is stable; as shown in Figures 6 and 7, no changes were observed in growth rate, production efficiency, and genetic make-up of the strain through 70 generations in a serial flask cultivation experiment.

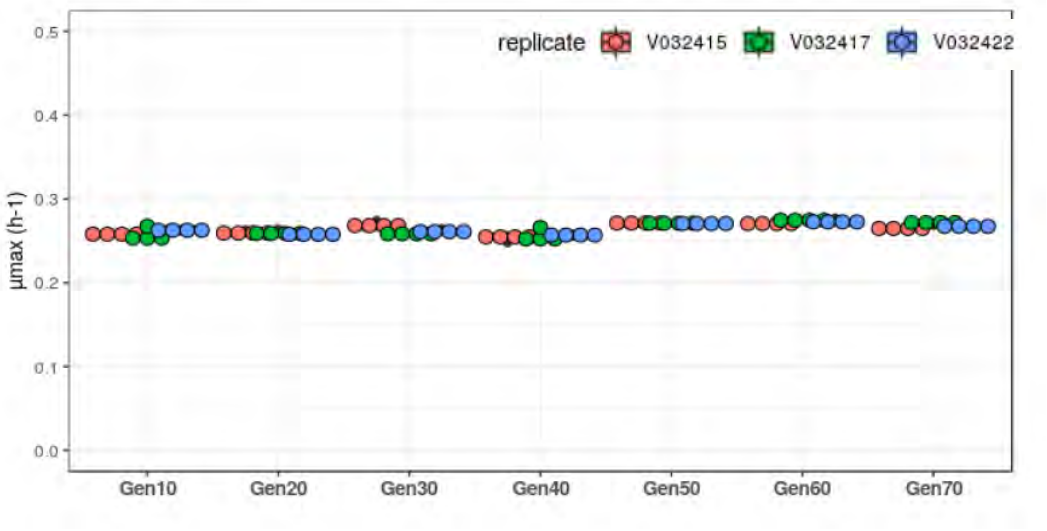


Figure 6: Growth Rate Assessment of Three Vials over 70 Generations

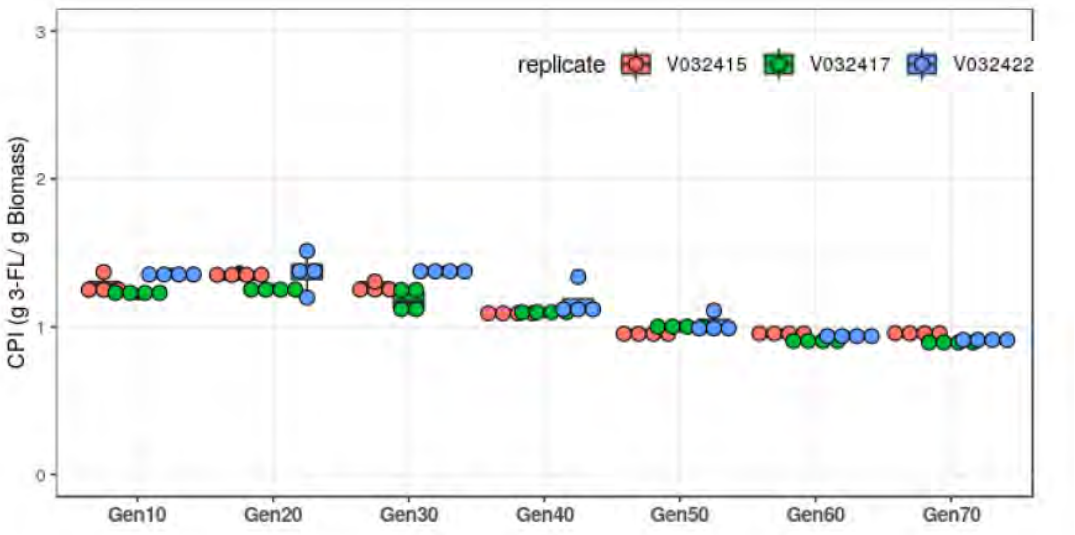


Figure 7: 3FL Production Assessment of Three Vials over 70 Generations

A comparison of next-generation sequencing (NGS) of the cultures and plasmid samples collected at the beginning and end (Gen 70) of the serial flask experiment detected no variants; the sINB008971 genome and the plasmid pINB003937 sequences were used as reference standards.

Production strain safety: No differences in pathogenicity are expected between the host strain and production strain, *E. coli* K12 MG1655 INB008971, as *E. coli* K-12 MG1655 is classified by ATCC as a Biosafety Level 1 microbial culture and the introduced genes are well characterized.

The absence of virulence factors unique to the production strain was verified by examining the whole genome sequencing (WGS) data of sINB008971 for the presence of genes coding for virulence factors using the Virulence Factors of Pathogenic Bacteria (VFDB) database and Virulence Finder analyzer tool. Virulence factors in the host strain, *E. coli* K-12 MG1655, were identified using the VFDB database and the Center for Genomic Epidemiology (CGE) Datasets. All virulence factors identified in the production strain were also present in the host strain.

No specific toxic or allergenic effects are expected from the proteins expressed by the introduced genes. These proteins are not secreted, and the cell mass is separated from the product during manufacturing.

Bioinformatic analyses were carried out to assess the expressed proteins for potential allergenic cross-reactivity risk. The Access Food Allergy Research and Resource Program (FARRP) database (Version 19, released February 10, 2019) was searched using the 80 amino acid sliding window and the 8 contiguous identical amino acid (FAO/WHO 2001) methods. No matches were found.

The toxicity of the expressed proteins was evaluated by their homology with known protein toxins. Swiss-Prot, a manually annotated and reviewed section of the UniProt Knowledgebase (UniProtKB) database, was used as the primary data source for scientific literature on toxicity. The toxin homology was performed with BLASTP® command line using the Entrez protein query "toxin" in

the source database. Apart from the flagging of the endogenous genes EcMdfA and EcLacY, no other matches were found. As EcMdfA and EcLacY are also present in the host strain, their presence does not raise safety concerns.

Intrinsic antimicrobial resistance to MLS (macrolide, lincosamide, and streptogramin B) is encoded by the EcMdfA gene inherent in *E. coli* K12. The *E. coli* K12 MG1655 INB008971 genome contains one extra copy of the endogenous MdfA gene, which serves as an efflux transporter of 3FL, allowing its removal from the intact cell. Analysis of the *E. coli* K12 MG1655 INB008971 genome against bioinformatic databases for antibiotic/antimicrobial resistance genes (ResFinder and CARD) revealed no additional resistance compared to *E. coli* K12 and host strains.

2.3.3. Specifications and Batch Analysis of 3FL

The product specifications for 3FL and analytical data for four batches of 3FL are shown in Tables 1, 2, 3, and 4, below. With the exception of the carbohydrate analysis and the appearance parameters, DuPont has utilized standard methods for analysis of 3FL. The HILIC method used for carbohydrate analysis has been validated for this specific application. The consistency of the batch data shows that the product is manufactured reproducibly and conforms to specifications.

Table 1. Appearance

Parameters	Specification	Method	Analyzed Batches			
			B90140	B90157	B90195	B90196
Appearance (Color)	White to ivory-colored	Visual	Pass	Pass	Pass	Pass
Appearance (Form)	Spray-dried powder	Visual	Pass	Pass	Pass	Pass
Appearance in solution	Clear, colorless to slightly yellow	Visual	Pass	Pass	Pass	Pass

Table 2. Carbohydrate Profile (HILIC method)

Parameters	Specification (Area under curve)	Analyzed Batches			
		B90140	B90157	B90195	B90196
3-Fucosyllactose	Min. 90%	97.2	95.0	97.0	96.3
Lactose	Max. 5%	< 0.2	2.6	< 0.2	0.4
Fucose	Max. 3%	1.0	0.8	0.8	0.9
Galactose/Glucose	Max. 3%	1.5	1.3	1.4	1.4
Other carbohydrates*	Max. 3%	0.4	0.3	0.8	1.0

*Note: Calculated by difference i.e. Sum of all peaks (100%) minus 3FL, lactose, fucose, and galactose/glucose

Table 3. Other Product Parameters & Impurities

Parameters	Specification	Method	Analyzed Batches			
			B90140	B90157	B90195	B90196
Water content	≤ 5.0%	Karl Fischer titration	4.4	3.88	4.39	3.8
Protein content	≤ 100 µg/g	Nanoquant (modified Bradford)	< 25	< 25	< 25	< 25
Total Ash	≤ 0.5%	NMKL 173:2005, mod	< 0.12	< 0.12	< 0.12	< 0.12
Arsenic	≤ 0.2 mg/kg	EN 15763:2010	< 0.1	< 0.1	< 0.1	< 0.1
Cadmium	≤ 0.05 mg/kg	EN 15763:2010	< 0.01	< 0.01	< 0.01	< 0.01
Lead	≤ 0.05 mg/kg	EN 15763:2010	< 0.02	< 0.02	0.04 (± 0.02)	< 0.02
Mercury	≤ 0.1 mg/kg	EN 15763:2010	< 0.005	< 0.005	< 0.005	< 0.005
Endotoxins	≤ 300 EU/g	Ph. Eur. 2.6.14 + Interference study	53	< 5.0	19	< 5.0
GMO detection (production strain rDNA)	Negative	PCR (internally validated; EFSA 2018)	Negative	Negative	Negative	Negative

Table 4. Microbiological Specifications

Parameters	Specification	Method	Analyzed Batches			
			B90140	B90157	B90195	B90196
Standard Plate Count	≤ 1000 cfu/g	ISO 4833-1	< 10	< 10	< 10	< 10
Yeast	≤ 100 cfu/g	NMKL 98	< 10	< 10	< 10	< 10
Mold	≤ 100 cfu/g	NMKL 98	< 10	< 10	< 10	< 10
Coliform/ Enterobacteriaceae	Absent in 10 g	ISO 21528-1	Absent	Absent	Absent	Absent
Salmonella	Absent in 100 g	NMKL 71	Absent	Absent	Absent	Absent
Cronobacter sakazakii	Absent in 100 g	ISO/TS 22964	Absent	Absent	Absent	Absent
Listeria monocytogenes	Absent in 25 g	BRD 07/04-09/98	Absent	Absent	Absent	Absent
Bacillus cereus	≤ 10 cfu/g	NMKL 67-M	< 10	< 10	< 10	< 10

2.3.4. Other Relevant Data

1. Biogenic amines

The potential to produce biogenic amines was evaluated using screening methods for amino acids and biogenic amines. None of the tested batches contained any of the 20 common amino acids or biogenic amines, namely phenylethylamine, spermidine, spermine, histamine, putrescine, cadaverine, tryptamine, or tyramine.

2. Allergens

None of the genes introduced into the production strain secreted proteins. Bioinformatic analysis of each of the gene sequences did not reveal a SignalP sequence. SignalP is the sequence for the signal peptide that targets protein excretion into the extracellular space (Nielsen, 2017; Petersen et al., 2011). Moreover, the cell mass is separated from the product during manufacture.

Batch data and other analyses demonstrate that DuPont's 3FL is consistently below levels of concern of proteins, bacteria, or bacterial endotoxins, residual recombinant DNA, and chemical sensitizers including metals. The final product contains lactose.

PART 3. DIETARY EXPOSURE

3.1. Current Dietary Exposure

There is no public information on the current dietary exposures of 3FL by children, teenagers and adults. As 3FL is currently not being added to infant formulas and toddler foods in the US, there is also no public information on the current dietary exposure resulting from addition of 3FL.

3.2. Estimated Daily Intake for Proposed Uses

For non-exempt term infant formula and formulas intended for your children 12 months of age and older, the maximum intended use level is 0.2 g/serving (2.0 g/L) as prepared.

3FL is also proposed for use in other foods and beverages with maximum proposed use levels ranging from 0.14 to 4.0 g/serving. The estimated daily intake (EDI) of 3FL was derived based on food consumption records collected in the *What We Eat in America* (WWEIA) dietary component of the *National Health and Nutrition Examination Survey* (NHANES) 2013-2016. The NHANES is a continuous survey that uses a complex multistage probability sample designed to be representative of the civilian U.S. population. The NHANES datasets provide nationally representative nutrition and health data and prevalence estimates for nutrition and health status measures in the U.S. Statistical weights are provided by the National Center for Health Statistics (NCHS) to adjust for the differential probabilities of selection and non-response.

As part of the examination, trained dietary interviewers collected detailed information on all foods and beverages consumed by respondents in the previous 24-hour time period (midnight to midnight). A second dietary recall was administered by telephone three to ten days after the first dietary interview, but not on the same day of the week as the first interview. The dietary component of the survey is conducted as a partnership between the U.S. Department of Agriculture (USDA) and the U.S. Department of Health and Human Services (DHHS). DHHS is responsible for the sample design and data collection, and USDA is responsible for the survey's dietary data collection methodology, maintenance of the databases used to code and process the data, and data review and processing.

Using the NHANES 2013-2014 consumption data, Exponent Inc. estimated the 2-day average daily intake on a *per user* basis. *Per user* estimates refer to those who reported consuming any of the select foods included in the analysis on either of the survey days. The analysis was limited to individuals who provided two complete and reliable dietary recalls as determined by NCHS. The 2-day average intakes by each individual were estimated using Exponent's Foods Analysis and Residue Evaluation Program (FARE® version 13.04) software.

Table 5 lists the proposed food applications for 3FL addition, their corresponding maximum use levels, and examples of representative foods in the NHANES 2013-2016 database for each food category.

Table 5: Proposed Food Applications and Use Levels of 3-FL

Food Category	Food Uses	3FL Maximum Proposed Use Level (g/serving)	3FL Maximum Proposed Use Level (g/kg)	Representative Foods in NHANES
Formula for infants and young children	Infant formula (0-12 months)	0.2	2.0	Infant formulas, ready-to-feed, prepared from powder/concentrate
	Formula targeted to young children aged 1-3 years	0.2	2.0	Toddler formulas, ready-to-feed, prepared from powder or concentrate
Foods for infants	Cereal & grain products, dry RTE (ready-to-eat) cereals, cookies, teething biscuit & toasts	0.14	20	Dry cereals (e.g., barley, mixed, oatmeal, rice, brown rice, multigrain), snack and sweets (e.g., cereal bars, cookies and teething cookies, crackers, puffs, crunchy snacks)
Foods for young children	Cereal & grain products, dry RTE (ready-to-eat) cereals, cookies, teething biscuit & toasts	0.4	20	Dry cereals (e.g., barley, mixed, oatmeal, rice, brown rice, multigrain), snack and sweets (e.g., cereal bars, cookies and teething cookies, crackers, puffs, crunchy snacks)
Juice and drinks for infants and young children	Juice/drinks	0.2	2.0	Juices including apple, mixed fruit, orange, pear, and grape juices
Baked goods & baking mixes	Cereal & nutrition bars	1.2	30	Cereal bars (e.g., Nutri-Grain bars, milk 'n cereal bar, granola bars) and nutrition bars (e.g., meal replacement bars, Cliff Bar, PowerBar, Slim Fast Bar, Zone Perfect Bar)
Beverages & beverage bases, nonalcoholic	Enhanced or fortified water	0.3	1.2	Enhanced or fortified waters (e.g., Propel Water, Glaceau Vitamin Water, SoBe Life Water)
	Energy, sports & isotonic drinks & mixes	0.3	1.2	Regular and low-calorie sport drinks (e.g., Gatorade, Powerade) and energy drinks (Full Throttle, Monster, NOS, Red Bull, Rockstar)
Breakfast cereals	Hot cereals	1.6	31	Oatmeal, cream of rice, cream of wheat, cream of rye, whole wheat hot cereal, oat bran hot cereal, grits, cornmeal mush
Breakfast cereals	RTE cereals	1.6	40	All types of RTE cereals

Food Category	Food Uses	3FL Maximum Proposed Use Level (g/serving)	3FL Maximum Proposed Use Level (g/kg)	Representative Foods in NHANES
Dairy product analogs	Milk substitutes, fluid	0.3	1.2	Soy milk, almond milk, rice milk, coconut milk (excluding coconut milk/cream used for cooking)
	Non-dairy yogurts	2.0	12	Soy and coconut milk yogurt
Milk products	Fermented milk, RTD (ready-to-drink) & mixes	0.3	1.2	Buttermilk and kefir
	Flavored Milk, RTD & mixes (including dairy-based beverages)	0.3	1.2	Flavored milk (e.g., chocolate and strawberry flavors), hot chocolate, milk shakes, malted milk drink
	Meal replacement beverages	1.2	5	Meal replacement beverages such as Carnation Instant Breakfast, Muscle Milk, Slim Fast, and high protein drinks
	Smoothies (dairy and non-dairy)	1.2	5	Fruit and/or vegetables smoothies (dairy and non-dairy types)
	Yogurt	1.2	12	Regular and Greek yogurt, all flavors, excluding frozen yogurt
Processed fruits and fruit juices	Fruit juices and nectars (including fruit-based beverages)	0.3	1.2	100% fruit juices (excluding lemon juice), fruit juice drinks, fruit and vegetable juice drinks, nectars, and coconut water
Processed vegetables and vegetable juices	Vegetable juice	0.3	1.2	100% vegetable juices
Foods for Special Dietary Use	Special dietary purpose ingredient in oral and enteral tube feeding ($\geq$ 11 years)	4.0	20	Not applicable

Per-capita and per-user mean and 90th percentile results for the U.S. population and selected sub-populations in g/day and g/kg-bw/day are provided in Tables 6 and 7.

Table 6: Per Capita Total 3FL Intake from All Proposed Uses by the Total U.S. Population and Subpopulations

Per Capita Population/ Subpopulation	N	% Users	Mean		90th Percentile	
			g/day	g/kg bw/day	g/day	g/kg bw/day
Total US Population	12130	81	1.7	0.03	4.5	0.08
Infants 0-6 mo	259	72	1.1	0.18	2.3	0.38
Infants 7-12 mo	294	97	1.7	0.19	3.1	0.34
Toddlers 13-35 mo	673	96	1.7	0.14	4.3	0.37
Children 3-12 y	2611	96	1.6	0.06	3.4	0.14
Adolescents 13-18 y	1421	87	1.5	0.02	3.4	0.06
Adults 19-49 y	3537	77	1.7	0.02	4.5	0.06
Adults 50+ y	3335	77	1.9	0.02	5.1	0.07

Table 7: Per User Total 3FL Intake from All Proposed Uses by the Total U.S. Population and Subpopulations

Per User Population/ Subpopulation	N	% Users	Mean		90th Percentile	
			g/day	g/kg bw/day	g/day	g/kg bw/day
Total US Population	12130	81	2.2	0.04	5.0	0.10
Infants 0-6 mo	259	72	1.5	0.24	2.5	0.40
Infants 7-12 mo	294	97	1.7	0.19	3.1	0.35
Toddlers 13-35 mo	673	96	1.8	0.15	4.4	0.38
Children 3-12 y	2611	96	1.6	0.06	3.5	0.14
Adolescents 13-18 y	1421	87	1.7	0.03	3.9	0.06
Adults 19-49 y	3537	77	2.2	0.03	5.2	0.07
Adults 50+ y	3335	77	2.5	0.03	5.9	0.08

81% of the U.S. population were identified as consumers of foods wherein addition of 3FL is proposed. The mean and 90th percentile per-user EDIs for 3FL for the total U.S. population were 2.2 g/day (0.04 g/kg bw/day) and 5.0 g/day (0.10 g/kg bw/day), respectively. Per-user mean intakes of 3FL from all proposed food uses range from 1.5 g/day among infants aged 0-6 months to 2.5 g/day among older adults. On a bodyweight basis, the highest 3FL per-user mean intake was for infants age 0-6 months at 0.24 g/kg bw/day.

Infants, especially the 0-6 months age group, are expected to consume either breast milk or formula. As such, no increase in overall 3FL dietary exposure is anticipated by adding 3FL to infant formula.

As a special dietary purpose ingredient in oral and enteral tube feeding (> 11 years), 3FL is intended to be added at levels not exceeding 4 g/serving. These specially formulated foods are intended to be consumed only as necessary, under the guidance and direction of a physician, by the targeted patient population (≥ 11 years) to address particular dietary needs. Up to 3 servings of these foods may be consumed daily. Therefore, the maximum anticipated daily intake from foods for the specified special dietary use is 12 g/person/day, equivalent to 0.21 g/kg bw/day for a 56-kg adolescent and 0.16 g/kg bw/day for a 75-kg adult. No increase in overall 3FL dietary exposure in conventional food is anticipated by the incorporation of 3FL as an ingredient in foods for the specified special dietary use.

Tarleton et al. (2013) observe that the addition of low-digestible carbohydrates (CHO) to enteral formulas is intended to normalize bowel function and improve feeding tolerance, but suggests that the presence of certain comorbidities (bowel ischemia or severe dysmotility) may contra-indicate such addition. These are both easily observable conditions, and it is likely that the health professional overseeing the administration of partial or total enteral nutrition would be aware of the patient's status. Further, there are many randomized clinical trials and open-label studies in which low-digestible carbohydrates were added to enteral feedings given to preterm infants, children, healthy adults, bed-ridden elderly adults, and patients hospitalized for a variety of serious medical conditions. The test articles include partially hydrolyzed guar gum (PHGG), galactomannan, fructooligosaccharides (from scFOS to long-chain inulin), galactooligosaccharides, and GOS/FOS blends, with ingestion levels often greater than 20 g/day and as high as 63 g/day. No adverse effects were reported in any study, suggesting that addition of not more than 4 g of 3FL per serving of enteral feeds is safe.

A possibility exists that manufacturers of infant formula, enteral feeding solutions, and conventional foods may choose to use 3FL in conjunction with other low-digestible carbohydrates. We expect infant formula manufacturers to use our 3FL either alone within the level specified in this GRAS notice or in conjunction with other commercially manufactured human milk oligosaccharides within the levels of total oligosaccharides found in human milk, which is inherently well tolerated. Manufacturers might also use our ingredient in conjunction with other indigestible carbohydrates within ranges already established as well tolerated as per clinical trials.

Manufacturers of infant formula, in order to comply with Section 412(d)(1) of the Food, Drug and Cosmetic Act, must—prior to marketing a new formulation—notify FDA and provide a basis for concluding that the formulation, including any content of indigestible carbohydrates, is safe, well tolerated, and able to support normal growth.

For other uses, for which target levels cannot be established based on natural occurrence, appropriate studies of tolerance may be recommended. Here it must be recognized that it is not in a food or enteral formula manufacturer's interest to market a product that causes gastro-intestinal intolerance symptoms such as bloating or flatulence or more serious adverse reactions such as diarrhea or constipation. Thus, it is unlikely that a manufacturer planning a total nondigestible carbohydrate level in excess of the levels of 3FL contemplated in this GRAS notice would proceed without first obtaining data to support the safety and tolerability of the target level.

3.3. Estimated Dietary Exposure of Other Substances

3.3.1. Carbohydrates Other than 3FL

The commercial specifications for 3FL allow for the presence of small amounts of lactose (≤ 5%), fucose (≤ 3%), galactose/glucose (≤ 3%) and other carbohydrates (≤ 3%). Assuming each of the sugars/other carbohydrates are present at their maximum level in the commercial product, the anticipated mean per-user intake by infants (0-6 months) would be a maximum of 0.075 g/day for lactose, 0.045 g/day each for fucose, galactose/glucose, and other carbohydrates as a group.

Most commercial formulas manufactured in the United States contain lactose (72-74 g/L) from cow's milk as the sole source of carbohydrate (Raiten et al., 1998). The lactose is broken down into glucose and galactose prior to intestinal absorption. By comparison, the contribution of lactose, glucose, and galactose from the commercial 3FL product to the overall intake of these sugars from formula is insignificant.

