Appendix qPCR

Evaluation of 2'-FL for absence of genes of the E. coli production strain

Appendix qPCR1
Summary of evaluation of 2'-FL for absence of genes of the E. coli production strain by qPCR

Appendix qPCR2
Validation study protocol of the Kanamycin resistance gene detection method by quantitative PCR

Appendix qPCR3
Validation final report of the Kanamycin resistance gene detection method by quantitative PCR

Appendix qPCR4
Validation study protocol of the E 638 ΔlacI-lacY gene and the E 638 ΔwcaJ gene detection method by quantitative PCR

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Appendix qPCR6
Validation report of the E 638 ΔwcaJ gene detection method by quantitative PCR

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qPCR Test Reports

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Eurofins MicroSafe Laboratories GMP- and GLP certificate

Appendix qPCR1

Summary of **Evaluation of 2'**-FL for absence of genes of the E. coli production strain by qPCR

Introduction

3 qPCR assays have been developed for unique chromosomal target regions of E. coli strain E997:

- Kanamycin resistance gene
- E 638 ΔlacI-lacY gene
- E 638 ΔwcaJ gene

Method validation

For each assay, the specificity and Limit of Detection is determined. This is described in the validation study protocols and the validation reports of the qPCR assays in this appendix.

Results

Samples of each lot of 2'-fucosyllactose have been sent to the laboratory to run the qPCR assays. The result reports can be found in this appendix. The overview of the results is given in the table below.

Table L1 **: Evaluation of 2'**-FL for absence of genes of the E. coli strain E997

Chromosomal target gene	C[DNA] LOD	**2'**-FL lot number				
		PMRS10	PMRS11	CMRS03	CMRS06	CMRS07
Kanamycin resistance gene	1.25 x10^{-5} ng/µL	<LOD	<LOD	<LOD	<LOD	<LOD
E638 ΔlacI–lacY gene	4.5 x 10^{-7} ng/µL	<LOD	<LOD	<LOD	<LOD	<LOD
E638 ΔwcaJ gene	4.5 x 10^{-4} ng/µL	<LOD	<LOD	<LOD	<LOD	<LOD

Source: FrieslandCampina Nederland B.V.
Note: Production strain E997 is E. coli K12 # E997
LOD = limit of detection for the individual genes; ng = nanogram; µL = microliter; qPCR = quantitative polymerase chain reaction

The analysis of these 5 batches of 2'-fucosyllactose demonstrate no detectable levels of residual DNA (limit of detection = 5 ppb) present in the final ingredient.

Appendix qPCR2
Validation study protocol of the Kanamycin resistance gene detection method by quantitative PCR

Sign off for review and approval			
Function	**Name**	**Signature**	**Date**
Author	M. Schrumpf	(b) (6)	22dec16
Manager QA	J.M. Klein		22 dec 2016
Sponsor	J Bastiaans		21/12016

Eurofins MicroSafe Laboratories
Study Protocol

Sponsor:
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Bronland 20
6708 WH Wageningen
The Netherlands

Testing Facility:
Eurofins MicroSafe Laboratories
Darwinweg 24
2333 CR Leiden
The Netherlands

Print date: 22-dec-2016
(b) (6)

1 Introduction

1.1 The customer has a product which is produced in a GMO *E.coli* cell line. The customer must show that host cell derived DNA is not present in the product. As the process is a not sterile one, it is imperative that the assays can discriminate between wild type *E.coli* DNA and DNA coming from the production strain.

1.2 During the feasibility study (T16K192) a PCR was set up using primer sequences from literature, which have been supplied by the sponsor.

1.3 The copy number for the kanamycin resistance gene is ~1/cell. The customer has supplied isolated *E.coli* DNA containing the gene of interest.

1.4 Limit of Detection: The parameter LOD will be performed to determine the Limit of Detection of the test method. The LOD is defined as the lowest concentration where ≥ 95% of the replicates tested show a qPCR product.

1.5 Specificity: The parameter specificity will be performed to demonstrate the ability of the selected primers to detect the kanamycin resistance gene. Four other bacteria and one fungus were selected to show the absence of cross detection of the kanamycin resistance primer set.

2 Purpose and Scope

2.1 This protocol describes the validation of the qPCR as a test method for the detection of the kanamycin resistance gene.

2.2 The scope of this validation is limited to the parameters Limit of Detection (LOD) and Specificity.

3 Abbreviations and Definitions

3.1	ATCC	American Type Culture Collection
3.2	*B. subtillis*	*Bacillus subtilis*
3.3	*C. albicans*	*Candida albicans*
3.4	*C. sporogenes*	*Clostridium sporogenes*
3.5	C_t	Threshold Cycle
3.6	DNA	Deoxyribose nucleic acid
3.7	*E. coli*	*Escherichia coli*
3.8	LOD:	The limit of detection is defined as the lowest concentration where ≥ 95% of the replicates tested show a detectable qPCR product.
3.9	NTC	No Template Control: This is a control reaction that contains all essential components of the amplification reaction except the template. This control monitors contamination and primer-dimer formation that could produce false positive results.
3.10	Positive control	Test to confirm the qPCR reaction mix has worked according to specification
3.11	Primers:	Short nucleotide sequences used in the qPCR to start the amplification process
3.12	qPCR	Real-time and quantitative Polymerase Chain Reaction
3.13	*S. aureus*	*Staphylococcus aureus*
3.14	Specificity	The ability to detect the specified gene of interest without cross-reaction with other micro-organisms.

(b) (6)

3.15 SYBR Green — Dye used as a nucleic acid stain in molecular biology. SYBR Green binds to double-stranded DNA. The resulting DNA-dye-complex absorbs blue light and emits green light. It is used to measure the increase in DNA in the qPCR reaction.

3.16 Template — The material that is amplified during the qPCR reaction.

4 Materials

4.1 Equipment
- 4.1.1 Pipettes
- 4.1.2 ABI 7500 QPCR (SDS version 1.4)
- 4.1.3 PCR hood

4.2 Consumables
- 4.2.1 Pipet tips
- 4.2.2 Optical 96-wells PCR plate
- 4.2.3 Optical adhesive film

4.3 Reagents
- 4.3.1 SYBR Green PCR Master Mix
- 4.3.2 Primers:
  - 4.3.2.1 Forward primer: KAN-F (10µM), 5'-CTC ACC TTG CTC CTG CCG AGA-3'
  - 4.3.2.2 Reverse primer: KAN-R (10µM), 5'-CGC CTT GAG CCT GGC GAA CAG-3'
- 4.3.3 DNase/RNase free water

4.4 Positive template DNA, diluted to 10 ng/µl:

MicroSafe sample number	Description	Lot number (10 ng/µl)
71161	pG217 DNA (191 µg/ml)	T16K192-B
71162	E997 DNA (contains PG217 @~40 copies/gene) 218 µg/ml	T16K192-A

4.5 Regular negative control DNA
- 4.5.1 *E. coli* DNA, lot number: T16K192-C
  Isolated according to LAB-MOLBIO-140 revision 1.0, from: *E. coli*, ATCC 8739, lot number: T16C318, DNA concentration: 35 ng/µl (NanoDrop measured)

4.6 Specificity negative control DNA
- 4.6.1 *S. aureus* DNA, lot number: T16K192-D
  Isolated according to LAB-MOLBIO-140 revision 1.0, from: *S. aureus*, ATCC 6538, lot number: T16K352, DNA concentration: 13 ng/µl (NanoDrop measured)
- 4.6.2 *B. subtilis* DNA, lot number: T16K192-F
  Isolated according to LAB-MOLBIO-140 revision 1.0, from: *B. subtilis*, ATCC 6633, lot number: T15I390, DNA concentration: 14 ng/µl (NanoDrop measured)
- 4.6.3 *C. sporogenes* DNA, lot number: T16K192-G
  Isolated according to LAB-MOLBIO-140 revision 1.0, from: *C. sporogenes*, ATCC 19404, lot number: T15E174, DNA concentration: 11 ng/µl (NanoDrop measured)
- 4.6.4 *C. albicans* DNA, lot number: T16K192-H
  Isolated according to LAB-MOLBIO-140 revision 1.0, from: *C. albicans*, ATCC 10231, lot number: T15E173, DNA concentration: 9 ng/µl (NanoDrop measured)

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5 Procedure

5.1 General workflow - amplifying DNA

5.1.1 The target DNA will be amplified using 2x SYBR Green master mix as supplied by Life Technologies. DNase/RNase free water and primers will be added to the mix and 11 µl mix will be pipetted into a 96 wells optical plate. 9 µl template DNA will be added to the mix and the 96 wells plate will be centrifuged for 1 minute at 1000 rpm to collect all the fluid in the bottom of the plate. Subsequently, the plate will be placed in an ABI 7500 qPCR system and the appropriate program will be chosen for amplification of the DNA.

5.2 Limit of Detection

5.2.1 To establish the LOD, a positive cut-off point will be determined. The positive cut-off point is the minimum number of target sequence copies per volume of sample that can be detected in 95% of experiments.

5.2.2 The LOD will be tested three times, by at least two different operators, on at least two different days.

5.2.3 E997 DNA and pG217 DNA (10 ng/µl) will be diluted to a concentration of 1 ng/µl. Subsequently, three separate ten-fold serial dilutions of the DNA will be prepared till 10^{-7}. Of each dilution series the dilutions 10^{-3} until 10^{-7} will be tested in duplicate, per experiment.

5.2.4 *E. coli* DNA will be diluted 10 times with DNase/RNase free water. This dilution will be used as a negative control.

5.2.5 DNase/RNase free water will be used as Negative Template Control (NTC).

5.3 Specificity

5.3.1 The specificity will be tested three times, by at least two different operators and on two different days.

5.3.2 The DNA of wild-type *E. coli, S. aureus, B. subtilis, C. sporogenes* and *C. albicans* will be diluted 10 times with DNase/RNase free water. These dilutions will be tested in duplicate, in order to prove that the PCR does not detect wild-type DNA from these species.

5.3.3 DNase/RNase free water will be used as a Negative Template Control (NTC).

6 Acceptance Criteria

6.1 A value equal or below 35 is considered a positive result. A signal above 35 is considered a negative result. The cut-off value was chosen based on various preliminary studies.

6.2 The qPCR is valid when:

6.2.1 NTC Ct value > 35 or undetermined

6.2.2 Positive control Ct value ≤35

6.3 Limit of detection

6.3.1 The parameter LOD is defined as the lowest concentration used where ≥ 95% of the replicated tested show a qPCR product (Ct-value ≤ 35).

6.4 Specificity

6.4.1 The parameter specificity will be performed to exclude the possibility of cross detection with wild type *E. coli*, three other bacteria and one fungus. The kanamycin primers should not generate a qPCR product in the these cases (Ct-value >35).

(b) (6)

7 References

7.1 The study will be performed in compliance with the agreed protocol and will be executed in accordance with MicroSafe Laboratories Standard Operating Procedures (SOP's). The execution of the study will conform to the principles of Good Manufacturing Practices of the European Community.

7.2 SOP's and other documentation covering all the techniques involved in this study are available for auditing by the Sponsor.

7.3 The Quality Assurance Unit of MicroSafe regularly inspects the performance of the techniques described in the relevant SOP's.

REVISION SHEET

DATE	REV	NATURE OF CHANGES	CHANGED BY
19DEC2016	1.0	Initial release	MESC

Appendix qPCR3

Validation report of the Kanamycin resistance gene detection method by quantitative PCR

Title: Validation of a PCR method for the sensitive detection of a Kanamycin resistance gene

Study number: SP082.71161 and 71162

Sample identification:

MicroSafe sample number	Sample name	Concentration
71161	pG217 DNA	191 µg/ml
71162	E997 DNA	218 µg/ml

Administrative details:

Sponsor: Sponsor representative: Sponsor number:	Friesland Campina Innovation Bronland 20 6708 WH Wageningen The Netherlands Mr. Jan-Willem Boots 2995
Testing facility:	Eurofins MicroSafe Laboratories Darwinweg 24 2333 CR Leiden The Netherlands
Test Schedule: Laboratory start date: Laboratory completion date:	 28 December 2016 05 January 2017
Raw data references:	00010584TR: T16L479, T17A051
Report revision note:	After the report was sent new information was provided by the customer. Due to this the conclusion of this report needs to be adjusted. The LOD of the method was calculated using qPCR results of the plasmid DNA (71161). Kanamycin resistance gene is on the chromosome, therefore the LOD needs to be calculated using results of the *E.coli* strain (71162). The conclusion was rewritten.

Study details:

Test method:	According to protocol TM-SP-082 revision code 1.0 with study specific supplement.
Sample preparation:	The given concentration of the samples is used to dilute to a concentration of 10 ng/µl.
Quantity tested:	5 µl template DNA is tested in the qPCR.
Specificity:	DNA of *E.coli, S. aureus, B. subtilis, C. sporogenes* and *C. albicans* were isolated, 10 times diluted in DNase/RNase free water and tested in duplicate by qPCR. Tests were performed in triplicate by at least two operators on at least two different days.
Limit of Detection	DNA of samples 71161 and 71162 were diluted to a concentration of 1 ng/µl using DNase/RNase free water. Three independent 10-fold series of dilutions (10^{-1} till 10^{-7}) were prepared. Dilutions 10^{-3} till 10^{-7} were tested in duplicate by qPCR. Tests were performed in triplicate by at least two operators on at least two different days.
Negative template control (NTC):	DNase/RNase free water
Protocol amendments:	A total reaction volume of 40 µl is used; 20 µl of SYBR Green, 13 µl of DNase/RNase free water, 1 µl per primer (0.25 µM) and a template volume of 5 µl.
Non conformance:	There was one non-conformance, refer to OOO-300-2016-12-30-B. In the initial validation a reaction volume of 20 µl was tested, by using 11 µl mix with 9 µl template DNA, according to the protocol TM-SP-082 revision 1.0. During the first two specificity runs, *C. albicans* DNA as template used in the assay resulted in a positive signal. During the feasibility a reaction volume of 40 µl was tested, using 35 µl mix with 5 µl template DNA. The first two validation runs were repeated using new isolated DNA of *C. albicans* and a reaction volume of 40 µl.

Results:

Table 1: Test for specificity

Item	Run	Ct	Evaluation
E. coli	1	Undetermined	Negative
	1	Undetermined	Negative
S. aureus	1	Undetermined	Negative
	1	38.0	Negative
B. subtilis	1	Undetermined	Negative
	1	39.4	Negative
C. sporogenes	1	Undetermined	Negative
	1	Undetermined	Negative
C. albicans	1	Undetermined	Negative
	1	Undetermined	Negative
E. coli	2	39.2	Negative
	2	Undetermined	Negative
S. aureus	2	39.0	Negative
	2	Undetermined	Negative
B. subtilis	2	Undetermined	Negative
	2	39.9	Negative

>35 or undetermined = negative
≤35 = positive

Table 1: Test for specificity (continued)

Item	Run	Ct	Evaluation
C. sporogenes	2	Undetermined	Negative
	2	Undetermined	Negative
C. albicans	2	Undetermined	Negative
	2	Undetermined	Negative
E. coli	3	Undetermined	Negative
	3	Undetermined	Negative
S. aureus	3	Undetermined	Negative
	3	Undetermined	Negative
B. subtilis	3	Undetermined	Negative
	3	39.3	Negative
C. sporogenes	3	Undetermined	Negative
	3	Undetermined	Negative
C. albicans	3	Undetermined	Negative
	3	Undetermined	Negative

>35 or undetermined = negative
≤35 = positive

Table 2: Test for Limit of Detection on pG217 DNA, dilution series 1

Concentration (ng/µl)	Run 1		Run 2		Run 3	
	Ct	Evaluation	Ct	Evaluation	Ct	Evaluation
10^{-3}	29.7	Positive	29.5	Positive	32.6	Positive
	30.0	Positive	29.9	Positive	32.5	Positive
10^{-4}	33.9	Positive	33.5	Positive	38.2	Negative
	34.6	Positive	35.0	Positive	37.4	Negative
10^{-5}	37.8	Negative	37.9	Negative	39.8	Negative
	Undetermined	Negative	Undetermined	Negative	Undetermined	Negative
10^{-6}	Undetermined	Negative	Undetermined	Negative	34.8	Positive
	Undetermined	Negative	Undetermined	Negative	39.1	Negative
10^{-7}	Undetermined	Negative	Undetermined	Negative	Undetermined	Negative
	Undetermined	Negative	Undetermined	Negative	Undetermined	Negative

>35 or undetermined = negative
≤35 = positive

Table 3: Test for Limit of Detection on pG217 DNA, dilution series 2

Concentration (ng/µl)	Run 1		Run 2		Run 3	
	Ct	Evaluation	Ct	Evaluation	Ct	Evaluation
10^{-3}	29.7	Positive	30.0	Positive	32.9	Positive
	29.6	Positive	29.7	Positive	32.4	Positive
10^{-4}	33.8	Positive	34.0	Positive	36.0	Negative
	33.9	Positive	32.7	Positive	36.2	Negative
10^{-5}	39.7	Negative	36.0	Negative	Undetermined	Negative
	36.3	Negative	37.0	Negative	Undetermined	Negative
10^{-6}	Undetermined	Negative	Undetermined	Negative	Undetermined	Negative
	Undetermined	Negative	Undetermined	Negative	Undetermined	Negative
10^{-7}	Undetermined	Negative	Undetermined	Negative	Undetermined	Negative
	Undetermined	Negative	Undetermined	Negative	Undetermined	Negative

>35 or undetermined = negative
≤35 = positive

Table 4: Test for Limit of Detection on pG217 DNA, dilution series 3

Concentration (ng/µl)	Run 1		Run 2		Run 3	
	Ct	Evaluation	Ct	Evaluation	Ct	Evaluation
10^{-3}	29.3	Positive	29.7	Positive	32.1	Positive
	29.2	Positive	29.3	Positive	31.8	Positive
10^{-4}	33.5	Positive	33.6	Positive	36.5	Negative
	33.5	Positive	33.2	Positive	36.3	Negative
10^{-5}	36.6	Negative	37.8	Negative	Undetermined	Negative
	36.2	Negative	34.6	Positive	Undetermined	Negative
10^{-6}	Undetermined	Negative	Undetermined	Negative	Undetermined	Negative
	Undetermined	Negative	Undetermined	Negative	Undetermined	Negative
10^{-7}	Undetermined	Negative	Undetermined	Negative	Undetermined	Negative
	Undetermined	Negative	Undetermined	Negative	Undetermined	Negative

>35 or undetermined = negative
≤35 = positive

Table 5: Test for Limit of Detection on E997 DNA, dilution series 1

Concentration (ng/µl)	Run 1		Run 2		Run 3	
	Ct	Evaluation	Ct	Evaluation	Ct	Evaluation
10^{-3}	29.7	Positive	29.8	Positive	28.9	Positive
	29.7	Positive	29.9	Positive	28.7	Positive
10^{-4}	33.1	Positive	33.8	Positive	33.1	Positive
	33.4	Positive	33.3	Positive	33.3	Positive
10^{-5}	37.1	Negative	Undetermined	Negative	38.6	Negative
	38.2	Negative	37.0	Negative	36.9	Negative
10^{-6}	Undetermined	Negative	Undetermined	Negative	Undetermined	Negative
	Undetermined	Negative	Undetermined	Negative	Undetermined	Negative
10^{-7}	Undetermined	Negative	Undetermined	Negative	Undetermined	Negative
	Undetermined	Negative	Undetermined	Negative	Undetermined	Negative

>35 or undetermined = negative
≤35 = positive

Table 6: Test for Limit of Detection on E997 DNA, dilution series 2

Concentration (ng/µl)	Run 1		Run 2		Run 3	
	Ct	Evaluation	Ct	Evaluation	Ct	Evaluation
10^{-3}	29.9	Positive	29.8	Positive	28.7	Positive
	30.1	Positive	30.0	Positive	29.0	Positive
10^{-4}	33.9	Positive	34.1	Positive	33.5	Positive
	34.9	Positive	34.5	Positive	33.4	Positive
10^{-5}	36	Negative	39.0	Negative	37.8	Negative
	39.3	Negative	38.1	Negative	37.5	Negative
10^{-6}	Undetermined	Negative	Undetermined	Negative	Undetermined	Negative
	Undetermined	Negative	Undetermined	Negative	Undetermined	Negative
10^{-7}	Undetermined	Negative	Undetermined	Negative	Undetermined	Negative
	Undetermined	Negative	Undetermined	Negative	Undetermined	Negative

>35 or undetermined = negative
≤35 = positive

Table 7: Test for Limit of Detection on E997 DNA, dilution series 3

Concentration (ng/µl)	Run 1		Run 2		Run 3	
	Ct	Evaluation	Ct	Evaluation	Ct	Evaluation
10^{-3}	29.9	Positive	30.1	Positive	29.2	Positive
	30.0	Positive	30.2	Positive	29.0	Positive
10^{-4}	34.2	Positive	34.4	Positive	33.6	Positive
	34.3	Positive	33.9	Positive	34.0	Positive
10^{-5}	39.3	Negative	38.0	Negative	38.1	Negative
	38.7	Negative	38.7	Negative	Undetermined	Negative
10^{-6}	Undetermined	Negative	Undetermined	Negative	Undetermined	Negative
	Undetermined	Negative	Undetermined	Negative	Undetermined	Negative
10^{-7}	Undetermined	Negative	Undetermined	Negative	Undetermined	Negative
	Undetermined	Negative	Undetermined	Negative	Undetermined	Negative

>35 or undetermined = negative
≤35 = positive

Evaluation of the results:

1. A positive signal could still be detected by using 5 µl of 10^{-4} ng/µl of the E997 DNA template DNA, using a total reaction volume of 40 µl.
2. A positive signal could still be detected by using 5 µl of 10^{-3} ng/µl of the pG217 DNA template DNA, using a total reaction volume of 40 µl.
3. Kanamycin resistance gene is on the chromosome. The study was performed before this information was provided by the customer. The LOD in the conclusion is calculated using results of the *E.coli* strain (71162). This new information indicates that it was not necessary to test the plasmid DNA (71161).

Conclusions:

1. The assay meets the criteria for a valid test.
2. The primers are specific in this assay, no positive signals are observed using DNA of *E.coli, S. aureus, B. subtilis, C. sporogenes* and *C. albicans*.
3. Kanamycin resistance gene is on the chromosome, therefore the LOD is calculated using results of the *E. coli* strain DNA (E997 DNA). The LOD of the assay based is $1.25*10^{-5}$ ng/µl reaction.

Quality Statement:
The study was performed in compliance with the agreed protocol and was executed in accordance with MicroSafe Laboratories Standard Operating Procedures except when clearly documented otherwise. The execution of the study conformed to the principles of Good Manufacturing Practices of the European Community.

Authorisation and approval:
Prepared by:

M. Schrumpf	(b) (6)	15 Mrt 17
Name	Signature	Date

Quality Assurance:

S. Man	(b) (6)	16 Mar 17
Name	Signature	Date

Appendix qPCR4

Validation study protocol of the E 638 Δ*lacI-lacY* gene and the E 638 Δ*wcaJ* gene detection method by quantitative PCR

Sign off for review and approval			
Function	**Name**	**Signature**	**Date**
Author	M. Schrumpf	(b) (6)	08feb17
Manager QA	J.M. Hom		08 feb 2017
Sponsor	J. Basticaans		13/2/2017

Eurofins MicroSafe Laboratories
Study Protocol

Sponsor:
Friesland Campina Innovation
Bronland 20
6708 WH Wageningen
The Netherlands

Testing Facility:
Eurofins MicroSafe Laboratories
Darwinweg 24
2333 CR Leiden
The Netherlands

1 Introduction

1.1 The customer has a product which is produced in a GMO *E.coli* cell line. The customer must show that host cell derived DNA is not present in the product. As the process is a not sterile one, it is imperative that the assays can discriminate between wild type *E.coli* DNA and DNA coming from the production strain.

1.2 During the feasibility study (LNB160231-016-018, LNB160231-025-026 and T17A406) a PCR was set up using primer sequences shown in section 4.3.2.

1.3 The customer has supplied isolated *E.coli* DNA containing the DNA sequences of interest (E638 wcaJ gen and F'402_E638 lacI-lacY gene).

1.4 Limit of Detection: The parameter LOD will be performed to determine the Limit of Detection of the test method. The LOD is defined as the lowest concentration where ≥ 95% of the replicates tested show a qPCR product.

1.5 Specificity: The parameter specificity will be performed to demonstrate the ability of the selected primers to detect the specific DNA sequences. Four other bacteria and one fungus were selected to show the absence of cross detection of the E638 wcaJ gen and F'402_E638 lacI-lacY gene primer sets.

2 Purpose and Scope

2.1 This protocol describes the validation of the qPCR as a test method for the detection of two specific DNA sequences.

2.2 The scope of this validation is limited to the parameters Limit of Detection (LOD) and Specificity.

3 Abbreviations and Definitions

3.1	ATCC	American Type Culture Collection
3.2	*B. subtillis*	*Bacillus subtilis*
3.3	*C. albicans*	*Candida albicans*
3.4	*C. sporogenes*	*Clostridium sporogenes*
3.5	C_t	Threshold Cycle
3.6	DNA	Deoxyribose nucleic acid
3.7	*E. coli*	*Escherichia coli*
3.8	LOD:	The limit of detection is defined as the lowest concentration where ≥ 95% of the replicates tested show a detectable qPCR product.
3.9	NTC	No Template Control: This is a control reaction that contains all essential components of the amplification reaction except the template. This control monitors contamination and primer-dimer formation that could produce false positive results.
3.10	Positive control	Test to confirm the qPCR reaction mix has worked according to specification
3.11	Primers:	Short nucleotide sequences used in the qPCR to start the amplification process
3.12	qPCR	Real-time and quantitative Polymerase Chain Reaction
3.13	*S. aureus*	*Staphylococcus aureus*
3.14	Specificity	The ability to detect the specified gene of interest without cross-reaction with other micro-organisms.

(b) (6)

3.15 SYBR Green — Dye used as a nucleic acid stain in molecular biology. SYBR Green binds to double-stranded DNA. The resulting DNA-dye-complex absorbs blue light and emits green light. It is used to measure the increase in DNA in the qPCR reaction.

3.16 Template — The material that is amplified during the qPCR reaction.

4 Materials

4.1 Equipment
- 4.1.1 Pipettes
- 4.1.2 ABI 7500 qPCR (SDS version 1.4)
- 4.1.3 PCR hood

4.2 Consumables
- 4.2.1 Pipet tips
- 4.2.2 Optical 96-wells PCR plate
- 4.2.3 Optical adhesive film

4.3 Reagents
- 4.3.1 SYBR Green PCR Master Mix
- 4.3.2 Primers
  - 4.3.2.1 Forward primer: E638 wcaJ-F2 (10µM), 5'- AGGAACAACGATGATTCCGGG-3'
  - 4.3.2.2 Reverse primer: E638 wcaJ-R2 (10µM), 5'- GCCGCTTTGTTAACTGTAGGC-3'
  - 4.3.2.3 Forward primer: E638 lacI-lacY-F2 (10µM), 5'- GCCCGGAAGAGAGTCAAGTG-3'
  - 4.3.2.4 Reverse primer: E638 lacI-lacY-F2 (10µM), 5'- TCCTCCTTAGTTCCTATTCCGAAG-3'
- 4.3.3 DNase/RNase free water

4.4 Positive template DNA, diluted to 10 ng/µl:

MicroSafe sample number	Description	Lot number (10 ng/µl)
71161	pG217 DNA (191 µg/ml)	T16K192-B
71162	E997 DNA (contains PG217 @~40 copies/gene) 218 µg/ml	T16K192-A

4.5 Regular negative control DNA
- 4.5.1 *E. coli* DNA, lot number: T16K192-C
  Isolated according to LAB-MOLBIO-140 revision 1.0, from: *E. coli*, ATCC 8739, lot number: T16C318, DNA concentration: 35 ng/µl (NanoDrop measured)

4.6 Specificity negative control DNA
- 4.6.1 *S. aureus* DNA, lot number: T16K192-D
  Isolated according to LAB-MOLBIO-140 revision 1.0, from: *S. aureus*, ATCC 6538, lot number: T16K352, DNA concentration: 13 ng/µl (NanoDrop measured)
- 4.6.2 *B. subtilis* DNA, lot number: T16K192-F
  Isolated according to LAB-MOLBIO-140 revision 1.0, from: *B. subtilis*, ATCC 6633, lot number: T15I390, DNA concentration: 14 ng/µl (NanoDrop measured)
- 4.6.3 *C. sporogenes* DNA, lot number: T16K192-G
  Isolated according to LAB-MOLBIO-140 revision 1.0, from: *C. sporogenes*, ATCC 19404, lot number: T15E174, DNA concentration: 11 ng/µl (NanoDrop measured)
- 4.6.4 *C. albicans* DNA, lot number: T16K192-H
  Isolated according to LAB-MOLBIO-140 revision 1.0, from: *C. albicans*, ATCC 10231, lot number: T15E173, DNA concentration: 9 ng/µl (NanoDrop measured)

(b) (6)

5 Procedure

5.1 General workflow - amplifying DNA

5.1.1 The target DNA will be amplified using 2x SYBR Green master mix as supplied by Life Technologies. Primers with (end concentration of 0.5 µM) will be added to the mix and 11 µl mix will be pipetted into a 96 wells optical plate. 9 µl template DNA will be added to the mix and the 96 wells plate will be centrifuged for 1 minute at 1000 rpm to collect all the fluid in the bottom of the plate. Subsequently, the plate will be placed in an ABI 7500 qPCR system and the appropriate program will be chosen for amplification of the DNA.

5.2 Limit of Detection

5.2.1 To establish the LOD, a positive cut-off point will be determined. The positive cut-off point is the minimum number of target sequence copies per volume of sample that can be detected in 95% of experiments.

5.2.2 The LOD will be tested three times, by at least two different operators, on at least two different days.

5.2.3 E997 DNA and pG217 DNA (10 ng/µl) will be diluted to a concentration of 1 ng/µl. Subsequently, three separate three-fold serial dilutions of the DNA will be prepared till 10^{-7}. Of each dilution series the dilutions 10^{-3} until 10^{-7} will be tested in duplicate, per experiment.

5.2.4 *E. coli* DNA will be diluted 10 times with DNase/RNase free water. This dilution will be used as a negative control.

5.2.5 DNase/RNase free water will be used as Negative Template Control (NTC).

5.3 Specificity

5.3.1 The specificity will be tested three times, by at least two different operators and on two different days.

5.3.2 The DNA of wild-type *E. coli, S. aureus, B. subtilis, C. sporogenes* and *C. albicans* will be diluted 10 times with DNase/RNase free water. These dilutions will be tested in duplicate, in order to prove that the PCR does not detect wild-type DNA from these species.

5.3.3 DNase/RNase free water will be used as a Negative Template Control (NTC).

6 Acceptance Criteria

6.1 A value equal or below 40 is considered a positive result. An undetermined result is considered a negative result. The cut-off value was chosen based on various preliminary studies.

6.2 The qPCR is valid when:

6.2.1	NTC	undetermined
6.2.2	Positive control	Ct value ≤ 40

6.3 Limit of detection

6.3.1 The parameter LOD is defined as the lowest concentration used where ≥ 95% of the replicated tested show a qPCR product (Ct-value ≤ 40).

6.4 Specificity

6.4.1 The parameter specificity will be performed to exclude the possibility of cross detection with wild type *E. coli*, three other bacteria and one fungus. The E638 wcaJ and E638 lacI-lacY primers should not generate a qPCR product in these cases (undetermined).

(b) (6)

7 References

7.1 The study will be performed in compliance with the agreed protocol and will be executed in accordance with MicroSafe Laboratories Standard Operating Procedures (SOP's). The execution of the study will conform to the principles of Good Manufacturing Practices of the European Community.

7.2 SOP's and other documentation covering all the techniques involved in this study are available for auditing by the Sponsor.

7.3 The Quality Assurance Unit of MicroSafe regularly inspects the performance of the techniques described in the relevant SOP's.