Fucose is naturally present in breast milk in free form at mean concentrations of 20-30 mg/L (Choi et al., 2015). Therefore, when consuming the average amount of breast milk (840 ml/day[1]) for their age group, infants aged 0-6 months would consume a mean concentration of 0.02-0.04 g/day of free fucose. This value is comparable to the conservative anticipated mean per user intake of fucose (0.045 g/day) from the commercial 3FL product.

3.3.2. Microbial Endotoxins

Internal specifications for lipopolysaccharides (i.e., endotoxins) originating from the fermentation organism have been established at ≤ 300 EU/g using the *Limulus* amebocyte lysate kinetic chromogenic assay described in the European Pharmacopoeia (Ph.Eur. 2.6.14 + Interference study). This level is consistent with the typical ranges of endotoxins detected in drinking water (Anderson et al., 2002; O'Toole et al., 2008), cow's milk (Gehring et al., 2008), and infant formula powder (Townsend et al., 2007). The analytical data from multiple batches (Part 2, Section 2.3.3) provides assurance that the 3FL product complies with the endotoxin specification.

3.3.3. Production Organism

The production microorganism is efficiently removed in the first step of the downstream processing. Various sequential purification processes are also applied to ensure microbiological purity.

The absence of residues of the production microorganism in the 3FL product is demonstrated by microbial testing for coliform and *Enterobacteriaceae* using internationally recognized methods (ISO 21528-1) and by residual bacterial rDNA analysis by quantitative PCR (qPCR) to confirm the absence of production organism DNA following EFSA guidance (EFSA 2018) on evaluation of

[1] American Academy of Pediatrics: "General Guidelines for Baby Feeding" and "Amount and Schedule of Formula Feedings."

fermentation derived products for the absence of production strain and presence of DNA from the production strain.

As noted in the product data reported in Part 2, Section 2.3.3, all four batches tested negative for recombinant DNA from the GMM production host. The Limit of Detection (LoD) for the PCR reactions was below 10 ng DNA/g 3FL product, consistent with the DNA detection threshold set forward in the EFSA guidelines.

PART 4. SELF-LIMITING LEVELS OF USE

The intended use of DuPont's 3FL is not self-limiting.

PART 5. EXPERIENCE BASED UPON COMMON USE IN FOOD BEFORE 1958

The conclusion that the intended use of 3FL is GRAS is based on scientific procedures rather than experience based on common use in food prior to 1958.

PART 6. NARRATIVE

The safety of 3FL and the information and data providing the basis for the conclusion that 3FL is GRAS for the intended conditions of use outlined in this dossier (Part 1, Section 1.3 and Part 3, Section 3.2) are discussed below.

6.1. Manufacturing and Purity of 3FL

3FL is a highly purified material, as established by the product specifications and documented by the batch analytical data. The primary components in the product are carbohydrates that historically present no cause for safety concerns. Post-fermentation down-stream processing steps effectively remove production host biomass and any associated rDNA, along with other impurities and contaminants. Screening of the 3FL production batches for bioamines, heavy metals, rDNA, endotoxins, and microbiological contaminants found these possible hazards to be either absent or within established product specifications (Part 2, Sections 2.3 and 2.4.)

The 3FL molecule, which makes up a minimum of 90% of the commercial 3FL product, is chemically and structurally identical to the 3FL molecule present in human breast milk. (See Part 2, Section 2.1.) The other minor carbohydrates, including lactose, fucose, glucose, and galactose, are normal components of the diet and are readily metabolized.

As noted in Part 3, Section 3.3.1., based upon the most conservative case where the maximum concentrations of these minor carbohydrates are present in the commercial 3FL product, the increase to the overall intake of 0.075 g/day lactose and 0.045 g/day each for glucose and galactose in cow's-milk-based infant formula is insignificant.

For fucose, the projected conservative intake of 0.045 g/day is comparable to mean per-user intakes (0.02-0.04 g/day) of breastfed infants aged 0-6 months consuming an average of 840 ml/day of breast milk.[1]

6.2. Safety of the Production Strain

E. coli K12 MG1655 INB008971 is a stable non-pathogenic and non-toxigenic strain with a safety profile comparable to its host, *E. coli* K12 MG1655, an ATCC Biosafety Level 1 microbial culture.

As noted in Part 2, Section 2.2., although bioinformatics analysis of sINB008971 WGS data found gene sequences coding for virulence factors and flagged the endogenous genes, EcMdfA and EcLacY, as toxic proteins, these genes are also present in *E. coli* K12 MG1655. Consequently, their presence in the production strain does not trigger a change in the biosafety level.

The introduced genes in the production strain express proteins facilitating 3FL production. None of these proteins is secreted into the fermenter. No gene sequences coding for allergenic proteins were identified when the sINB008971 WGS data examined against the FARRP database. Furthermore, no gene sequences coding for signal peptides were found, indicating that no protein excretion into the extracellular space occurred.

[1] American Academy of Pediatrics: "General Guidelines for Baby Feeding" and Amount and Schedule of Formula Feedings."

E. coli K12 MG1655 INB008971 contains one additional copy each of the EcMdfA and EcLacY genes. Insertion of the extra copy of these genes results in increases in the concentration of lactose permease and the MDR transport protein in the production strain cell membrane. (Both lactose permease and the MDR transport protein are naturally present in the host cell membrane.) However, these changes do not present a safety concern as both expressed proteins are removed during post-fermentation downstream processing. Several factors contribute to this certainty. First, both proteins are membrane bound and insoluble in water. Second, the molecular masses of lactose permease (~45 KDa) and MDR transport protein (~44 KDa) are individually 100-fold larger than 3FL (~0.5 KDa). And third, the microfiltration and/or ultrafiltration membranes used for this step of the process are specifically selected to remove cell biomass and large molecules (including proteins and endotoxins) from the 3FL product stream.

6.3. Experience Based on Common Use in Food

First isolated from human breast milk in the mid-1950s (Kunz 2012), 3FL belongs to a group of complex carbohydrates described as human milk oligosaccharides (HMOs) that are highly abundant in breast milk, representing approximately 20% of the total carbohydrate content of human milk (Urashima et al. 2012).

3FL concentrations in breast milk of healthy women vary across geographical regions, ethnicity, and lactation stage. Significantly higher average 3FL concentrations were reported by Thurl et al. (2010) in breast milk from women with Lewis blood group who lack the gene to secrete 2'-Fucosyllactose (2'FL). The biological reason for this variability in HMO concentrations is unknown.

A study reported by Erney et al. (2000), surveying 3FL concentrations in breast milk samples collected from 435 healthy women residing in 10 different countries, found that mean 3FL concentrations in breast milk samples collected from US women increased through the postpartum period, from a low of 1.03 g/L at 3-10 days, to 1.48 g/L at 11-30 days, reaching a high of 2.57 g/L at 31-217 days. The mean postpartum concentration was 1.84 g/L. Similar increases were observed in other regions: after 31 days, mean 3FL concentrations reached values of 2.15, 1.36, and 0.88 g/L in Asia, Europe, and Latin America, respectively.

Smilowitz et al. (2013) evaluated the compositional profile of breast milk collected at day 90 postpartum from 52 healthy US women and reported 3FL concentration levels ranging from 480 to 5600 μmol/L, with a mean of 2100 $\pm$ 1200 μmol/L (equivalent to 0.23 to 2.73 g/L and 1.03 $\pm$ 0.58 g/L), respectively.

Other studies reporting 3FL concentrations in breast milk collected from healthy women living other regions of the world include the following:

Reference	Milk donor description	No. of samples	Days postpartum	3FL concentration (g/L)	
				Mean	Range
Austin et al. 2016	540 mothers living in urban areas of China	446	5–11 12–30 31-60 61-120 >120	0.50 0.59 0.74 1.13 1.34	0.02[a] - 2.88 0.02[a] - 2.47 0.02[a] - 2.58 0.02[a] - 3.40 0.06 - 6.07
Gabrielli et al. 2011	18 mothers with Se^-/Le^+ genotype, living in Italy, who delivered preterm newborns (mean gestational age: 27.9 weeks)	72	4 10 20 30	1.92 2.05 1.34 2.22	
Reference	Milk donor description	No. of samples	Days postpartum	3FL concentration (g/L)	
Sumiyoshi et al. 2003	16 Japanese women	46	4 10 30 100	0.23 0.28 0.43 0.45	0.01 - 1.01 0.04 – 0.77 0.02 – 1.42 ND – 1.49

[a] Samples having concentrations >LoD, but <LoQ (43 mg/kg) were assigned a value of half the LoQ (22 mg/kg); Specific gravity of breast milk = 1.03 kg/L

6.4. Safety Studies with 3FL

In vitro, *in vivo*, and animal feeding studies were conducted to supplement the data collected for the safety assessment and to support planned clinical and efficacy studies (Pitt et al. 2019). Studies conducted included acute oral toxicity and a 90-day subchronic rodent feeding study using test material conforming to the product specifications, and *in vitro* and *in vivo* assessments of genetic toxicity. The weight of evidence from these studies supports the safe use of 3FL produced using biotechnology as an ingredient in foods.

The acute oral toxicity study using female Crl:CD®(SD) rats as test animals was conducted in accordance with appropriate testing guidelines (US EPA, 2002; US FDA, 2007; OECD, 2008). A limit dose of 5000 mg/kg bw administered orally was selected based on the history of consumption and lack of toxicity historically observed with HMOs. No deaths or clinical signs of toxicity were observed over the 14-day observation period and there were no macroscopic observations at necropsy.

The subchronic rodent feeding study was conducted essentially as a limit-test in accordance with OECD test guideline 408 (OECD, 1998) and FDA Redbook guidance (US FDA, 2007), as there was no expectation of adverse effects, even at very high mg/kg bw intakes. However, the study design included two concentration levels of test substance rather than a single maximum concentration, which is typical for a limit-dose test, to provide an appropriate balance between animal welfare,

regulatory expectations, and business needs. Consequently, male and female Crl:CD®(SD) rats (n = 10 rats/sex/group) were fed either the basal diet or diets containing 5% 3FL, 10% 3FL, or 10% FOS (w/w), *ad libitum* for at least 90 consecutive days. Subchronic exposure of the rats to 3FL at 5% and 10% dietary concentrations did not produce any statistically significant or biologically-relevant differences in growth, feed intake, or feed efficiency ratio. There were no adverse clinical observations, and no clinical or anatomic pathology changes were observed. The average daily intakes of 3FL were equivalent to 5.98 and 7.27 g/kg bw/day for males and females, respectively.

The genetic toxicity potential of 3FL was evaluated *in vitro* using the bacterial reverse mutation (Ames) test, the mammalian cell micronucleus test in Chinese hamster ovary cells, and the chromosomal aberration test in human lymphocytes. 3FL was also evaluated in mice using the mammalian erythrocyte micronucleus test. There was no evidence of genetic toxicity in the bacterial reverse mutation test and chromosomal aberration assay. While there was a repeatable statistically-significant trend in the 4-h S9-activated test conditions in the *in vitro* micronucleus assay, the confirmatory *in vivo* mouse micronucleus study was negative at all doses.

6.4.1. Absorption, Distribution, Metabolism and Excretion (ADME)

3FL and other neutral HMOs are not hydrolyzed to the constituent monomers in the upper gastrointestinal tract; rather, most of the 3FL consumed passes through the gastrointestinal tract undigested. A low level of absorption does occur; the presence of intact 3FL has been reported in the plasma and excreted in the urine of healthy breastfed infants. Systemic exposure resulting from 3FL supplementation of infant formula or food is consistent with levels resulting from breast milk consumption.

Goehring et al. (2014) analyzed urine and blood samples of 17 healthy formula-fed and 16 healthy breastfed infants; multiple small molecular weight HMOs were detected in the urine and plasma of breastfed infants. Levels of 2FL, 3FL, and lacto-N-neotetraose (LNnT) in both plasma and urine were positively correlated with corresponding concentrations in breast milk. Relative to absorption of 2'FL and 6'-Sialyllactose (6'SL), representatives of major fucosyllated and acidic glycans present in human milk, were low; 0.1% of milk levels for plasma and 4% of milk levels for urine. This study confirms low levels of systemic exposure to 3FL and other HMOs and is consistent with other studies reporting the presence of HMOs in urine samples of breastfed infants.

Pharmacokinetic data from the 90-day rat feeding study described above (Pitt et al. 2019) provides an indication of systemic exposure to 3FL in 3FL-supplemented diets. Evaluation of blood and urine samples from rats fed a 5% or 10% 3FL-supplemented diet for 12-weeks indicated low absorption and systemic exposure from dietary intake that was proportional to dose. Recovery of 3FL in urine was ~0.4% (as mol %) of the administered dose in both dose groups. Thus, while measurable 3FL concentrations in the serum and urine confirmed low level absorption (well below 1.0% of the daily dietary intake levels), systemic exposure is negligible.

An *in-vitro* digestion study (Engfer et al. 2000) to assess the extent to which selected HMOs are hydrolyzed by enzymes present in the gastrointestinal tract found that neutral HMOs are very resistant to hydrolysis by secreted human pancreatic glycosidases and the enzymes bound to porcine or human intestinal brush border membranes of the upper small intestine. The studied neutral HMOs, including a total fraction of neutral HMOs, 2'FL, 3FL, and other non-fucosyllated

neutral HMOs, were recovered intact after the digestion step. Further analysis by MALDI-MS and enzymatic assays found no evidence of hydrolysis breakdown by-products.

Chaturvedi et al. (2001) investigated the fate of major neutral HMOs during transit through the gastrointestinal tract. The similarity between the HMO profile in breast milk and the urinary and fecal oligosaccharide profiles led to the conclusion that breast milk was the major source of oligosaccharides found in the urine and feces of breastfed infants. Oligosaccharide concentrations in urine samples were 1% of that of breast milk and while concentrations in fecal samples were about 10-fold higher than in breast milk. The investigators suggested that the findings indicate that approximately 97% of the consumed neutral HMOs, including 3FL and 2'FL, pass intact into the feces, while approximately 0.5% are absorbed and excreted into the urine.

6.4.2. 3FL Consumption in Human Milk vs. Dietary Exposure from Intended Use

The estimated dietary exposure to 3FL by infants (0-6 months), the highest consumers on a body weight basis, is comparable to the 3FL concentrations breastfed infants consume through breast milk.

Published studies in the literature report 3FL concentrations ranging from non-detectable to as high as 5.9 g/kg (Austin et al. 2016), equivalent to 6.08 g/L and 0.82 g/kg bw/day, based upon an average consumption of 840 ml breast milk per day and a body weight of 6.25 kg for infants aged 0-6 months.

Similowitz et al. (2013) reported 3FL concentrations of 0.23-2.73 g/L among women residing in the US, while Erney et al. (2000) found that 3FL mean concentrations increased over the postpartum period, reaching a high of 2.57 g/L at 31-217 days. These values are equivalent to a range of 0.03-0.37 g/kg bw/day with mean = 0.35 g/kg bw/day.

As the highest consumers of 3FL on a body weight basis, infants (0-6 months) represent the most sensitive population. As noted in Part 3, Section 3.2, the mean per user intake for infants (0-6 months) was 0.24 g/kg bw/day for the proposed applications and use levels. As breast milk is recommended as a sole source of nutrition for infants of this age group, breastfed infants are unlikely to consume the formula and other foods containing the added 3FL. As such, the overall dietary exposure for infants (0-6 months) is expected to be equal to the estimated mean daily intake from the proposed uses i.e. 0.24 g/kg bw/day and is below the mean 3FL daily intake levels (0.35 g/kg bw/day) resulting from consumption of breast milk.

6.5. Safety Assessment and GRAS Conclusion

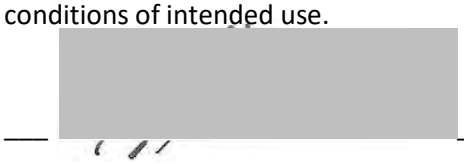
DuPont considers the following elements as key to our finding that 3FL is safe for use in the proposed food applications at the levels specified in Part 3, Section 3.2, and for reaching a GRAS conclusion under the conditions of intended use.

- 3FL is produced in accordance with good manufacturing practice in a food grade facility using food grade raw materials. It is a purified to yield a minimum 3FL assay of 90%; the 3FL molecule is chemically and structurally identical to that isolated from human breast milk. As evidenced by the batch data, the product can be manufactured reproducibly and consistently meets the specifications established to ensure product purity and safety.

- The production strain *E. coli* K12 MG1655 INB008971 is a stable non-pathogenic and non-toxigenic strain with a safety profile comparable to its host *E. coli* K12 MG1655, an ATCC Biosafety Level 1 microbial culture. The inserted genes are well characterized and synthetized *in-vitro* to avoid the potential introduction of undesirable genes from donor organisms.
- The in-vitro, in-vivo and animal feeding studies helps confirm that the biotechnologically produced 3FL product does not contain any impurities that would cause an adverse reaction when consumed at high levels.
- Absorption of 3FL is minimal; most of the ingested 3FL will be eliminated in the urine and feces. Systemic exposure resulting from 3FL added to infant formula and other food and beverages is expected to be very low and comparable with that in breastfed infants.
- At 0.24 g/kg bw/day, the mean (user only) dietary exposure levels of 3FL in the most sensitive subpopulation, Infants (0-6 months) is well within the 3FL intake range of breastfed infants (0.35 g/kg bw/day).
- The safety and ADME studies as well as the database and information to determine the dietary exposure and estimated daily intake for the proposed uses and by breastfed infants are publicly available.

6.6. Affirmative Statement Concerning Data & Information

I have reviewed the available data and information and am not aware of any data or information that are, or may appear to be, inconsistent with DuPont's conclusion of GRAS status under the conditions of intended use.



PART 7. LIST OF SUPPORTING DATA AND INFORMATION

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JHeimbach LLC

February 25, 2021

Ellen Anderson
Regulatory Review Scientist
Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
U.S. Food and Drug Administration

Dear Dr. Anderson:

On January 27, you sent me an email indicating that during review of DuPont Nutrition and Bioscience's GRAS notice GRN 000951 for the intended use of 3-fucosyllactose (3-FL), FDA had noted seventeen questions needing to be addressed. This letter provides these needed responses.

Before addressing FDA's questions, I need to inform you that this unit of DuPont has in the interim been acquired by International Flavors and Fragrances, Inc. (IFF). As a result, section 1.2 of GRN 000951 now reads as follows:

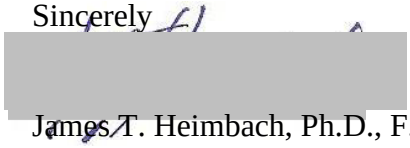
1.2. Name and Address of Notifier

Danisco USA Inc., a wholly owned subsidiary of IFF, Inc.
DuPont Experimental Station – E320
200 Powder Mill Road
Wilmington DE 19803

Notifier Contact
Angela Lim
Global Regulatory Strategy Lead (HMOs & Food Protection)
Tel: 302-695-6786
Angela.Lim@IFF.com

I trust that the following responses to your questions provide complete and fully satisfactory answers. If you require further information, please let me know.

Sincerely



James T. Heimbach, Ph.D., F.A.C.N.
President

Encl.

1. On page 7, DuPont provides the IUPAC name for 3-fucosyllactose (3-FL). We note that 3-FL is considered to be a branched oligosaccharide and, according to the IUPAC recommendations, terms designating branches should be enclosed in square brackets (https://doi.org/10.1016/S0065-2318(08)60090-6). Please provide the correct IUPAC name.

Q1 Response:
The correct IUPAC name is 6-Deoxy-α-L-galactopyranosyl-(1->3)-[β-D-galactopyranosyl-(1->4)]-D-glucopyranose. We apologize for the error.

2. On page 9, DuPont states that all ingredients and processing aids used in the fermentation medium are food grade and are GRAS substances or food additives. On the same page, DuPont states that all processing aids used in the post-fermentation processing are food grade. Please confirm that all ingredients and processing aids are approved for their respective use via a regulation in Part 21 of the U.S. Code of Federal Regulations, are the subject of an effective food contact notification, or are GRAS for that use in the U.S.

Q2 Response:
IFF confirms that all ingredients and processing aids are approved for their respective use via a regulation in Part 21 of the U.S. Code of Federal Regulations, are the subject of an effective food contact notification, or are GRAS for that use in the U.S.

3. On page 9, DuPont states the fermentation medium is supplemented with nutrients such as trace minerals, vitamins, and amino acids. However, OFAS notes that for certain trace minerals such as zinc, copper, manganese, and iron, which were reported by DuPont to be included in the fermentation medium, the Institute of Medicine has designated Tolerable Upper Intake Levels for infants (0–6 months; 7– 12 months), toddlers (1–3 years) or both (Institute of Medicine 2006. Dietary Reference Intakes: The Essential Guide to Nutrient Requirements. Washington, DC: The National Academies Press. https://doi.org/10.17226/11537). Given that the intended uses for 3-FL include infant formula and formula for young children (1–3years), please provide data from three (preferably five) nonconsecutive batches of 3- FL that demonstrate that these trace minerals are either removed or are present at levels that do not pose a safety concern. In addition, please specify an analytical method used to test for each trace mineral and indicate that the method(s) is validated for the intended purpose.

Q3 Response:
The data below demonstrate that the presence of these trace minerals is quite low, well below any TUL, and as such do not pose a safety concern.

Mineral	Batch# B90140	Batch # B90157	Batch # B90196	Method
Iron	0.6 (± 0.4) mg/kg	1.1 (± 0.5) mg/kg	0.8 (± 0.4) mg/kg	DIN EN ISO 17294-2 (2017-01), mod. ICP-MS
Copper	0.9 (± 0.2) mg/kg	0.8 (± 0.2) mg/kg	1.0 (± 0.2) mg/kg	DIN EN ISO 17294-2 (2017-01), mod. ICP-MS
Manganese	0.2 (± 0.1) mg/kg	<0.1 mg/kg	0.2 (± 0.1) mg/kg	DIN EN ISO 17294-2 (2017-01), mod. ICP-MS
Zinc	0.5 mg/kg	0.8 (± 0.4) mg/kg	1.2 (± 0.5) mg/kg	DIN EN ISO 17294-2 (2017-01), mod. ICP-MS

4. On page 16–18 (Tables 1–4), DuPont provides specifications for 3-FL along with the corresponding analytical methods, including some non-standard or modified methods (e.g., the Nordic Committee of Food Analysis method, or modified Bradford method). Please confirm that all analytical methods used to test for each specification parameter are validated for that purpose.

   Q4 Response:
   IFF confirms that all analytical methods used to test for each specification parameter are validated for that purpose.

5. DuPont states that 3-FL is intended for use in infant formula and formula for young children (1–3 years). Please specify the intended source of the protein base (e.g., milk, soy, etc.) of both formulas.

   Q5 Response:
   The intended sources of the protein base of both formulas include cow's milk and soybean.

6. On page 22, DuPont provides per capita (Table 6) and per user (Table 7) dietary exposure estimates for several subpopulations, as well as the corresponding number of individuals (N) and percent users (% Users) in each subpopulation. We note that the N and % Users are identical in both tables. We would expect the per capita N to be higher than the per user N. Also, in the case of the per capita exposure, the assumption is that 100% of the given population are users. Please provide clarification for N and % Users in Table 6.

   Q6 Response:
   The data in the two tables are correct, but the tables are perhaps misleadingly titled. Both tables report the total number of NHANES respondents in the age group and the percentage of them who reported consumption of at least one target food on at least one survey day. While Table 6 reports intakes by the total age group, both users and non-users, Table 7 reports intakes only for users. (Because the proportion of users is so high, particularly among infants aged 7-12 months, toddlers, and children, there is little difference between per capita and per user intakes.)

   It perhaps would have been clearer if Table 6 had omitted the "% Users" column and if Table 7 were titled, "Total 3FL Intake from All Proposed Uses by Those Individuals in the Total U.S. Population and Subpopulations Who Reported Consuming One or More of the Target Foods."

   We apologize for the confusion but, again, the intake data in the two tables are correct.