REVISION SHEET

DATE	REV	NATURE OF CHANGES	CHANGED BY
08FEB2017	1.0	Initial release	MESC

(b) (6)

Appendix qPCR5

Validation report of the E 638 ΔlacI-lacY gene detection method by quantitative PCR

Sample identification:

MicroSafe sample number	Sample name	Concentration
71161	pG217 DNA	191 µg/ml
71162	E997 DNA	218 µg/ml

Administrative details:

Sponsor: Sponsor representative: Sponsor number:	Friesland Campina Innovation Bronland 20 6708 WH Wageningen The Netherlands Mr. J. B. Bastiaans 2995
Testing facility:	Eurofins MicroSafe Laboratories Darwinweg 24 2333 CR Leiden The Netherlands
Test Schedule: Laboratory start date: Laboratory completion date:	 20 February 2017 27 February 2017
Raw data references:	LAB-GEN-700-TR01: T17B380, T17B381, T17B360, T17B465 T17B382, T17B383
Report revision note:	After the report was sent new information was provided by the customer. Due to this the conclusion of this report needs to be adjusted. The LOD of the method was calculated using qPCR results of the plasmid DNA (71161). LacI-lacY specific sequence is on the chromosome, therefore the LOD needs to be calculated using results of the *E.coli* strain (71162). The conclusion was rewritten.

Study details:

Test method:	According to protocol TM-SP-086 revision code 1.0 with study specific supplement.
Sample preparation:	The given concentration of the samples is used to dilute to a concentration of 10 ng/µl.
Quantity tested:	9 µl template DNA is tested in the qPCR.
Specificity:	DNA of *E.coli, S. aureus, B. subtilis, C. sporogenes* and *C. albicans* were isolated, 10 times diluted in DNase/RNase free water and tested in duplicate by qPCR. Tests were performed in triplicate by at least two operators on at least two different days.
Limit of Detection	DNA of samples 71161 and 71162 were diluted to a concentration of 1 ng/µl using DNase/RNase free water. Three independent 10-fold series of dilutions (10^{-1} till 10^{-7}) were prepared. Dilutions 10^{-3} till 10^{-7} were tested in duplicate by qPCR using E638 lacI-lacY specific primers. Tests were performed in triplicate by at least two operators on at least two different days.
Negative template control (NTC):	DNase/RNase free water
Protocol amendments:	In the first three runs one ten-fold serial dilution per sample was tested in duplicate. Therefore three extra runs were performed by preparing two extra independent ten-fold serial dilutions of the sample and these were tested in duplicate by PCR.
Non-conformances:	There were two non-conformances. OOO-200-2017-02-21-E: After the first run the *E. coli* DNA (T16K192-C) turned out to be not enough to perform all tests for the validation. Therefore a new *E.coli* DNA isolation was performed (T17B374) and the resulting DNA was used as a negative control in the last two runs. OOO-300-2017-02-24-B: Protocol TM-SP-086 revision code 1.0 describes that three independent serial dilutions per sample will be tested in duplicate per run. In the first three runs one ten-fold serial dilution per sample was tested in duplicate. Therefore three extra runs were performed by preparing two extra independent ten-fold serial dilutions of the sample and these were tested in duplicate by PCR. In the first extra run dilutions 10^{-2} to 10^{-6} ng/µl DNA was tested, instead of 10^{-3} to 10^{-7} ng/µl DNA. In the initial runs testing the first dilution series the reactions with an input of 9µl of 10^{-7} ng/µl were all negative. Therefore it can be concluded that the lack of these data has no impact on the determination of the LOD of the PCR.

Results:

Table 1: Test for E638 lacI-lacY PCR specificity

Item	Run	Ct	Evaluation
NTC	1	Undetermined	Negative
	1	Undetermined	Negative
E. coli	1	Undetermined	Negative
	1	Undetermined	Negative
S. aureus	1	Undetermined	Negative
	1	Undetermined	Negative
B. subtilis	1	Undetermined	Negative
	1	Undetermined	Negative
C. sporogenes	1	Undetermined	Negative
	1	Undetermined	Negative
C. albicans	1	Undetermined	Negative
	1	Undetermined	Negative
NTC	2	Undetermined	Negative
	2	Undetermined	Negative
E. coli	2	Undetermined	Negative
	2	Undetermined	Negative

Undetermined = negative
≤40 = positive

Table 1: Test for E638 lacI-lacY PCR specificity (continued)

Item	Run	Ct	Evaluation
S. aureus	2	Undetermined	Negative
	2	Undetermined	Negative
B. subtilis	2	Undetermined	Negative
	2	Undetermined	Negative
C. sporogenes	2	Undetermined	Negative
	2	Undetermined	Negative
C. albicans	2	Undetermined	Negative
	2	Undetermined	Negative
NTC	3	Undetermined	Negative
	3	Undetermined	Negative
E. coli	3	Undetermined	Negative
	3	Undetermined	Negative
S. aureus	3	Undetermined	Negative
	3	Undetermined	Negative
B. subtilis	3	Undetermined	Negative
	3	Undetermined	Negative

Undetermined = negative
≤40 = positive

Table 1: Test for E638 lacI-lacY PCR specificity (continued)

Item	Run	Ct	Evaluation
C. sporogenes	3	Undetermined	Negative
	3	Undetermined	Negative
C. albicans	3	Undetermined	Negative
	3	Undetermined	Negative

Undetermined = negative
≤40 = positive

Table 2: Test for E638 lacI-lacY PCR Limit of Detection on pG217 DNA, dilution series 1

Concentration (ng/µl)	Run 1		Run 2		Run 3	
	Ct	Evaluation	Ct	Evaluation	Ct	Evaluation
10^{-3}	27.2	Positive	26.9	Positive	25.2	Positive
	27.2	Positive	27.0	Positive	25.1	Positive
10^{-4}	31.1	Positive	30.4	Positive	29.0	Positive
	31.5	Positive	30.8	Positive	29.2	Positive
10^{-5}	35.6	Positive	34.5	Positive	33.5	Positive
	35.1	Positive	34.8	Positive	33.0	Positive
10^{-6}	39.3	Positive	38.3	Positive	38.0	Positive
	Undetermined	Negative	36.7	Positive	35.8	Positive
10^{-7}	Undetermined	Negative	Undetermined	Negative	Undetermined	Negative
	Undetermined	Negative	Undetermined	Negative	Undetermined	Negative

Undetermined = negative
≤40 = positive

Table 3: Test for E638 lacI-lacY PCR Limit of Detection on pG217 DNA, dilution series 2

Concentration (ng/µl)	Run 1		Run 2		Run 3	
	Ct	Evaluation	Ct	Evaluation	Ct	Evaluation
10^{-3}	27.1	Positive	27.2	Positive	27.5	Positive
	27.4	Positive	27.5	Positive	27.8	Positive
10^{-4}	31.2	Positive	31.3	Positive	31.2	Positive
	31.5	Positive	31.7	Positive	32.0	Positive
10^{-5}	34.7	Positive	36.1	Positive	36.5	Positive
	36.2	Positive	35.6	Positive	35.2	Positive
10^{-6}	Undetermined	Negative	Undetermined	Negative	Undetermined	Negative
	38.1	Positive	Undetermined	Negative	Undetermined	Negative
10^{-7}	Not applicable[a]	Not applicable[a]	Undetermined	Negative	Undetermined	Negative
	Not applicable[a]	Not applicable[a]	Undetermined	Negative	Undetermined	Negative

Undetermined = negative
≤40 = positive
[a] Refer to OOO-300-2017-02-24-B

Table 4: Test for E638 lacI-lacY PCR Limit of Detection on pG217 DNA, dilution series 3

Concentration (ng/µl)	Run 1		Run 2		Run 3	
	Ct	Evaluation	Ct	Evaluation	Ct	Evaluation
10^{-3}	27.1	Positive	27.8	Positive	27.9	Positive
	27.2	Positive	27.7	Positive	27.6	Positive
10^{-4}	31.1	Positive	32.0	Positive	32.2	Positive
	30.9	Positive	32.0	Positive	31.9	Positive
10^{-5}	35.1	Positive	35.5	Positive	37.2	Positive
	35.6	Positive	37.6	Positive	37.2	Positive
10^{-6}	39.4	Positive	Undetermined	Negative	Undetermined	Negative
	Undetermined	Negative	Undetermined	Negative	Undetermined	Negative
10^{-7}	Not applicable[a]	Not applicable[a]	Undetermined	Negative	Undetermined	Negative
	Not applicable[a]	Not applicable[a]	Undetermined	Negative	Undetermined	Negative

Undetermined = negative
≤40 = positive
[a] Refer to OOO-300-2017-02-24-B

Table 5: Test for E638 lacI-lacY PCR Limit of Detection on E997 DNA, dilution series 1

Concentration (ng/µl)	Run 1		Run 2		Run 3	
	Ct	Evaluation	Ct	Evaluation	Ct	Evaluation
10^{-3}	24.5	Positive	24.1	Positive	22.4	Positive
	24.5	Positive	24.1	Positive	22.4	Positive
10^{-4}	28.3	Positive	27.8	Positive	26.2	Positive
	28.5	Positive	27.7	Positive	26.3	Positive
10^{-5}	32.2	Positive	31.7	Positive	30.2	Positive
	32.7	Positive	31.7	Positive	30.0	Positive
10^{-6}	38.0	Positive	35.3	Positive	34.1	Positive
	35.6	Positive	35.3	Positive	33.7	Positive
10^{-7}	Undetermined	Negative	38.3	Positive	38.8	Positive
	Undetermined	Negative	Undetermined	Negative	Undetermined	Negative

Undetermined = negative
≤40 = positive

Table 6: Test for E638 lacI-lacY PCR Limit of Detection on E997 DNA, dilution series 2

Concentration (ng/µl)	Run 1		Run 2		Run 3	
	Ct	Evaluation	Ct	Evaluation	Ct	Evaluation
10^{-3}	24.1	Positive	25.2	Positive	25.1	Positive
	24.3	Positive	25.0	Positive	25.0	Positive
10^{-4}	28.2	Positive	29.1	Positive	29.1	Positive
	27.9	Positive	29.3	Positive	29.3	Positive
10^{-5}	32.6	Positive	33.5	Positive	33.4	Positive
	32.8	Positive	34.1	Positive	33.5	Positive
10^{-6}	35.6	Positive	39.0	Positive	39.2	Positive
	37.1	Positive	39.2	Positive	37.7	Positive
10^{-7}	Not applicable[a]	Not applicable[a]	Undetermined	Negative	Undetermined	Negative
	Not applicable[a]	Not applicable[a]	Undetermined	Negative	Undetermined	Negative

Undetermined = negative
≤40 = positive
[a] Refer to OOO-300-2017-02-24-B

Table 7: Test for E638 lacI-lacY PCR Limit of Detection on E997 DNA, dilution series 3

Concentration (ng/µl)	Run 1		Run 2		Run 3	
	Ct	Evaluation	Ct	Evaluation	Ct	Evaluation
10^{-3}	24.3	Positive	24.8	Positive	25.0	Positive
	24.5	Positive	24.8	Positive	25.0	Positive
10^{-4}	28.2	Positive	29.2	Positive	29.3	Positive
	28.5	Positive	29.0	Positive	29.2	Positive
10^{-5}	32.6	Positive	34.0	Positive	34.2	Positive
	32.8	Positive	33.0	Positive	34.1	Positive
10^{-6}	36.9	Positive	38.3	Positive	38.9	Positive
	36.0	Positive	38.7	Positive	38.2	Positive
10^{-7}	Not applicable[a]	Not applicable[a]	Undetermined	Negative	Undetermined	Negative
	Not applicable[a]	Not applicable[a]	Undetermined	Negative	Undetermined	Negative

Undetermined = negative
≤40 = positive
[a] Refer to OOO-300-2017-02-24-B

Evaluation of the results:

1. A positive signal could still be detected in the E638 lacI-lacY PCR by using 9 µl of 10^{-6} ng/µl of the E997 DNA template DNA, using a total reaction volume of 20 µl.
2. A positive signal could still be detected in the E638 lacI-lacY PCR by using 9 µl of 10^{-5} ng/µl of the pG217 DNA template DNA, using a total reaction volume of 20 µl.
3. LacI-lacY specific sequence is on the chromosome. The study was performed before this information was provided by the customer. The LOD in the conclusion is calculated using results of the *E.coli* strain (71162). This new information indicates that it was not necessary to test the plasmid DNA (71161).

Conclusions:

1. The assay meets the criteria for a valid test.
2. The primers are specific in this assay, no positive signals are observed using DNA of *E.coli, S. aureus, B. subtilis, C. sporogenes* and *C. albicans*.
3. LacI-lacY specific DNA sequence is on the chromosome, therefore the LOD is calculated using results of the *E. coli* strain (E997 DNA). The LOD of the assay is $4.5*10^{-7}$ ng/µl reaction.

Quality Statement:

The study was performed in compliance with the agreed protocol and was executed in accordance with MicroSafe Laboratories Standard Operating Procedures except when clearly documented otherwise. The execution of the study conformed to the principles of Good Manufacturing Practices of the European Community.

Authorisation and approval:

Prepared by:

Name	Signature	Date
M. Schrumpf	(b) (6)	15 Mrt 17

Quality Assurance:

Name	Signature	Date
S. Man	(b) (6)	16 Mart 7

Appendix qPCR6

Validation report of the E 638 ΔwcaJ gene gene detection method by quantitative PCR

Sample identification:

MicroSafe sample number	Sample name	Concentration
71161	pG217 DNA	191 µg/ml
71162	E997 DNA	218 µg/ml

Administrative details:

Sponsor: Sponsor representative: Sponsor number:	Friesland Campina Innovation Bronland 20 6708 WH Wageningen The Netherlands Mr. J. B. Bastiaans 2995
Testing facility:	Eurofins MicroSafe Laboratories Darwinweg 24 2333 CR Leiden The Netherlands
Test Schedule: Laboratory start date: Laboratory completion date:	 20 February 2017 02 March 2017
Raw data references:	LAB-GEN-700-TR01: T17B380, T17B381, T17B360, T17B465 T17B382, T17B383, T17C032, T17B487, T17B489
Report revision note:	After the report was sent new information was provided by the customer. Due to this the conclusion of this report needs to be adjusted. The LOD of the method was calculated using qPCR results of the plasmid DNA (71161). E638 wcaJ specific sequence is on the chromosome, therefore the LOD needs to be calculated using results of the *E.coli* strain (71162). The conclusion was rewritten.

Study details:

Test method:	According to protocol TM-SP-086 revision code 1.0 with study specific supplement.
Sample preparation:	The given concentration of the samples is used to dilute to a concentration of 10 ng/µl.
Quantity tested:	9 µl template DNA is tested in the qPCR.
Specificity:	DNA of *E.coli, S. aureus, B. subtilis, C. sporogenes* and *C. albicans* were isolated, 10 times diluted in DNase/RNase free water and tested in duplicate by qPCR. Tests were performed in triplicate by at least two operators on at least two different days.
Limit of Detection	DNA of samples 71161 and 71162 were diluted to a concentration of 1 ng/µl using DNase/RNase free water. Three independent 10-fold series of dilutions (10^{-1} till 10^{-7}) were prepared. Dilutions 10^{-3} till 10^{-7} were tested in duplicate by qPCR using E638 wcaJ specific primers. Tests were performed in triplicate by at least two operators on at least two different days.
Negative template control (NTC):	DNase/RNase free water
Protocol amendments:	In the first three runs one ten-fold serial dilution per sample was tested in duplicate. Therefore three extra runs were performed by preparing two extra independent ten-fold serial dilutions of the sample and these were tested in duplicate by PCR. When the pG217 DNA at dilution 10^{-3} is tested, a 95% positive signal could not be obtained. Therefore three runs, by two operators on at least two different days are repeated using 10^{-2} till 10^{-4} ng/µl pG217 DNA.
Non-conformances:	There were two non-conformances. OOO-200-2017-02-21-E: After the first run the *E. coli* DNA (T16K192-C) turned out to be not enough to perform all tests for the validation. Therefore a new *E.coli* DNA isolation was performed (T17B374) and the resulting DNA was used as a negative control in the last two runs. OOO-300-2017-02-24-B: Protocol TM-SP-086 revision code 1.0 describes that three independent serial dilutions per sample will be tested in duplicate per run. In the first three runs one ten-fold serial dilution per sample was tested in duplicate. Therefore three extra runs were performed by preparing two extra independent ten-fold serial dilutions of the sample and these were tested in duplicate by PCR. In the first extra run dilutions 10^{-2} to 10^{-6} ng/µl DNA was tested, instead of 10^{-3} to 10^{-7} ng/µl DNA. In the initial runs testing the first dilution series the reactions with an input of 9 µl of 10^{-5} ng/µl were all negative. Therefore it can be concluded that the lack of these data has no impact on the determination of the LOD of the PCR. When the pG217 DNA at dilution 10^{-3} is tested, a 95% positive signal could not be obtained. Therefore three runs, by two operators on at least two different days are repeated using 10^{-2} till 10^{-4} ng/µl pG217 DNA.

Results:

Table 1: Test for E638 wcaJ PCR specificity

Item	Run	Ct	Evaluation
NTC	1	Undetermined	Negative
	1	Undetermined	Negative
E. coli	1	Undetermined	Negative
	1	Undetermined	Negative
S. aureus	1	Undetermined	Negative
	1	Undetermined	Negative
B. subtilis	1	Undetermined	Negative
	1	Undetermined	Negative
C. sporogenes	1	Undetermined	Negative
	1	Undetermined	Negative
C. albicans	1	Undetermined	Negative
	1	Undetermined	Negative
NTC	2	Undetermined	Negative
	2	Undetermined	Negative
E. coli	2	Undetermined	Negative
	2	Undetermined	Negative

Undetermined = negative
≤40 = positive

Table 1: Test for E638 wcaJ PCR specificity (continued)

Item	Run	Ct	Evaluation
S. aureus	2	Undetermined	Negative
	2	Undetermined	Negative
B. subtilis	2	Undetermined	Negative
	2	Undetermined	Negative
C. sporogenes	2	Undetermined	Negative
	2	Undetermined	Negative
C. albicans	2	Undetermined	Negative
	2	Undetermined	Negative
NTC	3	Undetermined	Negative
	3	Undetermined	Negative
E. coli	3	Undetermined	Negative
	3	Undetermined	Negative
S. aureus	3	Undetermined	Negative
	3	Undetermined	Negative
B. subtilis	3	Undetermined	Negative
	3	Undetermined	Negative

Undetermined = negative
≤40 = positive

Table 1: Test for E638 wcaJ PCR specificity (continued)

Item	Run	Ct	Evaluation
C. sporogenes	3	Undetermined	Negative
	3	Undetermined	Negative
C. albicans	3	Undetermined	Negative
	3	Undetermined	Negative

Undetermined = negative
≤40 = positive

Table 2: Test for E638 wcaJ PCR Limit of Detection on pG217 DNA, dilution series 1

Concentration (ng/µl)	Run 1		Run 2		Run 3	
	Ct	Evaluation	Ct	Evaluation	Ct	Evaluation
10^{-3}	36.7	Positive	34.7	Positive	36.0	Positive
	36.4	Positive	36.9	Positive	35.8	Positive
10^{-4}	Undetermined	Negative	Undetermined	Negative	Undetermined	Negative
	37.3	Positive	Undetermined	Negative	Undetermined	Negative
10^{-5}	Undetermined	Negative	Undetermined	Negative	Undetermined	Negative
	Undetermined	Negative	Undetermined	Negative	Undetermined	Negative
10^{-6}	Undetermined	Negative	Undetermined	Negative	Undetermined	Negative
	Undetermined	Negative	Undetermined	Negative	Undetermined	Negative
10^{-7}	Undetermined	Negative	Undetermined	Negative	Undetermined	Negative
	Undetermined	Negative	Undetermined	Negative	Undetermined	Negative

Undetermined = negative
≤40 = positive

Table 3: Test for E638 wcaJ PCR Limit of Detection on pG217 DNA, dilution series 2

Concentration (ng/µl)	Run 1		Run 2		Run 3	
	Ct	Evaluation	Ct	Evaluation	Ct	Evaluation
10^{-3}	36.1	Positive	37.0	Positive	37.3	Positive
	37.2	Positive	39.5	Positive	36.7	Positive
10^{-4}	Undetermined	Negative	Undetermined	Negative	39.7	Positive
	Undetermined	Negative	Undetermined	Negative	Undetermined	Negative
10^{-5}	Undetermined	Negative	Undetermined	Negative	Undetermined	Negative
	Undetermined	Negative	Undetermined	Negative	Undetermined	Negative
10^{-6}	Undetermined	Negative	36.2	Positive	Undetermined	Negative
	Undetermined	Negative	Undetermined	Negative	Undetermined	Negative
10^{-7}	Not applicable[a]	Not applicable[a]	Undetermined	Negative	Undetermined	Negative
	Not applicable[a]	Not applicable[a]	Undetermined	Negative	Undetermined	Negative

Undetermined = negative
≤40 = positive
[a] Refer to OOO-300-2017-02-24-B

Table 4: Test for E638 wcaJ PCR Limit of Detection on pG217 DNA, dilution series 3

Concentration (ng/µl)	Run 1		Run 2		Run 3	
	Ct	Evaluation	Ct	Evaluation	Ct	Evaluation
10^{-3}	37.5	Positive	38.6	Positive	Undetermined	Negative
	37.5	Positive	Undetermined	Negative	37.8	Positive
10^{-4}	36.2	Positive	Undetermined	Negative	Undetermined	Negative
	Undetermined	Negative	Undetermined	Negative	Undetermined	Negative
10^{-5}	Undetermined	Negative	Undetermined	Negative	Undetermined	Negative
	Undetermined	Negative	Undetermined	Negative	Undetermined	Negative
10^{-6}	Undetermined	Negative	Undetermined	Negative	Undetermined	Negative
	Undetermined	Negative	Undetermined	Negative	Undetermined	Negative
10^{-7}	Not applicable[a]	Not applicable[a]	38.7	Positive	Undetermined	Negative
	Not applicable[a]	Not applicable[a]	37.2	Positive	Undetermined	Negative

Undetermined = negative
≤40 = positive
[a] Refer to OOO-300-2017-02-24-B

Table 5: Test for E638 wcaJ PCR Limit of Detection on pG217 DNA, dilution series 1 (Repeat test)

Concentration (ng/µl)	Run 1		Run 2		Run 3	
	Ct	Evaluation	Ct	Evaluation	Ct	Evaluation
10^{-2}	32.9	Positive	32.1	Positive	34.5	Positive
	34.0	Positive	32.2	Positive	33.9	Positive
10^{-3}	36.1	Positive	36.3	Positive	Undetermined	Negative
	37.2	Positive	37.2	Positive	Undetermined	Negative
10^{-4}	Undetermined	Negative	Undetermined	Negative	Undetermined	Negative
	Undetermined	Negative	Undetermined	Negative	Undetermined	Negative

Undetermined = negative
≤40 = positive

Table 6: Test for E638 wcaJ PCR Limit of Detection on pG217 DNA, dilution series 2 (Repeat test)

Concentration (ng/µl)	Run 1		Run 2		Run 3	
	Ct	Evaluation	Ct	Evaluation	Ct	Evaluation
10^{-2}	33.4	Positive	32.9	Positive	34.3	Positive
	33.5	Positive	32.9	Positive	34.4	Positive
10^{-3}	37.5	Positive	36.1	Positive	Undetermined	Negative
	37.5	Positive	35.8	Positive	38.4	Positive
10^{-4}	36.2	Positive	Undetermined	Negative	Undetermined	Negative
	Undetermined	Negative	37.2	Positive	Undetermined	Negative

Undetermined = negative
≤40 = positive

Table 7: Test for E638 wcaJ PCR Limit of Detection on pG217 DNA, dilution series 3 (Repeat test)

Concentration (ng/µl)	Run 1		Run 2		Run 3	
	Ct	Evaluation	Ct	Evaluation	Ct	Evaluation
10^{-2}	31.1	Positive	33.0	Positive	34.2	Positive
	32.0	Positive	32.4	Positive	35.3	Positive
10^{-3}	37.1	Positive	37.1	Positive	36.3	Positive
	36.0	Positive	35.7	Positive	Undetermined	Negative
10^{-4}	Undetermined	Negative	Undetermined	Negative	Undetermined	Negative
	Undetermined	Negative	40.0	Positive	Undetermined	Negative

Undetermined = negative
≤40 = positive

Table 8: Test for E638 wcaJ PCR Limit of Detection on E997 DNA, dilution series 1

Concentration (ng/µl)	Run 1		Run 2		Run 3	
	Ct	Evaluation	Ct	Evaluation	Ct	Evaluation
10^{-3}	36.3	Positive	33.3	Positive	32.5	Positive
	37.2	Positive	33.3	Positive	34.2	Positive
10^{-4}	Undetermined	Negative	38.0	Positive	36.6	Positive
	38.6	Positive	36.6	Positive	Undetermined	Negative
10^{-5}	Undetermined	Negative	Undetermined	Negative	Undetermined	Negative
	37.8	Positive	Undetermined	Negative	39.5	Positive
10^{-6}	Undetermined	Negative	Undetermined	Negative	Undetermined	Negative
	Undetermined	Negative	Undetermined	Negative	Undetermined	Negative
10^{-7}	Undetermined	Negative	Undetermined	Negative	Undetermined	Negative
	Undetermined	Negative	Undetermined	Negative	Undetermined	Negative

Undetermined = negative
≤40 = positive

Table 9: Test for E638 wcaJ PCR Limit of Detection on E997 DNA, dilution series 2

Concentration (ng/µl)	Run 1		Run 2		Run 3	
	Ct	Evaluation	Ct	Evaluation	Ct	Evaluation
10^{-3}	35.3	Positive	35.9	Positive	35.9	Positive
	34.5	Positive	36.2	Positive	38.2	Positive
10^{-4}	38.4	Positive	37.7	Positive	Undetermined	Negative
	37.2	Positive	39.4	Positive	Undetermined	Negative
10^{-5}	Undetermined	Negative	38.9	Positive	39.6	Positive
	Undetermined	Negative	37.9	Positive	37.7	Positive
10^{-6}	Undetermined	Negative	39.4	Positive	38.7	Positive
	Undetermined	Negative	Undetermined	Negative	Undetermined	Negative
10^{-7}	Not applicable[a]	Not applicable[a]	Undetermined	Negative	Undetermined	Negative
	Not applicable[a]	Not applicable[a]	Undetermined	Negative	Undetermined	Negative

Undetermined = negative
≤40 = positive
[a] Refer to OOO-300-2017-02-24-B

Table 10: Test for E638 wcaJ PCR Limit of Detection on E997 DNA, dilution series 3

Concentration (ng/µl)	Run 1		Run 2		Run 3	
	Ct	Evaluation	Ct	Evaluation	Ct	Evaluation
10^{-3}	31.3	Positive	34.6	Positive	36.5	Positive
	31.0	Positive	35.0	Positive	36.7	Positive
10^{-4}	35.2	Positive	Undetermined	Negative	37.8	Positive
	34.5	Positive	37.7	Positive	38.8	Positive
10^{-5}	Undetermined	Negative	Undetermined	Negative	Undetermined	Negative
	37.6	Positive	37.1	Positive	Undetermined	Negative
10^{-6}	Undetermined	Negative	Undetermined	Negative	Undetermined	Negative
	Undetermined	Negative	Undetermined	Negative	Undetermined	Negative
10^{-7}	Not applicable[a]	Not applicable[a]	Undetermined	Negative	Undetermined	Negative
	Not applicable[a]	Not applicable[a]	Undetermined	Negative	Undetermined	Negative

Undetermined = negative
≤40 = positive
[a] Refer to OOO-300-2017-02-24-B

Evaluation of the results:

1. A positive signal could still be detected in the E638 wcaJ PCR by using 9 µl of 10^{-3} ng/µl of the E997 DNA template DNA, using a total reaction volume of 20 µl.
2. A positive signal could still be detected in the E638 wcaJ PCR by using 9 µl of 10^{-2} ng/µl of the pG217 DNA template DNA, using a total reaction volume of 20 µl.
3. E638 wcaJ specific sequence is on the chromosome. The study was performed before this information was provided by the customer. The LOD in the conclusion is calculated using results of the *E.coli* strain (71162). This new information indicates that it was not necessary to test the plasmid DNA (71161).

Conclusions:

1. The assay meets the criteria for a valid test.
2. The primers are specific in this assay, no positive signals are observed using DNA of *E.coli, S. aureus, B. subtilis, C. sporogenes* and *C. albicans*.
3. E638 wcaJ specific DNA sequence is on the chromosome, therefore the LOD is calculated using results of the *E. coli* strain (E997 DNA). The LOD of the assay is $4.5*10^{-4}$ ng/µl reaction.

Quality Statement:
The study was performed in compliance with the agreed protocol and was executed in accordance with MicroSafe Laboratories Standard Operating Procedures except when clearly documented otherwise. The execution of the study conformed to the principles of Good Manufacturing Practices of the European Community.

Authorisation and approval:
Prepared by:

M. Schrumpf	(b) (6)	15Mrt17
Name	Signature	Date

Quality Assurance:

S. Man	(b) (6)	16 Mar 17
Name	Signature	Date

Appendix qPCR7:
qPCR Test Reports

Sample identification:

MicroSafe sample number	Sample name	Lotnumber
71895	Fucosyllactose	PMRS10

Administrative details:

Sponsor: Sponsor representative: Sponsor number:	Friesland Campina Innovation Bronland 20 6708 WH Wageningen The Netherlands Mr. Jan-Willem Boots 2995
Testing facility:	Eurofins MicroSafe Laboratories Darwinweg 24 2333 CR Leiden The Netherlands
Test Schedule: Laboratory start date: Laboratory completion date:	 25 January 2017 26 January 2017
Raw data references:	00010584TR: T17A404

Study details:

Sample preparation and test method:	Samples and controls are tested in duplicate. Matrix interference test is performed once. In order to obtain possible DNA present in the sample, the sample is isolated using PrepMan Ultra. The sample is also tested directly in the qPCR to check if isolation is needed. Both methods include matrix interference. For direct PCR 10 mg of sample is weighed and dissolved in 2 ml DNase/RNase free water. For matrix interference 5 µl of E997 DNA (0.1 ng/µl) is added. The DNA isolation is performed using PrepMan Ultra. 20 mg of sample is weighed and 400 µl of PrepMan Ultra is added. For matrix interference 10 µl of E997 DNA (0.1 ng/µl) is added. After incubation at 98 °C for 15 minutes, the sample is centrifuged (13.000 rpm, 5 minutes). 100 µl supernatant is transferred to an new tube and consists of the isolated DNA. The isolated DNA is diluted 10 times using DNase/RNase free water before it was used as template in the qPCR. For the qPCR the following primers (10 µM) are used: - KAN-F: CTC ACC TTG CTC CTG CCG AGA - KAN-R: CGC CTT GAG CCT GGC GAA CAG Per reaction a volume of 1 µl of each primer is added to 13 µl DNase/RNase free water and 20 µl of SYBR Green PCR Mastermix (2x). A total of 35 µl is filled out in a 96 wells PCR plate. A volume of 5 µl template is added resulting in a total reaction volume of 40 µl. The following qPCR program is performed: • 10 minutes on 95 °C • 15 seconds on 95 °C } 40 • 60 seconds on 60 °C } cycles + dissociation stage
Quantity tested:	20 mg of the sample is used for DNA isolation and 10 mg is used for direct qPCR.

Study details continued:

Non template control (NTC):	DNase/RNase free water
Positive Control:	E997 DNA (0.1 ng/µl)
Negative Control:	*E. coli* DNA
Protocol amendments:	Not applicable
Non-conformances:	Not applicable

Results:

Item	Ct	Acceptance criteria	Evaluation
NTC	Undetermined	> 35	Complies
Negative Control	37.2	> 35	Complies
Positive Control	19.5	≤ 35	Complies
71895 (direct PCR)	Undetermined	None	Negative
	Undetermined	None	Negative
71895 (DNA isolation)	Undetermined	None	Negative
	Undetermined	None	Negative
Matrix interference			
71895 (direct PCR)	35.4	≤ 35	Complies
71895 (DNA isolation)	29.4	≤ 35	Complies

Conclusions:

1. The assay meets the criteria for a valid test.
2. No Kanamycin resistance gene is detected in the sample.
3. The matrix interference is successfully performed using both methods (direct qPCR and with DNA isolation).
4. It is recommended performing a DNA isolation for this type of sample, because a lower Ct value is observed using this method compared to direct qPCR.

Quality Statement:
The study was performed according to the study specific supplement and MicroSafe Laboratories Standard Operating Procedures except when clearly documented otherwise. The execution of the study conformed to the principles of Good Manufacturing Practices of the European Community.

Authorisation and approval:

Prepared by:

M. Schrumpf — Name
(b) (6) — Signature
30 jan 17 — Date

Quality Assurance:

M. L. Fekkes — Name
(b) (6) — Signature
31 jan 17 — Date

Title: Performing a qPCR for the detection of a E638 lacI-lacY gene to confirm that host strain DNA is undetectable in the final product at a sensitivity level of 5 ppb

Study number: 2995A.72510

Sample identification:

MicroSafe sample number	Sample name	Lot number
72510	2'fucosyllactose	PMRS10

Administrative details:

Sponsor: Sponsor representative: Sponsor number:	Friesland Campina Innovation Bronland 20 6708 WH Wageningen The Netherlands Mr. J.B. Bastiaans 2995
Testing facility:	Eurofins MicroSafe Laboratories Darwinweg 24 2333 CR Leiden The Netherlands
Test Schedule: Laboratory start date: Laboratory completion date:	 05 May 2017 10 May 2017
Raw data references:	LAB-GEN-700-TR01: T17E125, T17E162

Study details:

Sample preparation and test method:	According to SOP LAB-MOLBIO-240 revision code [2.0] with study specific supplement. On request of the sponsor, the sample was tested in the lacI-lacY PCR to confirm that host strain DNA is undetectable in the final product at a sensitivity level of 5 ppb. Sample and controls were tested in duplicate for presence of the lacI-lacY gene by qPCR. Matrix validation was tested once. DNA isolation: In order to obtain possible DNA present in the sample, the sample is isolated using PrepMan Ultra. 20 mg of sample is weighed and 400 µl of PrepMan Ultra is added. For matrix interference 10 µl of E997 DNA (0.1 ng/µl) is added. After incubation at 98 °C for 15 minutes, the sample is centrifuged (13.000 rpm, 5 minutes). 100 µl supernatant is transferred to an new tube and consists of the isolated DNA. The isolated DNA is diluted 250 times using DNase/RNase free water before it was used as template in the qPCR. This makes the sensitivity level of the assay 5 ng/g (ppb). E638 lacI-lacY qPCR: For the qPCR the following primers (10 µM) are used: - E638 lacI-lacY-F2: GCC CGG AAG AGA GTC AAG TG - E638 lacI-lacY-R2: TCC TCC TTA GTT CCT ATT CCG AAG Per reaction a volume of 0.5 µl of each primer is added to 10 µl of SYBR Green PCR Mastermix (2x). A total of 11 µl is filled out in a 96 wells PCR plate. A volume of 9 µl template is added resulting in a total reaction volume of 20 µl. The following qPCR program is performed: • 2 minutes on 50 °C • 10 minutes on 95 °C • 15 seconds on 95 °C } 40 • 60 seconds on 60 °C } cycles + dissociation stage
Quantity tested:	20 mg of the sample is tested in duplicate 20 mg of the sample is used in the matrix interference test
Non template control (NTC):	DNase/RNase free water
Positive Control:	E997 DNA (0.1 ng/µl)
Negative Control:	*E. coli* DNA
Protocol amendments:	Not applicable
Non-conformances:	There was one non-conformance (OOO-300-2017-05-08-B). In the initial test, the NTC was positive in the lacI-lacY PCR. The test results were considered invalid and therefore the PCR was repeated. In the re-test, the results were valid. The results of the re-test are reported.

Results:

Item	Ct*	Acceptance criteria	Evaluation
NTC	Undetermined	> 40	Complies
Negative Control	Undetermined	> 40	Complies
	Undetermined	> 40	Complies
Positive Control	15.6	≤ 40	Complies
	15.7	≤ 40	Complies
72510	Undetermined	None	Negative
	Undetermined	None	Negative
Matrix interference			
72510	30.7	≤ 40	Complies

>40 or undetermined = negative
≤40 = positive
*Refer to OOO-300-2017-05-08-B

Conclusions:

1. The assay meets the criteria for a valid test.
2. No host strain DNA is detected in the final product at a sensitivity level of 5 ppb of the E638 lacI-lacY assay.

Quality Statement:

The study was performed according to the study specific supplement and MicroSafe Laboratories Standard Operating Procedures except when clearly documented otherwise. The execution of the study conformed to the principles of Good Manufacturing Practices of the European Community.

Authorisation and approval:

Prepared by:

M. Schrumpf
Name

(b) (6)
Signature

16 may 17
Date

Quality Assurance:

(b) (6)
Name

17 May 17
Date

Sample identification:

MicroSafe sample number	Sample name	Lot number
72510/72512	2'fucosyllactose	PMRS10

Administrative details:

Sponsor: Sponsor representative: Sponsor number:	Friesland Campina Innovation Bronland 20 6708 WH Wageningen The Netherlands Mr. J.B. Bastiaans 2995
Testing facility:	Eurofins MicroSafe Laboratories Darwinweg 24 2333 CR Leiden The Netherlands
Test Schedule: Laboratory start date: Laboratory completion date:	 20 March 2017 23 March 2017
Raw data references:	LAB-GEN-700-TR01: T17B484
Report revision note:	On request of the Sponsor separate reports are made for each PCR.

Study details:

Sample preparation and test method:	According to SOP LAB-MOLBIO-240 revision code [1.0] with study specific supplement. Samples and controls are tested in duplicate. Matrix interference is tested once. DNA isolation In order to obtain possible DNA present in the sample, the sample is isolated using PrepMan Ultra. 20 mg of sample is weighed and 400 µl of PrepMan Ultra is added. For matrix interference 10 µl of E997 DNA (0.1 ng/µl) is added. After incubation at 98 °C for 15 minutes, the sample is centrifuged (13.000 rpm, 5 minutes). 100 µl supernatant is transferred to an new tube and consists of the isolated DNA. The isolated DNA is diluted 10 times using DNase/RNase free water before it was used as template in the qPCR. E638 wcaJ qPCR: For the qPCR the following primers (10 µM) are used: - E638 wcaJ-F2: AGG AAC AAC GAT GAT TCC GGG - E638 wcaJ-R2: GCC GCT TTG TTA ACT GTA GGC Per reaction a volume of 0.5 µl of each primer is added to 10 µl of SYBR Green PCR Mastermix (2x). A total of 11 µl is filled out in a 96 wells PCR plate. A volume of 9 µl template is added resulting in a total reaction volume of 20 µl. The following qPCR program is performed: • 2 minutes on 50 °C • 10 minutes on 95 °C • 15 seconds on 95 °C } 40 • 60 seconds on 60 °C } cycles + dissociation stage

Study details continued:

Quantity tested:	20 mg of the sample is tested in duplicate. 20 mg of the sample is used in the matrix interference test.
Non template control (NTC):	DNase/RNase free water
Positive Control:	E997 DNA (0.1 ng/µl)
Negative Control:	*E. coli* DNA
Protocol amendments:	Not applicable
Non-conformances:	Not applicable

Results using E638 wcaJ primers:

Item	Ct	Acceptance criteria	Evaluation
NTC	Undetermined	Undetermined	Complies
Negative Control	Undetermined	Undetermined	Complies
	Undetermined	Undetermined	Complies
Positive Control	24.8	≤ 40	Complies
	25.1	≤ 40	Complies
72510	Undetermined	None	Negative
	Undetermined	None	Negative
72512	Undetermined	None	Negative
	Undetermined	None	Negative
Matrix Interference			
72510	34.2	≤ 40	Complies
72512	32.8	≤ 40	Complies

Undetermined = negative
≤40 = positive

Conclusions:

1. The assay meets the criteria for a valid test.
2. No inhibition was observed in the matrix interference test.
3. No E638 wcaJ gene is detected in the sample.

Quality Statement:

The study was performed according to the study specific supplement and MicroSafe Laboratories Standard Operating Procedures except when clearly documented otherwise. The execution of the study conformed to the principles of Good Manufacturing Practices of the European Community.

Authorisation and approval:

Prepared by:

Name	Signature	Date
M. Schrumpf	(b) (6)	03 May 17

Quality Assurance:

Name	Signature	Date
S Man	(b) (6)	04 May 17

Eurofins MicroSafe Laboratories
Final Report

Title: Detection of a Kanamycin resistance gene by qPCR
Study number: 2995.71896

Sample identification:

MicroSafe sample number	Sample name	Lotnumber
71896	Fucosyllactose	PMRS11

Administrative details:

Sponsor: Sponsor representative: Sponsor number:	Friesland Campina Innovation Bronland 20 6708 WH Wageningen The Netherlands Mr. Jan-Willem Boots 2995
Testing facility:	Eurofins MicroSafe Laboratories Darwinweg 24 2333 CR Leiden The Netherlands
Test Schedule: Laboratory start date: Laboratory completion date:	 25 January 2017 26 January 2017
Raw data references:	00010584TR: T17A404

Study details:

Sample preparation and test method:	Samples and controls are tested in duplicate. Matrix interference test is performed once. In order to obtain possible DNA present in the sample, the sample is isolated using PrepMan Ultra. The sample is also tested directly in the qPCR to check if isolation is needed. Both methods include matrix interference. For direct PCR 10 mg of sample is weighed and dissolved in 2 ml DNase/RNase free water. For matrix interference 5 µl of E997 DNA (0.1 ng/µl) is added. The DNA isolation is performed using PrepMan Ultra. 20 mg of sample is weighed and 400 µl of PrepMan Ultra is added. For matrix interference 10 µl of E997 DNA (0.1 ng/µl) is added. After incubation at 98 °C for 15 minutes, the sample is centrifuged (13.000 rpm, 5 minutes). 100 µl supernatant is transferred to an new tube and consists of the isolated DNA. The isolated DNA is diluted 10 times using DNase/RNase free water before it was used as template in the qPCR. For the qPCR the following primers (10 µM) are used: - KAN-F: CTC ACC TTG CTC CTG CCG AGA - KAN-R: CGC CTT GAG CCT GGC GAA CAG Per reaction a volume of 1 µl of each primer is added to 13 µl DNase/RNase free water and 20 µl of SYBR Green PCR Mastermix (2x). A total of 35 µl is filled out in a 96 wells PCR plate. A volume of 5 µl template is added resulting in a total reaction volume of 40 µl. The following qPCR program is performed: • 10 minutes on 95 °C • 15 seconds on 95 °C • 60 seconds on 60 °C } 40 cycles + dissociation stage
Quantity tested:	20 mg of the sample is used for DNA isolation and 10 mg is used for direct qPCR.

Study details continued:

Non template control (NTC):	DNase/RNase free water
Positive Control:	E997 DNA (0.1 ng/µl)
Negative Control:	*E. coli* DNA
Protocol amendments:	Not applicable
Non-conformances:	Not applicable

Results:

Item	Ct	Acceptance criteria	Evaluation
NTC	Undetermined	> 35	Complies
Negative Control	37.2	> 35	Complies
Positive Control	19.5	≤ 35	Complies
71896 (direct PCR)	Undetermined	None	Negative
	Undetermined	None	Negative
71896 (DNA isolation)	Undetermined	None	Negative
	Undetermined	None	Negative
Matrix interference			
71896 (direct PCR)	36.0	≤ 35	Does not comply
71896 (DNA isolation)	29.3	≤ 35	Complies

Conclusions:

1. The assay meets the criteria for a valid test.
2. No Kanamycin resistance gene is detected in the sample.
3. The matrix interference is successfully performed using DNA isolation.
4. It is recommended performing a DNA isolation for this type of sample, because no positive result is observed using direct qPCR in the matrix interference test.

Quality Statement:
The study was performed according to the study specific supplement and MicroSafe Laboratories Standard Operating Procedures except when clearly documented otherwise. The execution of the study conformed to the principles of Good Manufacturing Practices of the European Community.

Authorisation and approval:

Prepared by:

M Schrumpf — Name
(b) (6) — Signature
30 jan 17 — Date

Quality Assurance:

M.L. Fekkes — Name
(b) (6) — Signature
31 Jan 17 — Date

Title: Performing a qPCR for the detection of a E638 lacI-lacY gene to confirm that host strain DNA is undetectable in the final product at a sensitivity level of 5 ppb

Sample identification:

MicroSafe sample number	Sample name	Lot number
72509	2'fucosyllactose	PMRS11

Administrative details:

Sponsor: Sponsor representative: Sponsor number:	Friesland Campina Innovation Bronland 20 6708 WH Wageningen The Netherlands Mr. J.B. Bastiaans 2995
Testing facility:	Eurofins MicroSafe Laboratories Darwinweg 24 2333 CR Leiden The Netherlands
Test Schedule: Laboratory start date: Laboratory completion date:	 05 May 2017 10 May 2017
Raw data references:	LAB-GEN-700-TR01: T17E125, T17E162

Study details:

Sample preparation and test method:	According to SOP LAB-MOLBIO-240 revision code [2.0] with study specific supplement. On request of the sponsor, the sample was tested in the lacI-lacY PCR to confirm that host strain DNA is undetectable in the final product at a sensitivity level of 5 ppb. Sample and controls were tested in duplicate for presence of the lacI-lacY gene by qPCR. Matrix validation was tested once. DNA isolation: In order to obtain possible DNA present in the sample, the sample is isolated using PrepMan Ultra. 20 mg of sample is weighed and 400 µl of PrepMan Ultra is added. For matrix interference 10 µl of E997 DNA (0.1 ng/µl) is added. After incubation at 98 °C for 15 minutes, the sample is centrifuged (13.000 rpm, 5 minutes). 100 µl supernatant is transferred to an new tube and consists of the isolated DNA. The isolated DNA is diluted 250 times using DNase/RNase free water before it was used as template in the qPCR. This makes the sensitivity level of the assay 5 ng/g (ppb). E638 lacI-lacY qPCR: For the qPCR the following primers (10 µM) are used: - E638 lacI-lacY-F2: GCC CGG AAG AGA GTC AAG TG - E638 lacI-lacY-R2: TCC TCC TTA GTT CCT ATT CCG AAG Per reaction a volume of 0.5 µl of each primer is added to 10 µl of SYBR Green PCR Mastermix (2x). A total of 11 µl is filled out in a 96 wells PCR plate. A volume of 9 µl template is added resulting in a total reaction volume of 20 µl. The following qPCR program is performed: • 2 minutes on 50 °C • 10 minutes on 95 °C • 15 seconds on 95 °C } 40 • 60 seconds on 60 °C } cycles + dissociation stage
Quantity tested:	20 mg of the sample is tested in duplicate 20 mg of the sample is used in the matrix interference test
Non template control (NTC):	DNase/RNase free water
Positive Control:	E997 DNA (0.1 ng/µl)
Negative Control:	*E. coli* DNA
Protocol amendments:	Not applicable
Non-conformances:	There was one non-conformance (OOO-300-2017-05-08-B). In the initial test, the NTC was positive in the lacI-lacY PCR. The test results were considered invalid and therefore the PCR was repeated. In the re-test, the results were valid. The results of the re-test are reported.

Results:

Item	Ct*	Acceptance criteria	Evaluation
NTC	Undetermined	> 40	Complies
Negative Control	Undetermined	> 40	Complies
	Undetermined	> 40	Complies
Positive Control	15.6	≤ 40	Complies
	15.7	≤ 40	Complies
72509	Undetermined	None	Negative
	Undetermined	None	Negative
Matrix interference			
72509	31.4	≤ 40	Complies

>40 or undetermined = negative
≤40 = positive
*Refer to OOO-300-2017-05-08-B

Conclusions:

1. The assay meets the criteria for a valid test.
2. No host strain DNA is detected in the final product at a sensitivity level of 5 ppb of the E638 lacI-lacY assay.

Quality Statement:
The study was performed according to the study specific supplement and MicroSafe Laboratories Standard Operating Procedures except when clearly documented otherwise. The execution of the study conformed to the principles of Good Manufacturing Practices of the European Community.

Authorisation and approval:
Prepared by:

M. Schrumpf
Name

(b) (6)
Signature

16may17
Date

Quality Assurance:

Name

(b) (6)

17may17
Date

Sample identification:

MicroSafe sample number	Sample name	Lot number
72509/72511	2'fucosyllactose	PMRS11

Administrative details:

Sponsor: Sponsor representative: Sponsor number:	Friesland Campina Innovation Bronland 20 6708 WH Wageningen The Netherlands Mr. J.B. Bastiaans 2995
Testing facility:	Eurofins MicroSafe Laboratories Darwinweg 24 2333 CR Leiden The Netherlands
Test Schedule: Laboratory start date: Laboratory completion date:	 20 March 2017 23 March 2017
Raw data references:	LAB-GEN-700-TR01: T17B484
Report revision note:	On request of the Sponsor separate reports are made for each PCR.

Study details:

Sample preparation and test method:	According to SOP LAB-MOLBIO-240 revision code [1.0] with study specific supplement. Samples and controls are tested in duplicate. Matrix interference is tested once. DNA isolation In order to obtain possible DNA present in the sample, the sample is isolated using PrepMan Ultra. 20 mg of sample is weighed and 400 µl of PrepMan Ultra is added. For matrix interference 10 µl of E997 DNA (0.1 ng/µl) is added. After incubation at 98 °C for 15 minutes, the sample is centrifuged (13.000 rpm, 5 minutes). 100 µl supernatant is transferred to an new tube and consists of the isolated DNA. The isolated DNA is diluted 10 times using DNase/RNase free water before it was used as template in the qPCR. E638 wcaJ qPCR: For the qPCR the following primers (10 µM) are used: - E638 wcaJ-F2: AGG AAC AAC GAT GAT TCC GGG - E638 wcaJ-R2: GCC GCT TTG TTA ACT GTA GGC Per reaction a volume of 0.5 µl of each primer is added to 10 µl of SYBR Green PCR Mastermix (2x). A total of 11 µl is filled out in a 96 wells PCR plate. A volume of 9 µl template is added resulting in a total reaction volume of 20 µl. The following qPCR program is performed: • 2 minutes on 50 °C • 10 minutes on 95 °C • 15 seconds on 95 °C } 40 • 60 seconds on 60 °C } cycles + dissociation stage

Study details continued:

Quantity tested:	20 mg of the sample is tested in duplicate. 20 mg of the sample is used in the matrix interference test.
Non template control (NTC):	DNase/RNase free water
Positive Control:	E997 DNA (0.1 ng/µl)
Negative Control:	*E. coli* DNA
Protocol amendments:	Not applicable
Non-conformances:	Not applicable

Results using E638 wcaJ primers:

Item	Ct	Acceptance criteria	Evaluation
NTC	Undetermined	Undetermined	Complies
Negative Control	Undetermined	Undetermined	Complies
	Undetermined	Undetermined	Complies
Positive Control	24.8	≤ 40	Complies
	25.1	≤ 40	Complies
72509	Undetermined	None	Negative
	Undetermined	None	Negative
72511	Undetermined	None	Negative
	Undetermined	None	Negative
Matrix interference			
72509	34.6	≤ 40	Complies
72511	34.0	≤ 40	Complies

Undetermined = negative
≤40 = **positive**

Conclusions:

1. The assay meets the criteria for a valid test.
2. No inhibition was observed in the matrix interference test.
3. No E638 wcaJ gene is detected in the sample.

Quality Statement:

The study was performed according to the study specific supplement and MicroSafe Laboratories Standard Operating Procedures except when clearly documented otherwise. The execution of the study conformed to the principles of Good Manufacturing Practices of the European Community.

Authorisation and approval:

Prepared by:

M. Schrumpf	(b) (6)	03 May 17
Name	Signature	Date

Quality Assurance:

S Man	(b) (6)	04 May 17
Name	Signature	Date

Title: Detection of a Kanamycin resistance gene by qPCR
Study number: 2995.71893

Sample identification:

MicroSafe sample number	Sample name	Lotnumber
71893	Fucosyllactose	CMRS03

Administrative details:

Sponsor: Sponsor representative: Sponsor number:	Friesland Campina Innovation Bronland 20 6708 WH Wageningen The Netherlands Mr. Jan-Willem Boots 2995
Testing facility:	Eurofins MicroSafe Laboratories Darwinweg 24 2333 CR Leiden The Netherlands
Test Schedule: Laboratory start date: Laboratory completion date:	 25 January 2017 26 January 2017
Raw data references:	00010584TR: T17A404

Study details:

Sample preparation and test method:	Samples and controls are tested in duplicate. Matrix interference test is performed once. In order to obtain possible DNA present in the sample, the sample is isolated using PrepMan Ultra. The sample is also tested directly in the qPCR to check if isolation is needed. Both methods include matrix interference. For direct PCR 10 mg of sample is weighed and dissolved in 2 ml DNase/RNase free water. For matrix interference 5 µl of E997 DNA (0.1 ng/µl) is added. The DNA isolation is performed using PrepMan Ultra. 20 mg of sample is weighed and 400 µl of PrepMan Ultra is added. For matrix interference 10 µl of E997 DNA (0.1 ng/µl) is added. After incubation at 98 °C for 15 minutes, the sample is centrifuged (13.000 rpm, 5 minutes). 100 µl supernatant is transferred to an new tube and consists of the isolated DNA. The isolated DNA is diluted 10 times using DNase/RNase free water before it was used as template in the qPCR. For the qPCR the following primers (10 µM) are used: - KAN-F: CTC ACC TTG CTC CTG CCG AGA - KAN-R: CGC CTT GAG CCT GGC GAA CAG Per reaction a volume of 1 µl of each primer is added to 13 µl DNase/RNase free water and 20 µl of SYBR Green PCR Mastermix (2x). A total of 35 µl is filled out in a 96 wells PCR plate. A volume of 5 µl template is added resulting in a total reaction volume of 40 µl. The following qPCR program is performed: • 10 minutes on 95 °C • 15 seconds on 95 °C • 60 seconds on 60 °C } 40 cycles + dissociation stage
Quantity tested:	20 mg of the sample is used for DNA isolation and 10 mg is used for direct qPCR.

Study details continued:

Non template control (NTC):	DNase/RNase free water
Positive Control:	E997 DNA (0.1 ng/µl)
Negative Control:	*E. coli* DNA
Protocol amendments:	Not applicable
Non-conformances:	Not applicable

Results:

Item	Ct	Acceptance criteria	Evaluation
NTC	Undetermined	> 35	Complies
Negative Control	37.2	> 35	Complies
Positive Control	19.5	≤ 35	Complies
71893 (direct PCR)	Undetermined	None	Negative
	Undetermined	None	Negative
71893 (DNA isolation)	Undetermined	None	Negative
	Undetermined	None	Negative
Matrix interference			
71893 (direct PCR)	37.9	≤ 35	Does not comply
71893 (DNA isolation)	29.2	≤ 35	Complies

Conclusions:

1. The assay meets the criteria for a valid test.
2. No Kanamycin resistance gene is detected in the sample.
3. The matrix interference is successfully performed using DNA isolation.
4. It is recommended performing a DNA isolation for this type of sample, because no positive result is observed using direct qPCR in the matrix interference test.

Quality Statement:
The study was performed according to the study specific supplement and MicroSafe Laboratories Standard Operating Procedures except when clearly documented otherwise. The execution of the study conformed to the principles of Good Manufacturing Practices of the European Community.

Authorisation and approval:

Prepared by:

Name	Signature	Date
M. Schrumpf	(b) (6)	30 jan 17

Quality Assurance:

Name	Signature	Date
M. L. Fokkens	(b) (6)	31 jan 17

Title: Performing a qPCR for the detection of a E638 lacI-lacY gene to confirm that host strain DNA is undetectable in the final product at a sensitivity level of 5 ppb

Study number: 2995A.72515

Sample identification:

MicroSafe sample number	Sample name	Lot number
72515	2'fucosyllactose	CMRS03

Administrative details:

Sponsor: Sponsor representative: Sponsor number:	Friesland Campina Innovation Bronland 20 6708 WH Wageningen The Netherlands Mr. J.B. Bastiaans 2995
Testing facility:	Eurofins MicroSafe Laboratories Darwinweg 24 2333 CR Leiden The Netherlands
Test Schedule: Laboratory start date: Laboratory completion date:	 05 May 2017 10 May 2017
Raw data references:	LAB-GEN-700-TR01: T17E125, T17E162

Study details:

Sample preparation and test method:	According to SOP LAB-MOLBIO-240 revision code [2.0] with study specific supplement. On request of the sponsor, the sample was tested in the lacI-lacY PCR to confirm that host strain DNA is undetectable in the final product at a sensitivity level of 5 ppb. Sample and controls were tested in duplicate for presence of the lacI-lacY gene by qPCR. Matrix validation was tested once. DNA isolation: In order to obtain possible DNA present in the sample, the sample is isolated using PrepMan Ultra. 20 mg of sample is weighed and 400 µl of PrepMan Ultra is added. For matrix interference 10 µl of E997 DNA (0.1 ng/µl) is added. After incubation at 98 °C for 15 minutes, the sample is centrifuged (13.000 rpm, 5 minutes). 100 µl supernatant is transferred to an new tube and consists of the isolated DNA. The isolated DNA is diluted 250 times using DNase/RNase free water before it was used as template in the qPCR. This makes the sensitivity level of the assay 5 ng/g (ppb). E638 lacI-lacY qPCR: For the qPCR the following primers (10 µM) are used: - E638 lacI-lacY-F2: GCC CGG AAG AGA GTC AAG TG - E638 lacI-lacY-R2: TCC TCC TTA GTT CCT ATT CCG AAG Per reaction a volume of 0.5 µl of each primer is added to 10 µl of SYBR Green PCR Mastermix (2x). A total of 11 µl is filled out in a 96 wells PCR plate. A volume of 9 µl template is added resulting in a total reaction volume of 20 µl. The following qPCR program is performed: • 2 minutes on 50 °C • 10 minutes on 95 °C • 15 seconds on 95 °C } 40 • 60 seconds on 60 °C } cycles + dissociation stage
Quantity tested:	20 mg of the sample is tested in duplicate 20 mg of the sample is used in the matrix interference test
Non template control (NTC):	DNase/RNase free water
Positive Control:	E997 DNA (0.1 ng/µl)
Negative Control:	*E. coli* DNA
Protocol amendments:	Not applicable
Non-conformances:	There was one non-conformance (OOO-300-2017-05-08-B). In the initial test, the NTC was positive in the lacI-lacY PCR. The test results were considered invalid and therefore the PCR was repeated. In the re-test, the results were valid. The results of the re-test are reported.

Results:

Item	Ct*	Acceptance criteria	Evaluation
NTC	Undetermined	> 40	Complies
Negative Control	Undetermined	> 40	Complies
	Undetermined	> 40	Complies
Positive Control	15.6	≤ 40	Complies
	15.7	≤ 40	Complies
72515	Undetermined	None	Negative
	Undetermined	None	Negative
Matrix interference			
72515	30.5	≤ 40	Complies

>40 or undetermined = negative
≤40 = positive
*Refer to OOO-300-2017-05-08-B

Conclusions:

1. The assay meets the criteria for a valid test.
2. No host strain DNA is detected in the final product at a sensitivity level of 5 ppb of the E638 lacI-lacY assay.

Quality Statement:
The study was performed according to the study specific supplement and MicroSafe Laboratories Standard Operating Procedures except when clearly documented otherwise. The execution of the study conformed to the principles of Good Manufacturing Practices of the European Community.

Authorisation and approval:

Prepared by:

M. Schrumpf — Name

(b) (6) — Signature

16 may 17 — Date

Quality Assurance:

J. van Olphen — Name

(b) (6)

17 may 17 — Date

Sample identification:

MicroSafe sample number	Sample name	Lot number
72515/72516	2'fucosyllactose	CMRS03

Administrative details:

Sponsor: Sponsor representative: Sponsor number:	Friesland Campina Innovation Bronland 20 6708 WH Wageningen The Netherlands Mr. J.B. Bastiaans 2995
Testing facility:	Eurofins MicroSafe Laboratories Darwinweg 24 2333 CR Leiden The Netherlands
Test Schedule: Laboratory start date: Laboratory completion date:	 20 March 2017 23 March 2017
Raw data references:	LAB-GEN-700-TR01: T17B484
Report revision note:	On request of the Sponsor separate reports are made for each PCR.

Study details:

Sample preparation and test method:	According to SOP LAB-MOLBIO-240 revision code [1.0] with study specific supplement. Samples and controls are tested in duplicate. Matrix interference is tested once. DNA isolation In order to obtain possible DNA present in the sample, the sample is isolated using PrepMan Ultra. 20 mg of sample is weighed and 400 µl of PrepMan Ultra is added. For matrix interference 10 µl of E997 DNA (0.1 ng/µl) is added. After incubation at 98 °C for 15 minutes, the sample is centrifuged (13.000 rpm, 5 minutes). 100 µl supernatant is transferred to an new tube and consists of the isolated DNA. The isolated DNA is diluted 10 times using DNase/RNase free water before it was used as template in the qPCR. E638 wcaJ qPCR: For the qPCR the following primers (10 µM) are used: - E638 wcaJ-F2: AGG AAC AAC GAT GAT TCC GGG - E638 wcaJ-R2: GCC GCT TTG TTA ACT GTA GGC Per reaction a volume of 0.5 µl of each primer is added to 10 µl of SYBR Green PCR Mastermix (2x). A total of 11 µl is filled out in a 96 wells PCR plate. A volume of 9 µl template is added resulting in a total reaction volume of 20 µl. The following qPCR program is performed: • 2 minutes on 50 °C • 10 minutes on 95 °C • 15 seconds on 95 °C } 40 • 60 seconds on 60 °C } cycles + dissociation stage

Study details continued:

Quantity tested:	20 mg of the sample is tested in duplicate. 20 mg of the sample is used in the matrix interference test.
Non template control (NTC):	DNase/RNase free water
Positive Control:	E997 DNA (0.1 ng/µl)
Negative Control:	*E. coli* DNA
Protocol amendments:	Not applicable
Non-conformances:	Not applicable

Results using E638 wcaJ primers:

Item	Ct	Acceptance criteria	Evaluation
NTC	Undetermined	Undetermined	Complies
Negative Control	Undetermined	Undetermined	Complies
	Undetermined	Undetermined	Complies
Positive Control	24.8	≤ 40	Complies
	25.1	≤ 40	Complies
72515	Undetermined	None	Negative
	Undetermined	None	Negative
72516	Undetermined	None	Negative
	Undetermined	None	Negative
Matrix interference			
72515	33.3	≤ 40	Complies
72516	34.3	≤ 40	Complies

Undetermined = negative
≤40 = positive

Conclusions:

1. The assay meets the criteria for a valid test.
2. No inhibition was observed in the matrix interference test.
3. No E638 wcaJ gene is detected in the sample.

Quality Statement:

The study was performed according to the study specific supplement and MicroSafe Laboratories Standard Operating Procedures except when clearly documented otherwise. The execution of the study conformed to the principles of Good Manufacturing Practices of the European Community.

Authorisation and approval:

Prepared by:

M Schrumpf	(b) (6)	03May17
Name	Signature	Date

Quality Assurance:

S Man	(b) (6)	04 May17
Name	Signature	Date

Sample identification:

MicroSafe sample number	Sample name	Lot number
72708	2'fucosyllactose	CMRS06 A NIRO

Administrative details:

Sponsor: Sponsor representative: Sponsor number:	Friesland Campina Innovation Bronland 20 6708 WH Wageningen The Netherlands Mr. J.B. Bastiaans 2995
Testing facility:	Eurofins MicroSafe Laboratories Darwinweg 24 2333 CR Leiden The Netherlands
Test Schedule: Laboratory start date: Laboratory completion date:	 31 March 2017 03 April 2017
Raw data references:	LAB-GEN-700-TR01: T17C617
Report revision note:	On request of the Sponsor separate reports are made for each PCR.