7. On page 24, DuPont estimates dietary exposure to lactose, fucose, galactose/glucose, and other carbohydrates for infants aged 0–6 months. The estimates are based on the maximum level of sugars/other carbohydrates in 3-FL and the mean per-user consumption of 3-FL from the intended use in infant formula. Please provide the corresponding estimates based on the 90th percentile per-user consumption of 3-FL.

Q7 Response:
Based upon the 90th percentile per-user intake of 3-FL by infants aged 0-6 months (2.5 g/day), anticipated intake would be a maximum of 0.125 g/day for lactose and 0.050 g/day each for fucose, galactose/glucose, and other carbohydrates.

8. On page 32, DuPont provides the statement: "…based upon an average consumption of 840 ml breast milk per day and a body weight of 6.25 kg for infants aged 0–6 months…" Please provide a reference for the body weight of 6.25 kg.

Q8 Response:
A body weight of 6.25 kg is an approximate average weight for infants aged up to 6 months according to WHO Child Growth Standards, based upon average weight at birth (3.8 kg and 3.3. kg for boys and girls, respectively) to average weight at 6 months (7.9 kg and 7.3 kg), https://www.cdc.gov/growthcharts/who_charts.htm#The%20WHO%20Growth%20Charts).

9. The fermentation media contains lactose. Please clarify whether any proteins from a major allergen, including cow milk, will be present in the final ingredient.

Q9 response:
The specifications from the lactose supplier indicate some residual protein which is included in the 3FL product specification (See Table 3). Accordingly, there may be some cow's milk protein in the final ingredient. The ingredient will therefore be labeled as containing cow's milk protein.

10. Please clarify whether the provided specifications for Cronobacter sakazakii and Salmonella are performed using sample sizes for analysis of 100 g based on the cited methods ISO 22964 and NMKL 71. Please provide results from analyses of three non-consecutive batches in samples of 10 g for C. sakazakii and 25 g for Salmonella serovars, per the cited methods.

Q10 Response:
IFF confirms that the analysis was performed in compliance with ISO 22964 for *Cronobacter sakazakii* and NMKL 71 for *Salmonella*. Neither method specifies a volume of analyte. Additionally, we clarify that for these 2 parameters, our sampling size of 100 g is larger than the required sample size. This is clearly a more rigorous standard. By demonstrating absence in a larger sample, we have confirmed compliance with the requirement of absence of 10 g and 25 g respectively for *C. sakazakii* and *Salmonella*.

11. Please confirm that the manufacturer continuously monitors the fermentation process for contaminants and quality control procedures are taken upon observation of contamination.

Q11 Response:
Yes, there are standard operating procedures ensuring that the fermentation process is continuously monitored for contaminants and quality control procedures are in place to ensure that appropriate action is taken upon any observation of contamination.

12. On page 8, DuPont states, "The residual components were identified as mono-, di-, and trimeric carbohydrates with a polyol nature." Given that these "other carbohydrates" appear to comprise up to 3% of the final product (Table 2; page 17), please identify these polyols and/or discuss why they are not a safety concern.

Q12 Response:
There is no safety concern with intake of these other carbohydrates (CHOs) up to 3% based on the animal studies discussed in the notice (Pitt et al 2019). While the level of "other carbohydrates (CHO)" was different between the rat 90-day study material (1.4%) and the product specifications in the GRAS Notice (3%), the actual dosage of "other CHOs" is similar between the rat 90-day study and the anticipated 90th percentile. The highest dose level of the 90-day study, 10% 3-FL for female rats, can be converted to a human equivalent dose (HED) [1]. The HED of the rat study can then be compared to anticipated human exposure. The following assumptions are made for this calculation:

- 90th percentile consumption of 3-FL by infants (0-6 m): 0.4 g/kg bw/day (Table 7 of GRAS notice)
- 7.27 g/kg bw/day: highest dose in the rat 90-day study
- 1.4% "other carbohydrates" level in the rat 90-day study
- 3% "other CHOs" level in the commercial grade of 3-FL
- Anticipated human "other CHOs " exposure at the 90th percentile, 0.4 g 3-FL/kg bw/d * 3 % "other CHOs"/g 3FL * 1000mg/g = 12 mg "other CHOs"/kg bw/d
- "Other CHOs" exposure in the rat 90-day study: 7.27g 3-FL/kg bw/d * 6.2 (rat to human conversion) * 1000 mg/d = 1170 mg 3-FL/kg bw/d ∗ 1.4% = 16.4 mg "other CHOs"/kg bw/day

From this conversion exercise, it is clear that rodents were exposed to similar levels of the "other CHOs" as infants who would consume 3-FL at the ≤90th percentile per day. As there were no adverse effects observed in either the 90-day study or the piglet study, we would anticipate no adverse events and therefore no safety concerns in infants as a result of exposure to 3% "other CHOs."

1 Guidance for Industry: Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers. U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER). July 2005, Pharmacology and Toxicology.

13. On page 22, Section 3.2, DuPont asserts that "no increase in overall 3-FL dietary exposure is anticipated by adding 3-FL to infant formula." Given that 3-FL is not currently added to infant formula, OFAS understands this statement to mean that there is no increased exposure to 3-FL in comparison to the overall exposure to 3-FL in breastfed infants. Please confirm or provide an alternative explanation and/or clarification for this statement.

Q13 Response:
The statement in this section was made within the context of 3-FL exposure by breastfed infants. The sentence should be revised to "As such, in non-breastfed infants, dietary exposure resulting from addition of 3-FL to infant formula is anticipated to be comparable to 3-FL concentrations consumed by breastfed infants."

14. On page 23, DuPont discusses the intended use of 3-FL as a special dietary purpose ingredient in oral and enteral tube feeding for patient populations ≥ 11 years.

    a. Please provide examples of the patient populations expected to receive 3-FL through enteral tube feeding.

    b. DuPont states, "Further, there are many randomized clinical trials and open- label studies in which low-digestible carbohydrates were added to enteral feedings given to preterm infants, children, healthy adults, bed-ridden elderly adults, and patients hospitalized for a variety of serious medical conditions. The test articles include partially hydrolyzed guar gum (PHGG), galactomannan, fructooligosaccharides (from scFOS to long-chain inulin), galactooligosaccharides, and GOS/FOS blends, with ingestion levels often greater than 20 g/day and as high as 63 g/day. No adverse effects were reported in any study, suggesting that addition of not more than 4 g of 3-FL per serving of enteral feeds is safe." However, no references are provided to support these statements. Please provide a more detailed discussion, such as duration of the studies and any relevant information, positive or negative with appropriate citations, to support the safe use of 3-FL in oral and enteral tube feeding.

Q14a Response:
The patient populations expected to receive 3-FL through enteral tube feedings are those unable to consume sufficient calories and other nutrients by mouth, including the elderly (free living and bed-ridden), critically ill, and hospitalized individuals.

Q14b Response:
Table 1 below (References at end of response) provides a summary of the studies in which low-digestible carbohydrates were added to enteral feedings and given to a variety of different types of subjects. Details provided include study design, subjects, ingredient source, characteristics, and dose, duration and safety-related findings.

Table 1. Human Studies of Poorly Digested Carbohydrates in Enteral Formula.

Citation	Study Design	Subjects	Source, Characteristics, and Dose	Duration	Safety-Related Findings
Partially Hydrolyzed Guar Gum (PHGG)					
Lampe et al. (1992)	Prospective, randomized, double-blind cross-over design	11 healthy men	15 g PHGG/day in enteral formula	18 days	Stool weight and fecal consistency did not change significantly with any dietary treatment and no adverse effects were reported.
Homann et al. (1994)	Prospective randomized double-blind placebo-controlled trial	100 hospital patients; 30 receiving total enteral nutrition and 70 receiving enteral supplementation	20 g of PHGG per 1000 mL of formula; intake of TPN patients = 24 g PHGG/day; intake of enteral supplementation patients = 20 g PHGG/day	Not reported	No bloating or cramping was noted. 4 patients on the standard total enteral diet, but no patients receiving PHGG, had to be discontinued due to GI side effects. In the supplemental feeding groups, 8 control v. 2 PHGG patients had to discontinue feeding. "The total number of GI-side effects was not different in the two groups (17 in each group)."
Peters and Davidson (1996)	Prospective, randomized, double-blind cross-over study	12 patients with Type 1 diabetes receiving enteral feeding	Not reported	Not reported	No adverse effects were reported.
Spapen et al. (2001)	Prospective, randomized, double-blind, placebo-controlled study of enteral feeding	25 ICU patients (13 M, 12 F; mean age = 68.5±13.1 years) with severe sepsis and septic shock	22 g/L	At least 6 days	No significant effect on sepsis-related mortality (1 death in the test group, 4 in the control) or duration of stay in the intensive care unit. The authors concluded that "Fiber treatment was well-tolerated."

Table 1. Human Studies of Poorly Digested Carbohydrates in Enteral Formula.

Citation	Study Design	Subjects	Source, Characteristics, and Dose	Duration	Safety-Related Findings
Rushdi et al. (2004)	Prospective, randomized, double-blind, controlled study of enteral feeding	20 IBS patients (11 M, 9 F; aged 28-73 years with mean age = 57/5±13/8 years) on enteral nutrition with 3 or more liquid stools/day	2% (22 g/L); 22 to 37 g/day	4 days	The PHGG was well tolerated with fewer adverse GI symptoms than in the control group. "Throughout the course of this clinical trial, in the fiber-enriched feed group, only two patients complained of flatulence (20%). On the other hand, in the control group, four patients complained of flatulence (40%), two patients got vomiting (20%) and one case of constipation (10%) was reported. However, no statistical significance was found between both groups as regards incidence or severity of gastrointestinal symptoms. None of these symptoms was severe enough to necessitate therapeutic intervention."
Alam et al. (2015)	Prospective, randomized, double-blind, placebo-controlled trial of oral rehydration solution	126 mal-nourished children (67M, 59F) aged 6-36 months (mean age = 18.1± 10.7 months) with watery diarrhea	15 g PHGG/L	Until resolution of diarrhea or up to 7 days	6 children from the PHGG group and 5 controls withdrew prior to study completion, none for reasons associated with treatment. No adverse events were reported.
Rosli et al. (2020)	Randomized, double-blind, placebo-controlled trial of PHGG supplementation for radiation patients	30 patients aged 56.5±10.8 years undergoing pelvic radiation and receiving enteral feeds	20 g/day of PHGG or maltodextrin	28 days	No adverse effects were reported from the PHGG.

Table 1. Human Studies of Poorly Digested Carbohydrates in Enteral Formula.

Citation	Study Design	Subjects	Source, Characteristics, and Dose	Duration	Safety-Related Findings
Galactooligosaccharide (GOS)					
Nagafuchi et al. (2015)	Randomized, double-blind, controlled study of the effect of the addition of GOS to enteral formula for elderly in-patients	24 enterally fed in-patients aged ~80.3 years	400 mg GOS/ 100 kcal	14 weeks	No adverse effects were reported.
Fructooligosaccharide or Short-Chain Fructooligosaccharide (FOS or scFOS)					
Garleb et al. (1996)	Randomized, double-blind, controlled study of the effect of the addition of scFOS to enteral feeding formulas	27 apparently healthy male college students	0, 5, or 10 g/L of scFOS	14 days	The low and high scFOS groups had intakes of about 15 and 30 g scFOS/d. No change in body weight or deviations from the normal range of blood chemistry (glucose, BUN, creatinine, bilirubin, TC, TAG, protein, albumin, globulin, ALP, lactate dehydrogenase, ALT, AST, Ca, Na, K, chloride, Fe, P, GGT). Tolerance of the scFOS formula was good. Complaints of nausea, cramping, distension, vomiting, diarrhea, and regurgitation were similar across all groups and occurred on fewer than 5% of days. Flatus was more frequent inthose consuming 30 g scFOS/day, but most complaints occurred during the first 4 days. The authors concluded that “these results indicate that [scFOS] does not compromise serum chemistry profiles, is well tolerated particularly at an intake of 15 g/d and would serve as a bifidogenic factor when incorporated into a liquid enteral product.”

Table 1. Human Studies of Poorly Digested Carbohydrates in Enteral Formula.

Citation	Study Design	Subjects	Source, Characteristics, and Dose	Duration	Safety-Related Findings
Karakan et al. (2007)	Randomized, double-blind, placebo-controlled trial of adding scFOS to early enteral nutrition solution for feeding of patients with severe acute pancreatitis	30 patients aged 46.1±14.0 years with severe acute pancreatitis requiring stoppage of oral feeding	0 or 24 g fiber (about 50% scFOS)/day	48 hours	the authors reported that, "nasojejunal EN with pre-biotic fiber supplementation in severe AP improves hospital stay, duration of nutrition therapy, acute phase response and overall complications compared to standard EN therapy." Both enteral feeding solutions were well tolerated with no reported adverse effects.
Kapiki et al. (2007)	Randomized, double-blind, placebo-controlled trial of enterally fed preterm infants	56 preterm infants $\leq$36 weeks gestation receiving enteral feeds	400 mg/100 ml enteral solution of either FOS or maltodextrin	14 days	
Galactooligosaccharide/Fructooligosaccharide (GOS/FOS)					
Boehm et al. (2002)	Randomized, double-blind, placebo-controlled trial of enterally fed preterm infants	30 preterm infants <32 weeks gestation receiving enteral feeds	1 g/dl of GOS (90%)/FOS (10%) or maltodextrin	28 days	Enteral feed was well tolerated; no reports of vomiting, gastric aspirates, abdominal distension, or diarrhea. No change in time to full enteral feed.
Mihatsch et al. (2006)	Randomized, double-blind, placebo-controlled trial of enterally fed preterm infants	20 preterm infants <1500 g birth weight receiving enteral feeds	1 g/dl of GOS/ FOS or maltodextrin	15 days	Enteral feed was well tolerated; no reports of vomiting, gastric aspirates, abdominal distension, or diarrhea. No difference in volume of feed tolerated.
Indrio et al. (2009)	Randomized, double-blind, placebo-controlled trial of enterally fed preterm infants	20 healthy preterm infants	0.8 g/dl of GOS (90%)/FOS (10%) or maltodextrin	15 days	Enteral feed was well tolerated; no reports of vomiting, gastric aspirates, abdominal distension, or diarrhea; no symptoms of feed intolerance.

Table 1. Human Studies of Poorly Digested Carbohydrates in Enteral Formula.

Citation	Study Design	Subjects	Source, Characteristics, and Dose	Duration	Safety-Related Findings
Lactulose					
Zoppi et al. (2001)	Randomized, double-blind, placebo-controlled trial of enteral ceftriaxone with or w/o lactulose	51 children aged ~5.1 years admitted for respiratory tract infections	4 or 6.6 g/day (200 or 330 mg/kg bw/day)	Mean = 4.5 days	No participants dropped out of the study due to GI complications.No difference in intestinal complaints was observed among the various groups during treatment.

15. On page 31, DuPont discusses the results of the *in vitro* micronucleus test performed in CHO cells which was reported by Pitt et al., 2019 to be equivocal. Please provide a narrative that elaborates on why an equivocal result in this assay is not a safety concern and does not contradict your GRAS conclusion. Similar to the discussion in Pitt et al., 2019, such a narrative could include a discussion of the false positive rate of the assay, genotoxicity results of other structurally similar human milk oligosaccharides, the purity of the 3-FL ingredient., etc.

Q15 Response:

IFF would like to clarify that in the *in vitro* micronucleus study, there was no actual increase in micronuclei, but only a statistically identified trend towards an increase in micronuclei. Per the OECD 487 (2016) guidance, that study was described as equivocal, i.e., neither positive nor negative. However, the incidences of the micronuclei in all treated groups were within the 95% CI of the laboratory historical control database (HCD) indicating that there was no genotoxic increase in micronuclei. Additionally, Fowler et al. (2012) has demonstrated that the micronucleus assay with carbohydrate cells produces false positive at rates up to 53%, most likely due as a result of p53 deficiency in these cell lines. Since there was an equivocal *in vitro* test, a definitive *in vivo* mouse micronucleus test was conducted to further evaluate if 3-FL has the potential to induce micronuclei in whole animals. 3-FL did not increase the frequency of micronuclei in reticulocytes in blood from mice exposed to an oral dose ≤2000 mg/kg bw. Thus, 3-FL should be considered non-genotoxic in mammals. Published micronuclei studies (both *in vitro* and *in vivo*) on other human-milk oligosaccharides have shown these materials not to be genotoxic, as would be expected for a compound produced by lactating women (Coulet et al 2014, Kobayashi et al 2009, Patschat et al 2020, Phipps et al 2018).

References:

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16. Please confirm that a literature search was conducted to encompass all relevant published safety data on the intended uses of 3-FL. Please also state search terms, time frames, and databases utilized.

Q16 Response:
Google Scholar and the National Library of Medicine's PubMed were searched for published safety data on 3-FL. Search terms included 3-FL, 3-fucosyllactose, fucosyllactose, difucosyllactose, 2'-fucosyllactose, oligosaccharides, tetraose, neotetraose, fucopentaose, breast milk. Searches were performed multiple times between 2018 and 2019 with the last done in March 2019.

17. On page 19, DuPont states that for non-exempt term infant formula and formulas intended for children 12 months of age and older, the maximum intended use level of 3-FL is 2.0 g/L as prepared.

    a. On pages 29, 30, and 32 of the notice, DuPont provides data on the reported concentrations of 3-FL in human milk. Specifically, DuPont reports data from Erney et al., 2000; Smilowitz et al., 2013; Austin et al., 2016; Gabrielli et al., 2011; and Sumiyoshi et al., 2003. OFAS notes that DuPont did not reference or discuss a systematic review published by Thurl et al., 2017. In this study, the authors applied robust inclusion and exclusion criteria for the selection of 21 studies, 7 of which were used to report a mean and 95% confidence interval for the concentration of 3-FL in human milk for term deliveries. In DuPont's notice, only Smilowitz et al., 2013 met the Thurl et al., 2017 inclusion criteria and was included in the analysis for 3-FL.

       We note that two of the studies discussed by DuPont failed to meet the inclusion criteria and are excluded from the Thurl et al., 2017 analysis (see Tables S1 and S6):

       Austin et al., 2016
       Sumiyoshi et al., 2003

We further note that two studies met the inclusion criteria but were not included in the estimation of 3-FL concentrations in human milk from term deliveries (Tables 2 and 3):

Erney et al., 2011 (undefined secretor status for neutral HMOs)
Gabrielli et al., 2011 (preterm infants)

On page 33, DuPont states, "At 0.24 g/kg bw/d, the mean (user only) dietary exposure levels of 3-FL in the most sensitive subpopulation, infants (0-6 months) is well within the 3-FL intake range of breastfed infants (0.35 g/kg bw/d)." However, the intake level of 0.35 g/kg bw/d for breastfed infants is derived from reported 3-FL concentrations (i.e., 2.73 g/L and 2.57 g/L; page 32) that are higher than the mean and the value for the upper 95% confidence interval reported by Thurl et al., 2017. Furthermore, we note (see below) that the levels of 3-FL change with period of lactation, and it is not clear whether this was taken into account in your estimation. Please provide a clear, detailed rationale that discusses the specific studies, study data, and the analysis (if any) used by DuPont to justify the proposed use level of 2.0 g/L for 3-FL for all infants expected to consume this ingredient.

b. Data on 3-FL in human milk as reported in Thurl et al., 2017 (Table S2-A) indicate that the levels of 3-FL increase with period of lactation. Additionally, the mean and the upper 95% confidence limit for 3-FL levels in human milk from all lactation days (i.e., 0 to > 100 days) are less than the fixed use level of 2.0 g/L proposed by DuPont. Thus, it is not clear how the levels of 3-FL found in human milk support safety of 3-FL in very young term infants, especially when their gastrointestinal system is considered underdeveloped compared to those of older infants. Given the lack of clinical studies demonstrating the tolerability in younger infants, please provide DuPont's rationale that the proposed use level is tolerable to all infants.

Q 17 Response:
The careful review by FDA of the sources of data regarding 3-FL concentrations in breast milk identified an important reference that was inadvertently omitted, i.e., Thurl et al. 2017. We have considered FDA's view that the GRAS Panel may have been working with less comprehensive information which may have impacted the conclusion they reached.

For this reason, we felt that FDA's questions could only be addressed by returning to the GRAS Panel with complete information and asking them to re-evaluate their previous conclusion. The complete Q17 asked by FDA was provided to the GRAS Panel along with the attachments listed below.

1. The GRAS notice from April 2020
2. The Conclusion of the Expert Panel from April 2020
3. The five studies of the 3-FL content of breast milk cited in the GRAS notice
   a. Austin et al. 2016
   b. Erney et al. 2000
   c. Gabrielli et al. 2011
   d. Smilowitz et al. 2013
   e. Sumiyoshi et al. 2003
4. Thurl et al. 2017,

5. A comprehensive literature review of the studies of the 3FL content of breast milk published through January 2021, which included Table 2 below (references provided at the end of the response letter)

Table 2. 3-FL Content of Human Milk (based on literature review through January 2021).