Study details:

Sample preparation and test method:	According to SOP LAB-MOLBIO-240 revision code [1.0] with study specific supplement. Samples and controls are tested in duplicate for presence of the kanamycin resistance gene by qPCR. DNA isolation: In order to obtain possible DNA present in the sample, the sample is isolated using PrepMan Ultra. 20 mg of sample is weighed and 400 µl of PrepMan Ultra is added. After incubation at 98 °C for 15 minutes, the sample is centrifuged (13.000 rpm, 5 minutes). 100 µl supernatant is transferred to an new tube and consists of the isolated DNA. The isolated DNA is diluted 10 times using DNase/RNase free water before it was used as template in the qPCR. Kanamycin qPCR: For the qPCR the following primers (10 µM) are used: - KAN-F: CTC ACC TTG CTC CTG CCG AGA - KAN-R: CGC CTT GAG CCT GGC GAA CAG Per reaction a volume of 1 µl of each primer is added to 13 µl DNase/RNase free water and 20 µl of SYBR Green PCR Mastermix (2x). A total of 35 µl is filled out in a 96 wells PCR plate. A volume of 5 µl template is added resulting in a total reaction volume of 40 µl. The following qPCR program is performed: • 10 minutes on 95 °C • 15 seconds on 95 °C } 40 • 60 seconds on 60 °C } cycles + dissociation stage

Study details continued:

Quantity tested:	20 mg of the sample is tested in duplicate.
Non template control (NTC):	DNase/RNase free water
Positive Control:	E997 DNA (0.1 ng/µl)
Negative Control:	*E. coli* DNA
Protocol amendments:	Not applicable
Non-conformances:	There was one non-conformances. OOO-300-2017-04-05-B: In the test the controls were tested once instead of in duplicate. This had no impact on the outcome of the test.

Results using Kanamycin primers:

Item	Ct	Acceptance criteria	Evaluation
NTC	Undetermined	> 35	Complies
Negative Control	Undetermined	> 35	Complies
Positive Control	20.4	≤ 35	Complies
72708	Undetermined	None	Negative
	Undetermined	None	Negative

>35 or undetermined = negative
≤35 = positive

Conclusions:

1. The assay meets the criteria for a valid test.
2. No Kanamycin resistance gene is detected in the sample.

Quality Statement:
The study was performed according to the study specific supplement and MicroSafe Laboratories Standard Operating Procedures except when clearly documented otherwise. The execution of the study conformed to the principles of Good Manufacturing Practices of the European Community.

Authorisation and approval:
Prepared by:

M. Schrumpf — Name
(b) (6) — Signature
03may17 — Date

Quality Assurance:

S Man — Name
(b) (6) — Signature
04/may/17 — Date

Title: Performing a qPCR for the detection of a E638 lacI-lacY gene to confirm that host strain DNA is undetectable in the final product at a sensitivity level of 5 ppb

Study number: 2995A.72708

Sample identification:

MicroSafe sample number	Sample name	Lot number
72708	2'fucosyllactose	CMRS06 A NIRO

Administrative details:

Sponsor: Sponsor representative: Sponsor number:	Friesland Campina Innovation Bronland 20 6708 WH Wageningen The Netherlands Mr. J.B. Bastiaans 2995
Testing facility:	Eurofins MicroSafe Laboratories Darwinweg 24 2333 CR Leiden The Netherlands
Test Schedule: Laboratory start date: Laboratory completion date:	 05 May 2017 10 May 2017
Raw data references:	LAB-GEN-700-TR01: T17E125, T17E162

Study details:

Sample preparation and test method:	According to SOP LAB-MOLBIO-240 revision code [2.0] with study specific supplement. On request of the sponsor, the sample was tested in the lacI-lacY PCR to confirm that host strain DNA is undetectable in the final product at a sensitivity level of 5 ppb. Sample and controls were tested in duplicate for presence of the lacI-lacY gene by qPCR. Matrix validation was tested once. DNA isolation: In order to obtain possible DNA present in the sample, the sample is isolated using PrepMan Ultra. 20 mg of sample is weighed and 400 µl of PrepMan Ultra is added. For matrix interference 10 µl of E997 DNA (0.1 ng/µl) is added. After incubation at 98 °C for 15 minutes, the sample is centrifuged (13.000 rpm, 5 minutes). 100 µl supernatant is transferred to an new tube and consists of the isolated DNA. The isolated DNA is diluted 250 times using DNase/RNase free water before it was used as template in the qPCR. This makes the sensitivity level of the assay 5 ng/g (ppb). E638 lacI-lacY qPCR: For the qPCR the following primers (10 µM) are used: - E638 lacI-lacY-F2: GCC CGG AAG AGA GTC AAG TG - E638 lacI-lacY-R2: TCC TCC TTA GTT CCT ATT CCG AAG Per reaction a volume of 0.5 µl of each primer is added to 10 µl of SYBR Green PCR Mastermix (2x). A total of 11 µl is filled out in a 96 wells PCR plate. A volume of 9 µl template is added resulting in a total reaction volume of 20 µl. The following qPCR program is performed: • 2 minutes on 50 °C • 10 minutes on 95 °C • 15 seconds on 95 °C } 40 • 60 seconds on 60 °C } cycles + dissociation stage
Quantity tested:	20 mg of the sample is tested in duplicate 20 mg of the sample is used in the matrix interference test
Non template control (NTC):	DNase/RNase free water
Positive Control:	E997 DNA (0.1 ng/µl)
Negative Control:	*E. coli* DNA
Protocol amendments:	Not applicable
Non-conformances:	There was one non-conformance (OOO-300-2017-05-08-B). In the initial test, the NTC was positive in the lacI-lacY PCR. The test results were considered invalid and therefore the PCR was repeated. In the re-test, the results were valid. The results of the re-test are reported.

Results:

Item	Ct*	Acceptance criteria	Evaluation
NTC	Undetermined	> 40	Complies
Negative Control	Undetermined	> 40	Complies
	Undetermined	> 40	Complies
Positive Control	15.6	≤ 40	Complies
	15.7	≤ 40	Complies
72708	Undetermined	None	Negative
	Undetermined	None	Negative
Matrix interference			
72708	31.2	≤ 40	Complies

>40 or undetermined = negative
≤40 = positive
*Refer to OOO-300-2017-05-08-B

Conclusions:

1. The assay meets the criteria for a valid test.
2. No host strain DNA is detected in the final product at a sensitivity level of 5 ppb of the E638 lacI-lacY assay.

Quality Statement:
The study was performed according to the study specific supplement and MicroSafe Laboratories Standard Operating Procedures except when clearly documented otherwise. The execution of the study conformed to the principles of Good Manufacturing Practices of the European Community.

Authorisation and approval:
Prepared by:

M. Schrumpf
Name

(b) (6)
Signature

16may17
Date

Quality Assurance:

Name

(b) (6)

Date

Sample identification:

MicroSafe sample number	Sample name	Lot number
72708	2'fucosyllactose	CMRS06 A NIRO

Administrative details:

Sponsor: Sponsor representative: Sponsor number:	Friesland Campina Innovation Bronland 20 6708 WH Wageningen The Netherlands Mr. J.B. Bastiaans 2995
Testing facility:	Eurofins MicroSafe Laboratories Darwinweg 24 2333 CR Leiden The Netherlands
Test Schedule: Laboratory start date: Laboratory completion date:	 31 March 2017 03 April 2017
Raw data references:	LAB-GEN-700-TR01: T17C617
Report revision note:	On request of the Sponsor separate reports are made for each PCR.

Study details:

Sample preparation and test method:	According to SOP LAB-MOLBIO-240 revision code [1.0] with study specific supplement. Samples and controls are tested in duplicate for presence of the E638 wcaJ gene by qPCR. DNA isolation: In order to obtain possible DNA present in the sample, the sample is isolated using PrepMan Ultra. 20 mg of sample is weighed and 400 µl of PrepMan Ultra is added. After incubation at 98 °C for 15 minutes, the sample is centrifuged (13.000 rpm, 5 minutes). 100 µl supernatant is transferred to an new tube and consists of the isolated DNA. The isolated DNA is diluted 10 times using DNase/RNase free water before it was used as template in the qPCR. E638 wcaJ qPCR: For the qPCR the following primers (10 µM) are used: - E638 wcaJ-F2: AGG AAC AAC GAT GAT TCC GGG - E638 wcaJ-R2: GCC GCT TTG TTA ACT GTA GGC Per reaction a volume of 0.5 µl of each primer is added to 10 µl of SYBR Green PCR Mastermix (2x). A total of 11 µl is filled out in a 96 wells PCR plate. A volume of 9 µl template is added resulting in a total reaction volume of 20 µl. The following qPCR program is performed: • 2 minutes on 50 °C • 10 minutes on 95 °C • 15 seconds on 95 °C } 40 • 60 seconds on 60 °C } cycles + dissociation stage

Study details continued:

Quantity tested:	20 mg of the sample is tested in duplicate.
Non template control (NTC):	DNase/RNase free water
Positive Control:	E997 DNA (0.1 ng/µl)
Negative Control:	*E. coli* DNA
Protocol amendments:	Not applicable
Non-conformances:	There was one non-conformances. OOO-300-2017-04-05-B: In the test the controls were tested once instead of in duplicate. This had no impact on the outcome of the test.

Results using E638 wcaJ primers:

Item	Ct	Acceptance criteria	Evaluation
NTC	Undetermined	Undetermined	Complies
Negative Control	Undetermined	Undetermined	Complies
Positive Control	24.1	≤ 40	Complies
72708	Undetermined	None	Negative
	Undetermined	None	Negative

Undetermined = negative
≤40 = positive

Conclusions:

1. The assay meets the criteria for a valid test.
2. No E638 wcaJ gene is detected in the sample.

Quality Statement:
The study was performed according to the study specific supplement and MicroSafe Laboratories Standard Operating Procedures except when clearly documented otherwise. The execution of the study conformed to the principles of Good Manufacturing Practices of the European Community.

Authorisation and approval:
Prepared by:

M. Schrumpf — Name
(b) (6) — Signature
03may17 — Date

Quality Assurance:

S. Man — Name
(b) (6) — Signature
04may17 — Date

Sample identification:

MicroSafe sample number	Sample name	Lot number
73084	2'fucosyllactose	CMRS07

Administrative details:

Sponsor: Sponsor representative: Sponsor number:	Friesland Campina Innovation Bronland 20 6708 WH Wageningen The Netherlands Mr. J.B. Bastiaans 2995
Testing facility:	Eurofins MicroSafe Laboratories Darwinweg 24 2333 CR Leiden The Netherlands
Test Schedule: Laboratory start date: Laboratory completion date:	 05 May 2017 08 May 2017
Raw data references:	LAB-GEN-700-TR01: T17E125

Study details:

Sample preparation and test method:	According to SOP LAB-MOLBIO-240 revision code [2.0] with study specific supplement. Sample and controls were tested in duplicate for presence of the kanamycin resistance gene by qPCR. DNA isolation: In order to obtain possible DNA present in the sample, the sample is isolated using PrepMan Ultra. 20 mg of sample is weighed and 400 µl of PrepMan Ultra is added. After incubation at 98 °C for 15 minutes, the sample is centrifuged (13.000 rpm, 5 minutes). 100 µl supernatant is transferred to an new tube and consists of the isolated DNA. The isolated DNA is diluted 10 times using DNase/RNase free water before it was used as template in the qPCR. Kanamycin qPCR: For the qPCR the following primers (10 µM) are used: - KAN-F: CTC ACC TTG CTC CTG CCG AGA - KAN-R: CGC CTT GAG CCT GGC GAA CAG Per reaction a volume of 1 µl of each primer is added to 13 µl DNase/RNase free water and 20 µl of SYBR Green PCR Mastermix (2x). A total of 35 µl is filled out in a 96 wells PCR plate. A volume of 5 µl template is added resulting in a total reaction volume of 40 µl. The following qPCR program is performed: • 10 minutes on 95 °C • 15 seconds on 95 °C } 40 • 60 seconds on 60 °C } cycles + dissociation stage

Study details continued:

Quantity tested:	20 mg of the sample is tested in duplicate.
Non template control (NTC):	DNase/RNase free water
Positive Control:	E997 DNA (0.1 ng/µl)
Negative Control:	*E. coli* DNA
Protocol amendments:	Not applicable
Non-conformances:	Not applicable

Results:

Item	Ct	Acceptance criteria	Evaluation
NTC	Undetermined	> 35	Complies
Negative Control	Undetermined	> 35	Complies
	Undetermined	> 35	Complies
Positive Control	21.0	≤ 35	Complies
	21.0	≤ 35	Complies
73084	38.5	None	Negative
	Undetermined	None	Negative

>35 or undetermined = negative
≤35 = positive

Conclusions:

1. The assay meets the criteria for a valid test.
2. No kanamycin resistance gene is detected in the sample.

Quality Statement:
The study was performed according to the study specific supplement and MicroSafe Laboratories Standard Operating Procedures except when clearly documented otherwise. The execution of the study conformed to the principles of Good Manufacturing Practices of the European Community.

Authorisation and approval:

Prepared by:

M Schrumpf — Name
(b) (6) — Signature
11 May 17 — Date

Quality Assurance:

S Man — Name
(b) (6) — Signature
15 May 17 — Date

Title: Performing a qPCR for the detection of a E638 lacI-lacY gene to confirm that host strain DNA is undetectable in the final product at a sensitivity level of 5 ppb

Sample identification:

MicroSafe sample number	Sample name	Lot number
73084	2'fucosyllactose	CMRS07

Administrative details:

Sponsor: Sponsor representative: Sponsor number:	Friesland Campina Innovation Bronland 20 6708 WH Wageningen The Netherlands Mr. J.B. Bastiaans 2995
Testing facility:	Eurofins MicroSafe Laboratories Darwinweg 24 2333 CR Leiden The Netherlands
Test Schedule: Laboratory start date: Laboratory completion date:	 05 May 2017 10 May 2017
Raw data references:	LAB-GEN-700-TR01: T17E125, T17E162

Study details:

Sample preparation and test method:	According to SOP LAB-MOLBIO-240 revision code [2.0] with study specific supplement. On request of the sponsor, the sample was tested in the lacI-lacY PCR to confirm that host strain DNA is undetectable in the final product at a sensitivity level of 5 ppb. Sample and controls were tested in duplicate for presence of the lacI-lacY gene by qPCR. Matrix validation was tested once. DNA isolation: In order to obtain possible DNA present in the sample, the sample is isolated using PrepMan Ultra. 20 mg of sample is weighed and 400 µl of PrepMan Ultra is added. For matrix interference 10 µl of E997 DNA (0.1 ng/µl) is added. After incubation at 98 °C for 15 minutes, the sample is centrifuged (13.000 rpm, 5 minutes). 100 µl supernatant is transferred to an new tube and consists of the isolated DNA. The isolated DNA is diluted 250 times using DNase/RNase free water before it was used as template in the qPCR. This makes the sensitivity level of the assay 5 ng/g (ppb). E638 lacI-lacY qPCR: For the qPCR the following primers (10 µM) are used: - E638 lacI-lacY-F2: GCC CGG AAG AGA GTC AAG TG - E638 lacI-lacY-R2: TCC TCC TTA GTT CCT ATT CCG AAG Per reaction a volume of 0.5 µl of each primer is added to 10 µl of SYBR Green PCR Mastermix (2x). A total of 11 µl is filled out in a 96 wells PCR plate. A volume of 9 µl template is added resulting in a total reaction volume of 20 µl. The following qPCR program is performed: • 2 minutes on 50 °C • 10 minutes on 95 °C • 15 seconds on 95 °C } 40 • 60 seconds on 60 °C } cycles + dissociation stage
Quantity tested:	20 mg of the sample is tested in duplicate 20 mg of the sample is used in the matrix interference test
Non template control (NTC):	DNase/RNase free water
Positive Control:	E997 DNA (0.1 ng/µl)
Negative Control:	*E. coli* DNA
Protocol amendments:	Not applicable
Non-conformances:	There was one non-conformance (OOO-300-2017-05-08-B). In the initial test, the NTC was positive in the lacI-lacY PCR. The test results were considered invalid and therefore the PCR was repeated. In the re-test, the results were valid. The results of the re-test are reported.

Results:

Item	Ct*	Acceptance criteria	Evaluation
NTC	Undetermined	> 40	Complies
Negative Control	Undetermined	> 40	Complies
	Undetermined	> 40	Complies
Positive Control	15.6	≤ 40	Complies
	15.7	≤ 40	Complies
73084	Undetermined	None	Negative
	Undetermined	None	Negative
Matrix interference			
73084	31.5	≤ 40	Complies

>40 or undetermined = negative
≤40 = positive
*Refer to OOO-300-2017-05-08-B

Conclusions:

1. The assay meets the criteria for a valid test.
2. No host strain DNA is detected in the final product at a sensitivity level of 5 ppb of the E638 lacI-lacY assay.

Quality Statement:

The study was performed according to the study specific supplement and MicroSafe Laboratories Standard Operating Procedures except when clearly documented otherwise. The execution of the study conformed to the principles of Good Manufacturing Practices of the European Community.

Authorisation and approval:

Prepared by:

M. Schrumpf
Name

(b) (6)
Signature

16may17
Date

Quality Assurance:

Name

(b) (6)

17 May 17
Date

Sample identification:

MicroSafe sample number	Sample name	Lot number
73084	2'fucosyllactose	CMRS07

Administrative details:

Sponsor: Sponsor representative: Sponsor number:	Friesland Campina Innovation Bronland 20 6708 WH Wageningen The Netherlands Mr. J.B. Bastiaans 2995
Testing facility:	Eurofins MicroSafe Laboratories Darwinweg 24 2333 CR Leiden The Netherlands
Test Schedule: Laboratory start date: Laboratory completion date:	 05 May 2017 08 May 2017
Raw data references:	LAB-GEN-700-TR01: T17E125

Study details:

Sample preparation and test method:	According to SOP LAB-MOLBIO-240 revision code [2.0] with study specific supplement. Sample and controls were tested in duplicate for presence of the wcaJ gene by qPCR. DNA isolation: In order to obtain possible DNA present in the sample, the sample is isolated using PrepMan Ultra. 20 mg of sample is weighed and 400 µl of PrepMan Ultra is added. After incubation at 98 °C for 15 minutes, the sample is centrifuged (13.000 rpm, 5 minutes). 100 µl supernatant is transferred to an new tube and consists of the isolated DNA. The isolated DNA is diluted 10 times using DNase/RNase free water before it was used as template in the qPCR. E638 wcaJ qPCR: For the qPCR the following primers (10 µM) are used: - E638 wcaJ-F2: AGG AAC AAC GAT GAT TCC GGG - E638 wcaJ-R2: GCC GCT TTG TTA ACT GTA GGC Per reaction a volume of 0.5 µl of each primer is added to 10 µl of SYBR Green PCR Mastermix (2x). A total of 11 µl is filled out in a 96 wells PCR plate. A volume of 9 µl template is added resulting in a total reaction volume of 20 µl. The following qPCR program is performed: • 2 minutes on 50 °C • 10 minutes on 95 °C • 15 seconds on 95 °C } 40 • 60 seconds on 60 °C } cycles + dissociation stage

Study details continued:

Quantity tested:	20 mg of the sample is tested in duplicate.
Non template control (NTC):	DNase/RNase free water
Positive Control:	E997 DNA (0.1 ng/µl)
Negative Control:	*E. coli* DNA
Protocol amendments:	Not applicable
Non-conformances:	Not applicable

Results:

Item	Ct	Acceptance criteria	Evaluation
NTC	Undetermined	> 40	Complies
Negative Control	Undetermined	> 40	Complies
	Undetermined	> 40	Complies
Positive Control	24.4	≤ 40	Complies
	24.4	≤ 40	Complies
73084	Undetermined	None	Negative
	Undetermined	None	Negative

>40 or undetermined = negative
≤40 = positive

Conclusions:

1. The assay meets the criteria for a valid test.
2. No E628 wcaJ gene is detected in the sample.

Quality Statement:
The study was performed according to the study specific supplement and MicroSafe Laboratories Standard Operating Procedures except when clearly documented otherwise. The execution of the study conformed to the principles of Good Manufacturing Practices of the European Community.

Authorisation and approval:
Prepared by:

M. Schrumpf — Name
(b) (6) — Signature
16 may 17 — Date

Quality Assurance:

Name
(b) (6)
17 May 17 — Date

Appendix qPCR8
Eurofins MicroSafe Laboratories GMP- and GLP certificate

Health Care Inspectorate - Pharmaceutical Affairs and Medical Technology

CERTIFICATE NUMBER: ***NL/H 14/1002953***

CERTIFICATE OF GMP COMPLIANCE OF A MANUFACTURER [1, 2]

Part 1

Issued following an inspection in accordance with :
Art. 111(5) of Directive 2001/83/EC as amended

The competent authority of Netherlands confirms the following:
The manufacturer: ***Proxy Laboratories,locatie MicroSafe***
Site address: ***Darwinweg 24, Leiden, 2333CR, Netherlands***

Has been inspected under the national inspection programme in accordance with Art. 40 of Directive 2001/83/EC transposed in the following national legislation:
Art. 100 of the Medicines Act

From the knowledge gained during inspection of this manufacturer, the latest of which was conducted on ***2014-09-11*** , it is considered that it complies with :

- The principles and guidelines of Good Manufacturing Practice laid down in Directive 2003/94/EC [3]

This certificate reflects the status of the manufacturing site at the time of the inspection noted above and should not be relied upon to reflect the compliance status if more than three years have elapsed since the date of that inspection. However, this period of validity may be reduced or extended using regulatory risk management principles by an entry in the Restrictions or Clarifying remarks field. This certificate is valid only when presented with all pages and both Parts 1 and 2. The authenticity of this certificate may be verified in EudraGMDP. If it does not appear, please contact the issuing authority.

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[1] *The certificate referred to in paragraph 111(5) of Directive 2001/83/EC and 80(5) of Directive 2001/82/EC, shall also be required for imports coming from third countries into a Member State.*

[2] *Guidance on the interpretation of this template can be found in the Help menu of EudraGMDP database.*

[3] *These requirements fulfil the GMP recommendations of WHO.*

(b) (6)

Part 2

Human Medicinal Products

1 MANUFACTURING OPERATIONS	
1.1	**Sterile products**
	1.1.1 Aseptically prepared (processing operations for the following dosage forms) 1.1.1.4 Small volume liquids
1.3	**Biological medicinal products (list of product types)**
	1.3.1 Biological medicinal products (list of product types) 1.3.1.5 Biotechnology products
1.5	**Packaging**
	1.5.1 Primary Packing 1.5.1.5 Liquids for external use 1.5.1.6 Liquids for internal use
	1.5.2 Secondary packing
1.6	**Quality control testing**
	1.6.1 Microbiological: sterility *1.6.2 Microbiological: non-sterility* *1.6.4 Biological*

2 IMPORTATION OF MEDICINAL PRODUCTS	
2.1	**Quality control testing of imported medicinal products**
	2.1.1 Microbiological: sterility *2.1.2 Microbiological: non-sterility* *2.1.4 Biological*

(b) (6)

2016-07-25

Name and signature of the authorised person of the Competent Authority of Netherlands

(b) (6)

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ENDORSEMENT OF COMPLIANCE

WITH THE OECD PRINCIPLES OF GOOD LABORATORY PRACTICE

Pursuant to the Netherlands GLP Compliance Monitoring Programme and according to Directive 2004/9/EC the conformity with the OECD Principles of GLP was assessed on 3 – 4 March 2015 at

MicroSafe Laboratories
Darwinweg 24
2333 CR Leiden

It is herewith confirmed that the afore-mentioned test facility is currently operating in compliance with the OECD Principles of Good Laboratory Practice in the following area of expertise: Tests for microbial contamination and identification.

Utrecht, 14 April 2015

(b) (6)

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ESTIMATED DAILY INTAKE OF 2'-FL BY THE U.S. POPULATION FROM PROPOSED FOOD-USES (2013-2014 NHANES)

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21 September 2017

Estimated Daily Intake of 2'-FL by the U.S. Population from Proposed Food-Uses (2013-2014 NHANES)

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1.0 INTRODUCTION

Glycosyn LLC proposes to use 2'-fucosyllactose (2'-FL) as an ingredient in foods marketed in the United States (U.S.). Such foods include products falling under the following food categories: beverages and beverage bases, breakfast cereals, dairy product analogs, frozen dairy desserts and mixes, gelatins, puddings, and fillings, grain products and pastas, commercial jams and jellies, whole and skim milk, milk products, processed fruits and fruit juices, sweet sauces, toppings, and syrups, non-exempt infant and follow-on formula, and baby foods.

Estimates for the intake of 2'-FL from foods were based on the proposed food-uses and use levels for 2'-FL in conjunction with food consumption data included in the U.S. National Center for Health Statistics' (NCHS) National Health and Nutrition Examination Surveys (NHANES) 2013-2014 (CDC, 2015, 2016; USDA, 2016). Calculations for the mean and 90th percentile *per capita* and consumer-only intakes were performed for all proposed food-uses of 2'-FL and the percentage of consumers were determined. Similar calculations were used to estimate the intake of 2'-FL resulting from each individual proposed food-use, including the calculations of percent consumers. In both cases, the per person and per kilogram body weight intakes were reported for the following population groups:

Infants, ages 0 to 5 months;
Infants, ages 6 to 11 months;
Toddlers, ages 12 to 35 months;
Children, ages 3 to 11 years;
Female teenagers, ages 12 to 19 years;
Male teenagers, ages 12 to 19 years;
Women of child-bearing age, ages 16 to 45 years;
Female adults, ages 20 years and up;
Male adults, ages 20 years and up;
Elderly, ages 65 years and up; and
Total population (all age and gender groups combined).

In addition to the NHANES-based assessment of exposures from proposed food uses, exposure estimates of 2'-FL from proposed uses in medical foods were considered independently, based on the intended dosages for target populations for which these products were intended.

2.0 FOOD CONSUMPTION SURVEY DATA

2.1 Survey Description

NHANES for the years 2013-2014 are available for public use (CDC, 2015). NHANES are conducted as continuous, annual surveys, and are released in 2-year cycles. During each year of the ongoing NHANES program, individuals from the United States are sampled from up to 30 different study locations in a complex multi-stage probability design intended to ensure the data are a nationally representative sample of the U.S. population.

NHANES 2013-2014 dietary survey data were collected from individuals and households *via* 24-hour dietary recalls administered on 2 non-consecutive days (Day 1 and Day 2) throughout all 4 seasons of the year. Day 1 data were collected in-person, and Day 2 data were collected by telephone in the following 3 to 10 days, on different days of the week, to achieve the desired degree of statistical independence. The data were collected by first selecting Primary Sampling Units (PSUs), which were counties throughout the U.S., of which 30 PSUs are visited per year. Smaller contiguous counties were combined to attain a minimum population size. These PSUs were segmented and households were chosen within each segment. One or more participants within a household were interviewed. For NHANES 2013-2014, 14,332 individuals were selected for the sample, 10,175 were interviewed (71.0%) and 9,813 were examined (68.5%).

In addition to collecting information on the types and quantities of foods being consumed, NHANES 2013-2014 collected socio-economic, physiological and demographic information from individual participants in the survey, such as sex, age, body weight, and other variables (such as height and race-ethnicity) that may be useful in characterizing consumption. The inclusion of this information allows for further assessment of food intake based on consumption by specific population groups of interest within the total population. The primary sample design for NHANES 2013-2014 includes an oversample of Non-Hispanic Asian persons, Hispanic persons, non-Hispanic black persons, older adults, and "low income whites/others", however sample weights were incorporated to allow estimates from these subgroups to be combined to obtain national estimates that reflect the relative proportions of these groups in the population as a whole (CDC, 2015).

2.2 Statistical Methods

For the intake assessment, consumption data from individual dietary records, detailing food items ingested by each survey participant, were collated by computer and used to generate estimates for the intake of 2'-FL by the U.S. population[1]. Estimates for the daily intake of 2'-FL represent projected 2-day averages for each individual from Day 1 and Day 2 of NHANES 2013-2014; these average amounts comprised the distribution from which mean and percentile intake estimates were determined. Mean and percentile estimates were generated incorporating survey weights in order to provide representative intakes for the entire U.S. population. "*Per capita*" intake refers to the estimated intake of 2'-FL averaged over all individuals surveyed, regardless of whether they consumed food products in which 2'-FL is proposed for use, and therefore includes individuals with "zero" intakes (*i.e*. those who reported no intake of food products containing 2'-FL during the 2 survey days). "Consumer-only" intake refers to the estimated intake of 2'-FL by those individuals who reported consuming food products in which the use of 2'-FL is currently under consideration. Individuals were considered "consumers" if they reported consumption of 1 or more food products in which 2'-FL is proposed for use on either Day 1 or Day 2 of the survey.

Mean and 90th percentile intake estimates based on sample sizes of less than 30 and 80, respectively, may not be considered statistically reliable due to the limited sampling size (CDC, 2013). As such, the reliability of estimates for the intake of 2'-FL based on consumption estimates derived from individual population groups of a limited sample size should be interpreted with caution. These values are marked with an asterisk in the relevant data tables.

[1] Statistical analysis and data management were conducted in DaDiet Software (Dazult Ltd., 2017). DaDiet Software is a web-based software tool that allows accurate estimate of exposure to nutrients and to substances added to foods, including contaminants, food additives and novel ingredients. The main input components are concentration (use level) data and food consumption data. Data sets are combined in the software to provide accurate and efficient exposure assessments.

3.0 FOOD USAGE DATA

The individual proposed food-uses and use-levels for 2'-FL employed in the current intake analysis are summarized in Table 3-1. Food codes representative of each proposed food-use were chosen from the NHANES 2013-2014 (CDC, 2016). Food codes were grouped in food-use categories according to Title 21, Section §170.3 of the Code of Federal Regulations (CFR, 2017a). If necessary, adjustment factors were developed for composite foods/mixtures based on data provided in the Food and Nutrition Database for Dietary Studies (FNDDS) (USDA, 2016). All food codes included in the current intake assessment are listed in Appendix C.

Table 3-1 Summary of the Individual Proposed Food-Uses and Use-Levels for 2'-FL in the U.S.

Food Category (21 CFR 170.3)	Food-Uses	Maximum 2'-FL Level (g/serving)	RACC[a] (g or mL)	Maximum 2'-FL Use-Levels (g/100 g)
Beverages and Beverage Bases	Energy drinks	0.28	360	0.08
	Fitness water and third quenchers, sports and isotonic drinks	0.28	360	0.08
Breakfast Cereals	Ready-to-eat breakfast cereals for adults and children	1.2	15 (puffed) 40 (high-fiber) 60 (biscuit-types)	8.0 3.0 2.0
	Hot cereals for adults and children	1.2	40 (dry) ~ 250 (prepared)	0.48 (as consumed)
Dairy Product Analogs	Milk substitutes such as soy milk and imitation milks	0.28	240	0.12
Frozen Dairy Desserts and Mixes	Frozen desserts including ice creams* and frozen yogurts, frozen novelties	1.2	~ 70	1.7
Gelatins, puddings, and fillings	Dairy-based puddings, custards, and mousses	1.2	~ 70	1.7
	Fruit pie filling	1.2	85	1.41
	"Fruit prep" such as fruit filling in bars, cookies, yogurt, cakes	1.2	~ 40	3.0
Grain Products and Pastas	Bars, including snack bars, meal-replacement bars, breakfast bars	0.48	40	1.20
Jams and Jellies, Commercial	Jellies and jams, fruit preserves*, fruit butters	1.2	~ 20	6.0
Milk, Whole and Skim	All *acidophilus* or fortified milks, non-fat and low-fat fluid milks, including fluid milk and reconstituted milk powder*	0.28	240	0.12
Milk Products	Flavored milks, including chocolate milk, coffee drinks, cocoa, smoothies (dairy and fruit-based), other fruit and dairy combinations, yogurt drinks fermented milk drinks including kefir**	0.28	240	0.12
	Milk-based meal replacement beverages or diet beverages**	0.28	240	0.12
	Yogurt*, **	1.2	225	0.53
	Formula intended for pregnant women ("mum" formulas; -9 to 0 months)	1.2	200[b]	0.6
Processed Fruits and Fruit Juices	Fruit drinks, including vitamin and mineral-fortified products	0.28	240	0.12
	Fruit juices*	0.28	240	0.12

Table 3-1 Summary of the Individual Proposed Food-Uses and Use-Levels for 2'-FL in the U.S.

Food Category (21 CFR 170.3)	Food-Uses	Maximum 2'-FL Level (g/serving)	RACC[a] (g or mL)	Maximum 2'-FL Use-Levels (g/100 g)
Sweet Sauces, Toppings, and Syrups	Syrups used to flavor milk beverages	0.28	40	0.70
Other Categories				
Non-Exempt Infant and Follow-On Formula	Infant Formula (0 to 6 months), including ready-to-drink formula or formula prepared from powder	0.24	100[b]	0.24 (0.40 g/100 kcal)[c]
	Follow-On Formula (6 to 12 months), including ready-to-drink formula or formula prepared from powder	0.24	100[b]	0.24 (0.40 g/100 kcal)[c]
Baby Foods	Meal replacement products such as Pediasure	0.24	120[b]	0.2
	Growing-Up (Toddler) Milks (12 to 36 months)	0.24	120[b]	0.2
	Ready-to-eat, ready-to-serve, hot cereals	1.2	15 (dry) 110 (ready-to-serve)	1.09 (as consumed)
	Yogurt and juice beverages identified as "baby" drinks	1.2	120	1.0
	Desserts including fruit desserts, cobblers, yogurt / fruit combinations ("junior type" desserts)	1.2	110	1.09
	Baby crackers, pretzels, cookies, and snack items	0.4	7	5.7
Medical Foods	Oral nutritional supplements and enteral tube feeding (11 years and older)	4.0	200[b]	2.0

2'-FL = 2'-fucosyllactose; CFR = Code of Federal Regulations; RACC = Reference Amounts Customarily Consumed per Eating Occasion; U.S. = United States.

[a] RACC based on values established in 21 CFR §101.12 (U.S. FDA, 2016, CFR, 2017b). When a range of values is reported for a proposed food-use, particular foods within that food-use may differ with respect to their RACC.

[b] No RACC value exists; therefore, approximate serving sizes are provided according to the food manufacturer instructions.

[C] The intended use level in infant formula and baby meal replacement products is 2.4 g per L (0.24 g per 100 mL), or 0.40 g per 100 kcal. For a 100 mL formula that contains 60 kcal, the conversion is as follows:

$$\frac{100\ mL}{60\ kcal} \times \frac{0.24\ g}{100\ mL} = 0.004 \frac{g}{kcal}\ or\ 0.40 \frac{g}{100 kcal}$$

* 2'-FL is intended for use in unstandardized products when standards of identity do not permit its addition.

** Includes ready-to-drink and powder forms.

It is further noted that 2'-FL is intended for use in medical foods (oral nutritional supplements and enteral tube feeding) at maximum dosages of 4.0 g per product. The dietary exposures from these intended uses are considered separately from the NHANES-based assessment, as the conventional food consumption database would not adequately capture these target uses.

4.0 FOOD SURVEY RESULTS

Estimates for the total daily intakes of 2'-FL from proposed food-uses are provided in Tables 4.1-1 and 4.1-2. Estimates for the daily intake of 2'-FL from individual proposed food-uses in the U.S. are summarized in Tables A-1 to A-10 and B-1 to B-10 of Appendices A and B, respectively. Tables A-1 to A-10 provide estimates for the daily intake of 2'-FL on an absolute basis (g/person/day), whereas Tables B-1 to B-10 provide estimates for the daily intake of 2'-FL on a per kilogram body weight basis (mg/kg body weight/day).

4.1 Estimated Daily Intake of 2'-FL from All Proposed Food-Uses in the U.S.

Table 4.1-1 summarizes the estimated total intake of 2'-FL (g/person/day) from all proposed food-uses in the U.S. population group. Table 4.1-2 presents this data on a per kilogram body weight basis (mg/kg body weight/day). The percentage of consumers was high among all age groups evaluated in the current intake assessment; greater than 57.5% of the infant population and greater than 86.8% of the other population groups consisted of consumers of food products in which 2'-FL is currently proposed for use (Table 4.1-1). Owing to the proposed uses of 2'-FL in milks, juices, cereals, yogurts which are popular food items among toddlers, 100% of individuals aged 12 to 35 months simulated to consume foods in which 2'-FL is proposed for use. The consumer-only estimates are more relevant to risk assessments as they represent exposures in the target population; consequently, only the consumer-only intake results are discussed in detail herein.

Among the total population (all ages), the mean and 90th percentile consumer-only intakes of 2'-FL were determined to be 1.70 and 3.54 g/person/day, respectively. Of the individual population groups, older infants aged 6 to 11 months were determined to have the greatest mean consumer-only intakes of 2'-FL on an absolute basis, at 2.28 g/person/day, whereas male teenagers were estimated to have the highest 90th percentile intake of 2'-FL at 4.29 g/day. Females of childbearing age (16 to 45 years old) had the lowest estimated mean and 90th percentile consumer-only intakes of 1.36 and 2.87 g/person/day, respectively (Table 4.1-1).

Table 4.1-1 Summary of the Estimated Daily Intake of 2'-FL from Proposed Food-Uses in the U.S. by Population Group (2013-2014 NHANES Data)

Population Group	Age Group (Years)	*Per Capita* Intake (g/day)		Consumer-Only Intake (g/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
Infants	0 to 5 (months)	1.10	2.75	57.5	107	1.91	3.00
Infants	6 to 11 (months)	2.14	3.86	94.1	160	2.28	3.86
Toddlers	12 to 35 (months)	1.83	2.97	100.0	348	1.83	2.97
Children	3 to 11	1.96	3.53	99.7	1,277	1.97	3.53
Female Teenagers	12 to 19	1.47	2.95	94.7	544	1.55	2.95
Male Teenagers	12 to 19	1.85	4.16	92.5	526	2.00	4.29
Women of Child-Bearing Age	16 to 45	1.22	2.82	89.9	1,219	1.36	2.87
Female Adults	20 and up	1.32	2.96	91.9	2,169	1.44	3.05
Male Adults	20 and up	1.59	3.81	86.8	1,842	1.84	3.97
Elderly	65 and up	1.76	3.74	92.8	939	1.90	3.91
Total Population	All Ages	1.55	3.41	91.2	6,973	1.70	3.54

2'-FL = 2'-fucosyllactose; NHANES = National Health and Nutrition Examination Survey; U.S. = United States.

On a body weight basis, the total population (all ages) mean and 90th percentile consumer-only intakes of 2'-FL were determined to be 36 and 80 mg/kg body weight/day, respectively. Among the individual population groups, younger infants aged 0 to 5 months were identified as having the highest mean and 90th percentile consumer-only intakes of any population group, of 315 and 532 mg/kg body weight/day, respectively. Female adults and females of childbearing age were predicted to have the lowest mean and 90th percentile intakes at 20 and 43 mg/kg body weight/day, respectively (Table 4.1-2).

Table 4.1-2 Summary of the Estimated Daily Per Kilogram Body Weight Intake of 2'-FL from Proposed Food-Uses in the U.S. by Population Group (2013-2014 NHANES Data)

Population Group	Age Group (Years)	*Per Capita* Intake (mg/kg bw/day)		Consumer-Only Intake (mg/kg bw/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
Infants	0 to 5 (months)	181	477	57.5	107	315	532
Infants	6 to 11 (months)	244	441	94.1	160	259	447
Toddlers	12 to 35 (months)	148	243	100.0	346	148	243
Children	3 to 11	75	147	99.7	1,268	76	147
Female Teenagers	12 to 19	24	52	94.7	536	26	52
Male Teenagers	12 to 19	29	67	92.5	524	31	67
Women of Child-Bearing Age	16 to 45	18	42	89.9	1,209	20	43
Female Adults	20 and up	19	42	91.9	2,156	20	43
Male Adults	20 and up	19	46	86.7	1,833	22	48
Elderly	65 and up	24	53	92.6	928	26	54
Total Population	All Ages	32	76	91.1	6,930	36	80

2'-FL = 2'-fucosyllactose; bw = body weight; NHANES = National Health and Nutrition Examination Survey; U.S. = United States.

4.2 Estimated Daily Intake of 2'-FL from Specific Food Categories

4.2.1 Intake of 2'-FL from Infant and Follow-On Formula Among Non-Breastfeeding Infants and Toddlers

In order to consider the intake of 2'-FL from formula products, an additional assessment was undertaken in which the subpopulation of breastfed infants/toddlers were removed, and the intakes were examined by the remaining non-breastfed infants and toddlers to investigate whether 2'-FL intake was greater among this group. This reflected the intake models included under GRN 546 and GRN 571 (Glycom A/S, 2014; Environ International Corp., 2015). The anticipated intake of 2'-FL from (non-exempt) infant formula products among infants and toddlers who are not breastfed are presented in the table below on an absolute (g/day) and per kilogram body weight basis (mg/kg body weight/day). Mean intakes decreased with age from 2.14 to 0.39 g/day, or 354 to 40 mg/kg body weight/day, which is anticipated as children move on to a more varied diet over 6 months of age. Due to the low sample size, the 90th percentile results are only statistically reliable for infants aged 6 to 11 months, at 2.56 g/day or 311 mg/kg body weight/day.

Table 4.2.1-1 Estimated Daily Intake of 2'-FL from Non-Exempt Infant Formula Among Non-Breastfed Infants (2013-2014 NHANES Data)

Population Group	Age Group (Months)	Consumer-Only Intake of 2'-FL¥					
		%	n	g/day		mg/kg body weight/day	
				Mean	90th Percentile	Mean	90th Percentile
Infants	0 to 5	43.0	79	2.14	2.88*	354	498*
Infants	6 to 11	56.6	100	1.67	2.56	192	311
Toddlers	12 to 35	11.7	39	0.39	1.14*	40	101*

2'-FL = 2'-fucosyllactose; NHANES = National Health and Nutrition Examination Survey.

¥ Infants and toddlers recording a breastmilk consumption event in NHANES were removed from these analyses. The results represent intake of 2'-FL from non-exempt infant and follow-on formula among consumers of formula, by age group.

* Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements.

4.2.2 Intake of 2'-FL from Other Food Categories

Estimates for the mean and 90th percentile daily intakes of 2'-FL from each individual food category are summarized in Tables A-1 to A-10 and B-1 to B-10 on a g/day and mg/kg body weight/day basis, respectively. Among the non-infant population, individuals were identified as being significant consumers of milk products (46.2 to 89.1% consumers among the individual demographics), fruit juices (25.3 to 64.0% consumers), and ready-to-eat breakfast cereals (28.5 to 61.1% consumers).

In terms of contribution to total mean intake of 2'-FL among the non-infant population, ready-to-eat breakfast cereal accounted for 18.5 to 32.3% of total intakes, which were followed by frozen desserts (contributed 5.7 to 29.0% to total intakes) and milks (contributed 8.1 to 26.2% to total mean intakes). The other food categories accounted for less than 8.8% of the total 2'-FL intake (see Tables A-1 to A-10 and/or B-1 to B-10 for further details).

4.2.3 Intake of 2'-FL from Proposed Uses in Medical Foods

As noted in the introduction, 2'-FL is proposed for use in medical foods at maximum dosage levels of 4 g/serving, intended to be consumed by patients aged 11 years and older at no more than 3 servings per day. Medical foods containing 2'-FL will be used under the supervision of a physician for the dietary management of a disease or condition and therefore will not be combined with a diet containing 2'-FL from its conventional food uses described under Table 3-1. Therefore, the anticipated daily intake of 2'-FL from its proposed uses in medical foods is expected to be at a maximum of 12 g/person/day[2] among the target population. Using default body weight values for adolescents and adults as established in the U.S. Environmental Protection Agency's Exposure Factors Handbook (U.S. EPA, 2011), dosages are equivalent to 211 mg/kg body weight/day in a 56.8 kg adolescent and 150 mg/kg body weight/day in an 80.0 kg adult.

5.0 SUMMARY AND CONCLUSIONS

Consumption data and information pertaining to the individual proposed food-uses of 2'-FL were used to estimate the *per capita* and consumer-only intakes of 2'-FL for specific demographic groups and for the total U.S. population. There were a number of assumptions included in the assessment which render exposure estimates that may be considered suitably conservative. For example, it has been assumed in both exposure assessments that all food products within a food category contain 2'-FL at the maximum specified level of use. In reality, the levels added to specific foods will vary depending on the nature of the food product and it is unlikely that 2'-FL will have 100% market penetration in all identified food categories.

In summary, on consumer-only basis, the resulting mean and 90th percentile intakes of 2'-FL by the total (all ages) U.S. population from all proposed food-uses, were estimated to be 1.70 g/person/day (36 mg/kg body weight/day) and 3.54 g/person/day (80 mg/kg body weight/day), respectively. Among the individual population groups, older infants aged 6 to 11 months were determined to have the greatest mean consumer-only intakes of 2'-FL on an absolute basis, at 2.28 g/person/day (259 mg/kg body weight/day), whereas male teenagers were estimated to have the highest 90th percentile intake of 2'-FL at 4.29 g/day (67 mg/kg body weight/day). When intakes were expressed on a body weight basis, younger infants aged 0 to 5 months were identified as having the highest mean and 90th percentile consumer-only intakes of any population group, of 315 and 532 mg/kg body weight/day, respectively. When considering predicted intake of 2'-FL from formula products among non-breastfed infants, estimates of mean consumer-only intake

[2] Calculated as 4.0 g/serving x 3 servings/day = 12 g/day.

ranged from 0.39 g/day (40 mg/kg body weight/day) among toddlers, up to 2.14 g/day (354 mg/kg body weight/day) among young infants aged 0 to 5 months.

Uses of 2'-FL in medical foods at a dosage of 4 g/serving are expected to result in a maximum daily intake of 12 g/day of 2'-FL among its intended target patient population of individuals aged 11 years and older (equivalent to approximately 211 mg/kg body weight/day in adolescents and 150 mg/kg body weight/day in adults).

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Appendix A
Estimated Daily Intake of 2'-FL from Individual Proposed Food-Uses by Different Population Groups Within the U.S. (2013-2014 NHANES DATA)

Table A-1 Estimated Daily Intake of 2'-FL from Individual Proposed Food-Uses by Infants Aged 0 to 5 Months Within the U.S. (2013-2014 NHANES Data)

Food-Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake (g/day)		Consumer-Only Intake (g/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
All	***100***	***1.10***	***2.75***	***57.5***	***107***	***1.91***	***3.00***
Beverages and Beverage Bases							
Energy drinks	0	na	na	0	0	na	na
Fitness water and third quenchers, sports and isotonic drinks	0	na	na	0	0	na	na
Breakfast Cereals							
Ready-to-eat breakfast cereals for adults and children	<0.1	<0.01*	na	0.2	1	0.01*	0.01*
Hot cereals for adults and children	0.1	<0.01*	na	0.9	1	0.15*	0.15*
Dairy Product Analogs							
Milk substitutes such as soy milk and imitation milks	0	na	na	0	0	na	na
Frozen Dairy Desserts and Mixes							
Frozen desserts including ice creams and frozen yogurts, frozen novelties	0.3	<0.01*	na	0.9	1	0.32*	0.32*
Gelatins, Puddings, and Fillings							
Dairy-based puddings, custards, and mousses	0	na	na	0	0	na	na
Fruit pie filling	0	na	na	0	0	na	na
"Fruit prep"	0	na	na	0	0	na	na
Grain Products and Pastas							
Snack, breakfast, and meal replacement bars	0	na	na	0	0	na	na
Jams and Jellies, Commercial							
Jellies and jams, fruit preserves, fruit butters	0	na	na	0	0	na	na
Milk, Whole and Skim							
All acidophilus or fortified milks, non-fat and low-fat fluid milks, including fluid milk and reconstituted milk powder	<0.1	<0.01*	na	0.2	1	0.10*	0.09*
Milk Products							
Flavored milks	0	na	na	0	0	na	na
Milk-based meal replacement beverages or diet beverages	0	na	na	0	0	na	na
Yogurt	0	na	na	0	0	na	na
Processed Fruits and Fruit Juices							
Fruit drinks, including vitamin and mineral-fortified products	0	na	na	0	0	na	na
Fruit juices	0.3	<0.01*	na	2.1	5	0.16*	0.26*
Sweet Sauces, Toppings, and Syrups							
Syrups used to flavor milk beverages	0	na	na	0	0	na	na
Other							

Table A-1 Estimated Daily Intake of 2'-FL from Individual Proposed Food-Uses by Infants Aged 0 to 5 Months Within the U.S. (2013-2014 NHANES Data)

Food-Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake (g/day)		Consumer-Only Intake (g/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
Infant formula	92.3	1.02	2.63	52.4	102	1.94	2.78
Follow-on formula	0	na	na	0	0	na	na
Meal replacement products	0	na	na	0	0	na	na
Growing-up (toddler) milks	0	na	na	0	0	na	na
Ready-to-eat, ready-to-serve, hot cereals for babies	0.8	0.01*	na	2.4	4	0.37*	0.66*
Yogurt and juice beverages identified as "baby" drinks	3.0	0.03*	na	4.5	8	0.74*	1.79*
"Junior type" desserts	1.2	0.01*	na	6.5	6	0.20*	0.33*
Baby crackers, pretzels, cookies, and snack items	2.1	0.02*	na	2.6	6	0.86*	1.61*

2'-FL = 2'-fucosyllactose; na = not available; NHANES = National Health and Nutrition Examination Survey; U.S. = United States.
* Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements.

Table A-2 Estimated Daily Intake of 2'-FL from Individual Proposed Food-Uses by Older Infants Aged 6 to 11 Months Within the U.S. (2013-2014 NHANES Data)

Food-Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake (g/day)		Consumer-Only Intake (g/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
All	***100***	***2.14***	***3.86***	***94.1***	***160***	***2.28***	***3.86***
Beverages and Beverage Bases							
Energy drinks	0	na	na	0	0	na	na
Fitness water and third quenchers, sports and isotonic drinks	0.1	<0.01*	na	2.7	6	0.11*	0.10*
Breakfast Cereals							
Ready-to-eat breakfast cereals for adults and children	1.4	0.03*	0.12	15.9	22	0.19*	0.33*
Hot cereals for adults and children	1.5	0.03*	0.06	11.3	13	0.29*	0.48*
Dairy Product Analogs							
Milk substitutes such as soy milk and imitation milks	0.1	<0.01*	na	1.6	2	0.17*	0.17*
Frozen Dairy Desserts and Mixes							
Frozen desserts including ice creams and frozen yogurts, frozen novelties	0.2	<0.01*	na	4.3	8	0.10*	0.25*
Gelatins, puddings, and fillings							
Dairy-based puddings, custards, and mousses	0.6	0.01*	na	2.9	4	0.45*	0.56*
Fruit pie filling	0.2	0.01*	na	1.7	1	0.31*	0.31*
"Fruit prep"	0	na	na	0	0	na	na
Grain Products and Pastas							
Snack, breakfast, and meal replacement bars	0.5	0.01*	na	1.7	1	0.68*	0.68*
Jams and Jellies, Commercial							
Jellies and jams, fruit preserves, fruit butters	0	na	na	0	0	na	na
Milk, Whole and Skim							
All acidophilus or fortified milks, non-fat and low-fat fluid milks, including fluid milk and reconstituted milk powder	3.2	0.07	0.29*	15.9	33	0.43	0.73*
Milk Products							
Flavored milks	0.1	<0.01*	na	2.0	4	0.12*	0.19*
Milk-based meal replacement beverages or diet beverages	0	na	na	0	0	na	na
Yogurt	1.8	0.04*	0.02	10.6	18	0.37*	0.65*
Processed Fruits and Fruit Juices							
Fruit drinks, including vitamin and mineral-fortified products	0.1	<0.01*	na	2.1	5	0.08*	0.10*
Fruit juices	0.8	0.02*	0.06	14.0	29	0.12*	0.19*
Sweet Sauces, Toppings, and Syrups							
Syrups used to flavor milk beverages		na	na	0	0	na	na
Other							

Table A-2 Estimated Daily Intake of 2'-FL from Individual Proposed Food-Uses by Older Infants Aged 6 to 11 Months Within the U.S. (2013-2014 NHANES Data)

Food-Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake (g/day)		Consumer-Only Intake (g/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
Infant Formula	46.6	1.00	2.38	63.0	107	1.58	2.56
Follow-On Formula	0	na	na	0	0	na	na
Meal replacement products	0	na	na	0	0	na	na
Growing-Up (Toddler) Milks	3.0	0.06*	na	6.5	7	0.98*	1.43*
Ready-to-eat, ready-to-serve, hot cereals for babies	1.7	0.04*	na	5.0	9	0.74*	0.92*
Yogurt and juice beverages identified as "baby" drinks	18.6	0.40	1.24*	40.5	59	0.98	2.17*
"Junior Type" Desserts	11.2	0.24	0.62*	36.3	55	0.66	1.29*
Baby crackers, pretzels, cookies, and snack items	8.1	0.17	0.60	46.5	82	0.37	0.82

2'-FL = 2'-fucosyllactose; na = not available; NHANES = National Health and Nutrition Examination Survey; U.S. = United States.
* Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements.

Table A-3 Estimated Daily Intake of 2'-FL from Individual Proposed Food-Uses by Toddlers Aged 12 to 35 Months Within the U.S. (2013-2014 NHANES Data)

Food-Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake (g/day)		Consumer-Only Intake (g/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
All	***100***	***1.83***	***2.97***	***100***	***348***	***1.83***	***2.97***
Beverages and Beverage Bases							
Energy drinks	0	0	na	0	0	na	na
Fitness water and third quenchers, sports and isotonic drinks	0.8	0.01*	na	7.0	20	0.21*	0.40*
Breakfast Cereals							
Ready-to-eat breakfast cereals for adults and children	18.5	0.34	0.90	61.1	203	0.55	1.07
Hot cereals for adults and children	5.2	0.09	0.40*	17.7	66	0.53	0.87*
Dairy Product Analogs							
Milk substitutes such as soy milk and imitation milks	1.4	0.03*	na	7.5	22	0.35*	0.91*
Frozen Dairy Desserts and Mixes							
Frozen desserts including ice creams and frozen yogurts, frozen novelties	5.7	0.10	0.49*	17.1	69	0.61	1.21*
Gelatins, Puddings, and Fillings							
Dairy-based puddings, custards, and mousses	2.0	0.04*	na	5.6	24	0.64*	1.02*
Fruit pie filling	<0.1	<0.01*	na	0.5	1	0.16*	0.16*
"Fruit prep"	0.5	0.01*	na	5.6	14	0.17*	0.33*
Grain Products and Pastas							
Snack, breakfast, and meal replacement bars	1.5	0.03*	na	7.7	25	0.35*	0.58*
Jams and Jellies, Commercial							
Jellies and jams, fruit preserves, fruit butters	5.4	0.10	0.38*	19.9	51	0.50	0.90*
Milk, Whole and Skim							
All acidophilus or fortified milks, non-fat and low-fat fluid milks, including fluid milk and reconstituted milk powder	26.2	0.48	0.99	89.1	305	0.54	1.04
Milk Products							
Flavored milks	1.5	0.03	0.10*	15.9	59	0.17	0.34*
Milk-based meal replacement beverages or diet beverages	0.9	0.02*	na	4.6	7	0.37*	0.68*
Yogurt	6.9	0.13	0.41	29.3	95	0.43	0.90
Processed Fruits and Fruit Juices							
Fruit drinks, including vitamin and mineral-fortified products	3.7	0.07	0.22	30.1	128	0.23	0.43
Fruit juices	7.0	0.13	0.34	64.0	213	0.20	0.41
Sweet Sauces, Toppings, and Syrups							
Syrups used to flavor milk beverages	0.2	<0.01*	na	3.2	8	0.09*	0.12*
Other							

Table A-3 Estimated Daily Intake of 2'-FL from Individual Proposed Food-Uses by Toddlers Aged 12 to 35 Months Within the U.S. (2013-2014 NHANES Data)

Food-Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake (g/day)		Consumer-Only Intake (g/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
Infant formula	1.6	0.03*	na	4.0	13	0.75*	1.21*
Follow-on formula	<0.1	<0.01*	na	<0.1	1	0.58*	0.58*
Meal replacement products	0.9	0.02*	na	2.9	6	0.55*	1.22*
Growing-up (toddler) milks	0.5	0.01*	na	1.1	3	0.79*	0.92*
Ready-to-eat, ready-to-serve, hot cereals for babies	0.7	0.01*	na	1.9	5	0.69*	0.93*
Yogurt and juice beverages identified as "baby" drinks	3.6	0.07*	na	6.0	20	1.11*	2.02*
"Junior type" desserts	1.3	0.02*	na	4.1	12	0.58*	1.20*
Baby crackers, pretzels, cookies, and snack items	4.0	0.07	0.02*	11.5	31	0.65	1.63*

2'-FL = 2'-fucosyllactose; na = not available; NHANES = National Health and Nutrition Examination Survey; U.S. = United States.
* Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements.

Table A-4 Estimated Daily Intake of 2'-FL from Individual Proposed Food-Uses by Children Aged 3 to 11 Years Within the U.S. (2013-2014 NHANES Data)

Food-Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake (g/day)		Consumer-Only Intake (g/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
All	***100***	***1.96***	***3.53***	***99.7***	***1,277***	***1.97***	***3.53***
Beverages and Beverage Bases							
Energy drinks	<0.1	<0.01*	na	0.1	1	0.15*	0.15*
Fitness water and third quenchers, sports and isotonic drinks	1.4	0.03	0.07	12.2	128	0.22	0.43
Breakfast Cereals							
Ready-to-eat breakfast cereals for adults and children	27.1	0.53	1.47	59.0	777	0.90	1.74
Hot cereals for adults and children	2.4	0.05	na	8.3	117	0.57	1.19
Dairy Product Analogs							
Milk substitutes such as soy milk and imitation milks	0.2	<0.01	na	2.5	33	0.18	0.33*
Frozen Dairy Desserts and Mixes							
Frozen desserts including ice creams and frozen yogurts, frozen novelties	21.9	0.43	1.29	35.2	388	1.22	2.41
Gelatins, puddings, and fillings							
Dairy-based puddings, custards, and mousses	3.9	0.08	na	5.0	67	1.53	3.09*
Fruit pie filling	0.2	<0.01*	na	1.0	15	0.31*	0.42*
"Fruit prep"	0.2	<0.01	na	3.4	46	0.14	0.38*
Grain Products and Pastas							
Snack, breakfast, and meal replacement bars	1.5	0.03	0.13	11.9	128	0.24	0.45
Jams and Jellies, Commercial							
Jellies and jams, fruit preserves, fruit butters	4.9	0.10	0.38	17.2	224	0.56	1.13
Milk, Whole and Skim							
All acidophilus or fortified milks, non-fat and low-fat fluid milks, including fluid milk and reconstituted milk powder	13.2	0.26	0.57	80.7	1,041	0.32	0.63
Milk Products							
Flavored milks	3.8	0.08	0.26	35.1	473	0.22	0.44
Milk-based meal replacement beverages or diet beverages	0.1	<0.01*	na	1.4	10	0.21*	0.29*
Yogurt	5.6	0.11	0.45	23.3	260	0.47	0.90
Processed Fruits and Fruit Juices							
Fruit drinks, including vitamin and mineral-fortified products	6.2	0.12	0.34	50.6	665	0.24	0.44
Fruit juices	6.7	0.13	0.34	56.9	759	0.23	0.45
Sweet Sauces, Toppings, and Syrups							
Syrups used to flavor milk beverages	0.4	0.01	na	4.9	55	0.15	0.30*
Other							

Table A-4 Estimated Daily Intake of 2'-FL from Individual Proposed Food-Uses by Children Aged 3 to 11 Years Within the U.S. (2013-2014 NHANES Data)

Food-Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake (g/day)		Consumer-Only Intake (g/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
Infant Formula	<0.1	<0.01*	na	<0.1	1	0.44*	0.44*
Follow-On Formula	0	na	na	0	0	na	na
Meal replacement products	0.1	<0.01*	na	0.4	11	0.42*	0.99*
Growing-Up (Toddler) Milks	0	na	na	0	0	na	na
Ready-to-eat, ready-to-serve, hot cereals for babies	0	na	na	0	0	na	na
Yogurt and juice beverages identified as "baby" drinks	0.1	<0.01*	na	0.3	3	0.77*	0.90*
"Junior Type" Desserts	0	na	na	0	0	na	na
Baby crackers, pretzels, cookies, and snack items	0.1	<0.01*	na	0.2	2	0.82*	0.98*

2'-FL = 2'-fucosyllactose; na = not available; NHANES = National Health and Nutrition Examination Survey; U.S. = United States.
* Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements.

Table A-5 Estimated Daily Intake of 2'-FL from Individual Proposed Food-Uses by Female Teenagers Aged 12 to 19 Years Within the U.S. (2013-2014 NHANES Data)

Food-Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake (g/day)		Consumer-Only Intake (g/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
All	***100***	***1.47***	***2.95***	***94.7***	***544***	***1.55***	***2.95***
Beverages and Beverage Bases							
Energy drinks	0.3	<0.01*	na	1.9	7	0.25*	0.38*
Fitness water and third quenchers, sports and isotonic drinks	1.6	0.02	na	9.2	55	0.26	0.47*
Breakfast Cereals							
Ready-to-eat breakfast cereals for adults and children	32.3	0.47	1.49	43.0	230	1.10	1.99
Hot cereals for adults and children	2.3	0.03	na	4.9	38	0.69	1.22*
Dairy Product Analogs							
Milk substitutes such as soy milk and imitation milks	0.3	<0.01*	na	2.0	22	0.18*	0.40*
Frozen Dairy Desserts and Mixes							
Frozen desserts including ice creams and frozen yogurts, frozen novelties	21.3	0.31	1.21	24.3	125	1.29	2.04
Gelatins, puddings, and fillings							
Dairy-based puddings, custards, and mousses	3.9	0.06*	na	4.1	20	1.39*	1.85*
Fruit pie filling	0.3	<0.01*	na	0.7	10	0.60*	1.02*
"Fruit prep"	0.5	0.01*	na	3.0	23	0.24*	0.49*
Grain Products and Pastas							
Snack, breakfast, and meal replacement bars	3.7	0.05	0.26*	17.0	68	0.32	0.50*
Jams and Jellies, Commercial							
Jellies and jams, fruit preserves, fruit butters	4.2	0.06	na	9.7	44	0.63	0.84*
Milk, Whole and Skim							
All acidophilus or fortified milks, non-fat and low-fat fluid milks, including fluid milk and reconstituted milk powder	10.6	0.16	0.41	59.6	320	0.26	0.50
Milk Products							
Flavored milks	2.8	0.04	0.19	18.9	107	0.22	0.38
Milk-based meal replacement beverages or diet beverages	0.1	<0.01*	na	0.5	6	0.23*	0.29*
Yogurt	4.8	0.07	0.24*	12.8	53	0.54	1.01*
Processed Fruits and Fruit Juices							
Fruit drinks, including vitamin and mineral-fortified products	7.5	0.11	0.34	37.1	235	0.30	0.70
Fruit juices	3.6	0.05	0.20	25.3	189	0.21	0.42
Sweet Sauces, Toppings, and Syrups							
Syrups used to flavor milk beverages	0.2	<0.01*	na	1.9	6	0.13*	0.15*
Other							

Table A-5 Estimated Daily Intake of 2'-FL from Individual Proposed Food-Uses by Female Teenagers Aged 12 to 19 Years Within the U.S. (2013-2014 NHANES Data)

Food-Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake (g/day)		Consumer-Only Intake (g/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
Infant Formula	0	na	na	0	0	na	na
Follow-On Formula	0	na	na	0	0	na	na
Meal replacement products	<0.1	<0.01*	na	<0.1	1	1.51*	1.51*
Growing-Up (Toddler) Milks	0	na	na	0	0	na	na
Ready-to-eat, ready-to-serve, hot cereals for babies	0	na	na	0	0	na	na
Yogurt and juice beverages identified as "baby" drinks	0	na	na	0	0	na	na
"Junior Type" Desserts	0	na	na	0	0	na	na
Baby crackers, pretzels, cookies, and snack items	<0.1	<0.01*	na	<0.1	1	1.14*	1.14*

2'-FL = 2'-fucosyllactose; na = not available; NHANES = National Health and Nutrition Examination Survey; U.S. = United States.
* Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements.