Reference	Location	Gestation length	# of Milk samples analyzed	# of mothers	Duration of lactation (days)	3-FL concentration (g/L)	Additional information
International Studies							
Samuel *et al.*, 2019	Spain, France, Italy, Norway, Portugal, Romania, Sweden	Full and preterm	236	236	2	0.42	Q1-Q3: 3-FL = 0.16 - 0.42
			289	289	17	0.59	Q1-Q3: 3-FL = 0.24 - 0.70
			260	260	30	0.72	Q1-Q3: 3-FL = 0.30 - 0.87
			242	242	60	0.97	Q1-Q3: 3-FL = 0.48 - 1.26
			233	233	90	1.14	Q1-Q3: 3-FL = 0.67 - 1.53
			223	223	120	1.21	Q1-Q3: 3-FL = 0.66 - 1.64
McGuire *et al*., 2017	Spain	No details	41	41	14 - 153	0.10	Secretor 3-FL = 0.11 g/L
							Non-secretor 3-FL = 0.077 g/L[1]
	Sweden	No details	24	24		0.23	Secretor 3-FL = 0.27 g/L
							Non-secretor 3-FL = 0.077 g/L
	Peru	No details	43	43		0.10	Secretor 3-FL = 0.10 g/L
							Non-secretor 3-FL = 0.022 g/L
	US (Washington)	No details	41	41		0.06	Secretor 3-FL = 0.074 g/L
							Non-secretor 3-FL = 0.028 g/L
	US (S. California - Hispanic)	No details	19	19		0.19	Secretor 3-FL = 0.20 g/L
							Non-secretor 3-FL = 0.049 g/L
	Ethiopia [2]	No details	80	80		0.090 - 0.092	Secretor 3-FL = 0.068-0.10 g/L
							Non-secretor 3-FL = 0.055-0.14 g/L
	Gambia [2]	No details	80	80		0.050 - 0.079	Secretor 3-FL = 0.063-0.078 g/L
							Non-secretor 3-FL = 0.027-0.086 g/L
	Ghana	No details	40	40		0.094	Secretor 3-FL = 0.12 g/L
							Non-secretor 3-FL = 0.05g/L
	Kenya	No details	42	42		0.095	Secretor 3-FL = 0.11 g/L
							Non-secretor 3-FL = 0.04g/L
Erney *et al*., 2000	Asia (China, Philippines, and Singapore)	No details	80 [3]	80	0 - >31	1.59	Day 0-2: 0.68 g/L
							Day 3-10: 1.38 g/L
							Day 11-30: 1.76 g/L

Reference	Location	Gestation length	# of Milk samples analyzed	# of mothers	Duration of lactation (days)	3-FL concentration (g/L)	Additional information
							Day ≥ 31: 2.15g/L
	Europe (France, Germany, and Italy)	No details	68 [4]	68	0 - >31	1.16	Day 0-2: 1.3 g/L
							Day 3-10: 0.97 g/L
							Day 11-30: 0.7 g/L
							Day ≥ 31: 1.36 g/L
	Latin America (Chile, and Mexico)	No details	197	197	3 - 452	0.76	Day 3-10: 0.66 g/L
							Day 11-30: 0.75 g/L
							Day 31-452: 0.88 g/L
	United States	No details	36 [5]	36	2 - 217	1.84	Day 3-10: 1.03 g/L
							Day 11-30: 1.48 g/L
							Day 31-217: 2.57 g/L
	Global Average	No details	381	381	3 - 452	1.15	Day 0-2: 0.87 g/L
							Day 3-10: 1.11 g/L
							Day 11-30: 0.94 g/L [6]
							Day 31-452: 1.69 g/L
Countries in the EU							
Borewicz *et al*., 2020	Netherlands	Full term	24	24	14	0.52	
			24	24	42	0.78	
			24	24	84	1.00	
Lefebvre *et al*., 2020	Germany	No details	156	156	92	2.63	Maternal Secretor Se-/Le+
						0.17	Maternal Secretor Se+/Le-
						0.91	Maternal Secretor Se+/Le+
			122	156	183	3.26	Maternal Secretor Se-/Le+
						0.22	Maternal Secretor Se+/Le-
						1.26	Maternal Secretor Se+/Le+
			28	28	365	3.43	Maternal Secretor Se-/Le+
						0.31	Maternal Secretor Se+/Le-
						1.38	Maternal Secretor Se+/Le+
Coppa *et al*., 2011	Italy	Full term	20	10	3 - 35	0.36	Maternal secretor: Se+ / Le +
			38	19	3 - 35	0.40	Maternal secretor: Se- / Le +
			12	6	3 - 35	0.41	Maternal secretor: Se+ / Le -
			8	4	3 - 35	0.44	Maternal secretor: Se- / Le -
Thurl *et al*., 2010	Germany	No details	109	22	3 - 96	0.42	Maternal secretor: Le (a- b+)
							Day 3: 0.24 g/L

Reference	Location	Gestation length	# of Milk samples analyzed	# of mothers	Duration of lactation (days)	3-FL concentration (g/L)	Additional information
							Day 8: 0.26 g/L
							Day 15: 0.38 g/L
							Day 22: 0.44 g/L
							Day 30: 0.42 g/L
							Day 60: 0.56 g/L
							Day 90: 0.67 g/L
			28	5	3-96	1.79	Maternal secretor: Le (a+ b-)
			17	3	3-96	0.15	Maternal secretor: Le (a- b-)
United States							
Spevacek *et al*., 2015	US	Full term	15	15	0-5	0.44	
			14	14	14	0.58	
			15	15	28	0.77	
Alderete *et al*., 2015	US	Gestation length ≥ 37 weeks	25	25	30	0.16	
			25	25	60	0.52	
Bao *et al*., 2013	US	1 donor preterm (24 weeks)	NS	4	3 - 29	0.03 – 1.34	Day 3 milk was colostrum
		3 donors full term					
Smilowitz, *et al*., 2013	US	Full term	52	52	90	1.22 [7]	n=40 secretors, n=12 non-secretors [8]
China							
Wu et al., 2020	China	No details	25	25	3	0.25	Secretor, Le(a+b−)
			27	27	7	0.23	
			28	28	21	0.33	
			15	15	42	0.53	
			10	10	77	0.77	
			7	7	168	1.03	
			13	13	3	0.60	Non-secretor, Le(a−b+)
			12	12	7	0.60	
			10	10	21	0.87	
			10	10	42	1.30	
			4	4	77	1.58	
			2	2	168	1.64	
Huang *et al*., 2019	China	No details	33	33	1-7	0.35	
			33	33	8-15	0.48	
			33	33	28-34	0.73	
Zhang *et al*., 2019	China	No details	61	61	60 - 183	0.59	

Reference	Location	Gestation length	# of Milk samples analyzed	# of mothers	Duration of lactation (days)	3-FL concentration (g/L)	Additional information
Ma *et al*., 2018	China	Full term	20	20	14	0.54	
			20	20	30	0.89	
			20	20	60	1.16	
			20	20	90	1.37	
			20	20	120	1.43	
			20	20	180	1.48	
			20	20	240	1.59	
Austin *et al*., 2016	Mainland China	Full term	88	88	5 - 11	0.51 [9]	42% of infants - Cesarean delivery
			88	88	12 - 30	0.60 [9]	48% of infants - Cesarean delivery
			90	90	30-61	0.76 [9]	59% of infants - Cesarean delivery
			90	90	61 - 122	1.16 [9]	39% of infants - Cesarean delivery
			90	90	122 - 244	1.37 [9]	38% of infants - Cesarean delivery
Other Countries							
Austin *et al*., 2019	Switzerland	Full term	28	28	7	0.35	
			26	26	14	0.46	
			28	28	21	0.49	
			28	28	28	0.56	
			28	28	35	0.62	
			27	27	42	0.68	
			27	27	49	0.72	
			28	28	56	0.73	
McJarrow *et al*., 2019	United Arab Emirates	No details	41	41	5-15	0.58	Secretor 3-FL = 1.60 g/L [10]
							Non-secretor 3-FL = 0.21 g/L
			40	40	183	1.19	Secretor 3-FL = 2.53 g/L
							Non-secretor 3-FL = 0.69 g/L
Tonon *et al.*, 2019	Brazil	Full term	10	10	17 - 45	0.69	Range 3-FL: 0.041 – 2.30 g/L
Azad *et al*., 2018	Canada	Full term	307	307	112	0.27 [7]	Secretor = 0.31 g/L [11;] Non-secretor = 0.16 g/L (n=120)
Ma *et al*., 2018	Malaysia	Full term	25	25	2	0.43	
			26	26	60	0.76	
			26	26	180	1.15	
			26	26	365	1.14	
Leo *et al.,* 2010	Samoa	No details	8	8	5-10	1.67	
			8	8	22 - 155	2.35	
Newburg *et al.,* 2004	Mexico	No details	93	93	7 - 214	0.28 [7]	Maternal secretor: Le (a-b-) (26%), Le (a- b+) (74%) [12]

Reference	Location	Gestation length	# of Milk samples analyzed	# of mothers	Duration of lactation (days)	3-FL concentration (g/L)	Additional information
Sumiyoshi *et al*., 2003	Japan	No details	16	16	4	0.23	
			15	15	10	0.28	
			16	16	30	0.43	
			15	15	100	0.45	
Chaturvedi *et al*., 2001	Mexico	No details	84	12	7	0.30	
					343	1.10	
					7 - 343	0.86	
Preterm infants							
Austin *et al*., 2019	Switzerland	Preterm	25	25	7	0.46	
			25	25	14	0.48	
			25	25	21	0.57	
			24	24	28	0.63	
			25	25	35	0.73	
			24	24	42	0.69	
			24	24	49	0.76	
			24	24	56	0.83	
			23	23	70	0.87	
			21	21	84	1.07	
			21	21	98	1.21	
			19	19	112	1.04	
Spevacek *et al*., 2015	US	Preterm	10	10	0-5	0.48 [7]	
			10	10	14	0.59 [7]	
			6	6	28	0.97 [7]	
Gabrielli *et al*., 2011	Italy	Preterm (25-30 weeks gestation)	140	35	4 - 30	0.42 - 0.74	Maternal secretor: Se+ / Le +
			72	18		1.34 - 2.22	Maternal secretor: Se - / Le +
			28	7		0.32 - 0.38	Maternal secretor: Se+ / Le –
			12	4		0.30 - 0.52	Maternal secretor: Se- / Le –
Nakhla *et al*., 1999	US	Preterm (mean gestation: 29.5 weeks)	23	13	0-33	0.43	Lewis positive (5 mothers) = 0.49 g/L
		Full term	3	2	0-33	0.16	Lewis positive (1 mother) = 0.46 g/L
Colostrum							
Asakuma *et al*., 2008	Japan	Full term	36	12	0-3	0.20 - 0.28	Colostrum only (days 0-3)
Systematic Review							
Thurl *et al*., 2017 (systematic	Various locations	Full term	79	55	0 - 4	0.24	
			48	48	5 - 10	0.27	

Reference	Location	Gestation length	# of Milk samples analyzed	# of mothers	Duration of lactation (days)	3-FL concentration (g/L)	Additional information
review) [13]			107	66	11 - 30	0.34	
			30	30	31 - 60	0.64	
			68	68	61 - 100	0.67	
			33	11	>100	1.24	
		Preterm	68	68	0 - 4	0.24	
			44	44	5 - 10	0.34	
			115	71	11 - 30	0.32	
			3	3	31 - 60	0.54	

Expert Panel response:

Dr. George Fahey provided the following assessment with which Dr. Michael Pariza was in agreement:

1. *The concentrations of 3-FL vary widely in human milk.*
   a. *New literature review – 0.24 to 1.64 g/L (3.26 g/L in German women with the Se-/Le+ gene)*
   b. *Austin et al. – 0.05 to 5.9 g/L*
   c. *Erney et al. – 0.76 to 1.84 g/L*
   d. *Gabrielli et al. – 0.30 (Se-/Le- group) to 2.22 (Se-/Le+ group) g/L depending on sampling day*
   e. *Smilowitz et al. - 0.054 to 0.63 g/L*
   f. *Sumiyoshi et al. – 0.004 to 1.492 g/L*
2. *The methodology to extract 3-FL from the milieu of compounds found in human milk varies widely (storage times and conditions, temperatures, solvent used, labeling compounds, internal standards added, etc.) as does the instrumentation used to measure HMOs.*
3. *The new literature report indicates that the intake of 3-FL could be anywhere from 0.19 to 1.49 g/d assuming milk intakes of 800 to 1,200 mL/d (1 L average) by a 6.7 kg infant. European data suggest values of 1.31-1.97 g/d using the same assumptions. The example of German women with the Se-/Le+ gene results in values of 2.61 to 3.91 g 3-FL/d.*
4. *Given the information provided, I have no problem with the 2 g/L recommendation.*

Dr. Berthold Koletzko provided a more conservative opinion as follows:

It is true that 2g/L is within the range of reported human milk contents, but as FDA notes this is clearly higher than mean or median values. I wonder whether it might be an option to address the outspoken concerns of FDA … by calculation, e.g., from the new literature review the mean and median concentrations and the

95% CI and to stay within that range for infant formula, while higher ranges may be justified in products used beyond infancy?

In summary, Drs. Fahey and Pariza remain comfortable with their original conclusion that addition of 2.0 g 3-FL/L infant formula for neonates and older infants is safe and GRAS, while Dr. Koletzko expressed interest in examining the complete distribution of concentrations of 3-FL in breast milk and limiting the addition of 3-FL to approximately the 95th percentile of that distribution.

We followed Dr. Koletzko's suggestion and calculated the mean and standard deviation of the analyzed concentrations of 3-FL in the milk of mothers of term infants in the attached table. We also calculated the percentile represented by the intended addition level of 2.0 g/L.

The mean±SD concentration of 3-FL in the breast milk of mothers of term infants, based on the 24 published studies cited in the table, is 0.79±0.63 g/L. The intended addition level of 2.0 g/L lies at the 96.3rd percentile. While this is slightly higher than the 95th percentile suggested by Dr. Kolezko, we believe that this is within the spirit of his suggestion.

The important implication of Dr. Koletzko's comment is that the safety determination is not limited to the mean or median of the 3-FL concentration distribution, but rather that there is a reasonable certainty that levels in the upper tail of the distribution are safe.

In conclusion, the three members of the GRAS Panel are in agreement that the safety of the intended addition level of 2 g 3-FL/L is supported by the range of levels found in samples of breast milk from Se+/Le+, Se+/Le-, Se-/Le+, and Se-/Le- secreters worldwide. We have shared our response to FDA with the three members of the Expert Panel and they unanimously agree with it.

Further, the safety of this level of 3-FL addition is supported by acute and subchronic toxicity studies in male and female Crl:CD®(SD) rats. No indications of toxicity were seen at the limit dose of 5000 mg 3-FL/kg bw in the acute study or at the high dose of 5.98 and 7.27 g 3-FL/kg bw/day in male and female rats, respectively, in the subchronic study. Safety was further supported by testing for genetic toxicity using *in vitro* assays (bacterial reverse mutation [Ames] test, mammalian cell micronucleus assay in Chinese hamster ovary cells, and chromosomal aberration test in human lymphocytes) and *in vivo* testing in mice using the mammalian erythrocyte micronucleus test.

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JHeimbach LLC

April 27, 2021

Ellen Anderson
Regulatory Review Scientist
Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
U.S. Food and Drug Administration

Dear Dr. Anderson:

On April 9, you sent me an email with additional FDA questions regarding Danisco's GRAS notice GRN 000951 for the intended use of 3-fucosyllactose (3-FL). This letter provides responses to these questions.

There are the following attachments to this response:

- Danisco's responses to FDA's questions
- As requested, a new version of Part 1 reflecting the change of ownership of Danisco
- As also requested, a new Statement of the Conclusion of the GRAS Panel and signatures of all three members of the GRAS Panel
- Accreditation of Eurofins Microbiological Laboratory West
- Accreditation of Eurofins Scientific Finland
- Current scope of accreditation of Eurofins Scientific Finland
- Statement of Eurofins Scientific Finland

I trust that our responses to your questions, along with these additional attached documents, provide complete and fully satisfactory answers. If you require further information, please let me know.

Sincerely,

James T. Heimbach, Ph.D., F.A.C.N.
President

Encl.

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A. In the February amendment (response to Question #17), Danisco attempted to address the issues we raised1 regarding the intended use level of 3-fucosyllactose (3-FL) in infant formula by stating that the intended use level of 2.0 g/L lies at the 96.3 percentile of 3-FL concentrations in human milk based on Danisco's literature review and analysis of those studies published through January 2021 that measured the 3-FL content in human milk. Danisco also asserted that the safety of the intended use level was supported by acute and subchronic toxicity studies in rats.

We discussed Danisco's response during our meeting on March 24, 2021. We stated that, to our knowledge, no currently published studies or on-going clinical trials of 3-FL in infants exist, and no clinical studies of any kind exist in which a given human milk oligosaccharide (HMO) has been added to infant formula at levels as high as the upper limit of the 95% confidence interval for the mean. We also noted that levels of 3-FL in human milk generally increase as infants age, making it difficult to appropriately extrapolate a safe level and exposure to all infants expected to consume infant formula. We also mentioned that Danisco's February amendment did not provide a thorough explanation of their statistical methodology used to calculate the percentile (i.e., 96.3) of 3-FL concentrations in human milk represented by the intended use level of 2.0 g/L. Moreover, we stated it is not clear whether a complete description of the statistical methodology would satisfy the standard of general recognition given that the method has not been subject to peer review.

In your email dated March 24, 2021, and received after our meeting, you indicated that Danisco decided to reduce the intended use level of 3-FL from 2.0 g/L to 0.44 g/L in infant formula, to match the 3-FL use level indicated in GRN 000925. We do not have any questions regarding a conclusion that a 3-FL use level of 0.44 g/L is GRAS for its intended use in infant formula. For the administrative record, please confirm that Danisco intends to reduce the use level of 3-FL to 0.44 g/L in infant formula as consumed, which corresponds to the mean value reported by Thurl et al., 2017, and is the same use level as GRN 000925. Please also confirm that Danisco intends to reduce the use level of 3-FL to 0.44 g/L in toddler formula as well.

Response:

We hereby confirm that the maximum intended level of addition of 3-FL to infant formula and to toddler formula is 0.44 g/L as prepared, reduced from 2.00 g/L. This level is the mean value reported by Thurl et al. (2017) and matches that regarded as GRAS in GRN 000925.

Additionally, we have lowered intended use level for conventional foods by the same proportion (0.44/2.00). See new use levels in revised Table 5 below.

Revised Table 5: Proposed Food Applications and Use Levels of 3-FL.

Food Category	Food Uses	3FL Maximum Proposed Use Level (g/serving)	3FL Maximum Proposed Use Level (g/kg)	Representative Foods in NHANES
Formula for infants and young children	Infant formula (0-12 months)	0.04	0.44	Infant formulas, ready-to-feed, prepared from powder/concentrate
	Formula targeted to young children aged 1-3 years	0.04	0.44	Toddler formulas, ready-to-feed, prepared from powder or concentrate
Foods for infants	Cereal & grain products, dry RTE (ready-to-eat) cereals, cookies, teething biscuit & toasts	0.03	4.4	Dry cereals (e.g., barley, mixed, oatmeal, rice, brown rice, multigrain), snack and sweets (e.g., cereal bars, cookies and teething cookies, crackers, puffs, crunchy snacks)
Foods for young children	Cereal & grain products, dry RTE (ready-to-eat) cereals, cookies, teething biscuit & toasts	0.09	4.4	Dry cereals (e.g., barley, mixed, oatmeal, rice, brown rice, multigrain), snack and sweets (e.g., cereal bars, cookies and teething cookies, crackers, puffs, crunchy snacks)
Juice and drinks for infants and young children	Juice/drinks	0.04	0.44	Juices including apple, mixed fruit, orange, pear, and grape juices
Baked goods & baking mixes	Cereal & nutrition bars	0.26	6.6	Cereal bars (e.g., Nutri-Grain bars, milk 'n cereal bar, granola bars) and nutrition bars (e.g., meal replace-ment bars, Cliff Bar, PowerBar, Slim Fast Bar, Zone Perfect Bar)
Beverages & beverage bases, nonalcoholic	Enhanced or fortified water	0.07	0.26	Enhanced or fortified waters (e.g., Propel Water, Glaceau Vitamin Water, SoBe Life Water)
	Energy, sports & isotonic drinks & mixes	0.07	0.26	Regular and low-calorie sport drinks (e.g., Gatorade, Powerade) and energy drinks (Full Throttle, Monster, NOS, Red Bull, Rockstar)
Breakfast cereals	Hot cereals	0.35	6.8	Oatmeal, cream of rice, cream of wheat, cream of rye, whole wheat hot cereal, oat bran hot cereal, grits, cornmeal mush
Breakfast cereals	RTE cereals	0.35	8.8	All types of RTE cereals

B. Since the GRAS panel deliberations in the notice were based on an intended use level of 2.0 g/L, please provide a statement summarizing the GRAS panel's conclusion on the new intended use level of 0.44 g/L in infant formula and toddler formula.

Response:

We attach a revised Conclusion of the GRAS Panel. It includes the statement:

*"*Intended uses of 3FL include infant formula, formula intended for young children 12 months of age and older, and other foods and beverages that are consumed by toddlers under 3 years of age and by the general US population aged 3 years and above. Proposed use levels are up to 0.44 g/L in infant formula and formula intended for young children 12 months of age and older."

We also attach signature pages to the Conclusion, signed by Drs. George C. Fahey, Jr., Berthold V. Koletzko, and Michael W. Pariza. The signature page includes the statement:

"We unanimously conclude that the intended use of 3-fucosyllactose produced by *E. coli* K12 MG1655 INB008971, manufactured consistent with current good manufacturing practice (cGMP) and meeting the food-grade specifications presented in the monograph, is safe and is GRAS by scientific procedures for addition to infant formula at up to 0.44 g/L."

C. Please provide an updated dietary exposure reflective of the reduced use level of 3-FL in infant formula and toddler formula.

Response:

Question F requested revised Tables 6 and 7 due to confused titling and labeling. We present these revised tables in our response to Question F. These revised tables include updated dietary exposure estimates reflecting the reduced use levels of 3-FL in infant formula, toddler formula, and other foods.

D. In the February amendment, we were informed of a business acquisition that resulted in a change to the name and address of the notifier. Please provide a revised "Part 1- Signed statements and certification" in its entirety that contains the updated information.

Response:

A revised Part 1 is attached; the notifier is amended to "Danisco USA Inc., a wholly owned subsidiary of International Flavors and Fragrances, Inc." Further, all mentions of DuPont in Part 1 have been changed to Danisco (with the exception of the mailing address for the notifier, which remains the DuPont Experimental Station).

E. In the February amendment (response to Question #3), Danisco provided analytical data on trace minerals from three nonconsecutive batches of 3- FL. The method used to test for trace minerals in 3-FL is a modification of DIN EN ISO 17294-2 (2017-01). We note that Danisco did not provide the requested statement indicating that the modified method was validated for the intended purpose. Please provide the appropriate statement.

Response:

Danisco confirms that the modified method used to test for trace minerals in 3-FL has been validated for this purpose.

F. In the February amendment (response to Question #6), Danisco stated that the estimates of dietary exposure provided in Tables 6 and 7 on page 22 of the notice are correct and are expected to be similar for certain populations. We note that we did not question the estimates; rather, the purpose of Question #6 was to obtain clarification for the administrative record on why values in columns titled "N" and "% Users" in Tables 6 and 7 are identical. As we mentioned in our previous letter, it is assumed that 100% of the population are users for per capita estimates. As you have already suggested, it would be better to not include the "% Users" column in Table 6. In addition, we suggest including a clear description for "N" in each table indicating that N includes both users and non-users. Please provide revised Tables 6 and 7.

Response:

In addition to the wording changes and the deletion of the column of % Users from Table 6, revised Tables 6 and 7 also reflect the reduced intakes of 3FL due to the reduction in the maximum addition level to 0.44 g/L in infant and toddler formulas and proportional reduction in all other foods, as explained in our response to Question A.

Revised Table 6. Per Capita Total 3FL Intake from All Proposed Uses by the Total U.S. Population and Subpopulations

Population/ Subpopulation	Total respondents in population, including both users and nonusers	Mean		90th Percentile	
		g/day	g/kg bw/day	g/day	g/kg bw/day
Total US Population	12130	0.374	0.007	0.990	0.018
Infants 0-6 mo	259	0.242	0.040	0.506	0.084
Infants 7-12 mo	294	0.374	0.042	0.682	0.075
Toddlers 13-35 mo	673	0.374	0.031	0.946	0.081
Children 3-12 y	2611	0.352	0.013	0.748	0.031
Adolescents 13-18 y	1421	0.330	0.004	0.748	0.013
Adults 19-49 y	3537	0.374	0.004	0.990	0.013
Adults 50+ y	3335	0.418	0.004	1.122	0.015

Revised Table 7. Total 3FL Intake from All Proposed Uses by Those Individuals in the Total U.S. Population and Subpopulations Who Reported Consuming One or More of the Target Foods.

Population/ Subpopulation	Total respondents in population, including both users and nonusers	% Users	Mean		90th Percentile	
			g/day	g/kg bw/day	g/day	g/kg bw/day
Total US Population	12130	81	0.484	0.009	1.100	0.022
Infants 0-6 mo	259	72	0.330	0.053	0.550	0.088
Infants 7-12 mo	294	97	0.374	0.042	0.682	0.077
Toddlers 13-35 mo	673	96	0.396	0.033	0.968	0.084
Children 3-12 y	2611	96	0.352	0.013	0.770	0.031
Adolescents 13-18 y	1421	87	0.374	0.007	0.858	0.013
Adults 19-49 y	3537	77	0.484	0.007	1.144	0.015
Adults 50+ y	3335	77	0.550	0.007	1.298	0.018

G. In the February amendment (response to Question #7), Danisco stated that "based upon the 90th percentile per-user intake of 3-FL by infants aged 0-6 months (2.5 g/day), anticipated intake would be a maximum of 0.125 g/day for lactose and 0.050 g/day each for fucose, galactose/glucose, and other carbohydrates." We note that, considering the limits established for the content of fucose, galactose/glucose, and other carbohydrates (each ≤3%), the maximum exposure of 0.050 g/day provided for each of these components is incorrect. Please provide updated estimates for fucose, galactose/glucose, and other carbohydrates as well as lactose based on the reduced use level of 3-FL (i.e., 0.44 g/L).