Table A-6 Estimated Daily Intake of 2'-FL from Individual Proposed Food-Uses by Male Teenagers Aged 12 to 19 Years Within the U.S. (2013-2014 NHANES Data)

Food-Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake (g/day)		Consumer-Only Intake (g/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
All	***100***	***1.85***	***4.16***	***92.5***	***526***	***2.00***	***4.29***
Beverages and Beverage Bases							
Energy drinks	0.7	0.01*	na	4.0	8	0.31*	0.41*
Fitness water and third quenchers, sports and isotonic drinks	6.0	0.11	0.30	19.9	93	0.56	0.79
Breakfast Cereals							
Ready-to-eat breakfast cereals for adults and children	31.4	0.58	1.61	45.5	252	1.27	2.80
Hot cereals for adults and children	1.8	0.03*	na	3.9	28	0.86*	1.92*
Dairy Product Analogs							
Milk substitutes such as soy milk and imitation milks	0.1	<0.01*	na	0.9	11	0.25*	0.55*
Frozen Dairy Desserts and Mixes							
Frozen desserts including ice creams and frozen yogurts, frozen novelties	19.4	0.36	1.34	19.8	109	1.82	3.22
Gelatins, puddings, and fillings							
Dairy-based puddings, custards, and mousses	1.7	0.03*	na	2.5	12	1.26*	2.22*
Fruit pie filling	0.4	0.01*	na	2.2	6	0.34*	0.41*
"Fruit prep"	0.6	0.01*	na	2.0	14	0.52*	0.98*
Grain Products and Pastas							
Snack, breakfast, and meal replacement bars	2.1	0.04	0.14*	12.3	52	0.32	0.82*
Jams and Jellies, Commercial							
Jellies and jams, fruit preserves, fruit butters	4.4	0.08	na	6.8	50	1.21	2.25*
Milk, Whole and Skim							
All acidophilus or fortified milks, non-fat and low-fat fluid milks, including fluid milk and reconstituted milk powder	14.4	0.27	0.71	65.3	360	0.41	0.85
Milk Products							
Flavored milks	3.2	0.06	0.21	23.9	140	0.24	0.53
Milk-based meal replacement beverages or diet beverages	0.8	0.02*	na	1.8	9	0.84*	1.19*
Yogurt	1.3	0.02*	na	4.4	27	0.55*	0.92*
Processed Fruits and Fruit Juices							
Fruit drinks, including vitamin and mineral-fortified products	5.6	0.10	0.29	30.2	209	0.34	0.75
Fruit juices	5.8	0.11	0.30	40.6	224	0.27	0.54
Sweet Sauces, Toppings, and Syrups							
Syrups used to flavor milk beverages	0.3	0.01*	na	1.4	4	0.40*	0.48*
Other							

Table A-6 Estimated Daily Intake of 2'-FL from Individual Proposed Food-Uses by Male Teenagers Aged 12 to 19 Years Within the U.S. (2013-2014 NHANES Data)

Food-Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake (g/day)		Consumer-Only Intake (g/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
Infant Formula	0	na	na	0	0	na	na
Follow-On Formula	0	na	na	0	0	na	na
Meal replacement products	0	na	na	0	0	na	na
Growing-Up (Toddler) Milks	0	na	na	0	0	na	na
Ready-to-eat, ready-to-serve, hot cereals for babies	0	na	na	0	0	na	na
Yogurt and juice beverages identified as "baby" drinks	0	na	na	0	0	na	na
"Junior Type" Desserts	0	na	na	0	0	na	na
Baby crackers, pretzels, cookies, and snack items	0	na	na	0	0	na	na

2'-FL = 2'-fucosyllactose; na = not available; NHANES = National Health and Nutrition Examination Survey; U.S. = United States.
* Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements.

Table A-7 Estimated Daily Intake of 2'-FL from Individual Proposed Food-Uses by Women of Childbearing Age, 16 to 45 Years, Within the U.S. (2013-2014 NHANES Data)

Food-Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake (g/day)		Consumer-Only Intake (g/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
All	***100***	***1.22***	***2.82***	***89.9***	***1,219***	***1.36***	***2.87***
Beverages and Beverage Bases							
Energy drinks	0.5	0.01	na	2.5	31	0.22	0.41*
Fitness water and third quenchers, sports and isotonic drinks	1.3	0.02	na	5.8	72	0.27	0.50*
Breakfast Cereals							
Ready-to-eat breakfast cereals for adults and children	25.8	0.32	1.09	31.0	410	1.02	2.10
Hot cereals for adults and children	5.1	0.06	na	9.1	141	0.69	1.23
Dairy Product Analogs							
Milk substitutes such as soy milk and imitation milks	0.7	0.01	na	6.2	86	0.15	0.29
Frozen Dairy Desserts and Mixes							
Frozen desserts including ice creams and frozen yogurts, frozen novelties	23.0	0.28	0.96	21.8	261	1.28	2.94
Gelatins, puddings, and fillings							
Dairy-based puddings, custards, and mousses	2.9	0.04	na	3.6	54	1.00	1.68*
Fruit pie filling	0.7	0.01	na	2.0	31	0.42	0.65*
"Fruit prep"	1.1	0.01	na	4.2	62	0.32	1.10*
Grain Products and Pastas							
Snack, breakfast, and meal replacement bars	3.7	0.05	0.21	14.1	154	0.32	0.54
Jams and Jellies, Commercial							
Jellies and jams, fruit preserves, fruit butters	3.5	0.04	na	7.8	93	0.54	1.13
Milk, Whole and Skim							
All acidophilus or fortified milks, non-fat and low-fat fluid milks, including fluid milk and reconstituted milk powder	8.7	0.11	0.35	46.2	644	0.23	0.50
Milk Products							
Flavored milks	2.9	0.04	0.15	12.1	172	0.29	0.57
Milk-based meal replacement beverages or diet beverages	0.6	0.01*	na	2.4	27	0.32*	0.44*
Yogurt	7.2	0.09	0.38	15.1	178	0.58	1.05
Processed Fruits and Fruit Juices							
Fruit drinks, including vitamin and mineral-fortified products	7.0	0.09	0.30	27.4	395	0.31	0.65
Fruit juices	5.1	0.06	0.22	27.8	409	0.22	0.39
Sweet Sauces, Toppings, and Syrups							
Syrups used to flavor milk beverages	0.2	<0.01*	na	1.0	7	0.20*	0.25*
Other							

Table A-7 Estimated Daily Intake of 2'-FL from Individual Proposed Food-Uses by Women of Childbearing Age, 16 to 45 Years, Within the U.S. (2013-2014 NHANES Data)

Food-Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake (g/day)		Consumer-Only Intake (g/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
Infant Formula	0	na	na	0	0	na	na
Follow-On Formula	0	na	na	0	0	na	na
Meal replacement products	0	na	na	0	0	na	na
Growing-Up (Toddler) Milks	0	na	na	0	0	na	na
Ready-to-eat, ready-to-serve, hot cereals for babies	0	na	na	0	0	na	na
Yogurt and juice beverages identified as "baby" drinks	0	na	na	0	0	na	na
"Junior Type" Desserts	0	na	na	0	0	na	na
Baby crackers, pretzels, cookies, and snack items	<0.1	<0.01*	na	<0.1	1	1.14*	1.14*

2'-FL = 2'-fucosyllactose; na = not available; NHANES = National Health and Nutrition Examination Survey; U.S. = United States.
* Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements.

Table A-8 Estimated Daily Intake of 2'-FL from Individual Proposed Food-Uses by Female Adults Aged 20 and Over Within the U.S. (2013-2014 NHANES Data)

Food-Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake (g/day)		Consumer-Only Intake (g/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
All	***100***	***1.32***	***2.96***	***91.9***	***2,169***	***1.44***	***3.05***
Beverages and Beverage Bases							
Energy drinks	0.2	<0.01	na	1.4	32	0.21	0.40*
Fitness water and third quenchers, sports and isotonic drinks	0.9	0.01	na	3.7	78	0.31	0.50*
Breakfast Cereals							
Ready-to-eat breakfast cereals for adults and children	22.3	0.30	1.03	31.0	711	0.95	1.85
Hot cereals for adults and children	7.3	0.10	0.42	13.6	394	0.71	1.20
Dairy Product Analogs							
Milk substitutes such as soy milk and imitation milks	1.1	0.01	na	7.5	176	0.20	0.48
Frozen Dairy Desserts and Mixes							
Frozen desserts including ice creams and frozen yogurts, frozen novelties	22.7	0.30	1.21	24.9	547	1.20	2.56
Gelatins, puddings, and fillings							
Dairy-based puddings, custards, and mousses	5.2	0.07	na	5.5	133	1.26	2.26
Fruit pie filling	2.0	0.03	na	5.1	101	0.51	1.17
"Fruit prep"	1.3	0.02	na	6.3	150	0.28	0.56
Grain Products and Pastas							
Snack, breakfast, and meal replacement bars	2.7	0.04	0.14	10.7	211	0.34	0.60
Jams and Jellies, Commercial							
Jellies and jams, fruit preserves, fruit butters	4.4	0.06	0.09	10.7	243	0.55	1.13
Milk, Whole and Skim							
All acidophilus or fortified milks, non-fat and low-fat fluid milks, including fluid milk and reconstituted milk powder	8.1	0.11	0.35	48.9	1,179	0.22	0.47
Milk Products							
Flavored milks	1.9	0.03	na	9.3	243	0.28	0.52
Milk-based meal replacement beverages or diet beverages	1.1	0.01	na	4.2	77	0.34	0.60*
Yogurt	8.8	0.12	0.45	19.1	379	0.61	1.05
Processed Fruits and Fruit Juices							
Fruit drinks, including vitamin and mineral-fortified products	5.4	0.07	0.24	22.3	558	0.32	0.63
Fruit juices	4.5	0.06	0.21	28.4	727	0.21	0.39
Sweet Sauces, Toppings, and Syrups							
Syrups used to flavor milk beverages	<0.1	<0.01*	na	0.6	12	0.25*	0.36*
Other							

Table A-8 Estimated Daily Intake of 2'-FL from Individual Proposed Food-Uses by Female Adults Aged 20 and Over Within the U.S. (2013-2014 NHANES Data)

Food-Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake (g/day)		Consumer-Only Intake (g/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
Infant Formula	0	na	na	0	0	na	na
Follow-On Formula	0	na	na	0	0	na	na
Meal replacement products	0	na	na	0	0	na	na
Growing-Up (Toddler) Milks	0	na	na	0	0	na	na
Ready-to-eat, ready-to-serve, hot cereals for babies	0	na	na	0	0	na	na
Yogurt and juice beverages identified as "baby" drinks	0	na	na	0	0	na	na
"Junior Type" Desserts	<0.1	<0.01*	na	<0.1	1	1.85*	1.85*
Baby crackers, pretzels, cookies, and snack items	0	na	na	0	0	na	na

2'-FL = 2'-fucosyllactose; na = not available; NHANES = National Health and Nutrition Examination Survey; U.S. = United States.
* Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements.

Table A-9 Estimated Daily Intake of 2'-FL from Individual Proposed Food-Uses by Male Adults Aged 20 Years and Over Within the U.S. (2013-2014 NHANES Data)

Food-Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake (g/day)		Consumer-Only Intake (g/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
All	***100***	***1.59***	***3.81***	***86.8***	***1,842***	***1.84***	***3.97***
Beverages and Beverage Bases							
Energy drinks	0.7	0.01	na	4.2	90	0.28	0.40
Fitness water and third quenchers, sports and isotonic drinks	2.0	0.03	na	8.1	163	0.40	0.77
Breakfast Cereals							
Ready-to-eat breakfast cereals for adults and children	25.0	0.40	1.29	28.5	574	1.40	2.55
Hot cereals for adults and children	6.3	0.10	0.28	10.4	292	0.97	1.94
Dairy Product Analogs							
Milk substitutes such as soy milk and imitation milks	0.6	0.01	na	4.2	95	0.22	0.44
Frozen Dairy Desserts and Mixes							
Frozen desserts including ice creams and frozen yogurts, frozen novelties	24.0	0.38	1.57	24.5	448	1.56	2.96
Gelatins, puddings, and fillings							
Dairy-based puddings, custards, and mousses	4.9	0.08	na	4.7	97	1.64	3.06
Fruit pie filling	1.4	0.02	na	4.5	86	0.51	0.97
"Fruit prep"	1.3	0.02	na	7.0	134	0.29	0.53
Grain Products and Pastas							
Snack, breakfast, and meal replacement bars	2.5	0.04	0.14	12.3	186	0.32	0.67
Jams and Jellies, Commercial							
Jellies and jams, fruit preserves, fruit butters	5.9	0.09	0.38	12.2	253	0.77	1.26
Milk, Whole and Skim							
All acidophilus or fortified milks, non-fat and low-fat fluid milks, including fluid milk and reconstituted milk powder	9.6	0.15	0.44	48.3	1,019	0.32	0.67
Milk Products							
Flavored milks	1.6	0.03	na	8.6	177	0.30	0.54
Milk-based meal replacement beverages or diet beverages	1.1	0.02	na	3.6	65	0.50	1.16*
Yogurt	4.4	0.07	0.28	11.0	201	0.65	1.30
Processed Fruits and Fruit Juices							
Fruit drinks, including vitamin and mineral-fortified products	4.3	0.07	0.26	18.9	448	0.36	0.67
Fruit juices	4.4	0.07	0.27	26.1	615	0.27	0.48
Sweet Sauces, Toppings, and Syrups							
Syrups used to flavor milk beverages	<0.1	<0.01*	na	0.6	9	0.12*	0.17*
Other							

Table A-9 Estimated Daily Intake of 2'-FL from Individual Proposed Food-Uses by Male Adults Aged 20 Years and Over Within the U.S. (2013-2014 NHANES Data)

Food-Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake (g/day)		Consumer-Only Intake (g/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
Infant Formula	0	na	na	0	0	na	na
Follow-On Formula	0	na	na	0	0	na	na
Meal replacement products	0	na	na	0	0	na	na
Growing-Up (Toddler) Milks	0	na	na	0	0	na	na
Ready-to-eat, ready-to-serve, hot cereals for babies	0	na	na	0	0	na	na
Yogurt and juice beverages identified as "baby" drinks	0	na	na	0	0	na	na
"Junior Type" Desserts	0	na	na	0	0	na	na
Baby crackers, pretzels, cookies, and snack items	0	na	na	0	0	na	na

2'-FL = 2'-fucosyllactose; na = not available; NHANES = National Health and Nutrition Examination Survey; U.S. = United States.
* Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements.

Table A-10 Estimated Daily Intake of 2'-FL from Individual Proposed Food-Uses by the Elderly Aged 65 Years and Over Within the U.S. (2013-2014 NHANES Data)

Food-Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake (g/day)		Consumer-Only Intake (g/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
All	***100***	***1.76***	***3.74***	***92.8***	***939***	***1.90***	***3.91***
Beverages and Beverage Bases							
Energy drinks	<0.1	<0.01*	na	0.1	3	0.18*	0.19*
Fitness water and third quenchers, sports and isotonic drinks	0.3	0.01*	na	2.1	21	0.27*	0.39*
Breakfast Cereals							
Ready-to-eat breakfast cereals for adults and children	22.5	0.40	1.28	39.7	359	1.00	1.81
Hot cereals for adults and children	8.1	0.14	0.58	17.1	224	0.83	1.69
Dairy Product Analogs							
Milk substitutes such as soy milk and imitation milks	0.7	0.01	na	5.8	63	0.21	0.49*
Frozen Dairy Desserts and Mixes							
Frozen desserts including ice creams and frozen yogurts, frozen novelties	29.0	0.51	1.65	35.5	306	1.44	2.70
Gelatins, puddings, and fillings							
Dairy-based puddings, custards, and mousses	5.9	0.10	na	8.0	78	1.30	2.07*
Fruit pie filling	2.9	0.05	na	7.9	68	0.64	1.17*
"Fruit prep"	1.8	0.03	0.05	11.3	88	0.28	0.67
Grain Products and Pastas							
Snack, breakfast, and meal replacement bars	1.0	0.02	na	7.0	51	0.24	0.32*
Jams and Jellies, Commercial							
Jellies and jams, fruit preserves, fruit butters	4.9	0.09	0.30	13.8	138	0.62	1.20
Milk, Whole and Skim							
All acidophilus or fortified milks, non-fat and low-fat fluid milks, including fluid milk and reconstituted milk powder	8.6	0.15	0.45	59.7	574	0.25	0.55
Milk Products							
Flavored milks	1.0	0.02	na	6.4	74	0.27	0.49*
Milk-based meal replacement beverages or diet beverages	1.4	0.02	na	6.1	45	0.40	0.68*
Yogurt	4.9	0.09	0.45	15.9	136	0.55	0.90
Processed Fruits and Fruit Juices							
Fruit drinks, including vitamin and mineral-fortified products	2.9	0.05	0.20	19.2	198	0.27	0.55
Fruit juices	4.1	0.07	0.25	34.1	360	0.21	0.39
Sweet Sauces, Toppings, and Syrups							
Syrups used to flavor milk beverages	0.1	<0.01*	na	0.7	7	0.26*	0.42*
Other							

Table A-10 Estimated Daily Intake of 2'-FL from Individual Proposed Food-Uses by the Elderly Aged 65 Years and Over Within the U.S. (2013-2014 NHANES Data)

Food-Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake (g/day)		Consumer-Only Intake (g/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
Infant Formula	0	na	na	0	0	na	na
Follow-On Formula	0	na	na	0	0	na	na
Meal replacement products	0	na	na	0	0	na	na
Growing-Up (Toddler) Milks	0	na	na	0	0	na	na
Ready-to-eat, ready-to-serve, hot cereals for babies	0	na	na	0	0	na	na
Yogurt and juice beverages identified as "baby" drinks	0	na	na	0	0	na	na
"Junior Type" Desserts	0.1	<0.01*	na	0.1	1	1.85*	1.85*
Baby crackers, pretzels, cookies, and snack items	0	na	na	0	0	na	na

2'-FL = 2'-fucosyllactose; na = not available; NHANES = National Health and Nutrition Examination Survey; U.S. = United States.
* Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements.

Table A-11 Estimated Daily Intake of 2'-FL from Individual Proposed Food-Uses by the Total U.S. Population (2013-2014 NHANES Data)

Food-Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake (g/day)		Consumer-Only Intake (g/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
All	***100***	***1.55***	***3.41***	***91.2***	***6,973***	***1.70***	***3.54***
Beverages and Beverage Bases							
Energy drinks	0.4	0.01	na	2.4	138	0.26	0.41
Fitness water and third quenchers, sports and isotonic drinks	1.7	0.03	na	7.5	543	0.36	0.62
Breakfast Cereals							
Ready-to-eat breakfast cereals for adults and children	24.6	0.38	1.21	35.2	2,770	1.08	2.08
Hot cereals for adults and children	5.5	0.08	0.25	10.9	949	0.78	1.36
Dairy Product Analogs							
Milk substitutes such as soy milk and imitation milks	0.7	0.01	na	5.0	361	0.21	0.44
Frozen Dairy Desserts and Mixes							
Frozen desserts including ice creams and frozen yogurts, frozen novelties	21.9	0.34	1.26	25.1	1,695	1.35	2.75
Gelatins, puddings, and fillings							
Dairy-based puddings, custards, and mousses	4.4	0.07	na	4.9	357	1.41	2.83
Fruit pie filling	1.2	0.02	na	3.9	220	0.50	0.99
"Fruit prep"	1.0	0.02	na	5.7	381	0.27	0.53
Grain Products and Pastas							
Snack, breakfast, and meal replacement bars	2.4	0.04	0.14	11.6	671	0.32	0.60
Jams and Jellies, Commercial							
Jellies and jams, fruit preserves, fruit butters	5.0	0.08	0.19	11.8	865	0.65	1.20
Milk, Whole and Skim							
All acidophilus or fortified milks, non-fat and low-fat fluid milks, including fluid milk and reconstituted milk powder	10.4	0.16	0.46	54.3	4,258	0.30	0.61
Milk Products							
Flavored milks	2.2	0.03	0.15	13.4	1,203	0.25	0.47
Milk-based meal replacement beverages or diet beverages	0.9	0.01	na	3.3	174	0.41	0.87
Yogurt	5.9	0.09	0.41	15.6	1,033	0.58	1.05
Processed Fruits and Fruit Juices							
Fruit drinks, including vitamin and mineral-fortified products	5.1	0.08	0.28	25.5	2,248	0.31	0.61
Fruit juices	4.8	0.07	0.26	32.0	2,761	0.23	0.45
Sweet Sauces, Toppings, and Syrups							
Syrups used to flavor milk beverages	0.1	0.00	na	1.3	94	0.17	0.42
Other							

Table A-11 Estimated Daily Intake of 2'-FL from Individual Proposed Food-Uses by the Total U.S. Population (2013-2014 NHANES Data)

Food-Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake (g/day)		Consumer-Only Intake (g/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
Infant Formula	1.0	0.02	na	0.9	223	1.62	2.64
Follow-On Formula	<0.1	<0.01*	na	<0.1	1	0.58*	0.58*
Meal replacement products	<0.1	<0.01*	na	0.1	18	0.52*	1.41*
Growing-Up (Toddler) Milks	<0.1	<0.01*	na	0.1	10	0.91*	1.04*
Ready-to-eat, ready-to-serve, hot cereals for babies	<0.1	<0.01*	na	0.1	18	0.66*	0.93*
Yogurt and juice beverages identified as "baby" drinks	0.3	0.01	na	0.5	90	0.99	2.07
"Junior Type" Desserts	0.2	<0.01	na	0.4	74	0.63	1.25*
Baby crackers, pretzels, cookies, and snack items	0.2	<0.01	na	0.7	122	0.52	1.62

2'-FL = 2'-fucosyllactose; na = not available; NHANES = National Health and Nutrition Examination Survey; U.S. = United States.
* Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements.

Appendix B
Estimated Daily Per Kilogram Body Weight Intake of 2'-FL from Individual Proposed Food-Uses by Different Population Groups Within the U.S. (2013-2014 NHANES Data)

Table B-1 Estimated Daily Per Kilogram Body Weight Intake of 2'-FL from Individual Proposed Food-Uses by Infants Aged 0 to 5 Months Within the U.S. (2013-2014 NHANES Data)

Food-Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake (mg/kg bw/day)		Consumer-Only Intake (mg/kg bw/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
All	***100***	***181***	***477***	***57.5***	***107***	***315***	***532***
Beverages and Beverage Bases							
Energy drinks	0	na	na	0	0	na	na
Fitness water and third quenchers, sports and isotonic drinks	0	na	na	0	0	na	na
Breakfast Cereals							
Ready-to-eat breakfast cereals for adults and children	<0.1	<1*	na	0.2	1	<1*	1*
Hot cereals for adults and children	0.1	<1*	na	0.9	1	25*	25*
Dairy Product Analogs							
Milk substitutes such as soy milk and imitation milks	0	na	na	0	0	na	na
Frozen Dairy Desserts and Mixes							
Frozen desserts including ice creams and frozen yogurts, frozen novelties	0.3	<1*	na	0.9	1	54*	54*
Gelatins, Puddings, and Fillings							
Dairy-based puddings, custards, and mousses	0	na	na	0	0	na	na
Fruit pie filling	0	na	na	0	0	na	na
"Fruit prep"	0	na	na	0	0	na	na
Grain Products and Pastas							
Snack, breakfast, and meal replacement bars	0	na	na	0	0	na	na
Jams and Jellies, Commercial							
Jellies and jams, fruit preserves, fruit butters	0	na	na	0	0	na	na
Milk, Whole and Skim							
All acidophilus or fortified milks, non-fat and low-fat fluid milks, including fluid milk and reconstituted milk powder	<0.1	<1*	na	0.2	1	12*	10*
Milk Products							
Flavored milks	0	na	na	0	0	na	na
Milk-based meal replacement beverages or diet beverages	0	na	na	0	0	na	na
Yogurt	0	na	na	0	0	na	na
Processed Fruits and Fruit Juices						na	
Fruit drinks, including vitamin and mineral-fortified products	0	na	na	0	0	na	na
Fruit juices	0.3	<1*	na	2.1	5	22*	37*
Sweet Sauces, Toppings, and Syrups							
Syrups used to flavor milk beverages	0	na	na	0	0	na	na
Other							

Table B-1 Estimated Daily Per Kilogram Body Weight Intake of 2'-FL from Individual Proposed Food-Uses by Infants Aged 0 to 5 Months Within the U.S. (2013-2014 NHANES Data)

Food-Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake (mg/kg bw/day)		Consumer-Only Intake (mg/kg bw/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
Infant formula	92.3	167	441	52.4	102	319	482
Follow-on formula	0	na	na	0	0	na	na
Meal replacement products	0	na	na	0	0	na	na
Growing-up (toddler) milks	0	na	na	0	0	na	na
Ready-to-eat, ready-to-serve, hot cereals for babies for babies	0.6	1*	na	2.4	4	45*	76*
Yogurt and juice beverages identified as "baby" drinks	3.5	6*	na	4.5	8	141*	315*
"Junior type" desserts	0.9	2*	na	6.5	6	26*	40*
Baby crackers, pretzels, cookies, and snack items	2.0	4*	na	2.6	6	138*	263*

2'-FL = 2'-fucosyllactose; bw = body weight; na = not available; NHANES = National Health and Nutrition Examination Survey; U.S. = United States.

* Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements.

Table B-2 Estimated Daily Per Kilogram Body Weight Intake of 2'-FL from Individual Proposed Food-Uses by Older Infants Aged 6 to 11 Months Within the U.S. (2013-2014 NHANES Data)

Food-Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake (mg/kg bw/day)		Consumer-Only Intake (mg/kg bw/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
All	***100***	***244***	***441***	***94.1***	***160***	***259***	***447***
Beverages and Beverage Bases							
Energy drinks	0	na	na	0	0	na	na
Fitness water and third quenchers, sports and isotonic drinks	0.1	<1*	na	2.7	6	11*	11*
Breakfast Cereals							
Ready-to-eat breakfast cereals for adults and children	1.4	3*	14*	15.9	22	21*	47*
Hot cereals for adults and children	1.5	4*	8*	11.3	13	33*	54*
Dairy Product Analogs							
Milk substitutes such as soy milk and imitation milks	0.1	<1*	na	1.6	2	22*	22*
Frozen Dairy Desserts and Mixes							
Frozen desserts including ice creams and frozen yogurts, frozen novelties	0.2	<1*	na	4.3	8	11*	30*
Gelatins, Puddings, and Fillings							
Dairy-based puddings, custards, and mousses	0.6	2*	na	2.9	4	53*	73*
Fruit pie filling	0.3	<1*	na	1.7	1	40*	40*
"Fruit prep"	0	na	na	0	0	na	na
Grain Products and Pastas							
Snack, breakfast, and meal replacement bars	0.6	1*	na	1.7	1	87*	87*
Jams and Jellies, Commercial							
Jellies and jams, fruit preserves, fruit butters	0	na	na	0	0	na	na
Milk, Whole and Skim							
All acidophilus or fortified milks, non-fat and low-fat fluid milks, including fluid milk and reconstituted milk powder	3.1	7	30*	15.9	33	47	85*
Milk Products							
Flavored milks	0.1	<1*	na	2.0	4	14*	23*
Milk-based meal replacement beverages or diet beverages	0	na	na	0	0	na	na
Yogurt	1.9	5*	2*	10.6	18	43*	81*
Processed Fruits and Fruit Juices							
Fruit drinks, including vitamin and mineral-fortified products	0.1	<1*	na	2.1	5	9*	11*
Fruit juices	0.8	2*	6*	14	29	13*	19*
Sweet Sauces, Toppings, and Syrups							
Syrups used to flavor milk beverages	0	na	na	0	0	na	na

Table B-2 Estimated Daily Per Kilogram Body Weight Intake of 2'-FL from Individual Proposed Food-Uses by Older Infants Aged 6 to 11 Months Within the U.S. (2013-2014 NHANES Data)

Food-Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake (mg/kg bw/day)		Consumer-Only Intake (mg/kg bw/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
Other							
Infant formula	47.1	115	284	63	107	183	307
Follow-on formula	0	na	na	0	0	na	na
Meal replacement products	0	na	na	0	0	na	na
Growing-up (toddler) milks	3.0	7*	na	6.5	7	113*	163*
Ready-to-eat, ready-to-serve, hot cereals for babies for babies	1.8	4*	na	5.0	9	87*	116*
Yogurt and juice beverages identified as "baby" drinks	18.7	46	141*	40.5	59	113	240*
"Junior type" desserts	10.9	27	76*	36.3	55	73	152*
Baby crackers, pretzels, cookies, and snack items	7.7	19	64	46.5	82	41	91

2'-FL = 2'-fucosyllactose; bw = body weight; na = not available; NHANES = National Health and Nutrition Examination Survey; U.S. = United States.

* Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements.

Table B-3 Estimated Daily Per Kilogram Body Weight Intake of 2'-FL from Individual Proposed Food-Uses by Toddlers Aged 12 to 35 Months Within the U.S. (2013-2014 NHANES Data)

Food-Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake (mg/kg bw/day)		Consumer-Only Intake (mg/kg bw/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
All	***100***	***148***	***243***	***100***	***346***	***148***	***243***
Beverages and Beverage Bases							
Energy drinks	0	na	na	0	0	na	na
Fitness water and third quenchers, sports and isotonic drinks	0.8	1*	na	7.0	20	17*	31*
Breakfast Cereals							
Ready-to-eat breakfast cereals for adults and children	18.2	27	69	60.7	201	44	88
Hot cereals for adults and children	5.3	8	33*	17.9	66	44	75*
Dairy Product Analogs							
Milk substitutes such as soy milk and imitation milks	1.5	2*	na	7.6	22	30*	81*
Frozen Dairy Desserts and Mixes							
Frozen desserts including ice creams and frozen yogurts, frozen novelties	4.7	7	28*	16.6	68	42	92*
Gelatins, Puddings, and Fillings							
Dairy-based puddings, custards, and mousses	2.0	3*	na	5.6	24	52*	83*
Fruit pie filling	<0.1	<1*	na	0.5	1	15*	15*
"Fruit prep"	0.5	1*	na	5.7	14	14*	32*
Grain Products and Pastas							
Snack, breakfast, and meal replacement bars	1.4	2*	na	7.8	25	27*	49*
Jams and Jellies, Commercial							
Jellies and jams, fruit preserves, fruit butters	4.5	7	22*	19.4	50	34	63*
Milk, Whole and Skim							
All acidophilus or fortified milks, non-fat and low-fat fluid milks, including fluid milk and reconstituted milk powder	26.7	40	83	89.0	303	44	85
Milk Products							
Flavored milks	1.4	2	7*	15.9	58	13	23*
Milk-based meal replacement beverages or diet beverages	0.9	1*	na	4.7	7	30*	52*
Yogurt	7.1	11	32	29.5	95	35	78
Processed Fruits and Fruit Juices							
Fruit drinks, including vitamin and mineral-fortified products	3.8	6	16	30.4	128	19	34
Fruit juices	6.8	10	26	63.7	211	16	34
Sweet Sauces, Toppings, and Syrups							
Syrups used to flavor milk beverages	0.2	<1*	na	3.2	8	8*	9*
Other							

Table B-3 Estimated Daily Per Kilogram Body Weight Intake of 2'-FL from Individual Proposed Food-Uses by Toddlers Aged 12 to 35 Months Within the U.S. (2013-2014 NHANES Data)

Food-Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake (mg/kg bw/day)		Consumer-Only Intake (mg/kg bw/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
Infant formula	1.9	3*	na	4.0	13	71*	111*
Follow-on formula	<0.1	<1*	na	<0.1	1	48*	48*
Meal replacement products	1.3	2*	na	3.0	6	64*	203*
Growing-up (toddler) milks	0.6	1*	na	1.1	3	85*	102*
Ready-to-eat, ready-to-serve, hot cereals for babies for babies	1.0	1*	na	1.9	5	77*	122*
Yogurt and juice beverages identified as "baby" drinks	3.9	6*	na	6.1	20	96*	195*
"Junior type" desserts	1.4	2*	na	4.2	12	50*	104*
Baby crackers, pretzels, cookies, and snack items	4.3	6	2*	11.4	30	56	112*

2'-FL = 2'-fucosyllactose; bw = body weight; na = not available; NHANES = National Health and Nutrition Examination Survey; U.S. = United States.

* Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements.

Table B-4 Estimated Daily Per Kilogram Body Weight Intake of 2'-FL from Individual Proposed Food-Uses by Children Aged 3 to 11 Years Within the U.S. (2013-2014 NHANES Data)

Food-Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake (mg/kg bw/day)		Consumer-Only Intake (mg/kg bw/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
All	***100***	***75***	***147***	***99.7***	***1,268***	***76***	***147***
Beverages and Beverage Bases							
Energy drinks	<0.1	<1*	na	0.1	1	4*	4*
Fitness water and third quenchers, sports and isotonic drinks	1.2	1	3	12.4	128	8	16
Breakfast Cereals							
Ready-to-eat breakfast cereals for adults and children	27.7	21	59	59.0	771	36	73
Hot cereals for adults and children	2.9	2	na	8.4	117	27	59
Dairy Product Analogs							
Milk substitutes such as soy milk and imitation milks	0.3	<1	na	2.6	33	8	15*
Frozen Dairy Desserts and Mixes							
Frozen desserts including ice creams and frozen yogurts, frozen novelties	19.9	15	48	35.3	386	42	90
Gelatins, Puddings, and Fillings							
Dairy-based puddings, custards, and mousses	4.2	3	na	5.1	67	63	127*
Fruit pie filling	0.2	<1*	na	1.0	15	12*	17*
"Fruit prep"	0.2	<1	na	3.4	45	5	12*
Grain Products and Pastas							
Snack, breakfast, and meal replacement bars	1.4	1	4	12.1	128	9	14
Jams and Jellies, Commercial							
Jellies and jams, fruit preserves, fruit butters	4.9	4	14	16.9	221	22	49
Milk, Whole and Skim							
All acidophilus or fortified milks, non-fat and low-fat fluid milks, including fluid milk and reconstituted milk powder	13.5	10	23	80.6	1,034	13	25
Milk Products							
Flavored milks	3.8	3	10	35.5	472	8	16
Milk-based meal replacement beverages or diet beverages	0.1	<1*	na	1.4	10	6*	8*
Yogurt	6.2	5	18	23.6	258	20	46
Processed Fruits and Fruit Juices							
Fruit drinks, including vitamin and mineral-fortified products	5.8	4	13	50.4	660	9	17
Fruit juices	6.8	5	14	56.8	753	9	18
Sweet Sauces, Toppings, and Syrups							
Syrups used to flavor milk beverages	0.5	<1	na	4.9	54	7	14*
Other							

Table B-4 Estimated Daily Per Kilogram Body Weight Intake of 2'-FL from Individual Proposed Food-Uses by Children Aged 3 to 11 Years Within the U.S. (2013-2014 NHANES Data)

Food-Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake (mg/kg bw/day)		Consumer-Only Intake (mg/kg bw/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
Infant formula	<0.1	<1*	na	<0.1	1	22*	22*
Follow-on formula	0	na	na	0	0	na	na
Meal replacement products	0.1	<1*	na	0.4	11	21*	60*
Growing-up (toddler) milks	0	na	na	0	0	na	na
Ready-to-eat, ready-to-serve, hot cereals for babies for babies	0	na	na	0	0	na	na
Yogurt and juice beverages identified as "baby" drinks	0.1	<1*	na	0.3	3	36*	44*
"Junior type" desserts	0	na	na	0	0	na	na
Baby crackers, pretzels, cookies, and snack items	0.1	<1*	na	0.2	2	50*	69*

2'-FL = 2'-fucosyllactose; bw = body weight; na = not available; NHANES = National Health and Nutrition Examination Survey; U.S. = United States.

* Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements.

Table B-5 Estimated Daily Per Kilogram Body Weight Intake of 2'-FL from Individual Proposed Food-Uses by Female Teenagers Aged 12 to 19 Years Within the U.S. (2013-2014 NHANES Data)

Food-Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake (mg/kg bw/day)		Consumer-Only Intake (mg/kg bw/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
All	***100***	***24***	***52***	***94.7***	***536***	***26***	***52***
Beverages and Beverage Bases							
Energy drinks	0.3	<1*	na	1.9	7	4*	7*
Fitness water and third quenchers, sports and isotonic drinks	1.4	<1	na	9.0	53	4	6*
Breakfast Cereals							
Ready-to-eat breakfast cereals for adults and children	32.4	8*	24	43.3	228	18*	30*
Hot cereals for adults and children	2.4	1	na	5.0	38	12	21*
Dairy Product Analogs							
Milk substitutes such as soy milk and imitation milks	0.3	<1*	na	2.1	22	3*	6*
Frozen Dairy Desserts and Mixes							
Frozen desserts including ice creams and frozen yogurts, frozen novelties	21.1	5	19	24.3	122	21	40
Gelatins, Puddings, and Fillings							
Dairy-based puddings, custards, and mousses	4.0	1*	na	4.1	20	24*	32*
Fruit pie filling	0.3	<1*	na	0.7	10	12*	20*
"Fruit prep"	0.5	<1*	na	3.0	23	4*	10*
Grain Products and Pastas							
Snack, breakfast, and meal replacement bars	3.7	1	4*	17.2	68	5	9*
Jams and Jellies, Commercial							
Jellies and jams, fruit preserves, fruit butters	4.3	1	na	9.7	42	11	21*
Milk, Whole and Skim							
All acidophilus or fortified milks, non-fat and low-fat fluid milks, including fluid milk and reconstituted milk powder	10.8	3	7	59.4	315	4	8
Milk Products							
Flavored milks	2.6	1	3	18.9	106	3	6
Milk-based meal replacement beverages or diet beverages	0.1	<1*	na	0.5	6	4*	6*
Yogurt	4.7	1	4*	12.8	52	9	18*
Processed Fruits and Fruit Juices							
Fruit drinks, including vitamin and mineral-fortified products	7.6	2	5	37.0	231	5	13
Fruit juices	3.4	1	3	25.3	188	3	6
Sweet Sauces, Toppings, and Syrups							
Syrups used to flavor milk beverages	0.2	<1*	na	1.7	5	2*	3*

Table B-5 Estimated Daily Per Kilogram Body Weight Intake of 2'-FL from Individual Proposed Food-Uses by Female Teenagers Aged 12 to 19 Years Within the U.S. (2013-2014 NHANES Data)

Food-Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake (mg/kg bw/day)		Consumer-Only Intake (mg/kg bw/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
Other							
Infant formula	0	na	na	0	0	na	na
Follow-on formula	0	na	na	0	0	na	na
Meal replacement products	0	na	na	0	0	na	na
Growing-up (toddler) milks	0	na	na	0	0	na	na
Ready-to-eat, ready-to-serve, hot cereals for babies for babies	0	na	na	0	0	na	na
Yogurt and juice beverages identified as "baby" drinks	0	na	na	0	0	na	na
"Junior type" desserts	0	na	na	0	0	na	na
Baby crackers, pretzels, cookies, and snack items	<0.1	<1*	na	<0.1	1	15*	15*

2'-FL = 2'-fucosyllactose; bw = body weight; na = not available; NHANES = National Health and Nutrition Examination Survey; U.S. = United States.

* Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements.

Table B-6 Estimated Daily Per Kilogram Body Weight Intake of 2'-FL from Individual Proposed Food-Uses by Male Teenagers Aged 12 to 19 Years Within the U.S. (2013-2014 NHANES Data)

Food-Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake (mg/kg bw/day)		Consumer-Only Intake (mg/kg bw/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
All	***100***	***29***	***67***	***92.5***	***524***	***31***	***67***
Beverages and Beverage Bases							
Energy drinks	0.4	<1*	na	4.0	8	3*	4*
Fitness water and third quenchers, sports and isotonic drinks	5.2	1	3	20.1	93	7	14
Breakfast Cereals							
Ready-to-eat breakfast cereals for adults and children	32.2	9	27	45.9	252	20	42
Hot cereals for adults and children	2.0	1*	na	3.9	28	15*	36*
Dairy Product Analogs							
Milk substitutes such as soy milk and imitation milks	0.1	<1*	na	0.9	11	3*	7*
Frozen Dairy Desserts and Mixes							
Frozen desserts including ice creams and frozen yogurts, frozen novelties	18.4	5	22	19.1	107	28	48
Gelatins, Puddings, and Fillings							
Dairy-based puddings, custards, and mousses	2.3	1*	na	2.5	12	26*	56*
Fruit pie filling	0.3	<1*	na	2.3	6	4*	9*
"Fruit prep"	0.6	<1*	na	2.0	14	9*	20*
Grain Products and Pastas							
Snack, breakfast, and meal replacement bars	2.1	1	2*	12.4	52	5	9*
Jams and Jellies, Commercial							
Jellies and jams, fruit preserves, fruit butters	4.1	1	na	6.9	50	17	32*
Milk, Whole and Skim							
All acidophilus or fortified milks, non-fat and low-fat fluid milks, including fluid milk and reconstituted milk powder	14.8	4	11	65.8	359	6	14
Milk Products							
Flavored milks	3.3	1	4	24.1	140	4	7
Milk-based meal replacement beverages or diet beverages	0.7	<1*	na	1.8	9	11*	15*
Yogurt	1.6	<1*	na	4.5	27	10*	20*
Processed Fruits and Fruit Juices							
Fruit drinks, including vitamin and mineral-fortified products	5.6	2	5	30.4	208	5	11
Fruit juices	5.8	2	5	40.9	223	4	9
Sweet Sauces, Toppings, and Syrups							
Syrups used to flavor milk beverages	0.3	<1*	na	1.4	4	6*	7*

Table B-6 Estimated Daily Per Kilogram Body Weight Intake of 2'-FL from Individual Proposed Food-Uses by Male Teenagers Aged 12 to 19 Years Within the U.S. (2013-2014 NHANES Data)

Food-Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake (mg/kg bw/day)		Consumer-Only Intake (mg/kg bw/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
Other							
Infant formula	0	na	na	0	0	na	na
Follow-on formula	0	na	na	0	0	na	na
Meal replacement products	0	na	na	0	0	na	na
Growing-up (toddler) milks	0	na	na	0	0	na	na
Ready-to-eat, ready-to-serve, hot cereals for babies for babies	0	na	na	0	0	na	na
Yogurt and juice beverages identified as "baby" drinks	0	na	na	0	0	na	na
"Junior type" desserts	0	na	na	0	0	na	na
Baby crackers, pretzels, cookies, and snack items	0	na	na	0	0	na	na

2'-FL = 2'-fucosyllactose; bw = body weight; na = not available; NHANES = National Health and Nutrition Examination Survey; U.S. = United States.
* Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements.

Table B-7 Estimated Daily Per Kilogram Body Weight Intake of 2'-FL from Individual Proposed Food-Uses by Women of Childbearing Age, 16 to 45 Years, Within the U.S. (2013-2014 NHANES Data)

Food-Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake (mg/kg bw/day)		Consumer-Only Intake (mg/kg bw/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
All	***100***	***18***	***42***	***89.9***	***1,209***	***20***	***43***
Beverages and Beverage Bases							
Energy drinks	0.5	<1	na	2.6	31	4	7*
Fitness water and third quenchers, sports and isotonic drinks	1.2	<1	na	5.8	70	4	7*
Breakfast Cereals							
Ready-to-eat breakfast cereals for adults and children	26.1	5	16	31.1	407	15	30
Hot cereals for adults and children	5.0	1	na	9.0	140	10	19
Dairy Product Analogs							
Milk substitutes such as soy milk and imitation milks	0.8	<1	na	6.2	86	2	5
Frozen Dairy Desserts and Mixes							
Frozen desserts including ice creams and frozen yogurts, frozen novelties	23.2	4	13	21.9	258	19	40
Gelatins, Puddings, and Fillings							
Dairy-based puddings, custards, and mousses	2.9	1	na	3.5	53	15	30*
Fruit pie filling	0.7	<1	na	2.0	30	6	12*
"Fruit prep"	1.0	<1	na	4.1	61	4	16*
Grain Products and Pastas							
Snack, breakfast, and meal replacement bars	3.9	1	3	14.2	153	5	9
Jams and Jellies, Commercial							
Jellies and jams, fruit preserves, fruit butters	3.3	1	na	7.8	91	8	12
Milk, Whole and Skim							
All acidophilus or fortified milks, non-fat and low-fat fluid milks, including fluid milk and reconstituted milk powder	8.9	2	5	46.1	637	3	7
Milk Products							
Flavored milks	2.9	1	2	12.2	172	4	9
Milk-based meal replacement beverages or diet beverages	0.6	<1*	na	2.4	27	5*	8*
Yogurt	7.0	1	5	15.1	177	8	18
Processed Fruits and Fruit Juices							
Fruit drinks, including vitamin and mineral-fortified products	6.8	1	4	27.4	392	4	9
Fruit juices	5.1	1	3	27.8	407	3	6
Sweet Sauces, Toppings, and Syrups							
Syrups used to flavor milk beverages	0.2	<1*	na	1.1	7	4*	5*

Table B-7 Estimated Daily Per Kilogram Body Weight Intake of 2'-FL from Individual Proposed Food-Uses by Women of Childbearing Age, 16 to 45 Years, Within the U.S. (2013-2014 NHANES Data)

Food-Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake (mg/kg bw/day)		Consumer-Only Intake (mg/kg bw/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
Other							
Infant formula	0	na	na	0	0	na	na
Follow-on formula	0	na	na	0	0	na	na
Meal replacement products	0	na	na	0	0	na	na
Growing-up (toddler) milks	0	na	na	0	0	na	na
Ready-to-eat, ready-to-serve, hot cereals for babies for babies	0	na	na	0	0	na	na
Yogurt and juice beverages identified as "baby" drinks	0	na	na	0	0	na	na
"Junior type" desserts	0	na	na	0	0	na	na
Baby crackers, pretzels, cookies, and snack items	<0.1	<1*	na	<0.1	1	15*	15*

2'-FL = 2'-fucosyllactose; bw = body weight; na = not available; NHANES = National Health and Nutrition Examination Survey; U.S. = United States.

* Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements.

Table B-8 Estimated Daily Per Kilogram Body Weight Intake of 2'-FL from Individual Proposed Food-Uses by Female Adults Aged 20 Years and Over Within the U.S. (2013-2014 NHANES Data)

Food-Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake (mg/kg bw/day)		Consumer-Only Intake (mg/kg bw/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
All	***100***	***19***	***42***	***91.9***	***2,156***	***20***	***43***
Beverages and Beverage Bases							
Energy drinks	0.2	<1	na	1.4	32	3	6*
Fitness water and third quenchers, sports and isotonic drinks	0.9	<1	na	3.8	78	4	7*
Breakfast Cereals							
Ready-to-eat breakfast cereals for adults and children	22.2	4	14	31.0	707	13	25
Hot cereals for adults and children	7.2	1	5	13.4	389	10	19
Dairy Product Analogs							
Milk substitutes such as soy milk and imitation milks	1.2	<1	na	7.5	175	3	7
Frozen Dairy Desserts and Mixes							
Frozen desserts including ice creams and frozen yogurts, frozen novelties	22.9	4	14	24.9	544	17	38
Gelatins, Puddings, and Fillings							
Dairy-based puddings, custards, and mousses	5.0	1	na	5.4	132	17	32
Fruit pie filling	2.1	<1	na	5.1	99	8	17
"Fruit prep"	1.3	<1	na	6.3	149	4	9
Grain Products and Pastas							
Snack, breakfast, and meal replacement bars	2.8	1	2	10.7	209	5	10
Jams and Jellies, Commercial							
Jellies and jams, fruit preserves, fruit butters	4.5	1	1	10.7	242	8	14
Milk, Whole and Skim							
All acidophilus or fortified milks, non-fat and low-fat fluid milks, including fluid milk and reconstituted milk powder	8.3	2	5	48.8	1,172	3	7
Milk Products							
Flavored milks	1.9	<1	na	9.3	243	4	8
Milk-based meal replacement beverages or diet beverages	1.1	<1	na	4.2	77	5	11*
Yogurt	8.5	2	7	19.1	378	8	15
Processed Fruits and Fruit Juices							
Fruit drinks, including vitamin and mineral-fortified products	5.0	1	3	22.3	556	4	8
Fruit juices	4.5	1	3	28.4	724	3	6
Sweet Sauces, Toppings, and Syrups							
Syrups used to flavor milk beverages	0.1	<1*	na	0.6	12	4*	5*

Table B-8 Estimated Daily Per Kilogram Body Weight Intake of 2'-FL from Individual Proposed Food-Uses by Female Adults Aged 20 Years and Over Within the U.S. (2013-2014 NHANES Data)

Food-Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake (mg/kg bw/day)		Consumer-Only Intake (mg/kg bw/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
Other							
Infant formula	0	na	na	0	0	na	na
Follow-on formula	0	na	na	0	0	na	na
Meal replacement products	0	na	na	0	0	na	na
Growing-up (toddler) milks	0	na	na	0	0	na	na
Ready-to-eat, ready-to-serve, hot cereals for babies for babies	0	na	na	0	0	na	na
Yogurt and juice beverages identified as "baby" drinks	0	na	na	0	0	na	na
"Junior type" desserts	<0.1	<1*	na	<0.1	1	51*	51*
Baby crackers, pretzels, cookies, and snack items	0	na	na	0	0	na	na

2'-FL = 2'-fucosyllactose; bw = body weight; na = not available; NHANES = National Health and Nutrition Examination Survey; U.S. = United States.

* Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements.

Table B-9 Estimated Daily Per Kilogram Body Weight Intake of 2'-FL from Individual Proposed Food-Uses by Male Adults Aged 20 Years and Over Within the U.S. (2013-2014 NHANES Data)

Food-Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake (mg/kg bw/day)		Consumer-Only Intake (mg/kg bw/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
All	***100***	***19***	***46***	***86.7***	***1,833***	***22***	***48***
Beverages and Beverage Bases							
Energy drinks	0.7	<1	na	4.3	90	3	5
Fitness water and third quenchers, sports and isotonic drinks	2.0	<1	na	8.0	161	5	9
Breakfast Cereals							
Ready-to-eat breakfast cereals for adults and children	25.2	5	16	28.6	569	17	30
Hot cereals for adults and children	6.8	1	3	10.5	291	12	28
Dairy Product Analogs							
Milk substitutes such as soy milk and imitation milks	0.6	<1	0	4.3	95	3	6
Frozen Dairy Desserts and Mixes							
Frozen desserts including ice creams and frozen yogurts, frozen novelties	23.3	4	17	24.1	443	18	33
Gelatins, Puddings, and Fillings							
Dairy-based puddings, custards, and mousses	5.0	1	na	4.8	97	20	38
Fruit pie filling	1.4	<1	na	4.4	85	6	10
"Fruit prep"	1.3	<1	na	7.1	133	3	7
Grain Products and Pastas							
Snack, breakfast, and meal replacement bars	2.5	<1	2	12.4	186	4	7
Jams and Jellies, Commercial							
Jellies and jams, fruit preserves, fruit butters	5.6	1	4	12.3	253	9	15
Milk, Whole and Skim							
All acidophilus or fortified milks, non-fat and low-fat fluid milks, including fluid milk and reconstituted milk powder	9.8	2	5	48.6	1,015	4	8
Milk Products							
Flavored milks	1.6	<1	na	8.7	176	3	6
Milk-based meal replacement beverages or diet beverages	1.0	<1	na	3.2	64	6	15*
Yogurt	4.6	1	3	11.1	201	8	15
Processed Fruits and Fruit Juices							
Fruit drinks, including vitamin and mineral-fortified products	4.1	1	3	19.1	448	4	8
Fruit juices	4.3	1	3	25.7	611	3	7
Sweet Sauces, Toppings, and Syrups							
Syrups used to flavor milk beverages	<0.1	<1*	na	0.6	9	1*	2*

Table B-9 Estimated Daily Per Kilogram Body Weight Intake of 2'-FL from Individual Proposed Food-Uses by Male Adults Aged 20 Years and Over Within the U.S. (2013-2014 NHANES Data)

Food-Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake (mg/kg bw/day)		Consumer-Only Intake (mg/kg bw/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
Other							
Infant formula	0	na	na	0	0	na	na
Follow-on formula	0	na	na	0	0	na	na
Meal replacement products	0	na	na	0	0	na	na
Growing-up (toddler) milks	0	na	na	0	0	na	na
Ready-to-eat, ready-to-serve, hot cereals for babies for babies	0	na	na	0	0	na	na
Yogurt and juice beverages identified as "baby" drinks	0	na	na	0	0	na	na
"Junior type" desserts	0	na	na	0	0	na	na
Baby crackers, pretzels, cookies, and snack items	0	na	na	0	0	na	na

2'-FL = 2'-fucosyllactose; bw = body weight; na = not available; NHANES = National Health and Nutrition Examination Survey; U.S. = United States.
* Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements.

Table B-10 Estimated Daily Per Kilogram Body Weight Intake of 2'-FL from Individual Proposed Food-Uses by the Elderly Aged 65 Years and Over Within the U.S. (2013-2014 NHANES Data)

Food-Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake (mg/kg bw/day)		Consumer-Only Intake (mg/kg bw/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
All	***100***	***24***	***53***	***92.6***	***928***	***26***	***54***
Beverages and Beverage Bases							
Energy drinks	<0.1	<1*	na	0.1	3	3*	3*
Fitness water and third quenchers, sports and isotonic drinks	0.3	<1*	na	1.7	19	4*	6*
Breakfast Cereals							
Ready-to-eat breakfast cereals for adults and children	22.4	5	18	40.3	356	14	23
Hot cereals for adults and children	8.3	2	8	17.2	220	12	25
Dairy Product Analogs							
Milk substitutes such as soy milk and imitation milks	0.7	<1	na	5.9	62	3	7*
Frozen Dairy Desserts and Mixes							
Frozen desserts including ice creams and frozen yogurts, frozen novelties	28.9	7	22	35.0	304	20	38
Gelatins, Puddings, and Fillings							
Dairy-based puddings, custards, and mousses	6.0	1	na	8.2	78	18	33*
Fruit pie filling	2.9	1	na	7.6	66	9	17*
"Fruit prep"	1.7	<1	1	11.4	87	4	10
Grain Products and Pastas							
Snack, breakfast, and meal replacement bars	1.0	<1	na	7.1	50	3	5*
Jams and Jellies, Commercial							
Jellies and jams, fruit preserves, fruit butters	4.9	1	4	14.1	138	9	16
Milk, Whole and Skim							
All acidophilus or fortified milks, non-fat and low-fat fluid milks, including fluid milk and reconstituted milk powder	8.9	2	6	60.6	570	4	8
Milk Products							
Flavored milks	0.9	<1	na	6.5	73	4	7*
Milk-based meal replacement beverages or diet beverages	1.1	<1	na	5.0	44	5	14*
Yogurt	5.0	1	6	16.1	135	8	13
Processed Fruits and Fruit Juices							
Fruit drinks, including vitamin and mineral-fortified products	2.7	1	3	19.5	196	3	7
Fruit juices	4.0	1	3	33.4	355	3	6
Sweet Sauces, Toppings, and Syrups							
Syrups used to flavor milk beverages	0.1	<1*	na	0.7	7	4*	5*

Table B-10 Estimated Daily Per Kilogram Body Weight Intake of 2'-FL from Individual Proposed Food-Uses by the Elderly Aged 65 Years and Over Within the U.S. (2013-2014 NHANES Data)

Food-Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake (mg/kg bw/day)		Consumer-Only Intake (mg/kg bw/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
Other							
Infant formula	0	na	na	0	0	na	na
Follow-on formula	0	na	na	0	0	na	na
Meal replacement products	0	na	na	0	0	na	na
Growing-up (toddler) milks	0	na	na	0	0	na	na
Ready-to-eat, ready-to-serve, hot cereals for babies for babies	0	na	na	0	0	na	na
Yogurt and juice beverages identified as "baby" drinks	0	na	na	0	0	na	na
"Junior type" desserts	<0.1	<1*	na	0.1	1	51*	51*
Baby crackers, pretzels, cookies, and snack items	0	na	na	0	0	na	na

2'-FL = 2'-fucosyllactose; bw = body weight; na = not available; NHANES = National Health and Nutrition Examination Survey; U.S. = United States.

* Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements.

Table B-11 Estimated Daily Per Kilogram Body Weight Intake of 2'-FL from Individual Proposed Food-Uses by the Total U.S. Population (2013-2014 NHANES Data)

Food-Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake (mg/kg bw/day)		Consumer-Only Intake (mg/kg bw/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
All	***100***	***32***	***76***	***91.1***	***6,930***	***36***	***80***
Beverages and Beverage Bases							
Energy drinks	0.2	<1	na	2.4	138	3	6
Fitness water and third quenchers, sports and isotonic drinks	1.3	<1	na	7.5	539	6	12
Breakfast Cereals							
Ready-to-eat breakfast cereals for adults and children	22.7	7	23	35.3	2,751	21	45
Hot cereals for adults and children	4.7	2	3	10.9	943	14	30
Dairy Product Analogs							
Milk substitutes such as soy milk and imitation milks	0.7	<1	na	5.1	360	4	8
Frozen Dairy Desserts and Mixes							
Frozen desserts including ice creams and frozen yogurts, frozen novelties	17.5	6	20	24.9	1,679	23	47
Gelatins, Puddings, and Fillings							
Dairy-based puddings, custards, and mousses	3.8	1	na	4.9	356	25	57
Fruit pie filling	0.8	<1	na	3.8	217	7	15
"Fruit prep"	0.7	<1	na	5.7	378	4	10
Grain Products and Pastas							
Snack, breakfast, and meal replacement bars	2.0	1	2	11.7	669	5	11
Jams and Jellies, Commercial							
Jellies and jams, fruit preserves, fruit butters	4.4	1	3	11.8	858	12	26
Milk, Whole and Skim							
All acidophilus or fortified milks, non-fat and low-fat fluid milks, including fluid milk and reconstituted milk powder	12.1	4	9	54.3	4,232	7	15
Milk Products							
Flavored milks	2.2	1	2	13.4	1,199	5	10
Milk-based meal replacement beverages or diet beverages	0.6	<1	na	3.2	173	7	15
Yogurt	5.7	2	6	15.7	1,029	12	23
Processed Fruits and Fruit Juices							
Fruit drinks, including vitamin and mineral-fortified products	4.6	1	5	25.5	2,236	6	13
Fruit juices	5.0	2	5	31.9	2,744	5	11
Sweet Sauces, Toppings, and Syrups							
Syrups used to flavor milk beverages	0.2	<1	na	1.3	92	5	9
Other							

Table B-11 Estimated Daily Per Kilogram Body Weight Intake of 2'-FL from Individual Proposed Food-Uses by the Total U.S. Population (2013-2014 NHANES Data)

Food-Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake (mg/kg bw/day)		Consumer-Only Intake (mg/kg bw/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
Infant formula	6.5	2	na	1.0	223	222	401
Follow-on formula	<0.1	<1*	na	<0.1	1	48*	48*
Meal replacement products	0.2	<1*	na	0.1	17	48*	185*
Growing-up (toddler) milks	0.2	<1*	na	0.1	10	103*	122*
Ready-to-eat, ready-to-serve, hot cereals for babies for babies	0.2	<1*	na	0.1	18	76*	133*
Yogurt and juice beverages identified as "baby" drinks	1.7	1	na	0.5	90	104	204
"Junior type" desserts	0.8	<1	na	0.4	74	62	106*
Baby crackers, pretzels, cookies, and snack items	1.1	<1	na	0.7	121	50	110

2'-FL = 2'-fucosyllactose; bw = body weight; na = not available; NHANES = National Health and Nutrition Examination Survey; U.S. = United States.

* Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements.

Appendix C
Representative Food Codes for Proposed Food-Uses of 2'-FL in the U.S. (2013-2014 NHANES Data)

Representative Food Codes for Proposed Food and Beverage-Uses of 2'-FL in the U.S. (U.S. NHANES 2013-2014)

Beverages and Beverage Bases

Energy Drinks
[2'-FL] = 0.08 g/100 g

93301216	Vodka and energy drink
95310200	Full Throttle Energy Drink
95310400	Monster Energy Drink
95310500	Mountain Dew AMP Energy Drink
95310550	No Fear Energy Drink
95310555	No Fear Motherload Energy Drink
95310560	NOS Energy Drink
95310600	Red Bull Energy Drink
95310700	Rockstar Energy Drink
95310750	SoBe Energize Energy Juice Drink
95310800	Vault Energy Drink
95311000	Energy Drink
95312400	Monster Energy Drink, Lo Carb
95312500	Mountain Dew AMP Energy Drink, sugar-free
95312550	No Fear Energy Drink, sugar-free
95312555	NOS Energy Drink, sugar-free
95312560	Ocean Spray Cran-Energy Cranberry Energy Juice Drink
95312600	Red Bull Energy Drink, sugar-free
95312700	Rockstar Energy Drink, sugar-free
95312800	Vault Zero Energy Drink
95312900	XS Energy Drink
95312905	XS Gold Plus Energy Drink
95313200	Energy drink, sugar free

Sports Drinks
[2'-FL] = 0.08 g/100 g

94210100	Propel Water
94220100	Propel Zero Water
94220110	Propel Zero Calcium Water
95320200	Gatorade G sports drink
95320500	Powerade sports drink
95321000	Sports drink, not further specified (NFS)
95322200	Gatorade G2 sports drink, low calorie
95322500	Powerade Zero sports drink, low calorie
95323000	Sports drink, low calorie
95330100	Fluid replacement, electrolyte solution
95330500	Fluid replacement, 5% glucose in water

Not Reconstituted Sports Drinks
(Adjusted for not being reconstituted, 16 g of powder to 240 mL of water)
[2'-FL] = 1.28 g/100 g

92900300	Sports drink, dry concentrate, not reconstituted

Breakfast Cereals

Ready-to-Eat Breakfast Cereals for Adults and Children

[2'-FL] = 2.0 to 8.0 g/100 g

57000000	Cereal, NFS
57000050	Kashi cereal, not specified (NS) as to ready to eat or cooked
57000100	Oat cereal, NFS
57100100	Cereal, ready-to-eat, NFS
57101000	All-Bran
57102000	Alpen
57103000	Alpha-Bits
57103020	Alpha-bits with marshmallows
57103100	Apple Cinnamon Cheerios
57104000	Apple Jacks
57106050	Banana Nut Crunch Cereal (Post)
57106060	Banana Nut Cheerios
57106100	Basic 4
57106250	Berry Berry Kix
57106260	Berry Burst Cheerios
57106530	Blueberry Morning, Post
57107000	Booberry
57110000	All-Bran Bran Buds, Kellogg's (formerly Bran Buds)
57117000	Cap'n Crunch
57117500	Cap'n Crunch's Christmas Crunch
57119000	Cap'n Crunch's Crunch Berries
57120000	Cap'n Crunch's Peanut Butter Crunch
57123000	Cheerios
57124000	Chex cereal, NFS
57124030	Chex Chocolate
57124050	Chex Cinnamon
57124100	Chocolate Cheerios
57124200	Chocolate flavored frosted puffed corn cereal
57124300	Chocolate Lucky Charms
57124900	Cinnabon cereal
57125000	Cinnamon Toast Crunch
57125010	Cinnamon Toast Crunch Reduced Sugar
57125900	Honey Nut Clusters (formerly called Clusters)
57126000	Cocoa Krispies
57127000	Cocoa Pebbles
57128000	Cocoa Puffs
57128005	Cocoa Puffs, reduced sugar
57130000	Cookie-Crisp
57131000	Crunchy Corn Bran, Quaker
57132000	Corn Chex
57134000	Corn flakes, NFS
57135000	Corn flakes, Kellogg's
57137000	Corn Puffs
57139000	Count Chocula
57143000	Cracklin' Oat Bran
57143500	Cranberry Almond Crunch, Post
57144000	Crisp Crunch
57148000	Crispix
57148500	Crispy Brown Rice Cereal
57151000	Crispy Rice
57201900	Dora the Explorer Cereal