Response:

The reduction in the intended use level of 3-FL from 2.0 g/L to 0.44 g/L results in a concomitant reduction in the intake of 3-FL and of other constituents of the product. With this reduction, the 90th-percentile per-user intakes of 3-FL by infants age 0-6 months, and of lactose, fucose, galactose/glucose, and other carbohydrates are as follows:

3-FL: 0.55 g/day

Lactose: 0.03 g/day

Fucose: 0.01 g/day

Galactose/glucose: 0.01 g/day

Other carbohydrates: 0.01 g/day

H. In the February amendment (response to Question #8), Danisco explained that the body weight of 6.25 kg was calculated based on the average body weight at birth to average body weight at 6 months reported in the WHO Growth Standards https://www.cdc.gov/growthcharts/who_charts.htm#The%20WHO%20Growth%20Charts). We discussed Danisco's response during our meeting on March 24, 2021. We noted that the body weights at birth stated by Danisco (3.8 kg and 3.3 kg for boys and girls, respectively) are not reported in the referenced data tables (https://www.cdc.gov/growthcharts/who/boys_length_weight.htm and https://www.cdc.gov/growthcharts/who/girls_length_weight.htm). In your email dated March 24, 2021, and received after our meeting, you indicated that the 6.25 kg body weight was calculated from the intake data reported in Table 7 of Section 3.2 of the notice. For the administrative record, please provide a detailed explanation of how the average body weight of 6.25 kg was determined.

Response:

As we indicated in our letter, we calculated the average body weight of infants aged 0-6 months as 6.25 as follows: Based on NHANES data reported in the original Table 7, the mean intake of 3FL by infants aged 0-6 months was 1.5 g/day or 0.24 g/kg bw/day. (The per kg weights are based on individual weights measured or reported during the NHANES data collection.) The average body weight of infants consuming formula was calculated as:

$$1.5\ g / 0.24\ g/kg = 6.25\ kg$$

I. In the February amendment (response to Question #10), Danisco stated that using a 100 g volume of analyte for *Cronobacter sakazakii* and *Salmonella* serovar testing provides "a more rigorous standard" than a smaller sample size of 10 g and 25 g respectively for *C. sakazakii* and *Salmonella*. Please correct this statement and provide information that the analyses performed for *C. sakazakii* and *Salmonella* used the sample sizes specified in the cited methods and provide results from analyses of three nonconsecutive batches, or provide evidence and rationale that the methods used to analyze for *C. sakazakii* and *Salmonella* are validated for the stated sample sizes and for their stated purposes.

Response:

Based on FDA's comments, we would like to withdraw our statement that using a 100 g volume of analyte for Cronobacter sakazakii *and* Salmonella *serovar testing provides "a more rigorous standard" than a smaller sample size of 10 g and 25 g respectively for* C. sakazakii *and* Salmonella.

Please see the attached documents from the testing laboratories, Eurofins Scientific Finland Ltd, Raisio, Finland, and Eurofins Laboratoires De Microbiologie Ouest, Nantes, France, which include a statement, accreditation and scope for EUROFINS LABORATOIRES DE MICROBIOLOGIE OUEST (note translation on p 6/13 for Cronobacter*), accreditation for Eurofins Finland, and scope for Eurofins Finland. The statement indicates that the cited methods for* C. sakazakii *and* Salmonella *have been validated for their stated purpose and provide an explanation that neither method specifies a sample size.*

J. In the February amendment (response to Question #12), Danisco provided a mathematical conversion, as described in a document from the Center for Drug Evaluation and Research (CDER), to convert a 3-FL dose in rats into a human equivalent dose to address the safety of polyols in the notified 3-FL ingredient. We note that this type of calculation is not relevant for assessing the safety of food ingredients. Please provide a brief safety narrative that addresses the question as originally stated.

Response:

FDA's Question 12 asked, "On page 8, DuPont states, 'The residual components were identified as mono-, di-, and trimeric carbohydrates with a polyol nature.' Given that these 'other carbohydrates' appear to comprise up to 3% of the final product (Table 2; page 17), please identify these polyols and/or discuss why they are not a safety concern."

According to Cavalli et al., (2006) human milk contains approximately 327 mcg/ml of a variety of polyols.[1] Based on these human milk levels and infant's body weights (per the CDC infant growth charts[2]), it is possible to determine an approximate exposure to these polyols for infants age 0-months to 6.5 months. The following assumptions were used to calculate exposure to polyols via human milk and formula made using 3-FL:

- *260 ml human milk/kg BW/day[3]*
- *260 ml formula/kg BW/day*
- *Average infant body weight in the 3rd and 97th percentiles (per CDC growth charts)*
- *0.44 g 3-FL/L formula*
- *3% Polyol content in 3-FL*

Example calculation for determining Polyol exposure via formula:

$$260\ ml\ (breast\ milk/kg\ BW)/day * 2.4\ kg\ BW\ \div 1000\ ml/L = 0.624\ L\ human\ milk/day$$

$$0.624\ L/day * 0.44\ g\ 3FL/L\ formula = 0.275\ g\ 3FL/day$$

$$0.275\ g\ 3\ FL/day * 0.03\ g\ Polyol/g\ 3FL = 0.00825\ g\ Polyol/day$$

$$0.00825\ g\ Polyol/day * 1000\ mg/g = 8.25\ mg\ Polyol/day$$

Comparing potential exposure from human milk and formula for small and large infants provides the following table:

[1] Cavalli C, Teng C, Battaglia FC, Bevilacqua G. 2006. Free sugar and sugar alcohol concentrations in human breast milk. *J Pediatr Gastroenterol Nutr* 42(2):215-221. doi: 10.1097/01.mpg.0000189341.38634.77

[2] https://www.cdc.gov/growthcharts/html_charts/wtageinf.htm#males

[3] EFSA Scientific Committee. 2017. Guidance on the risk assessment of substances present in food intended for infants below 16 weeks of age. *EFSA J* 15(5):4849 https://doi.org/10.2903/j.efsa.2017.4849

Age (in months) 3rd percentile BW	Total Polyols in Breast Milk (mg)	Total Polyols in 3-FL (mg)	Relative Difference		Age (in months) 97th percentile BW	Total Polyols in Breast Milk (mg)	Total Polyols in 3-FL (mg)	Relative Difference
0	205,022	8.3	24,745		**0**	361,355	14.6	24,745
0.5	234,135	9.5	24,745		**0.5**	402,855	16.3	24,745
1.5	288,944	11.7	24,745		**1.5**	480,461	19.4	24,745
2.5	339,519	13.7	24,745		**2.5**	551,391	22.3	24,745
3.5	386,193	15.6	24,745		**3.5**	616,240	24.9	24,745
4.5	429,264	17.3	24,745		**4.5**	675,567	27.3	24,745
5.5	469,006	19.0	24,745		**5.5**	729,893	29.5	24,745
6.5	505,675	20.4	24,745		**6.5**	779,704	31.5	24,745

The contribution of polyols from 3-FL in formula is >24,000 times less than the total amount of polyol in human milk based on Cavalli et al. (2006). These low amounts of polyols available from 3-FL are unlikely to produce the major disturbances of polyol overexposure, bloating and diarrhea[4], based on comparison to exposure from breast milk. A wide variety of polyols has been identified in the urine of 3-month-old infants.[5] The total polyol urinary levels are higher in breast-fed infants than formula-fed infants (both milk- and soy-based formula). As infants have historically been fed human milk or infant formula, one can infer that these polyols derived from either human milk or formula have no adverse impact on infants and thus the small additional contribution from the 3% polyol in 3-FL would also have no adverse impact on infants.

[4] Mäkinen KK. 2016. gastrointestinal disturbances associated with the consumption of sugar alcohols with special consideration of xylitol: Scientific review and instructions for dentists and other health-care professionals. *Int J Dent* 5967907. https://doi.org/10.1155/2016/5967907.

[5] Rosa F, Mercer KE, Lin H, Sims CR, Pack LM, Goode G, Badger T, Andres A, Yeruva L. 2020. Early infant formula feeding impacts urinary metabolite profile at 3 months of age. *Nutrients* 12:3552; doi:10.3390/nu12113552.

PART 1. SIGNED STATEMENTS AND CERTIFICATIONS

1.1. GRAS Notice Submission

In accordance with 21 CFR 170.255, Danisco USA Inc. (a wholly owned subsidiary of International Flavors and Fragrances, Inc.) submits this GRAS notice through its agent James T. Heimbach, president of JHeimbach LLC, for 3-fucosyllactose (3FL) produced by a genetically engineered *Escherichia coli* K12 MG1655 production strain.

1.2. Name and Address of Notifier

Danisco USA Inc., a wholly owned subsidiary of International Flavors and Fragrances, Inc.
DuPont Experimental Station – E320
200 Powder Mill Road
Wilmington DE 19803

Notifier Contact
Angela Lim
Global Regulatory Strategy Lead (HMOs & Food Protection)
Tel: 302-695-6786
angela.lim@iff.com

Agent Contact
James T. Heimbach, Ph.D., F.A.C.N.
President
JHeimbach LLC
923 Water Street #66
Port Royal VA 22535
Tel: 804-742-5543
jh@jheimbach.com

1.3. Name of the Notified Substance

3-fucosyllactose (3FL)

1.4. Intended Conditions of Use of the Notified Substance

3FL is intended be used as a food ingredient in nonexempt term infant formulas, formula intended for young children 12 months of age and older, and other foods and beverages that are consumed by toddlers under 3 years of age and by the general US population aged 3 years and above. Proposed use levels range from 0.044 g/serving (0.44 g/L) in infant formula and formula intended for young children 12 months of age and older to levels ranging from 0.03 to 0.88 g/serving (0.26 to 8.8 g/kg) in other foods and beverages.

1.5. Statutory Basis for Conclusion of GRAS status

Danisco has concluded that the notified substance, 3-fucosyllactose (3FL), as described herein is generally recognized as safe (GRAS) under the conditions of its intended use. This GRAS conclusion was reached through scientific procedures and in concert with the views of a panel of experts who are qualified by

scientific training and experience to evaluate the safety of substances added to foods, in accordance with 21 CFR 170.30(a) and (b).

1.6. Claim of Exclusion from the Requirement for Premarket Approval

Based upon Danisco's GRAS conclusion as stated in Part 1, Section 1.5 above, it is Danisco's view that 3FL is not subject to the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act.

1.7. Availability of Data and Information

Danisco agrees to make available the data and information that are the basis for Danisco's 3FL GRAS conclusion available for review and copying at FDA's request during customary business hours at the office listed in Part 1, Section 1.2 above. A complete copy of the data and information will be provided to FDA upon request.

1.8. Disclosure under the Freedom of Information Act

This GRAS notice does not contain data and information that are exempt from disclosure under the Freedom of Information Act (FOIA), USC 552.

1.9. Certification Statement

To the best of our knowledge, this dossier/notice presents a complete, representative, and balanced submission that includes unfavorable information, as well as favorable information, known to us and pertinent to the evaluation of the safety and GRAS status of the intended use of 3FL.

1.10. FSIS Statement

Not applicable.

1.11. Name, Position, and Signature of Notifier

James T. Heimbach, Ph.D., F.A.C.N.
President
JHeimbach LLC
Agent to Danisco USA, Inc., a wholly owned subsidiary of International Flavors and Fragrances, Inc.

CONCLUSION OF THE GRAS PANEL:

DETERMINATION OF THE GRAS STATUS OF THE USE OF 3-FUCOSYLLACTOSE PRODUCED BY GENETICALLY ENGINEERED *ESCHERICHIA COLI* K12 MG1655 IN TERM INFANT AND TODDLER FORMULAS AND IN FOODS AND BEVERAGES FOR TODDLERS <3 YEARS AND THE US POPULATION ≥3 YEARS

Prepared for:
International Flavors and Fragrances, Inc.

April 2021

CONCLUSION OF THE GRAS PANEL:

We, the members of the GRAS Panel, have individually and collectively critically evaluated the publicly available information on 3-fucosyllactose produced by a genetically engineered *Escherichia coli* K12 MG1655 production strain summarized in a monograph, *Conclusion of the Expert Panel: Determination of the GRAS Status of the Use of 3-Fucosyllactose Produced by Genetically Engineered Escherichia coli K12 MG1655 in Term Infant and Toddler Formulas and in Foods and Beverages for Toddlers <3 Years and the US Population ≥3 Years* (April, 2020), and other material deemed appropriate or necessary. Our evaluation included critical evaluation of the identity and physical-chemical properties of the substance, production methods, potential exposure resulting from the intended use of 3-fucosyllactose, and published research bearing on the safety of 3-fucosyllactose produced by the genetically engineered *Escherichia coli* K12 MG1655 production strain. Our summary and conclusion resulting from this critical evaluation are presented below.

Summary

- 3-fucosyllactose, abbreviated 3FL, is a milk oligosaccharide composed of a lactose core of D-glucose and D-galactose units linked via an α(1-3) bond to L-fucose, belonging to a group of complex carbohydrates described as human milk oligosaccharides (HMOs). HMOs are the third largest component in breast milk, totaling on average 12.9 g/L in mature milk and 20.9 g/L at 4 days post-partum.
- The 3FL that is the subject of this GRAS determination is produced by using a modified *E. coli* K12 host production strain. Nuclear magnetic resonance spectroscopy comparing three batches of *E. coli*-derived 3FL to 3FL isolated from human milk showed that there is no significant difference between 3FL derived from *E. coli* K12 MG1655 sINB008971 and 3FL isolated from human milk.
- *E. coli* K12 MG1655 was derived from the well-known *E. coli* K12 strain via standard classical mutagenesis steps and is classified by ATCC as a Biosafety Level 1 microorganism. The complete genome of this strain has been sequenced and published (GenBank Entry U00096.3).
- The production strain, *E. coli* K12 MG1655 INB008971, was constructed to produce high amounts of 3FL, making use of endogenous production of GDP-fucose and transfer of this nucleotide-activated sugar to the substrate lactose. Sucrose is used as a starting molecule in the production of GDP-fucose. Two heterologous genes, EcCscB and BaSP, were inserted to enable growth on sucrose and to generate fructose. The fructose is converted to fructose-6-phosphate by fructokinase, encoded by the heterologous gene ZmFrk, and subsequently to GDP-fucose.
- Genes encoding for unwanted proteins, including those for enzymes that interfere with the desired 3FL metabolic pathway, were deleted. These deletions help to make the strain more robust in industrial production settings, increase 3FL production, and improve strain stability. The genetic make-up, growth rate, and production efficiency of the strain have been shown to be stable through 70 generations.
- No differences in pathogenicity are expected between the host strain and production strain, *E. coli* K12 MG1655 INB008971. The absence of virulence factors unique to the production strain was verified by examining the whole-genome sequencing data of

sINB008971 for the presence of genes coding for virulence factors; All virulence factors identified in the production strain were also present in the host strain. Analysis of test batches did not disclose the presence of any biogenic amines.

- No specific toxic or allergenic effects are expected from the proteins expressed by the introduced genes. These proteins are not secreted, and the cell mass is separated from the product during manufacturing. Bioinformatic analyses were carried out to assess the expressed proteins for potential allergenic cross-reactivity risk and no matches were found. The toxicity of the expressed proteins was evaluated by their homology with known protein toxins; no matches were found other than the endogenous genes EcMdfA and EcLacY.
- 3FL is produced in accordance with current good manufacturing practice (cGMP) in a food-grade facility using food-grade raw materials. It is purified to yield a minimum 3FL assay of 90%; analytical batch data demonstrate that the product is manufactured reproducibly and consistently meets the specifications established to ensure product purity and safety.
- Intended uses of 3FL include infant formula, formula intended for young children 12 months of age and older, and other foods and beverages that are consumed by toddlers under 3 years of age and by the general US population aged 3 years and above. Proposed use levels are up to 0.44 g/L in infant formula and formula intended for young children 12 months of age and older.
- On a bodyweight basis, the highest level of estimated intake of 3FL is 0.053 g/kg bw/day by infants aged 0-6 months. This is less than the amount of 3FL consumed by breast-feeding infants.
- Acute oral toxicity of 3FL derived from genetically engineered *E. coli* K12 MG1655 was assessed in female Crl:CD®(SD) rats and subchronic oral toxicity was evaluated in Crl:CD®(SD) rats of both sexes. No indications of toxicity were reported, and the NOAEL in the subchronic study was set at 5.98 and 7.27 g/kg bw/day for males and females, respectively. No signs of genetic toxicity were reported in the bacterial reverse mutation (Ames) test, chromosomal aberration test in human lymphocytes, or mammalian erythrocyte micronucleus test.

Conclusion

We, the undersigned members of the GRAS Panel, are qualified by scientific education and experience to evaluate the safety of food ingredients, including those intended for addition to infant formula. We have individually and collectively critically evaluated the materials summarized above.

3-fucosyllactose produced by *E. coli* K12 MG1655 INB008971 has been sufficiently characterized to ensure that it is a food-grade product and that no toxicity concerns from impurities exist. The 3-fucosyllactose that is the subject of this GRAS review is substantially equivalent to 3-fucosyllactose occurring in breast milk. Ingestion of 3-fucosyllactose from its intended use results in levels of intake that is within safe limits established by published animal toxicity studies and a long history of safe consumption from breast milk.

It is also our opinion that other qualified and competent scientists reviewing the same publicly available information would reach the same conclusion.

We unanimously conclude that the intended use of 3-fucosyllactose produced by *E. coli* K12 MG1655 INB008971, manufactured consistent with current good manufacturing practice (cGMP) and meeting the food-grade specifications presented in the monograph, is safe and is GRAS by scientific procedures for addition to infant formula at up to 0.44 g/L.

George C. Fahey, Jr., Ph.D.
Professor Emeritus
University of Illinois
Urbana, Illinois

Signature: _____ Date: _____

Berthold V. Koletzko, Dr med, Dr med habil (M.D., Ph.D.)
Professor of Pediatrics
University of Munich
Munich, Germany

Signature: _____ Date: _____

Michael W. Pariza, Ph.D.
Professor Emeritus
University of Wisconsin—Madison
Madison, Wisconsin
Signature: _____ Date: _____

Conclusion

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Professor Emeritus
University of Illinois
Urbana, Illinois

Signature: [redacted] Date: 4/20/21

Berthold V. Koletzko, Dr med, Dr med habil (M.D., Ph.D.)
Professor of Pediatrics
University of Munich
Munich, Germany

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George C. Fahey, Jr., Ph.D.
Professor Emeritus
University of Illinois
Urbana, Illinois

Signature: _____ Date: _____

Berthold V. Koletzko, Dr med, Dr med habil (M.D., Ph.D.)
Professor of Pediatrics
University of Munich
Munich, Germany

Signature: _____ Date: 20 April 2021

Michael W. Pariza, Ph.D.
Professor Emeritus
University of Wisconsin—Madison
Madison, Wisconsin
Signature: _____ Date: _____

Conclusion

We, the undersigned members of the GRAS Panel, are qualified by scientific education and experience to evaluate the safety of food ingredients, including those intended for addition to infant formula. We have individually and collectively critically evaluated the materials summarized above.

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Professor Emeritus
University of Illinois
Urbana, Illinois

Signature: _____ Date: _____

Berthold V. Koletzko, Dr med, Dr med habil (M.D., Ph.D.)
Professor of Pediatrics
University of Munich
Munich, Germany

Signature: _____ Date: _____

Michael W. Pariza, Ph.D.
Professor Emeritus
University of Wisconsin—Madison
Madison, Wisconsin
Signature: _____ Date: April 20, 2021

Section Laboratoires

ATTESTATION D'ACCREDITATION

ACCREDITATION CERTIFICATE

N° 1-1830 rév. 16

Le Comité Français d'Accréditation (Cofrac) atteste que :
The French Committee for Accreditation (Cofrac) certifies that :

EUROFINS LABORATOIRES DE MICROBIOLOGIE OUEST
N° SIREN : 492441001

Satisfait aux exigences de la norme **NF EN ISO/IEC 17025 : 2017**
Fulfils the requirements of the standard

et aux règles d'application du Cofrac pour les activités d'analyses/essais/étalonnages en :
and Cofrac rules of application for the activities of testing/calibration in:

ENVIRONNEMENT / QUALITE DE L'EAU
ENVIRONMENT / WATER QUALITY
AGROALIMENTAIRE / DIVERS ALIMENTS
FOOD AND FOOD PRODUCTS / FOODSTUFFS
PRODUITS CHIMIQUES ET BIOLOGIQUES, EQUIPEMENTS MEDICAUX / MILIEUX DE CULTURE
CHEMICAL AND BIOLOGICAL PRODUCTS, MEDICAL DEVICES / CULTURE MEDIA

réalisées par / *performed by* :

EUROFINS LABORATOIRES DE MICROBIOLOGIE OUEST
11, rue Pierre Adolphe Bobierre
CS 12325
44300 NANTES

et précisément décrites dans l'annexe technique jointe
and precisely described in the attached technical appendix

L'accréditation suivant la norme internationale homologuée NF EN ISO/IEC 17025 est la preuve de la compétence technique du laboratoire dans un domaine d'activités clairement défini et du bon fonctionnement dans ce laboratoire d'un système de management adapté (cf. communiqué conjoint ISO-ILAC-IAF en vigueur disponible sur le site internet du Cofrac *www.cofrac.fr*)

Accreditation in accordance with the recognised international standard NF EN ISO/IEC 17025 demonstrates the technical competence of the laboratory for a defined scope and the proper operation in this laboratory of an appropriate management system (see current Joint ISO-ILAC-IAF Communiqué available on Cofrac web site www.cofrac.fr).

Le Cofrac est signataire de l'accord multilatéral d'EA pour l'accréditation, pour les activités objets de la présente attestation.
Cofrac is signatory of the European co-operation for Accreditation (EA) Multilateral Agreement for accreditation for the activities covered by this certificate.

Date de prise d'effet / *granting date :* **01/01/2021**
Date de fin de validité / *expiry date :* **31/05/2021**

Pour le Directeur Général et par délégation
On behalf of the General Director

La Responsable du Pôle Biologie-Agroalimentaire,
Pole manager - Biology-Agri-food,

Safaa KOBBI ABIL

La présente attestation n'est valide qu'accompagnée de l'annexe technique.
This certificate is only valid if associated with the technical appendix.

L'accréditation peut être suspendue, modifiée ou retirée à tout moment. Pour une utilisation appropriée, la portée de l'accréditation et sa validité doivent être vérifiées sur le site internet du Cofrac (www.cofrac.fr).
The accreditation can be suspended, modified or withdrawn at any time. For a proper use, the scope of accreditation and its validity should be checked on the Cofrac website (www.cofrac.fr).

Cette attestation annule et remplace l'attestation N° 1-1830 Rév 15.
This certificate cancels and replaces the certificate N° 1-1830 Rév 15.

Seul le texte en français peut engager la responsabilité du Cofrac.
The Cofrac's liability applies only to the french text.

Comité Français d'Accréditation - 52, rue Jacques Hillairet 75012 PARIS
Tél. : +33 (0)1 44 68 82 20 – Fax : 33 (0)1 44 68 82 21 Siret : 397 879 487 00031 www.cofrac.fr

cofrac

Section Laboratoires

ANNEXE TECHNIQUE

à l'attestation N° 1-1830 rév. 16

L'accréditation concerne les prestations réalisées par :

EUROFINS LABORATOIRES DE MICROBIOLOGIE OUEST
11, rue Pierre Adolphe Bobierre
CS 12325
44300 NANTES

Dans son unité :

- LABORATOIRE DE MICROBIOLOGIE ALIMENTAIRE
- PRELEVEMENTS

Elle porte sur :

Unité Technique : LABORATOIRE DE MICROBIOLOGIE ALIMENTAIRE

PORTÉE FIXE

# **Agroalimentaire / Divers aliments / Analyses microbiologiques** *(Analyses microbiologiques des produits et environnement agro-alimentaires – LAB GTA 59)*			
OBJET	**CARACTERISTIQUE MESUREE OU RECHERCHEE**	**PRINCIPE DE LA METHODE**	**REFERENCE DE LA METHODE**
Tous produits d'alimentation humaine	*Vibrio parahaemolyticus et Vibrio cholerae*	Recherche : Enrichissement, Isolement sur milieu sélectif	Méthode interne T-LN01-W08629
Mollusques bivalves	*Escherichia coli* - β - glucuronidase positive	Dénombrement des colonies sur milieu chromogénique sélectif TBX	Méthode interne T-LN01W025253

Portée fixe : Le laboratoire est reconnu compétent pour pratiquer les essais en respectant strictement les méthodes mentionnées dans la portée d'accréditation. Les modifications techniques du mode opératoire ne sont pas autorisées.

# **Agroalimentaire / Divers aliments / Analyses microbiologiques** *(Analyses microbiologiques des produits et environnement agro-alimentaires – LAB GTA 59)*			
OBJET	**CARACTERISTIQUE MESUREE OU RECHERCHEE**	**PRINCIPE DE LA METHODE**	**REFERENCE DE LA METHODE**
Produits destinés à la consommation humaine et à l'alimentation animale, et aux échantillons d'environnement prélevés dans la zone de production et de traitement des produits alimentaires	*Vibiro parahaemolyticus* et *Vibrio cholerae*	Recherche Isolement / Identification et confirmation	XP ISO/TS 21872-1 Juin 2017 Norme annulée
Produits destinés à la consommation humaine ou à l'alimentation animale	Staphylocoques à coagulase positive	Dénombrement des colonies à 37°C et confirmation	NF V08-057-1 Norme annulée

Portée fixe : Le laboratoire est reconnu compétent pour pratiquer les méthodes décrites en respectant strictement les méthodes reconnues mentionnées dans la portée d'accréditation.

PORTÉE FLEX 1

# **Agroalimentaire / Divers aliments / Analyses microbiologiques** *(Analyses microbiologiques des produits et environnement agro-alimentaires – LAB GTA 59)*			
OBJET	**CARACTERISTIQUE MESUREE OU RECHERCHEE**	**PRINCIPE DE LA METHODE**	**REFERENCE DE LA METHODE**
Produits destinés à la consommation humaine, aux aliments pour animaux et aux échantillons de l'environnement	Micro-organismes	Dénombrement des colonies à 30°C par la technique d'ensemencement en profondeur	NF EN ISO 4833-1
Produits destinés à la consommation humaine, aux aliments pour animaux et aux échantillons de l'environnement	Micro-organismes	Dénombrement des colonies à 30°C par la technique d'ensemencement en surface	NF EN ISO 4833-2
Produits destinés à la consommation humaine ou à l'alimentation animale, aux échantillons d'environnement du secteur agro-alimentaire	Micro-organismes	Ensemencement en surface et dénombrement des colonies à 30°C par méthode spirale	XP V08-034
Produits destinés à la consommation humaine ou à l'alimentation animale, aux échantillons d'environnement du secteur agro-alimentaire	Coliformes	Dénombrement des colonies à 30°C (ou 37°C)	NF ISO 4832
Produits destinés à la consommation humaine ou à l'alimentation animale	Coliformes présumés	Dénombrement des colonies à 30°C	NF V08-050
Produits destinés à la consommation humaine ou à l'alimentation animale, aux échantillons d'environnement du secteur agro-alimentaire	Coliformes	Recherche Enrichissement / Isolement et confirmation	NF ISO 4831
Produits destinés à la consommation humaine ou à l'alimentation animale	Coliformes thermotolérants	Dénombrement des colonies à 44°C	NF V08-060
Produits destinés à la consommation humaine ou à l'alimentation animale	Entérobactéries présumées	Dénombrement des colonies à 30°C ou 37°C	NF V08-054
Produits destinés à la consommation humaine ou à l'alimentation animale, aux échantillons d'environnement du secteur agro-alimentaire	*Enterobacteriaceae*	Recherche et dénombrement par technique NPP avec pré-enrichissement à 30°C ou 37°C	NF EN ISO 21528-1
Produits destinés à la consommation humaine ou à l'alimentation animale, aux échantillons d'environnement du secteur agro-alimentaire	*Enterobacteriaceae*	Dénombrement des colonies à 37°C (ou 30°C)	NF EN ISO 21528-2
Produits destinés à la consommation humaine ou à l'alimentation animale	*Escherichia coli* - β - glucuronidase positive	Dénombrement des colonies à 44°C	NF ISO 16649-2
Produits destinés à la consommation humaine ou à l'alimentation animale, aux échantillons d'environnement du secteur agro-alimentaire	*Escherichia coli* - β - glucuronidase positive	Recherche Enrichissement / isolement	NF EN ISO 16649-3
Produits destinés à la consommation humaine ou à l'alimentation animale, aux échantillons d'environnement du secteur agro-alimentaire	*Escherichia coli* - β - glucuronidase positive	Dénombrement par technique NPP à 37°C puis 44°C	NF EN ISO 16649-3

# **Agroalimentaire / Divers aliments / Analyses microbiologiques** *(Analyses microbiologiques des produits et environnement agro-alimentaires – LAB GTA 59)*			
OBJET	**CARACTERISTIQUE MESUREE OU RECHERCHEE**	**PRINCIPE DE LA METHODE**	**REFERENCE DE LA METHODE**
Produits destinés à la consommation humaine ou à l'alimentation animale, aux échantillons d'environnement du secteur agro-alimentaire	*Escherichia coli* présumés	Recherche Enrichissement / Isolement et confirmation	NF ISO 7251
Produits destinés à la consommation humaine ou à l'alimentation animale	*Escherichia coli* O157	Enrichissement Séparation / Concentration Isolement - Confirmation	NF EN ISO 16654
Produits destinés à la consommation humaine ou à l'alimentation animale	*Escherichia coli* - β - glucuronidase positive	Dénombrement des colonies à 44°C au moyen de membranes et milieu chromogénique	NF ISO 16649-1
Produits destinés à la consommation humaine ou à l'alimentation animale	Staphylocoques à coagulase positive	Dénombrement des colonies à 35°C ou 37°C par utilisation du milieu gélosé de Baird Parker	NF EN ISO 6888-1
Produits destinés à la consommation humaine ou à l'alimentation animale	Staphylocoques à coagulase positive	Dénombrement des colonies en aérobiose à 35°C ou 37°C par utilisation du milieu gélosé au plasma de lapin et au fibrinogène	NF EN ISO 6888-2
Produits destinés à la consommation humaine ou à l'alimentation animale, aux échantillons d'environnement du secteur agro-alimentaire	Staphylocoques à coagulase positive	Recherche par technique NPP pour les faibles nombres	NF EN ISO 6888-3
Produits destinés à la consommation humaine ou à l'alimentation animale, aux échantillons d'environnement du secteur agro-alimentaire	Bactéries sulfito-réductrices se développant en conditions anaérobies	Dénombrement des colonies à 37°C	NF ISO 15213
Produits destinés à la consommation humaine ou à l'alimentation animale	Bactéries sulfito-réductrices	Dénombrement des colonies à 46°C en anaérobiose	NF V08-061
Produits destinés à la consommation humaine ou à l'alimentation animale, aux échantillons d'environnement du secteur agro-alimentaire	Clostridium perfringens	Dénombrement des colonies à 37°C et confirmation	NF EN ISO 7937
Produits destinés à la consommation humaine ou à l'alimentation animale, aux échantillons d'environnement du secteur agro-alimentaire	Spores de *Clostridium perfringens*	Dénombrement des colonies 37°C après traitement thermique	NF EN ISO 7937 et NF V08-250
Produits destinés à la consommation humaine ou à l'alimentation animale, aux échantillons d'environnement du secteur agro-alimentaire	*Bacillus cereus* présomptifs	Dénombrement des colonies à 30°C	NF EN ISO 7932
Tous produits d'alimentation humaine et animale	*Bacillus cereus* présomptifs	Dénombrement par milieu chromogénique COMPASS® *Bacillus cereus* Agar	BKR 23/06-02/10
Produits destinés à la consommation humaine ou à l'alimentation animale	Bactéries lactiques mésophiles	Dénombrement des colonies à 30°C	NF ISO 15214
Produits destinés à la consommation humaine ou à l'alimentation animale	Bactéries lactiques mésophiles	Dénombrement des colonies à 30°C par la technique d'ensemencement en surface	NF ISO 15214 / Méthode spirale

# **Agroalimentaire / Divers aliments / Analyses microbiologiques** *(Analyses microbiologiques des produits et environnement agro-alimentaires – LAB GTA 59)*			
OBJET	**CARACTERISTIQUE MESUREE OU RECHERCHEE**	**PRINCIPE DE LA METHODE**	**REFERENCE DE LA METHODE**
Produits et ingrédients alimentaires destinés à la consommation humaine et à l'alimentation animale, échantillons environnementaux prélevés dans les secteurs de la production et de la manutention des aliments	*Cronobacter spp*	Recherche Isolement / Identification et confirmation	NF EN ISO 22964
Produits et ingrédients alimentaires destinés à la consommation humaine et à l'alimentation animale, échantillons environnementaux prélevés dans les secteurs de la production et de la manutention des aliments	*Cronobacter spp*	Recherche / Isolement Confirmation par spectrométrie de masse MALDI-TOF (Bruker MALDI Biotyper)	NF EN ISO 22964 2017LR72 Méthode certifiée par Microval
Viandes et produits à base de viande	*Pseudomonas* spp	Dénombrement des colonies à 25°C	NF EN ISO 13720
Produits carnés	*Pseudomonas* spp. présomptifs	Dénombrement sur milieu chromogénique	BKR 23-09/05-15 A
Produits laitiers	*Pseudomonas* spp.	Dénombrement sur milieu chromogénique	BKR 23-09/05-15 B
Produits destinés à la consommation humaine ou à l'alimentation animale	Levures et moisissures	Dénombrement des colonies à 25°C	NF V08-059
Produits destinés à la consommation humaine ou à l'alimentation animale	Levures et moisissures se développant sur un milieu à faible activité de l'eau	Dénombrement des colonies à 25°C	NF V08-036
Produits destinés à la consommation humaine ou à l'alimentation animale	Levures et moisissures	Dénombrement des Levures et Moisissures	3M 01/13-07/14
Produits destinés à la consommation humaine ou à l'alimentation animale à activité d'eau supérieure à 0,95	Levures et moisissures	Dénombrement des colonies à 25°C	NF ISO 21527-1
Produits destinés à la consommation humaine ou à l'alimentation animale à activité d'eau inférieure ou égale à 0,95	Levure osmophiles et moisissures xérophiles	Dénombrement des colonies à 25°C	NF ISO 21527-2
Lait et produits laitiers	Levures et moisissures	Dénombrement des colonies à 25°C	ISO 6611
Tous produits d'alimentation humaine et produits d'alimentation animale	Levures et moisissures	Dénombrement des colonies à 25°C sur milieu Symphony	BKR 23/11-12/18
Produits destinés à la consommation humaine ou à l'alimentation animale, aux échantillons d'environnement du secteur agro-alimentaire	*Salmonella* spp. dont *Salmonella* Typhi et *Salmonella* Paratyphi	Recherche Isolement / Identification et confirmation	NF EN ISO 6579-1

# **Agroalimentaire / Divers aliments / Analyses microbiologiques** *(Analyses microbiologiques des produits et environnement agro-alimentaires – LAB GTA 59)*			
OBJET	**CARACTERISTIQUE MESUREE OU RECHERCHEE**	**PRINCIPE DE LA METHODE**	**REFERENCE DE LA METHODE**
Produits destinés à la consommation humaine ou à l'alimentation animale, aux échantillons d'environnement du secteur agro-alimentaire	*Salmonella* spp. dont *Salmonella* Typhi et *Salmonella* Paratyphi	Recherche /Isolement Confirmation par spectrométrie de masse MALDI-TOF (Bruker MALDI Biotyper)	NF EN ISO 6579-1 2017LR73 Méthode certifiée par Microval
Tous produits d'alimentation humaine et animale	*Salmonella*	Recherche par milieu chromogénique RAPID *Salmonella*	BRD 07/11-12/05
Tous produits d'alimentation humaine et animale et échantillons d'environnement	*Salmonella*	Recherche par milieu chromogénique IRIS *Salmonella*®	BKR 23/07-10/11
Tous produits d'alimentation humaine et animale et prélèvements de l'environnement (hors environnement d'élevage)	*Salmonella*	Recherche par PCR en temps réel IQ-Check™ *Salmonella* II	BRD 07/06-07/04
Cultures pures de *Salmonella* spp	*Salmonella* spp	Sérotypie	FD CEN ISO/TR 6579-3
Produits destinés à la consommation humaine ou à l'alimentation animale et échantillons de l'environnement de production et de distribution des aliments	*Listeria monocytogenes* et *Listeria* spp	Recherche Isolement / Identification et confirmation	NF EN ISO 11290-1
Produits destinés à la consommation humaine ou à l'alimentation animale et échantillons de l'environnement de production et de distribution des aliments	*Listeria monocytogenes* et *Listeria* spp	Recherche / Isolement Confirmation par spectrométrie de masse MALDI-TOF (Bruker MALDI Biotyper)	NF EN ISO 11290-1 2017LR75 Méthode certifiée par Microval
Produits destinés à la consommation humaine ou à l'alimentation animale et échantillons de l'environnement de production et de distribution des aliments	*Listeria monocytogenes* et *Listeria* spp	Dénombrement des colonies à 37°C et confirmation	NF EN ISO 11290-2
Produits d'alimentation humaine et prélèvements d'environnement	*Listeria monocytogenes* et *Listeria* spp.	Recherche par milieu chromogénique RAPID'L. mono	BRD 07/4-09/98
Produits d'alimentation humaine et prélèvements d'environnement	*Listeria monocytogenes* et *Listeria* spp.	Recherche à 37°C par milieu chromogénique ALOA ONE DAY™	AES 10/03-09/00
Produits d'alimentation humaine et prélèvements d'environnement	*Listeria monocytogenes* et *Listeria* spp.	Recherche à 37°C par milieu chromogénique ALOA ONE DAY™ Confirmation par spectrométrie de masse MALDI-TOF (Bruker MALDI Biotyper)	AES 10/03-09/00 2017LR75 Méthode certifiée par Microval
Produits d'alimentation humaine et prélèvements d'environnement	*Listeria monocytogenes*	Dénombrement par milieu chromogénique RAPID' L. mono	BRD 07/05-09/01
Tous produits d'alimentation humaine	*Listeria monocytogenes*	Dénombrement à 37°C par milieu chromogénique ALOA COUNT™	AES 10/05-09/06
Produits d'alimentation humaine et prélèvements d'environnement	*Listeria monocytogenes*	Recherche en 24h par milieu chromogénique COMPASS® *Listeria* Agar	BKR 23/02-11/02

# **Agroalimentaire / Divers aliments / Analyses microbiologiques** *(Analyses microbiologiques des produits et environnement agro-alimentaires – LAB GTA 59)*			
OBJET	**CARACTERISTIQUE MESUREE OU RECHERCHEE**	**PRINCIPE DE LA METHODE**	**REFERENCE DE LA METHODE**
Produits d'alimentation humaine et prélèvements d'environnement	*Listeria monocytogenes*	Dénombrement en 24 à 48h par milieu chromogénique COMPASS® *Listeria* Agar	BKR 23/05-12/07
Produits destinés à la consommation humaine ou à l'alimentation animale, aux échantillons d'environnement du secteur agro-alimentaire	*Campylobacter* spp.	Recherche Isolement / Confirmation du genre	NF EN ISO 10272-1
Produits destinés à la consommation humaine ou à l'alimentation animale, aux échantillons d'environnement du secteur agro-alimentaire	*Campylobacter* spp.	Recherche / Isolement Confirmation par spectrométrie de masse MALDI-TOF (Bruker MALDI Biotyper)	NF EN ISO 10272-1 2017LR74 Méthode certifiée par Microval
Produits destinés à la consommation humaine ou à l'alimentation animale, aux échantillons d'environnement du secteur agro-alimentaire	*Campylobacter* spp.	Dénombrement des colonies à 41,5°C	NF EN ISO 10272-2
Produits destinés à la consommation humaine ou à l'alimentation animale, aux échantillons d'environnement du secteur agro-alimentaire	*Campylobacter* spp.	Dénombrement des colonies à 41,5°C Confirmation par spectrométrie de masse MALDI-TOF (Bruker MALDI Biotyper)	NF EN ISO 10272-2 2017LR74 Méthode certifiée par Microval
Viandes, Volailles et prélèvement de d'environnement	*Campylobacter* spp.	Dénombrement par la méthode CampyFood ID Agar	2009LR28 Méthode certifiée par MICROVAL
Produits destinés à la consommation humaine et à l'alimentation animale, et aux échantillons environnementaux dans le domaine de la production et de la manipulation de denrées alimentaires	*Vibrio parahaemolyticus* ~~et~~ *Vibrio cholerae* et *Vibrio vulnificus* potentiellement entéropathogènes	Recherche Isolement / Identification et confirmation	NF EN ISO 21872-1
Produits appertisés et assimilés	Stabilité	Incubation, pH, examen macroscopique et microscopique	NF V08-408
Produits alimentaires en conserves	pH	Potentiométrie	NF V08-409

Le laboratoire est reconnu compétent pour pratiquer les essais en suivant les méthodes référencées et leurs révisions ultérieures

PORTEE FLEX 2

Portée générale 1

# **Agroalimentaire / Divers aliments / Analyses microbiologiques** *(Analyses microbiologiques des produits et environnement agro-alimentaires – LAB GTA 59)*		
OBJET	**CARACTERISTIQUE MESUREE OU RECHERCHEE**	**PRINCIPE DE LA METHODE**
Produits agro-alimentaire (selon domaine d'application)	*Microorganisme*	Recherche par réaction immuno-enzymatique (ELFA) Système automatisé « VIDAS » -

Le laboratoire est reconnu compétent pour adopter toute méthode reconnue dans le domaine couvert par la portée générale.

*Portée détaillée**

# **Agroalimentaire / Divers aliments / Analyses microbiologiques** *(Analyses microbiologiques des produits et environnement agro-alimentaires – LAB GTA 59)*			
OBJET	**CARACTERISTIQUE MESUREE OU RECHERCHEE**	**PRINCIPE DE LA METHODE**	**REFERENCE DE LA METHODE**
Produits carnés crus, végétaux crus, lait cru, produits laitiers à base de lait cru et échantillons de l'environnement de production industrielle	*Escherichia coli* O157	Recherche par réaction immuno-enzymatique (ELFA) Système automatisé VIDAS® UP *E.coli O157 including H7* (VIDAS ECPT)	BIO 12/25-05/09
Tous produits d'alimentation humaine et animale et prélèvements de l'environnement (hors environnement d'élevage	*Salmonella*	Recherche par réaction immuno-enzymatique (ELFA) Système automatisé VIDAS EASY *Salmonella*	BIO 12/16-09/05
Tous produits d'alimentation humaine et animale et prélèvements de l'environnement (hors environnement d'élevage	*Salmonella*	Recherche par réaction immuno-enzymatique (ELFA) Système automatisé VIDAS EASY *Salmonella/* Confirmation par spectrométrie de masse MALDI-TOF (Bruker MALDI Biotyper)	BIO 12/16-09/05 2017LR73 Méthode certifiée par Microval
Produits d'alimentation humaine et prélèvements d'environnement	*Listeria monocytogenes et Listeria* spp.	Recherche par réaction immuno-enzymatique (ELFA) Système automatisé VIDAS® Listeria Duo	BIO 12/18-03/06
Produits d'alimentation humaine et prélèvements d'environnement	*Listeria monocytogenes et Listeria* spp.	Recherche par réaction immuno-enzymatique (ELFA) Système automatisé VIDAS® Listeria Duo Confirmation par spectrométrie de masse MALDI-TOF (Bruker MALDI Biotyper)	BIO 12/18-03/06 2017LR75 Méthode certifiée par Microval
Produits d'alimentation humaine et prélèvements d'environnement	*Listeria* spp.	Recherche par réaction immuno-enzymatique (ELFA) Système automatisé VIDAS® Listeria (VIDAS LIS)	BIO 12/02-06/94

# **Agroalimentaire / Divers aliments / Analyses microbiologiques** *(Analyses microbiologiques des produits et environnement agro-alimentaires – LAB GTA 59)*			
OBJET	**CARACTERISTIQUE MESUREE OU RECHERCHEE**	**PRINCIPE DE LA METHODE**	**REFERENCE DE LA METHODE**
Produits d'alimentation humaine et prélèvements d'environnement	*Listeria* spp.	Recherche par réaction immuno-enzymatique (ELFA) Système automatisé VIDAS® Listeria (VIDAS LIS) Confirmation par spectrométrie de masse MALDI-TOF (Bruker MALDI Biotyper)	BIO 12/02-06/94 2017LR75 Méthode certifiée par Microval
Tous produits d'alimentation humaine (sauf produits crus) et prélèvements de l'environnement	*Listeria monocytogenes*	Recherche par réaction immuno-enzymatique (ELFA) Etape d'enrichissement à 30°C Système automatisé VIDAS Listeria monocytogenes 2	BIO 12/09-07/02
Tous produits d'alimentation humaine (sauf produits crus) et prélèvements de l'environnement	*Listeria monocytogenes*	Recherche par réaction immuno-enzymatique (ELFA) Etape d'enrichissement à 30°C Système automatisé VIDAS Listeria monocytogenes 2 Confirmation par spectrométrie de masse MALDI-TOF (Bruker MALDI Biotyper)	BIO 12/09-07/02 2017LR75 Méthode certifiée par Microval
Produits d'alimentation humaine et prélèvements d'environnement	*Listeria monocytogenes*	Recherche par réaction immuno-enzymatique (ELFA) Etape d'enrichissement à 37°C Système automatisé VIDAS Listeria monocytogenes 2	BIO 12/11-03/04

* La liste exhaustive des analyses proposées sous accréditation est tenue à jour par le laboratoire.

Portée générale 2

# **Agroalimentaire / Divers aliments / Analyses microbiologiques** *(Analyses microbiologiques des produits et environnement agro-alimentaires – LAB GTA 59)*		
OBJET	**CARACTERISTIQUE MESUREE OU RECHERCHEE**	**PRINCIPE DE LA METHODE**
Produits agro-alimentaire (selon domaine d'application)	Microorganismes	Recherche par méthode PCR automatisé Système PALL GENE - Méthode en simple caractéristique recherchée

Le laboratoire est reconnu compétent pour adopter toute méthode reconnue dans le domaine couvert par la portée générale.

Portée détaillée 2*

# **Agroalimentaire / Divers aliments / Analyses microbiologiques** *(Analyses microbiologiques des produits et environnement agro-alimentaires – LAB GTA 59)*			
OBJET	**CARACTERISTIQUE MESUREE OU RECHERCHEE**	**PRINCIPE DE LA METHODE**	**REFERENCE DE LA METHODE**
Viandes crues de bœuf, produits laitiers et produits végétaux	*Escherichia coli O157 : H7*	Recherche par PCR en temps réel GeneDisc E. coli O157 : H7 Extraction Pack Food 1 / disques 06 et 12	GEN 25/06-11/08
Tous produits d'alimentation humaine et animale	*Salmonella*	Recherche par PCR GeneDisc Salmonella spp Extraction Pack Food 1 / disques 06 et 12	GEN 25/05-11/08

* La liste exhaustive des analyses proposées sous accréditation est tenue à jour par le laboratoire.

Portée générale 3

# **Agroalimentaire / Divers aliments / Analyses microbiologiques** *(Analyses microbiologiques des produits et environnement agro-alimentaires – LAB GTA 59)*		
OBJET	**CARACTERISTIQUE MESUREE OU RECHERCHEE**	**PRINCIPE DE LA METHODE**
Produits agro-alimentaire (selon domaine d'application)	Microorganismes	Recherche par méthode PCR automatisé Système BAX - Méthode en simple caractéristique recherchée

Le laboratoire est reconnu compétent pour adopter toute méthode reconnue dans le domaine couvert par la portée générale.

Portée détaillée 3*

# **Agroalimentaire / Divers aliments / Analyses microbiologiques** *(Analyses microbiologiques des produits et environnement agro-alimentaires – LAB GTA 59)*			
OBJET	**CARACTERISTIQUE MESUREE OU RECHERCHEE**	**PRINCIPE DE LA METHODE**	**REFERENCE DE LA METHODE**
Viandes crues, lait cru, fruits, végétaux, et plats cuisinés	*Escherichia coli O157 : H7*	Recherche par PCR BAX® E.coli O157 : H7 MP (automatisé)	QUA 18/04-03/08
Tous produits d'alimentation humaine et animale et prélèvements de l'environnement (hors environnement d'élevage)	*Salmonella*	Recherche par PCR BAX™ Salmonella (automatisé)	QUA 18/3-11/02

* La liste exhaustive des analyses proposées sous accréditation est tenue à jour par le laboratoire.

Portée générale 4

# **Agroalimentaire / Divers aliments / Analyses microbiologiques** *(Analyses microbiologiques des produits et environnement agro-alimentaires – LAB GTA 59)*		
OBJET	**CARACTERISTIQUE MESUREE OU RECHERCHEE**	**PRINCIPE DE LA METHODE**
Produits agro-alimentaire (selon domaine d'application)	Microorganismes	Recherche par méthode PCR automatisé Système BACGene

Le laboratoire est reconnu compétent pour adopter toute méthode reconnue dans le domaine couvert par la portée générale.

Portée détaillée 4*

# **Agroalimentaire / Divers aliments / Analyses microbiologiques** *(Analyses microbiologiques des produits et environnement agro-alimentaires – LAB GTA 59)*			
OBJET	**CARACTERISTIQUE MESUREE OU RECHERCHEE**	**PRINCIPE DE LA METHODE**	**REFERENCE DE LA METHODE**
Tous produits d'alimentation humaine et échantillons d'environnement de production	*Listeria monocytogenes* et *Listeria* spp	Recherche par PCR en temps réel BAC*Gene Listeria* Multiplex.	EGS 38/05-03/17
Tous produits d'alimentation humaine et échantillons d'environnement de production	*Listeria* spp	Recherche par PCR en temps réel BAC*Gene Listeria* spp	EGS 38/02-01/17
Tous produits d'alimentation humaine et échantillons d'environnement de production	*Listeria monocytogenes*	Recherche par PCR en temps réel BAC*Gene Listeria monocytogenes*	EGS 38/03-01/17
Tous produits d'alimentation humaine, produits pour l'alimentation animale, échantillons d'environnement de production	*Salmonella* spp	Recherche par PCR en temps réel BAC*Gene Salmonella* spp.	EGS 38/01-03/15
Cacao	*Salmonella* spp	Recherche par PCR en temps réel BAC*Gene Salmonella* spp.	Méthode validée AOAC 121501

* La liste exhaustive des analyses proposées sous accréditation est tenue à jour par le laboratoire.

Unité Technique : PRELEVEMENTS

PORTEE FIXE

Agroalimentaire / Divers aliments / Echantillonnage – Prélèvement* *(Prélèvement d'objets agroalimentaires – LAB GTA 59)*			
OBJET	**CARACTERISTIQUE MESUREE OU RECHERCHEE**	**PRINCIPE DE LA METHODE**	**REFERENCE DE LA METHODE**
Surface environnement Agroalimentaire	Prélèvements en vue d'analyses microbiologiques	Prélèvement instantané sur une surface	NF ISO 18593 Mode opératoire QT MO 003
Produits agroalimentaires hors carcasses et produits congelés en pain	Prélèvements en vue d'analyses microbiologiques	Prélèvement instantané	Mode opératoire QT MO 004

Le laboratoire est reconnu compétent pour pratiquer les échantillonnages en respectant strictement les méthodes mentionnées dans la portée d'accréditation. Les modifications techniques du mode opératoire ne sont pas autorisées.

*Le laboratoire a satisfait les exigences relatives au prélèvement d'objets en vue des essais sus cités.

PORTEE FLEX 1

Des préleveurs délocalisés sont basés à Rennes (35) et Lorient (56).

# **Environnement / Qualité de l'eau / Echantillonnage – Prélèvement** *(Echantillonnage d'eau en vue d'analyses physico-chimiques et microbiologiques – LAB GTA 29)*			
OBJET	**CARACTERISTIQUE MESUREE OU RECHERCHEE**	**PRINCIPE DE LA METHODE**	**REFERENCE DE LA METHODE**
Eaux destinées à la consommation humaine	Echantillonnage en vue d'analyses physico-chimiques et microbiologiques Echantillonnage - à la ressource - en production - en distribution	Echantillonnage instantané (prise d'un échantillon unique)	FD T 90-520 NF EN ISO 19458
Eaux de tours aéroréfrigérantes (IRDEFA)	Echantillonnage pour la recherche de Légionelles	Echantillonnage instantané (prise d'un échantillon unique)	FD T 90-522 NF EN ISO 19458 Circulaire Légionelles n° 2002/243 du 22/04/2002 Arrêté ministériel rubrique n° 2921
Eaux de réseaux sanitaires froides et chaudes	Echantillonnage pour la recherche de Légionelles	Echantillonnage instantané (prise d'un échantillon unique)	FD T 90-522 NF EN ISO 19458 Circulaire Légionelles n° 2002/243 du 22/04/2002 Arrêté ministériel du 01/02/2010 et Circulaire Légionelles n° 2010/448 du 21/12/2010

Le laboratoire est reconnu compétent pour pratiquer les échantillonnages en suivant les méthodes référencées et leurs révisions ultérieures.

PORTEE FIXE

# **Environnement / Qualité de l'eau / Echantillonnage – Prélèvement** *(Essais physico-chimiques des eaux sur site – LAB GTA 29)*			
OBJET	**CARACTERISTIQUE MESUREE OU RECHERCHEE**	**PRINCIPE DE LA METHODE**	**REFERENCE DE LA METHODE**
Eaux douces	Température (mesure instantanée)	Méthode à la sonde	Méthode interne QT MO 032

Le laboratoire est reconnu compétent pour pratiquer les échantillonnages en respectant strictement les méthodes mentionnées dans la portée d'accréditation. Les modifications techniques du mode opératoire ne sont pas autorisées.

[#] *Accréditation rendue obligatoire dans le cadre réglementaire français précisé par le texte cité en référence dans le document Cofrac LAB INF 99 disponible sur www.cofrac.fr*

Date de prise d'effet : **01/01/2021** Date de fin de validité : **31/05/2021**

La Responsable d'accréditation
The Accreditation Manager

Sonia LIBERSOU

Cette annexe technique annule et remplace l'annexe technique 1-1830 Rév. 15.

Comité Français d'Accréditation - 52, rue Jacques Hillairet 75012 PARIS
Tél. : +33 (0)1 44 68 82 20 – Fax : 33 (0)1 44 68 82 21 Siret : 397 879 487 00031 www.cofrac.fr

AKKREDITOINTITODISTUS

ACCREDITATION CERTIFICATE

EUROFINS SCIENTIFIC FINLAND OY

T089

FINAS-akkreditointipalvelun akkreditoima testauslaboratorio T089
Akkreditointipäätöksen viimeinen voimassaolopäivä: 14.03.2025
Pätevyysalue, toimipaikat ja akkreditoinnin voimassaolo: www.finas.fi

Testing laboratory No. T089 accredited by FINAS Finnish Accreditation Service
Date of expiry of the accreditation decision: 14.03.2025
Scope of accreditation, sites and current status of the accreditation: www.finas.fi

Toimielin täyttää seuraavan standardin vaatimukset:
The above body conforms of the requirements of the following standard:

SFS-EN ISO/IEC 17025:2017

Helsinki 03.02.2021

Risto Suominen

AKKREDITOITU TESTAUSLABORATORIO

ACKREDITERAT TESTNINGSLABORATORIUM

ACCREDITED TESTING LABORATORY

FINAS
Finnish Accreditation Service
T089 (EN ISO/IEC 17025)

EUROFINS SCIENTIFIC FINLAND OY

EUROFINS SCIENTIFIC FINLAND LTD

Tunnus Nummer *Code*	**Laboratorio** Laboratorium *Laboratory*	**Osoite** Adress *Address*	**www** www *www*
T089	**Eurofins Scientific Finland Oy, Raision yksikkö**	**(Raisionkaari 55, rakennus 750, ovi 6)** **PL 75** **21201 RAISIO**	**www.eurofins.fi**
	Eurofins Scientific Finland Oy, Raisio enhet	(Raisionkaari 55, rakennus 750, ovi 6) PB 75 21201 RAISIO	**www.eurofins.fi**
	Eurofins Scientific Finland Ltd, Raisio unit	*(Raisionkaari 55, building 750, door 1)* *P.O. BOX 75* *FI-21201 RAISIO* *FINLAND*	*www.eurofins.fi*
	Eurofins Scientific Finland Oy, Helsingin yksikkö (ei laboratoriotoimintoja)	**Viikinportti 2** **00790 HELSINKI**	**www.eurofins.fi**
	Eurofins Scientific Finland Oy, Helsingfors enhet (ingen laboratorieverksamhet)	Viksporten 2 00790 HELSINGFORS	www.eurofins.fi
	Eurofins Scientific Finland Ltd, Helsinki unit (no laboratory services)	*Viikinportti 2* *FI-00790 HELSINKI* *FINLAND*	*www.eurofins.fi*

Testausalat Testningsområde *Fields of testing*
Elintarviketestaus Testning av livsmedel *Testing of food*
Rehutestaus Testning av foder *Testing of feed*
Ympäristötestaus Testning av miljö *Testing of environment*

PÄTEVYYSALUE KOMPETENSOMRÅDE *SCOPE OF ACCREDITATION*		
Testattava materiaali / tuote Testat material / produkt *Material / product tested*	**Testityyppi, mittausalue** Testningstyp, mätområde *Type of test, measured range*	**Testausmenetelmä** Testningsmetod *Test method*
Elintarvike- ja rehutestaus, Kemia, Gravimetriset menetelmät Testning av livsmedel och foder, Kemi, Gravimetriska metoder *Testing of food and feed, Chemistry, Gravimetric methods*		
Yhdistelmäelintarvikkeet, Kasvikset, hedelmät, marjat ja viljat ja näistä tehdyt valmisteet, Maito ja maidosta tehdyt valmisteet, Kala ja äyriäiset ja näistä tehdyt valmisteet, Lihat ja munat ja näistä tehdyt valmisteet, Kasviperäiset rehut, eläinperäiset rehut ja rehuseokset Kombinationslivsmedel, Grönsaker, frukt, bär, spannmål samt produkter av dessa, Mjölk och mjölkprodukter, Fisk och skaldjur samt produkter av dessa, Kött och ägg samt produkter av dessa, Foder av vegetabiliskt ursprung, foder av animaliskt ursprung och foderblandningar *Composite foods, Vegetables, fruits, berries and cereals and products thereof, Milk and milk products, Fish and crustaceans and products thereof, Meats and eggs and products thereof, Feeds of plant origin, feed of animal origin and feed mix*	**Kosteus** Fukthalt *Moisture*	**NMKL 23:1991, muunneltu** / modifierad / *modified*
	Tuhka Aska *Ash*	**NMKL 173:2005, muunneltu** / modifierad / *modified*
Yhdistelmäelintarvikkeet, Kasvikset, hedelmät, marjat ja viljat ja näistä tehdyt valmisteet, Maito ja maidosta tehdyt valmisteet, Kala ja äyriäiset ja näistä tehdyt valmisteet, Lihat ja munat ja näistä tehdyt valmisteet Kombinationslivsmedel, Grönsaker, frukt, bär, spannmål samt produkter av dessa, Mjölk och mjölkprodukter, Fisk och skaldjur samt produkter av dessa, Kött och ägg samt produkter av dessa *Composite foods, Vegetables, fruits, berries and cereals and products thereof, Milk and milk products, Fish and crustaceans and products thereof, Meats and eggs and products thereof*	**Rasvapitoisuus** Fetthalt *Fat content*	**NMKL 160:1998, muunneltu** / modifierad / *modified*
Elintarvike- ja rehutestaus, Kemia, Ioni-/nestekromatografiset menetelmät Testning av livsmedel och foder, Kemi, Ion-/vätskekromatografiska metoder *Testing of food and feed, Chemistry, Liquid chromatography methods*		

PÄTEVYYSALUE KOMPETENSOMRÅDE *SCOPE OF ACCREDITATION*		
Testattava materiaali / tuote Testat material / produkt *Material / product tested*	**Testityyppi, mittausalue** Testningstyp, mätområde *Type of test, measured range*	**Testausmenetelmä** Testningsmetod *Test method*
Yhdistelmäelintarvikkeet, Kasvikset, hedelmät, marjat ja viljat ja näistä tehdyt valmisteet, Maito ja maidosta tehdyt valmisteet, Kasviperäiset rehut, eläinperäiset rehut ja rehuseokset Kombinationslivsmedel, Grönsaker, frukt, bär, spannmål samt produkter av dessa, Mjölk och mjölkprodukter, Foder av vegetabiliskt ursprung, foder av animaliskt ursprung och foderblandningar *Composite foods, Vegetables, fruits, berries and cereals and products thereof, Milk and milk products, Feeds of plant origin, feed of animal origin and feed mix*	**Laktoosi** Laktos *Lactose*	**Sisäinen menetelmä, perustuu AOAC 982.14** Egen metod, baserad på AOAC 982.14 *In-house method, based on AOAC 982.14*
Elintarvike- ja rehutestaus, Kemia, Kaasukromatografiset menetelmät Testning av livsmedel och foder, Kemi, Gaskromatografiska metoder *Testing of food and feed, Chemistry, Gas chromatography methods*		
Sterolirikastetut tuotteet, Luontaisia sterolipitoisuuksia sisältävät tuotteet, Rasvat ja öljyt sekä Kasvistanoli- ja kasvisteroliainesosat Sterolberikade produkter, Naturlikt förekommande sterolinnehåll i produkter, Fetter och oljor samt växtstanol- och växtsterolingredienser *Sterol enriched products, Natural sterol contents containing products, Fat and oils and Plant stanol and plant sterol ingredients*	**Kasvisterolien, kasvistanolien ja kolesterolin määrittäminen** Bestämning av växtsteroler, växtstanoler och kolesterol *Determination of plant sterols, plant stanols and cholesterol*	**NMKL 198:2014**

PÄTEVYYSALUE KOMPETENSOMRÅDE *SCOPE OF ACCREDITATION*		
Testattava materiaali / tuote Testat material / produkt *Material / product tested*	**Testityyppi, mittausalue** Testningstyp, mätområde *Type of test, measured range*	**Testausmenetelmä** Testningsmetod *Test method*
Rasvat ja öljyt, Yhdistelmäelintarvikkeet, Kasvikset, hedelmät, marjat ja viljat ja näistä tehdyt valmisteet, Maito ja maidosta tehdyt valmisteet, Kala ja äyriäiset ja näistä tehdyt valmisteet, Lihat ja munat ja näistä tehdyt valmisteet, Kasviperäiset rehut, Eläinperäiset rehut ja rehuseokset Oljor och fetter, Kombinationslivsmedel, Grönsaker, frukt, bär, spannmål samt produkter av dessa, Mjölk och mjölkprodukter, Fisk och skaldjur samt produkter av dessa, Kött och ägg samt produkter av dessa, Foder av vegetabiliskt ursprung, Foder av animaliskt ursprung och foderblandningar *Fats and oils, Composite foods Vegetables, Fruits, berries and cereals and products thereof, Milk and milk products, Fish and crustaceans and products thereof, Meats and eggs and products thereof, Feeds of plant origin, Feed of animal origin and feed mix*	**Rasvahappokoostumus** Fettsyrasammansättning *Fatty acid composition*	**Sisäinen menetelmä LAB-M1836, perustuu ISO 12966-1:2014** Intern metod LAB-M1836, baserad på ISO 12966-1:2014 *In-house method LAB-M1836, based on ISO 12966-1:2014*
Elintarvike- ja rehutestaus, Kemia, Titrimetriset menetelmät Testning av livsmedel och foder, Kemi, Titrimetriska metoder *Testing of food and feed, Chemistry, Titrimetric methods*		
Rasvat ja öljyt, Yhdistelmäelintarvikkeet, Kasvikset, hedelmät, marjat ja viljat ja näistä tehdyt valmisteet, Maito ja maidosta tehdyt valmisteet, Kala ja äyriäiset ja näistä tehdyt valmisteet, Lihat ja munat ja näistä tehdyt valmisteet, Kasviperäiset rehut, eläinperäiset rehut ja rehuseokset Oljor och fetter, Kombinationslivsmedel, Grönsaker, frukt, bär, spannmål samt produkter av dessa, Mjölk och mjölkprodukter, Fisk och skaldjur samt produkter av dessa, Kött och ägg samt produkter av dessa, Foder av vegetabiliskt ursprung, foder av animaliskt ursprung och foderblandningar *Fats and oils, Composite foods, Vegetables, fruits, berries and cereals and products thereof, Milk and milk products, Fish and crustaceans and products thereof, Meats and eggs and products thereof, feeds of plant origin, feed of animal origin and feed mix*	**Peroksidilukumääritys, visuaalinen titraus ja potentiometrinen titraus** Bestämninga av peroxidtal, visuell titrering och potentiometrisk titrering *Determination of peroxide value, visual titration and potentiometric titration*	**AOCS Cd 8b-90:2017, muunneltu** / modifierad / *modified*
	Vapaat rasvahapot (FFA-%) Fria fettsyror *Free fatty acids*	**AOCS Ca 5a-40:2017, muunneltu** / modifierad / *modified*

PÄTEVYYSALUE KOMPETENSOMRÅDE *SCOPE OF ACCREDITATION*		
Testattava materiaali / tuote Testat material / produkt *Material / product tested*	**Testityyppi, mittausalue** Testningstyp, mätområde *Type of test, measured range*	**Testausmenetelmä** Testningsmetod *Test method*
Elintarvike- ja rehutestaus, Kemia, Muut menetelmät Testning av livsmedel och foder, Kemi, Andra metoder *Testing of food and feed, Chemistry, Other methods*		
Yhdistelmäelintarvikkeet, Kasvikset, hedelmät, marjat ja viljat ja näistä tehdyt valmisteet, Maito ja maidosta tehdyt valmisteet, Kala ja äyriäiset ja näistä tehdyt valmisteet, Lihat ja munat ja näistä tehdyt valmisteet, Kasviperäiset rehut, eläinperäiset rehut ja rehuseokset Kombinationslivsmedel, Grönsaker, frukt, bär, spannmål samt produkter av dessa, Mjölk och mjölkprodukter, Fisk och skaldjur samt produkter av dessa, Kött och ägg samt produkter av dessa, Foder av vegetabiliskt ursprung, foder av animaliskt ursprung och foderblandningar *Composite foods, Vegetables, fruits, berries and cereals and products thereof, Milk and milk products, Fish and crustaceans and products thereof, Meats and eggs and products thereof, Feeds of plant origin, feed of animal origin and feed mix*	**Proteiinipitoisuuden määritys** Bestämning av protein *Determination of protein*	**NMKL 6:2003, muunneltu** / modifierad / *modified*
Elintarvike- ja rehutestaus, Mikrobiologia, Viljelymenetelmät, kvantitatiiviset Testning av livsmedel och foder, Mikrobiologi, Odlingsmetoder, kvantitativa *Testing of food and feed, Microbiology, Culture methods*		
Yhdistelmäelintarvikkeet, Kasvikset, hedelmät, marjat ja viljat ja näistä tehdyt valmisteet, Maito ja maidosta tehdyt valmisteet, Kala ja äyriäiset ja näistä tehdyt valmisteet, Lihat ja munat ja näistä tehdyt valmisteet, rehuseokset ja kasviperäiset rehut Kombinationslivsmedel, Grönsaker, frukt, bär, spannmål samt produkter av dessa, Mjölk och mjölkprodukter, Fisk och skaldjur samt produkter av dessa, Kött och ägg samt produkter av dessa, foderblandningar och foder av vegetabiliskt ursprung *Composite foods, Vegetables, fruits, berries and cereals and products thereof, Milk and milk*	**Aerobiset mikro-organismit, 30 °C** Aerobiska mikroorganismer, 30°C *Aerobic micro-organisms, 30 °C*	**NMKL 86:2013**
	Aerobiset mikro-organismit, 30 °C Aerobiska mikroorganismer, 30°C *Aerobic micro-organisms, 30 °C*	**ISO 4833: 2013**

PÄTEVYYSALUE KOMPETENSOMRÅDE *SCOPE OF ACCREDITATION*		
Testattava materiaali / tuote Testat material / produkt *Material / product tested*	**Testityyppi, mittausalue** Testningstyp, mätområde *Type of test, measured range*	**Testausmenetelmä** Testningsmetod *Test method*
products, Fish and crustaceans and products thereof, Meats and eggs and products thereof, feed mix and feed of plant origin	**Enterobakteerit** *Enterobakterier* *Enterobacteriaceae*	**NMKL 144:2005**
	Hiivat ja homeet Jäst och mögel *Yeasts and molds*	**NMKL 98:2005, muunneltu** / modifierad / *modified*
	Kolimuotoiset bakteerit 37 ˚C Koliforma bakterier 37 °C *Coliform bacteria 37 ˚C*	**NMKL 44:2004**
Yhdistelmäelintarvikkeet, Kasvikset, hedelmät, marjat ja viljat ja näistä tehdyt valmisteet, Maito ja maidosta tehdyt valmisteet, Kala ja äyriäiset ja näistä tehdyt valmisteet, Lihat ja munat ja näistä tehdyt valmisteet Kombinationslivsmedel, Grönsaker, frukt, bär, spannmål samt produkter av dessa, Mjölk och mjölkprodukter, Fisk och skaldjur samt produkter av dessa, Kött och ägg samt produkter av dessa *Composite foods, Vegetables, fruits, berries and cereals and products thereof, Milk and milk products, Fish and crustaceans and products thereof, Meats and eggs and products thereof*	**Alustava *Bacillus cereus*** *Presumtiva Bacillus cereus* *Presumptive Bacillus cereus*	**NMKL 67:2010, muunneltu** / modifierad / *modified*
	Escherichia coli	**ISO 16649-2:2001**
	Koagulaasipositiiviset stafylokokit ja *Staphylococcus aureus* Koagulaspositiva stafylokocker och *Staphylococcus aureus* *Coagulase positive staphylococci and Staphylococcus aureus*	**Sisäinen menetelmä, petrifilmi** Egen metod, petrifilm *In-house method, petrifilm*

PÄTEVYYSALUE KOMPETENSOMRÅDE *SCOPE OF ACCREDITATION*		
Testattava materiaali / tuote Testat material / produkt *Material / product tested*	**Testityyppi, mittausalue** Testningstyp, mätområde *Type of test, measured range*	**Testausmenetelmä** Testningsmetod *Test method*
	***Listeria monocytogenes* lukumäärä** Enumerering av *Listeria monocytogenes* *Enumeration of Listeria monocytogenes*	**Sisäinen menetelmä, RAPID'L.mono** Egen metod, RAPID'L.mono *In-house method, RAPID'L.mono*
Elintarvike- ja rehutestaus, Mikrobiologia, Kvalitatiiviset Testning av livsmedel och foder, Mikrobiologi, Kvalitativa *Testing of food and feed, Microbiology, Qualitative*		
Yhdistelmäelintarvikkeet, Kasvikset, hedelmät, marjat ja viljat ja näistä tehdyt valmisteet, Maito ja maidosta tehdyt valmisteet, Kala ja äyriäiset ja näistä tehdyt valmisteet, Lihat ja munat ja näistä tehdyt valmisteet ja tuotantoympäristö Kombinationslivsmedel, Grönsaker, frukt, bär, spannmål samt produkter av dessa, Mjölk och mjölkprodukter, Fisk och skaldjur samt produkter av dessa, Kött och ägg samt produkter av dessa och produktionsmiljö *Composite foods, Vegetables, fruits, berries and cereals and products thereof, Milk and milk products, Fish and crustaceans and products thereof, Meats and eggs and products thereof and production environment*	***Listeria spp.* ja *L. monocytogenes*, osoittaminen** Detektering av *Listeria spp.* och *L. monocytogenes* *Detection of Listeria spp. and L. monocytogenes*	**Sisäinen menetelmä, RAPID'L.mono** Egen metod, RAPID'L.mono *In-house method, RAPID'L.mono*

PÄTEVYYSALUE
KOMPETENSOMRÅDE
SCOPE OF ACCREDITATION

Testattava materiaali / tuote Testat material / produkt *Material / product tested*	**Testityyppi, mittausalue** Testningstyp, mätområde *Type of test, measured range*	**Testausmenetelmä** Testningsmetod *Test method*
Yhdistelmäelintarvikkeet, Kasvikset, hedelmät, marjat ja viljat ja näistä tehdyt valmisteet, Maito ja maidosta tehdyt valmisteet, Kala ja äyriäiset ja näistä tehdyt valmisteet, Lihat ja munat ja näistä tehdyt valmisteet, Suklaa ja suklaata sisältävät tuotteet, kasviperäiset rehut, eläinperäiset rehut, rehuseokset, hapotetut kasviperäiset rehut ja tuotantoympäristönäytteet Kombinationslivsmedel, Grönsaker, frukt, bär, spannmål samt produkter av dessa, Mjölk och mjölkprodukter, Fisk och skaldjur samt produkter av dessa, Kött och ägg samt produkter av dessa, Choklad och chokladprodukter, foder av vegetabiliskt ursprung, foder av animaliskt ursprung, foderblandningar, surgjort foder av vegetabiliskt ursprung och prover från produktionsmiljö *Composite foods, Vegetables, fruits, berries and cereals and products thereof, Milk and milk products, Fish and crustaceans and products thereof, Meats and eggs and products thereof, Chocolate and chocolate products, feed of plant origin, feed of animal origin, feed mix, acidified feed of plant origin and samples from production environmental*	***Salmonella*, osoittaminen** *Salmonella*, detektion *Salmonella, detection*	**NMKL 71:1999**
Elintarvike- ja rehutestaus, Mikrobiologia, Molekyylibiologiset menetelmät Testning av livsmedel och foder, Mikrobiologi, Molekylärbiologiska metoder *Testing of food and feed, Microbiology, Molecular biological methods*		

PÄTEVYYSALUE KOMPETENSOMRÅDE *SCOPE OF ACCREDITATION*		
Testattava materiaali / tuote Testat material / produkt *Material / product tested*	**Testityyppi, mittausalue** Testningstyp, mätområde *Type of test, measured range*	**Testausmenetelmä** Testningsmetod *Test method*
Yhdistelmäelintarvikkeet, Kasvikset, hedelmät, marjat ja viljat ja näistä tehdyt valmisteet, Maito ja maidosta tehdyt valmisteet, Kala ja äyriäiset ja näistä tehdyt valmisteet, Lihat ja munat ja näistä tehdyt valmisteet, Suklaa ja suklaata sisältävät tuotteet, kasviperäiset rehut, eläinperäiset rehut, rehuseokset, hapotetut kasviperäiset rehut ja tuotantoympäristönäytteet Kombinationslivsmedel, Grönsaker, frukt, bär, spannmål samt produkter av dessa, Mjölk och mjölkprodukter, Fisk och skaldjur samt produkter av dessa, Kött och ägg samt produkter av dessa, Choklad och chokladprodukter,foder av vegetabiliskt ursprung, foder av animaliskt ursprung, foderblandningar, surgjort foder av vegetabiliskt ursprung och prover från produktionsmiljö *Composite foods, Vegetables, fruits, berries and cereals and products thereof, Milk and milk products, Fish and crustaceans and products thereof, Meats and eggs and products thereof, Chocolate and chocolate products, feed of plant origin, feed of animal origin, feed mix, acidified feed of plant origin and samples from production environmental*	***Salmonella*, osoittaminen** *Salmonella*, detektion *Salmonella, detection*	**Sisäinen menetelmä, qPCR, BACGene** Egen metod, qPCR, BACGene *In-house method, qPCR, BACGene*

PÄTEVYYSALUE KOMPETENSOMRÅDE *SCOPE OF ACCREDITATION*		
Testattava materiaali / tuote Testat material / produkt *Material / product tested*	**Testityyppi, mittausalue** Testningstyp, mätområde *Type of test, measured range*	**Testausmenetelmä** Testningsmetod *Test method*
Ympäristötestaus, Mikrobiologia, Viljelymenetelmät, kvantitatiiviset Testning av miljö, Mikrobiologi, Odlingsmetoder, kvantitativa *Testing of environment, Microbiology, Culture methods*		
Talousvesi Hushållsvatten *Drinking water*	**Kolimuotoiset bakteerit ja *Escherichia coli*** Koliforma bakterier och *Escherichia coli* *Coliform bacteria and Escherichia coli*	**SFS-EN ISO 9308-1:2014 / A1:2017**
Talousvesi Hushållsvatten *Drinking water*	**Pesäkkeiden lukumäärä 22 °C ja 36 °C** Antal odlingsbara mikro-organismer 22 °C och 36 °C *Colony count 22 °C and 36 °C*	**SFS-EN ISO 6222:1999**
Talousvesi ja pakattu vesi Hushållsvatten och buteljerat vatten *Drinking water and bottled water*	***Pseudomonas aeruginosa***	**SFS-EN ISO 16266:2008**
Talousvesi Hushållsvatten *Drinking water*	**Suolistoperäiset enterokokit** Intestinala enterokocker *Intestinal enterococci*	**SFS-EN ISO 7899-2:2000**

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Statement of Eurofins Scientific Finland for request to provide information about analyses performed for *C. sakzakii* and *Salmonella,* regarding the sample sizes used in the methods

Eurofins Scientific Finland Ltd, Raisio, Finland and Eurofins Laboratoires De Microbiologie Ouest, Nantes, France performing analyses for *C. sakzakii* and *Salmonella* are both accredited according to standard ISO/IEC 17025:2017. Eurofins Scientific Finland accredited by FINAS Finnish Accreditation Service and date of expiry of the accreditation decision is 14th March 2025. Eurofins Laboratoires De Microbiologie Ouest is accredited by The French Committee for Accreditation (Cofrac) and date of expiry of the accreditation decision is 31st May 2021.

Analytical tests for *C. sakazakii* (Eurofins test code LN0S9) and *Salmonella* (Eurofins test code UML69) have been validated according to standard methods EN ISO 22964:2017 and NMKL 71:1999 requirements and accreditated by government inspection agencies (FINAS Finnish Accreditation Service in case of standard method NMKL 71:1999 and The French Committee for Accreditation (Cofrac) in case of standard method EN ISO 22964:2017). The scopes, sites and current status of the accreditations of laboratories can be found in attachments 1, 2, and 3.

Standard methods EN ISO 22964:2017 for *Cronobacter spp.* detection and NMKL 71:1999 for *Salmonella* detection do not specify any specific sample size to be used in analyses. In the standard method EN ISO 22964:2017 Chapter 9.1 Test Portion it is stated word by word as follows: "*To prepare the primary solution, add x g of the test sample (Clause 8) to 9 times x ml of pre-enrichment medium (5.2), which is the ratio of test sample to pre-enrichment medium specified in this method.*" When reporting results it is stated in the Method: "*Specify the final test result per mass (in grams) or per volume (in millimiters) of the analysed sample.*" In the standard method NMKL 71:1999 it is stated: "*This method is a qualitative method only, and the result is reported as: Salmonella detected/not detected in the amount of sample taken.*"

Raisio, 20th April 2021

Jaana Renko
Quality Manager
Eurofins Scientific Finland

Attachements:

1. Accreditation Certificate of Eurofins Scientific Finland
2. Current scope of accreditation of Eurofins Scientific Finland
3. Accreditation certificate and current scope of accreditation of Eurofins Laboratoires De Microbiologie Ouest

JHeimbach LLC

June 2, 2021

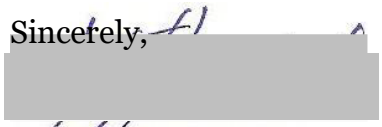
Ellen Anderson
Regulatory Review Scientist
Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
U.S. Food and Drug Administration

Dear Dr. Anderson:

On May 26, you sent me an email with additional FDA questions regarding Danisco's GRAS notice GRN 000951 for the intended use of 3-fucosyllactose (3-FL). This letter provides responses to these questions. We have started each question and response on a new page.

I trust that our responses to your questions provide complete and fully satisfactory answers. If you require further information, please let me know.

Sincerely,



James T. Heimbach, Ph.D., F.A.C.N.
President

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Question A.
We note that the revised Table 5 in the April amendment does not list all food categories listed in Table 5 in the notice. We presume that a part of the table was accidentally omitted. Please provide a complete revised Table 5.

Response:
See below for revised Table 5 which includes all food categories included in the original Table 5 in the notice.

Food Category	Food Uses	3FL Maximum Proposed Use Level (g/serving)	3FL Maximum Proposed Use Level (g/kg)	Representative Foods in NHANES
Formula for infants and young children	Infant formula (0-12 months)	0.04	0.44	Infant formulas, ready-to-feed, prepared from powder/concentrate
	Formula targeted to young children aged 1-3 years	0.04	0.44	Toddler formulas, ready-to-feed, prepared from powder or concentrate
Foods for infants	Cereal & grain products, dry RTE (ready-to-eat) cereals, cookies, teething biscuit & toasts	0.03	4.40	Dry cereals (e.g., barley, mixed, oatmeal, rice, brown rice, multigrain), snack and sweets (e.g., cereal bars, cookies and teething cookies, crackers, puffs, crunchy snacks)
Foods for young children	Cereal & grain products, dry RTE (ready-to-eat) cereals, cookies, teething biscuit & toasts	0.09	4.40	Dry cereals (e.g., barley, mixed, oatmeal, rice, brown rice, multigrain), snack and sweets (e.g., cereal bars, cookies and teething cookies, crackers, puffs, crunchy snacks)
Juice and drinks for infants and young children	Juice/drinks	0.04	0.44	Juices including apple, mixed fruit, orange, pear, and grape juices
Baked goods & baking mixes	Cereal & nutrition bars	0.26	6.60	Cereal bars (e.g., Nutri-Grain bars, milk 'n cereal bar, granola bars) and nutrition bars (e.g., meal replace-ment bars, Cliff Bar, PowerBar, Slim Fast Bar, Zone Perfect Bar)
Beverages & beverage bases, nonalcoholic	Enhanced or fortified water	0.07	0.26	Enhanced or fortified waters (e.g., Propel Water, Glaceau Vitamin Water, SoBe Life Water)
	Energy, sports & isotonic drinks & mixes	0.07	0.26	Regular and low-calorie sport drinks (e.g., Gatorade, Powerade) and energy drinks (Full Throttle, Monster, NOS, Red Bull, Rockstar)

Breakfast cereals	Hot cereals	0.35	6.80	Oatmeal, cream of rice, cream of wheat, cream of rye, whole wheat hot cereal, oat bran hot cereal, grits, cornmeal mush
Breakfast cereals	RTE cereals	0.35	8.80	All types of RTE cereals
Dairy product analogs	Milk substitutes, fluid	0.07	0.26	Soy milk, almond milk, rice milk, coconut milk (excluding coconut milk/cream used for cooking)
	Non-dairy yogurts	0.44	2.64	Soy and coconut milk yogurt
Milk products	Fermented milk, RTD (ready-to-drink) & mixes	0.07	.026	Buttermilk and kefir
	Flavored Milk, RTD & mixes (including dairy-based beverages)	0.07	0.26	Flavored milk (e.g., chocolate and strawberry flavors), hot chocolate, milk shakes, malted milk drink
	Meal replacement beverages	.026	1.10	Meal replacement beverages such as Carnation Instant Breakfast, Muscle Milk, Slim Fast, and high protein drinks
	Smoothies (dairy and non-dairy)	0.26	1.10	Fruit and/or vegetables smoothies (dairy and non-dairy types)
	Yogurt	0.26	2.64	Regular and Greek yogurt, all flavors, excluding frozen yogurt
Processed fruits and fruit juices	Fruit juices and nectars (including fruit-based beverages)	0.07	0.26	100% fruit juices (excluding lemon juice), fruit juice drinks, fruit and vegetable juice drinks, nectars, and coconut water
Processed vegetables and vegetable juices	Vegetable juice	0.07	0.26	100% vegetable juices
Foods for special dietary use	Special dietary purpose ingredient in oral and enteral tube feeding (> 11 years)	0.88	4.40	Not applicable

Question G.
The estimates provided by Danisco for fucose, galactose/glucose, and other carbohydrates are each 0.01 g/day. We note that the correct estimates after rounding to two decimal places should be 0.02 g/day. Please confirm that 0.02 g/day is the correct estimate.

Response*:*
Danisco confirms that the correct estimates for fucose, galactose/glucose and other carbohydrates are each 0.02 g/day.

Question I.
In response to Question I., Danisco stated the methods used for analyses of *Cronobactersakazakii* and *Salmonella* have been validated for their purposes and the methods do notspecify a sample size. We do not need any additional information regarding Question I. However, we wish to note for the administrative record that the cited method ISO 22964:2017 specifies a sample size for which the method has been validated. ISO 22964: 2017 in 10.1 states, " This document has been validated for test portions of 10 g. A smaller sizeof the test portion may be used without the need of additional validation/verification providing that the same ratio between pre-enrichment broth and test portion is maintained. A larger test portion than that initially validated may be used, if a validation/verification study has shown that there are no negative effects on thedetection of *Cronobacter* spp."

Response:
We make note of the clarification of the cited method for Cronobacter sakazakii.

Question J.

Danisco's response to question "J" includes a table which compares the exposure to polyols from human milk and formula for small and large infants. We note the following:

- In this table, the units for the columns titled "Total Polyols in Breast Milk" are indicated as milligrams (mg). However, our calculations indicate that the units should be micrograms (mcg or µg).

- The "Relative Difference" values in the table are identical for all infants regardlessof weight and age although they were provided with precision to five significant figures. Based on the estimates provided in the table, we would expect that there should be small differences among the "Relative Difference" values.

Please confirm the correct units for the "Total Polyols in Breast Milk" columns and update the data in the "Relative Difference" columns, if necessary. If the dietary exposure estimates for polyols in breast milk are updated, please confirm that this new dietary exposure estimate would not change the overall GRAS conclusion.

Response

We note that the units in the table should be µg. We have provided updated calculations for the polyol exposure from 3-FL in infant formula as well. Please see revised response below with new text in red which addresses the additional questions from the agency:

According to Cavalli et al. (2006), a number of polyols are present in human breast milk.[1] These include the following sugar alcohols with concentrations:

	µM	**µg/ml**
Inositol	1172	211.15
Glycerol	822	75.7
Erythritol	0	0
Arabitol	238	36.2
Ribitol	0	0
Mannitol	19.7	3.59

Based on these breast milk levels and infants' body weights (per the CDC infant growth charts[2]), it is possible to determine an approximate exposure to these polyols for infants aged 0 to 6.5 months.

[1] Cavalli, C; Teng, C; Battaglia, FC; Bevilacqua, G. (2006). Free sugar and sugar alcohol concentrations in human breast milk. *J Pediatr Gastroenterol Nutr* 42(2): 215-221 doi: 10.1097/01.mpg.0000189341.38634.77

[2] https://www.cdc.gov/growthcharts/html_charts/wtageinf.htm#males

The following assumptions were used to calculate exposure to polyols via breast milk and formula made using 3-FL:

- *260 ml breast milk/kg BW/day*[3]
- *260 ml formula/kg BW/day*
- *Average infant body weight in the 3rd and 97th percentiles (per CDC growth charts)*
- *0.44 g 3-FL/L formula*
- *3% polyol content in 3-FL*

Example calculation for determining polyol exposure via formula:

$260\ ml\ (breast\ milk/kg\ BW)/day * 2.4\ kg\ BW\ \div 1000\ ml/L = 0.628\ L\ breast\ milk/day$

$0.628\ L/day * 0.44\ g\ 3FL/L\ formula = 0.276\ g\ 3FL/day$

$0.276\ g\ 3\ FL/day * 0.03\ g\ polyol/g\ 3FL = 0.00829\ g\ polyol/day$

$0.00829\ g\ polyol/day * 10^6\ \mu g/g = 8{,}290\ \mu g\ polyol/day$

Comparing potential exposure from breast milk and formula for small and large infants provides the following table:

Age in months 3rd per-centile BW	Total Polyols in Breast Milk (µg)	Total Polyols in 3-FL (µg)*	Relative difference		Age in months 97th per-centile BW	Total Polyols in Breast Milk (µg)	Total Polyols in 3-FL (µg)	Relative difference
0	205,022	8,285	25		**0**	361,355	14,603	25
0.5	234,135	9,462	25		**0.5**	402,855	16,280	25
1.5	288,944	11,677	25		**1.5**	480,461	19,416	25
2.5	339,519	13,720	25		**2.5**	551,391	22,283	25
3.5	386,193	15,607	25		**3.5**	616,240	24,903	25
4.5	429,264	17,347	25		**4.5**	675,567	27,301	25
5.5	469,006	18,953	25		**5.5**	729,893	29,496	25
6.5	505,675	20,435	25		**6.5**	779,704	31,509	25

*Differences in the example calculation above and values in the table are a result of rounding and significant figures in the software used to calculate the values.

[3] EFSA Scientific Committee (2017) Guidance on the risk assessment of substances present in food intended for infants below 16 weeks of age. *EFSA Journal* 15(5):4849 https://doi.org/10.2903/j.efsa.2017.4849

The relative exposure of polyols from breast milk as compared to formula supplemented with 3-FL remains the same regardless of age or body weight since the polyol concentration is on a volume per kg body weight basis as per the above assumptions.

The amount of polyols from formula containing 3-FL is >25 times less than the amount of polyol in breast milk based on Cavalli et al. These low amounts of polyols available from 3-FL are unlikely to produce the major disturbances of polyol overexposure, bloating and diarrhea[4], based on comparison to exposure from breast milk. A wide variety of polyols (lactitol, hexitol, galactinol, myo-inositol, glycerol, ribitol, lyxitol, and mannitol) have been identified in the urine of 3-month-old infants.[5] For breast milk fed infants, lactitol, hexitol, galactinol, myo-inositol, and glycerol urinary levels were significantly higher than formula fed infants (either cow's milk or soy protein). For other polyols, ribitol, lyxitol, and mannitol, there were similar or higher urinary levels in human milk fed infants as compared to soy or milk-based formula fed infants, respectively. As infants have historically been fed breast milk or infant formula , one can infer that these polyols derived from either breast milk or formula have no adverse impact on infants and thus the small additional contribution from 3% polyol in 3-FL would also have no adverse impact on infants.

[4] Mäkinen K. K. (2016). Gastrointestinal disturbances associated with the consumption of sugar alcohols with special consideration of xylitol: Scientific review and instructions for dentists and other health-care professionals. *Int J Dentistry* 5967907. https://doi.org/10.1155/2016/5967907

[5] Rosa F, Mercer KE, Lin H, Sims CR, Pack LM, Goode G, Badger T, Andres A, Yeruva L. (2020). Early infant formula feeding impacts urinary metabolite profile at 3 months of age. *Nutrients* 12:3552; doi:10.3390/nu12113552

From: jheimbach@va.metrocast.net
To: Anderson, Ellen
Subject: RE: [EXTERNAL] RE: GRN 951 - final clarifications
Date: Wednesday, July 07, 2021 8:18:03 PM
Attachments: image001.png

Dear Ellen—

I am once again impressed with how carefully your team reviews these GRAS submissions and responses to questions. Your statement of the intended use levels is correct—these numbers should both be 0.26 rather than 0.026.

Regards,
Jim

James T. Heimbach, Ph.D., F.A.C.N.
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From: Anderson, Ellen <Ellen.Anderson@fda.hhs.gov>
Sent: Wednesday, July 7, 2021 6:00 PM
To: jheimbach@va.metrocast.net
Subject: FW: [EXTERNAL] RE: GRN 951 - final clarifications

Hello Jim,

I hope you are doing well. We are finishing up our review of GRN 951 and have a minor clarification to ask of you for the administrative record.

We note that in Table 5 in the amendment dated June 2, 2021, you provided the following revised use levels of 3-FL:

- Fermented milk, RTD (ready-to-drink) & mixes: 0.026 g/kg
- Meal replacement beverages: 0.026 g/serving

We believe that these use levels are incorrect due to typographical errors. Please confirm that the correct use levels are 0.26 g/kg and 0.26 g/serving, respectively.

Thank you,

Ellen
Ellen Anderson
Regulatory Review Scientist

Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
U.S. Food and Drug Administration
Tel: 240-402-1309
ellen.anderson@fda.hhs.gov

Pronouns: she/her/hers

From: jheimbach@va.metrocast.net <jheimbach@va.metrocast.net>
Sent: Wednesday, June 02, 2021 10:42 AM
To: Anderson, Ellen <Ellen.Anderson@fda.hhs.gov>; jh@jheimbach.com
Subject: [EXTERNAL] RE: GRN 951 - final clarifications

Dear Ellen—

Our responses to the FDA questions posed in the attachment to your e-mail are attached. Thank you for your patience.

Regards,
Jim

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From: Anderson, Ellen <Ellen.Anderson@fda.hhs.gov>
Sent: Wednesday, May 26, 2021 9:39 AM
To: jheimbach@va.metrocast.net; jh@jheimbach.com
Subject: GRN 951 - final clarifications

Hello Dr. Heimbach,

Please see the attached letter regarding GRN 951.

Sincerely,
Ellen

Ellen Anderson
Regulatory Review Scientist

Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
U.S. Food and Drug Administration
Tel: 240-402-1309
ellen.anderson@fda.hhs.gov

Pronouns: she/her/hers

From: James Heimbach
To: Anderson, Ellen
Subject: [EXTERNAL] RE: GRN 951 - final clarification
Date: Friday, July 23, 2021 10:46:21 AM
Attachments: image001.png

Dear Ellen—

I've done the same calculation as FDA did and get the same answer. Therefore, I can confirm that the revised estimates are as you suggested--2.64 g/p/d (0.047 g/kg bw/d for a 56-kg adolescent and 0.035 g/kg bw/d for a 75-kg adult).

Regards,
Jim

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From: Anderson, Ellen <Ellen.Anderson@fda.hhs.gov>
Sent: Friday, July 23, 2021 9:36 AM
To: 'jheimbach@va.metrocast.net' <jheimbach@va.metrocast.net>
Subject: GRN 951 - final clarification

Good morning, Jim,

I hope this email finds you well. We are in the home-stretch of finishing up our review of GRN 951, and we have one more clarification to confirm with you.

On page 23 of the notice, a dietary exposure to 3-FL from the intended use in oral and enteral tube feeding formulas is provided (12 g/p/day, equivalent to 0.21 g/kg bw/day for a 56-kg adolescent and 0.16 g/kg bw/day for a 75-kg adult). In an amendment received on June 2, 2021, the notifier confirmed that the intended use level of 3-FL in oral and enteral tube feeding formulas is 0.88 g/serving, reduced from 4.0 g/serving. We note that the notifier did not provide a revised dietary exposure to 3-FL from the intended use in oral and enteral tube feeding formulas to

reflect the reduction in use level. Please confirm that the revised estimates are 2.64 g/p/d (0.047 g/kg bw/d for a 56-kg adolescent and 0.035 g/kg bw/d for a 75-kg adult).

Thank you,
Ellen

Ellen Anderson
Regulatory Review Scientist

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Pronouns: she/her/hers