57206000	Familia
57206700	Fiber One
57206705	Fiber One Caramel Delight
57206710	Fiber One Honey Clusters
57206715	Fiber One Raisin Bran Clusters
57206800	Fiber 7 Flakes, Health Valley
57207000	Bran Flakes, NFS (formerly 40% Bran Flakes, NFS)
57208000	All-Bran Complete Wheat Flakes, Kellogg's
57209000	Natural Bran Flakes, Post (formerly called 40% Bran Flakes, Post)
57211000	Frankenberry
57213000	Froot Loops
57213010	Froot Loops Marshmallow
57213850	Frosted Cheerios
57214000	Frosted Mini-Wheats
57214100	Frosted Wheat Bites
57215000	Frosty O's
57216000	Frosted rice, NFS
57218000	Frosted Rice Krispies, Kellogg's
57219000	Fruit & Fibre (fiber), NFS
57221000	Fruit & Fibre (fiber) with dates, raisins, and walnuts
57221700	Fruit Rings, NFS
57221800	Fruit Whirls
57221810	Fruity Cheerios
57223000	Fruity Pebbles
57224000	Golden Grahams
57227000	Granola, NFS
57228000	Granola, homemade
57229000	Granola, lowfat, Kellogg's
57229500	Granola with Raisins, lowfat, Kellogg's
57230000	Grape-Nuts
57231000	Grape-Nuts Flakes
57231100	Grape-Nuts Trail Mix Crunch
57231200	Great Grains, Raisin, Date, and Pecan Whole Grain Cereal, Post
57231250	Great Grains Double Pecan Whole Grain Cereal, Post
57237100	Honey Bunches of Oats Honey Roasted Cereal
57237200	Honey Bunches of Oats with Vanilla Clusters, Post
57237300	Honey Bunches of Oats with Almonds, Post
57237310	Honey Bunches of Oats with Pecan Bunches
57237900	Honey Bunches of Oats Just Bunches
57238000	Honeycomb, plain
57239000	Honeycomb, strawberry
57239100	Honey Crunch Corn Flakes, Kellogg's
57240100	Honey Nut Chex
57241000	Honey Nut Cheerios
57241200	Honey Nut Shredded Wheat, Post
57243000	Honey Smacks, Kellogg's (formerly Smacks; Honey Smacks)
57301500	Kashi, Puffed
57301505	Kashi Autumn Wheat
57301510	Kashi GOLEAN
57301511	Kashi GOLEAN Crunch
57301512	Kashi GOLEAN Crunch Honey Almond Flax
57301520	Kashi Good Friends
57301530	Kashi Heart to Heart Honey Toasted Oat
57301535	Kashi Heart to Heart Oat Flakes and Blueberry Clusters

57301540	Kashi Honey Sunshine
57302100	King Vitaman
57303100	Kix
57303105	Honey Kix
57304100	Life (plain and cinnamon)
57305100	Lucky Charms
57305150	Frosted oat cereal with marshmallows
57305160	Malt-O-Meal Blueberry Muffin Tops
57305165	Malt-O-Meal Cinnamon Toasters
57305170	Malt-O-Meal Coco-Roos
57305174	Malt-O-Meal Colossal Crunch
57305175	Malt-O-Meal Cocoa Dyno-Bites
57305180	Malt-O-Meal Corn Bursts
57305200	Malt-O-Meal Crispy Rice
57305210	Malt-O-Meal Frosted Flakes
57305215	Malt-O-Meal Frosted Mini Spooners
57305300	Malt-O-Meal Fruity Dyno-Bites
57305400	Malt-O-Meal Honey Graham Squares
57305500	Malt-O-Meal Honey and Nut Toasty O's
57305600	Malt-O-Meal Marshmallow Mateys
57306100	Malt-O-Meal Puffed Rice
57306120	Malt-O-Meal Puffed Wheat
57306130	Malt-O-Meal Raisin Bran
57306500	Malt-O-Meal Golden Puffs (formerly Sugar Puffs)
57306700	Malt-O-Meal Toasted Oat Cereal
57306800	Malt-O-meal Tootie Fruities
57307010	Maple Pecan Crunch Cereal, Post
57307500	Millet, puffed
57308150	Mueslix cereal, NFS
57308190	Muesli, dried fruit and nuts (formerly Muesli with raisins, dates, and almonds)
57308400	MultiGrain Cheerios
57309100	Nature Valley Granola, with fruit and nuts
57316200	Nutty Nuggets, Ralston Purina
57316300	Oat Bran Flakes, Health Valley
57316380	Oat Cluster Cheerios Crunch
57316450	Oatmeal Crisp with Almonds
57316500	Oatmeal Crisp, Raisin (formerly Oatmeal Raisin Crisp)
57316710	Oh's, Honey Graham
57319000	100% Natural Cereal, plain, Quaker
57320500	100 % Natural Cereal, with oats, honey and raisins, Quaker
57321500	100 % Natural Wholegrain Cereal with raisins, lowfat, Quaker
57321900	Organic Flax Plus, Nature's Path
57321905	Organic Flax Plus, Pumpkin Granola, Nature's Path
57323000	Sweet Crunch, Quaker (formerly called Popeye)
57325000	Product 19
57326000	Puffins Cereal
57327450	Quaker Oat Bran Cereal
57327500	Quaker Oatmeal Squares (formerly Quaker Oat Squares)
57328000	Quisp
57329000	Raisin bran, NFS
57330000	Raisin Bran, Kellogg's
57330010	Raisin Bran Crunch, Kellogg's
57331000	Raisin Bran, Post
57332050	Raisin Bran, Total

57332100	Raisin Nut Bran
57335550	Reese's Peanut Butter Puffs cereal
57336000	Rice Chex
57337000	Rice Flakes, NFS
57339000	Rice Krispies, Kellogg's
57339500	Rice Krispies Treats Cereal, Kellogg's
57340000	Rice, puffed
57341000	Shredded Wheat'N Bran
57341200	Smart Start Strong Heart Antioxidants Cereal, Kellogg's
57344000	Special K
57344001	Special K Blueberry
57344005	Special K Chocolatey Delight
57344007	Special K Low Fat Granola
57344010	Special K Red Berries
57344015	Special K Fruit & Yogurt
57344020	Special K Vanilla Almond
57344025	Special K Cinnamon Pecan, Kellogg's
57346500	Oatmeal Honey Nut Heaven, Quaker (formerly Toasted Oatmeal, Honey Nut)
57347000	Corn Pops
57348000	Frosted corn flakes, NFS
57349000	Frosted Flakes, Kellogg's
57349020	Reduced Sugar Frosted Flakes Cereal, Kellogg's
57355000	Golden Crisp (Formerly called Super Golden Crisp)
57401100	Toasted oat cereal
57406100	Total
57407100	Trix
57407110	Trix, reduced sugar
57408100	Uncle Sam Cereal (formerly Uncle Sam's Hi Fiber Cereal)
57409100	Waffle Crisp, Post
57410000	Weetabix Whole Wheat Cereal
57411000	Wheat Chex
57412000	Wheat germ, plain
57413000	Wheat germ, with sugar and honey
57416000	Wheat, puffed, plain
57416010	Wheat, puffed, presweetened with sugar
57417000	Shredded Wheat, 100%
57418000	Wheaties
57419000	Yogurt Burst Cheerios

Hot Cereals for Adults and Children

[2'-FL] = 0.48 g/100 g

56200300	Cereal, cooked, NFS
56200350	Cereal, cooked, instant, NS as to grain
56200390	Barley, cooked, NS as to fat added in cooking
56200400	Barley, cooked, fat not added in cooking
56200490	Buckwheat groats, cooked, NS as to fat added in cooking
56200500	Buckwheat groats, cooked, fat not added in cooking
56200510	Buckwheat groats, cooked, fat added in cooking
56200990	Grits, cooked, corn or hominy, NS as to regular, quick, or instant, NS as to fat added in cooking
56201000	Grits, cooked, corn or hominy, NS as to regular, quick, or instant, fat not added in cooking
56201010	Grits, cooked, corn or hominy, regular, fat not added in cooking
56201020	Grits, cooked, corn or hominy, regular, fat added in cooking
56201030	Grits, cooked, corn or hominy, regular, NS as to fat added in cooking
56201040	Grits, cooked, corn or hominy, NS as to regular, quick, or instant, fat added in cooking

56201060	Grits, cooked, corn or hominy, with cheese, NS as to regular, quick, or instant, NS as to fat added in cooking
56201061	Grits, cooked, corn or hominy, with cheese, NS as to regular, quick, or instant, fat not added in cooking
56201062	Grits, cooked, corn or hominy, with cheese, NS as to regular, quick, or instant, fat added in cooking
56201070	Grits, cooked, corn or hominy, with cheese, regular, NS as to fat added in cooking
56201071	Grits, cooked, corn or hominy, with cheese, regular, fat not added in cooking
56201072	Grits, cooked, corn or hominy, with cheese, regular, fat added in cooking
56201080	Grits, cooked, corn or hominy, with cheese, quick, NS as to fat added in cooking
56201081	Grits, cooked, corn or hominy, with cheese, quick, fat not added in cooking
56201082	Grits, cooked, corn or hominy, with cheese, quick, fat added in cooking
56201090	Grits, cooked, corn or hominy, with cheese, instant, NS as to fat added in cooking
56201091	Grits, cooked, corn or hominy, with cheese, instant, fat not added in cooking
56201092	Grits, cooked, corn or hominy, with cheese, instant, fat added in cooking
56201110	Grits, cooked, corn or hominy, quick, fat not added in cooking
56201120	Grits, cooked, corn or hominy, quick, fat added in cooking
56201130	Grits, cooked, corn or hominy, quick, NS as to fat added in cooking
56201210	Grits, cooked, corn or hominy, instant, fat not added in cooking
56201220	Grits, cooked, corn or hominy, instant, fat added in cooking
56201230	Grits, cooked, corn or hominy, instant, NS as to fat added in cooking
56201240	Grits, cooked, flavored, corn or hominy, instant, fat not added in cooking
56201250	Grits, cooked, flavored, corn or hominy, instant, fat added in cooking
56201260	Grits, cooked, flavored, corn or hominy, instant, NS as to fat added in cooking
56201296	Grits, cooked, corn or hominy, NS as to regular, quick, or instant, made with milk, fat added in cooking
56201298	Grits, cooked, corn or hominy, NS as to regular, quick, or instant, made with milk, fat not added in cooking
56201300	Grits, cooked, corn or hominy, NS as to regular, quick, or instant, made with milk, NS as to fat added in cooking
56201320	Grits, cooked, corn or hominy, regular, made with milk, fat added in cooking
56201322	Grits, cooked, corn or hominy, regular, made with milk, fat not added in cooking
56201324	Grits, cooked, corn or hominy, regular, made with milk, NS as to fat added in cooking
56201330	Grits, cooked, corn or hominy, quick, made with milk, fat added in cooking
56201332	Grits, cooked, corn or hominy, quick, made with milk, fat not added in cooking
56201334	Grits, cooked, corn or hominy, quick, made with milk, NS as to fat added in cooking
56201340	Grits, cooked, corn or hominy, instant, made with milk, fat added in cooking
56201342	Grits, cooked, corn or hominy, instant, made with milk, fat not added in cooking
56201344	Grits, cooked, corn or hominy, instant, made with milk, NS as to fat added in cooking
56201510	Cornmeal mush, made with water
56201520	Cornmeal mush, fried
56201530	Cornmeal mush, made with milk
56201540	Cornmeal, made with milk and sugar, Puerto Rican Style (Harina de maiz)
56201600	Cornmeal, lime-treated, cooked (Masa harina)
56201700	Cornstarch with milk, eaten as a cereal (2 tbsp cornstarch in 2-1/2 cups milk)
56201990	Millet, cooked, NS as to fat added in cooking
56202000	Millet, cooked, fat not added in cooking
56202100	Millet, cooked, fat added in cooking
56202900	Oatmeal, cooked, from fast food
56202960	Oatmeal, cooked, NS as to regular, quick or instant; NS as to fat added in cooking
56202970	Oatmeal, cooked, quick (1 or 3 minutes), NS as to fat added in cooking
56202980	Oatmeal, cooked, regular, NS as to fat added in cooking
56203000	Oatmeal, cooked, NS as to regular, quick or instant, fat not added in cooking
56203010	Oatmeal, cooked, regular, fat not added in cooking
56203020	Oatmeal, cooked, quick (1 or 3 minutes), fat not added in cooking

56203030	Oatmeal, cooked, instant, fat not added in cooking
56203040	Oatmeal, cooked, NS as to regular, quick, or instant, fat added in cooking
56203050	Oatmeal, cooked, regular, fat added in cooking
56203060	Oatmeal, cooked, quick (1 or 3 minutes), fat added in cooking
56203070	Oatmeal, cooked, instant, fat added in cooking
56203080	Oatmeal, cooked, instant, NS as to fat added in cooking
56203110	Oatmeal with maple flavor, cooked
56203200	Oatmeal with fruit, cooked
56203210	Oatmeal, NS as to regular, quick, or instant, made with milk, fat not added in cooking
56203211	Oatmeal, cooked, regular, made with milk, fat not added in cooking
56203212	Oatmeal, cooked, quick (1 or 3 minutes), made with milk, fat not added in cooking
56203213	Oatmeal, cooked, instant, made with milk, fat not added in cooking
56203220	Oatmeal, NS as to regular, quick, or instant, made with milk, fat added in cooking
56203221	Oatmeal, cooked, regular, made with milk, fat added in cooking
56203222	Oatmeal, cooked, quick (1 or 3 minutes), made with milk, fat added in cooking
56203223	Oatmeal, cooked, instant, made with milk, fat added in cooking
56203230	Oatmeal, NS as to regular, quick, or instant, made with milk, NS as to fat added in cooking
56203231	Oatmeal, cooked, regular, made with milk, NS as to fat added in cooking
56203232	Oatmeal, cooked, quick (1 or 3 minutes), made with milk, NS as to fat added in cooking
56203233	Oatmeal, cooked, instant, made with milk, NS as to fat added in cooking
56203540	Oatmeal, made with milk and sugar, Puerto Rican style
56203600	Oatmeal, multigrain, cooked, NS as to fat added in cooking
56203610	Oatmeal, multigrain, cooked, fat not added in cooking
56203620	Oatmeal, multigrain, cooked, fat added in cooking
56206970	Wheat, cream of, cooked, quick, NS as to fat added in cooking
56206980	Wheat, cream of, cooked, regular, NS as to fat added in cooking
56206990	Wheat, cream of, cooked, NS as to regular, quick, or instant, NS as to fat added in cooking
56207000	Wheat, cream of, cooked, NS as to regular, quick, or instant, fat not added in cooking
56207010	Wheat, cream of, cooked, regular, fat not added in cooking
56207020	Wheat, cream of, cooked, quick, fat not added in cooking
56207030	Wheat, cream of, cooked, instant, fat not added in cooking
56207050	Wheat, cream of, cooked, made with milk and sugar, Puerto Rican style
56207060	Wheat, cream of, cooked, instant, fat added in cooking
56207070	Wheat, cream of, cooked, instant, NS as to fat added in cooking
56207080	Wheat, cream of, cooked, NS as to regular, quick, or instant, fat added in cooking
56207082	Wheat, cream of, cooked, NS as to regular, quick, or instant, made with milk, fat added in cooking
56207083	Wheat, cream of, cooked, NS as to regular, quick, or instant, made with milk, fat not added in cooking
56207084	Wheat, cream of, cooked, NS as to regular, quick, or instant, made with milk, NS as to fat added in cooking
56207086	Wheat, cream of, cooked, regular, made with milk, fat added in cooking
56207087	Wheat, cream of, cooked, regular, made with milk, fat not added in cooking
56207088	Wheat, cream of, cooked, regular, made with milk, NS as to fat added in cooking
56207091	Wheat, cream of, cooked, quick, made with milk, fat added in cooking
56207092	Wheat, cream of, cooked, quick, made with milk, fat not added in cooking
56207093	Wheat, cream of, cooked, quick, made with milk, NS as to fat added in cooking
56207094	Wheat, cream of, cooked, instant, made with milk, fat added in cooking
56207095	Wheat, cream of, cooked, instant, made with milk, fat not added in cooking
56207096	Wheat, cream of, cooked, instant, made with milk, NS as to fat added in cooking
56207100	Wheat, rolled, cooked, fat not added in cooking
56207110	Bulgur, cooked or canned, fat not added in cooking
56207120	Bulgur, cooked or canned, fat added in cooking
56207130	Bulgur, cooked or canned, NS as to fat added in cooking
56207140	Wheat, rolled, cooked, NS as to fat added in cooking
56207190	Whole wheat cereal, cooked, NS as to fat added in cooking

56207200 Whole wheat cereal, cooked, fat not added in cooking
56207210 Whole wheat cereal, cooked, fat added in cooking
56207212 Whole wheat cereal, cooked, made with milk
56207220 Wheat, cream of, cooked, regular, fat added in cooking
56207230 Wheat, cream of, cooked, quick, fat added in cooking
56207300 Whole wheat cereal, wheat and barley, cooked, fat not added in cooking
56207330 Whole wheat cereal, wheat and barley, cooked, fat added in cooking
56207340 Whole wheat cereal, wheat and barley, cooked, NS as to fat added in cooking
56207342 Whole wheat cereal, wheat and barley, cooked, made with milk
56207350 Wheat cereal, chocolate flavored, cooked, made with milk
56207360 Wheat cereal, chocolate flavored, cooked, fat not added in cooking
56207365 Wheat cereal, chocolate flavored, cooked, fat added in cooking
56207370 Wheat cereal, chocolate flavored, cooked, NS as to fat added in cooking
56208500 Oat bran cereal, cooked, fat not added in cooking
56208510 Oat bran cereal, cooked, fat added in cooking
56208520 Oat bran cereal, cooked, NS as to fat added in cooking
56208530 Oat bran cereal, cooked, made with milk, fat not added in cooking
56208540 Oat bran cereal, cooked, made with milk, fat added in cooking
56208550 Oat bran cereal, cooked, made with milk, NS as to fat added in cooking
56209000 Rye, cream of, cooked
56210000 Nestum cereal

Uncooked Hot Cereals
{Adjusted for not being cooked, approximately 15 g uncooked oats or bran into 150 mL of milk)
[2'-FL] = 4.8 g/100 g
57601100 Wheat bran, unprocessed
57602100 Oats, raw
57602500 Oat bran, uncooked

Dairy Product Analogs

Milk Substitutes
[2'-FL] = 0.12 g/100 g
11320000 Soy milk
11320100 Soy milk, light
11320200 Soy milk, nonfat
11321000 Soy milk, chocolate
11321100 Soy milk, light, chocolate
11321200 Soy milk, nonfat, chocolate
11340000 Imitation milk, non-soy, sweetened
11350000 Almond milk, sweetened
11350010 Almond milk, sweetened, chocolate
11350020 Almond milk, unsweetened
11350030 Almond milk, unsweetened, chocolate
11360000 Rice milk
11370000 Coconut milk

Mixtures Containing Milk Substitutes
(Adjusted for a Milk Substitute content ranging from 42.2 to 95.7%)
[2'-FL] = 0.05 to 0.11 g/100 g
11512030 Hot chocolate / Cocoa, ready to drink, made with non-dairy milk
11512120 Hot chocolate / Cocoa, ready to drink, made with non-dairy milk and whipped cream

11513310	Chocolate milk, made from dry mix with non-dairy milk
11513375	Chocolate milk, made from reduced sugar mix with non-dairy milk
11513385	Nesquik, chocolate milk, made from dry mix with non-dairy milk
11513395	Nesquik, chocolate milk, made from no sugar added dry mix with non-dairy milk
11514150	Hot chocolate / Cocoa, made with dry mix and non-dairy milk
11514360	Hot chocolate / Cocoa, made with no sugar added dry mix and non-dairy milk
11519215	Strawberry milk, non-dairy
92101903	Coffee, Latte, with non-dairy milk
92101906	Coffee, Latte, with non-dairy milk, flavored
92101913	Coffee, Latte, decaffeinated, with non-dairy milk
92101919	Coffee, Latte, decaffeinated, with non-dairy milk, flavored
92101923	Frozen coffee drink, with non-dairy milk
92101928	Frozen coffee drink, with non-dairy milk and whipped cream
92101933	Frozen coffee drink, decaffeinated, with non-dairy milk
92101938	Frozen coffee drink, decaffeinated, with non-dairy milk and whipped cream
92101960	Coffee, Cafe Mocha, with non-dairy milk
92101975	Coffee, Cafe Mocha, decaffeinated, with non-dairy milk
92102020	Frozen mocha coffee drink, with non-dairy milk
92102050	Frozen mocha coffee drink, with non-dairy milk and whipped cream
92102080	Frozen mocha coffee drink, decaffeinated, with non-dairy milk
92102110	Frozen mocha coffee drink, decaffeinated, with non-dairy milk and whipped cream
92102502	Coffee, Iced Latte, with non-dairy milk
92102505	Coffee, Iced Latte, with non-dairy milk, flavored
92102512	Coffee, Iced Latte, decaffeinated, with non-dairy milk
92102515	Coffee, Iced Latte, decaffeinated, with non-dairy milk, flavored
92102602	Coffee, Iced CafÃ© Mocha, with non-dairy milk
92102612	Coffee, Iced CafÃ© Mocha, decaffeinated, with non-dairy milk
92161002	Coffee, Cappuccino, with non-dairy milk
92162002	Coffee, Cappuccino, decaffeinated, with non-dairy milk
11513750	Chocolate milk, made from syrup with non-dairy milk
11513805	Chocolate milk, made from light syrup with non-dairy milk
11513855	Chocolate milk, made from sugar free syrup with non-dairy milk

Frozen Dairy Desserts and Mixes

Frozen Desserts

[2'-FL] = 1.7 g/100 g

11459990	Yogurt, frozen, NS as to flavor, NS as to type of milk
11460000	Yogurt, frozen, flavors other than chocolate, NS as to type of milk
11460100	Yogurt, frozen, chocolate, NS as to type of milk
11460150	Yogurt, frozen, NS as to flavor, lowfat milk
11460160	Yogurt, frozen, chocolate, lowfat milk
11460170	Yogurt, frozen, flavors other than chocolate, lowfat milk
11460190	Yogurt, frozen, NS as to flavor, nonfat milk
11460200	Yogurt, frozen, chocolate, nonfat milk
11460250	Yogurt, frozen, flavors other than chocolate, with sorbet or sorbet-coated
11460300	Yogurt, frozen, flavors other than chocolate, nonfat milk
11460400	Yogurt, frozen, chocolate, nonfat milk, with low-calorie sweetener
11460410	Yogurt, frozen, flavors other than chocolate, nonfat milk, with low-calorie sweetener
11460420	Yogurt, frozen, NS as to flavor, whole milk
11460430	Yogurt, frozen, chocolate, whole milk
11460440	Yogurt, frozen, flavors other than chocolate, whole milk

11461000	Yogurt, frozen, chocolate-coated
11461200	Yogurt, frozen, sandwich
11461250	Yogurt, frozen, cone, chocolate
11461260	Yogurt, frozen, cone, flavors other than chocolate
11461270	Yogurt, frozen, cone, flavors other than chocolate, lowfat milk
11461280	Yogurt, frozen, cone, chocolate, lowfat milk
13110000	Ice cream, NFS
13110100	Ice cream, regular, flavors other than chocolate
13110110	Ice cream, regular, chocolate
13110120	Ice cream, rich, flavors other than chocolate
13110130	Ice cream, rich, chocolate
13110140	Ice cream, rich, NS as to flavor
13110200	Ice cream, soft serve, flavors other than chocolate
13110210	Ice cream, soft serve, chocolate
13110220	Ice cream, soft serve, NS as to flavor
13110310	Ice cream, no sugar added, NS as to flavor
13110320	Ice cream, no sugar added, flavors other than chocolate
13110330	Ice cream, no sugar added, chocolate
13120050	Ice cream bar or stick, not chocolate covered or cake covered
13120100	Ice cream bar or stick, chocolate covered
13120110	Ice cream bar or stick, chocolate or caramel covered, with nuts
13120120	Ice cream bar or stick, rich chocolate ice cream, thick chocolate covering
13120121	Ice cream bar or stick, rich ice cream, thick chocolate covering
13120130	Ice cream bar or stick, rich ice cream, chocolate covered, with nuts
13120140	Ice cream bar or stick, chocolate ice cream, chocolate covered
13120300	Ice cream bar, cake covered
13120310	Ice cream bar, stick or nugget, with crunch coating
13120400	Ice cream bar or stick with fruit
13120500	Ice cream sandwich
13120550	Ice cream cookie sandwich
13120700	Ice cream cone with nuts, flavors other than chocolate
13120710	Ice cream cone, chocolate covered, with nuts, flavors other than chocolate
13120720	Ice cream cone, chocolate covered or dipped, flavors other than chocolate
13120730	Ice cream cone, no topping, flavors other than chocolate
13120740	Ice cream cone, no topping, NS as to flavor
13120750	Ice cream cone with nuts, chocolate ice cream
13120760	Ice cream cone, chocolate covered or dipped, chocolate ice cream
13120770	Ice cream cone, no topping, chocolate ice cream
13120780	Ice cream cone, chocolate covered, with nuts, chocolate ice cream
13120790	Ice cream sundae cone
13120800	Ice cream soda, flavors other than chocolate
13120810	Ice cream soda, chocolate
13121000	Ice cream sundae, NS as to topping, with whipped cream
13121100	Ice cream sundae, fruit topping, with whipped cream
13121200	Ice cream sundae, prepackaged type, flavors other than chocolate
13121300	Ice cream sundae, chocolate or fudge topping, with whipped cream
13121400	Ice cream sundae, not fruit or chocolate topping, with whipped cream
13121500	Ice cream sundae, fudge topping, with cake, with whipped cream
13122100	Ice cream pie, no crust
13122500	Ice cream pie, with cookie crust, fudge topping, and whipped cream
13126000	Ice cream, fried
13127000	Dippin' Dots, flash frozen ice cream snacks, flavors other than chocolate
13127010	Dippin' Dots, flash frozen ice cream snacks, chocolate
13130100	Light ice cream, NS as to flavor (formerly ice milk)

13130300	Light ice cream, flavors other than chocolate (formerly ice milk)
13130310	Light ice cream, chocolate (formerly ice milk)
13130320	Light ice cream, no sugar added, NS as to flavor
13130330	Light ice cream, no sugar added, flavors other than chocolate
13130340	Light ice cream, no sugar added, chocolate
13130590	Light ice cream, soft serve, NS as to flavor (formerly ice milk)
13130600	Light ice cream, soft serve, flavors other than chocolate (formerly ice milk)
13130610	Light ice cream, soft serve, chocolate (formerly ice milk)
13130620	Light ice cream, soft serve cone, flavors other than chocolate (formerly ice milk)
13130630	Light ice cream, soft serve cone, chocolate (formerly ice milk)
13130640	Light ice cream, soft serve cone, NS as to flavor (formerly ice milk)
13130700	Light ice cream, soft serve, blended with candy or cookies
13135000	Ice cream sandwich, made with light ice cream, flavors other than chocolate
13135010	Ice cream sandwich, made with light chocolate ice cream
13136000	Ice cream sandwich, made with light, no sugar added ice cream
13140100	Light ice cream, bar or stick, chocolate-coated (formerly ice milk)
13140110	Light ice cream, bar or stick, chocolate covered, with nuts (formerly ice milk)
13140450	Light ice cream, cone, NFS (formerly ice milk)
13140500	Light ice cream, cone, flavors other than chocolate (formerly ice milk)
13140550	Light ice cream, cone, chocolate (formerly ice milk)
13140570	Light ice cream, no sugar added, cone, NS as to flavor
13140575	Light ice cream, no sugar added, cone, flavors other than chocolate
13140580	Light ice cream, no sugar added, cone, chocolate
13140600	Light ice cream, sundae, soft serve, chocolate or fudge topping, with whipped cream (formerly ice milk)
13140630	Light ice cream, sundae, soft serve, fruit topping, with whipped cream (formerly ice milk)
13140650	Light ice cream, sundae, soft serve, not fruit or chocolate topping, with whipped cream (formerly ice milk)
13140660	Light ice cream, sundae, soft serve, chocolate or fudge topping (without whipped cream) (formerly ice milk)
13140670	Light ice cream, sundae, soft serve, fruit topping (without whipped cream) (formerly ice milk)
13140680	Light ice cream, sundae, soft serve, not fruit or chocolate topping (without whipped cream) (formerly ice milk)
13140700	Light ice cream, creamsicle or dreamsicle (formerly ice milk)
13140710	Light ice cream, creamsicle or dreamsicle, no sugar added
13140900	Light ice cream, fudgesicle (formerly ice milk)
13142000	Milk dessert bar or stick, frozen, with coconut
13150000	Sherbet, all flavors
13160150	Fat free ice cream, no sugar added, chocolate
13160160	Fat free ice cream, no sugar added, flavors other than chocolate
13160400	Fat free ice cream, flavors other than chocolate
13160410	Fat free ice cream, chocolate
13160420	Fat free ice cream, NS as to flavor
13161000	Milk dessert bar, frozen, made from lowfat milk
13161500	Milk dessert sandwich bar, frozen, made from lowfat milk
13161520	Milk dessert sandwich bar, frozen, with low-calorie sweetener, made from lowfat milk
13161600	Milk dessert bar, frozen, made from lowfat milk and low calorie sweetener
13161630	Light ice cream, bar or stick, with low-calorie sweetener, chocolate-coated (formerly ice milk)
13170000	Baked Alaska
91611050	Ice pop filled with ice cream, all flavor varieties

Gelatins, Puddings, and Fillings

Dairy-Based Puddings, Custards, and Mousses

[2'-FL] = 1.7 g/100 g

13200110	Pudding, NFS
13210110	Pudding, bread
13210150	Puerto Rican bread pudding made with evaporated milk
13210160	Diplomat pudding, Puerto Rican style (Budin Diplomatico)
13210180	Pudding, Mexican bread (Capirotada)
13210190	Pudding, Mexican bread (Capirotada), lower fat
13210220	Pudding, chocolate, NS as to from dry mix or ready-to-eat
13210250	Pudding, chocolate, low calorie, containing artificial sweetener, NS as to from dry mix or ready-to-eat
13210260	Rice flour cream, Puerto Rican style (manjar blanco)
13210270	Custard, Puerto Rican style (Maicena, Natilla)
13210280	Pudding, flavors other than chocolate, NS as to from dry mix or ready-to-eat
13210290	Pudding, flavors other than chocolate, low calorie, containing artificial sweetener, NS as to from dry mix or ready-to-eat
13210300	Custard
13210350	Flan
13210410	Pudding, rice
13210450	Pudding, rice flour, with nuts (Indian dessert)
13210520	Pudding, tapioca, made from dry mix, made with milk
13210530	Pudding, tapioca, chocolate, made with milk
13210610	Pudding, coconut
13210710	Pudding, Indian (milk, molasses and cornmeal-based pudding)
13210750	Pudding, pumpkin
13210810	Puerto Rican pumpkin pudding (Flan de calabaza)
13210820	Fresh corn custard, Puerto Rican style (Mazamorra, Mundo Nuevo)
13220110	Pudding, flavors other than chocolate, prepared from dry mix, milk added
13220120	Pudding, chocolate, prepared from dry mix, milk added
13220210	Pudding, flavors other than chocolate, prepared from dry mix, low calorie, containing artificial sweetener, milk added
13220220	Pudding, chocolate, prepared from dry mix, low calorie, containing artificial sweetener, milk added
13220230	Pudding, ready-to-eat, chocolate, reduced fat
13220235	Pudding, ready-to-eat, chocolate, fat free
13220240	Pudding, ready-to-eat, flavors other than chocolate, reduced fat
13220245	Pudding, ready-to-eat, flavors other than chocolate, fat free
13230110	Pudding, ready-to-eat, flavors other than chocolate
13230120	Pudding, ready-to-eat, low calorie, containing artificial sweetener, flavors other than chocolate
13230130	Pudding, ready-to-eat, chocolate
13230140	Pudding, ready-to-eat, low calorie, containing artificial sweetener, chocolate
13230200	Pudding, ready-to-eat, chocolate and non-chocolate flavors combined
13230500	Pudding, ready-to-eat, tapioca
13230510	Pudding, ready-to-eat, tapioca, fat free
13241000	Pudding, with fruit and vanilla wafers
13250000	Mousse, chocolate
13250100	Mousse, not chocolate
13250200	Mousse, chocolate, lowfat, reduced calorie, prepared from dry mix, water added
13252100	Coconut custard, Puerto Rican style (Flan de coco)
13252200	Milk dessert or milk candy, Puerto Rican style (Dulce de leche)
13252500	Barfi or Burfi, Indian dessert, made from milk and/or cream and/or Ricotta cheese
13252600	Tiramisu
91501010	Gelatin dessert
91501015	Gelatin snacks
91501020	Gelatin dessert with fruit

91501030 Gelatin dessert with whipped cream
91501040 Gelatin dessert with fruit and whipped cream
91501050 Gelatin dessert with cream cheese
91501060 Gelatin dessert with sour cream
91501070 Gelatin dessert with fruit and sour cream
91501080 Gelatin dessert with fruit and cream cheese
91501090 Gelatin dessert with fruit, vegetable, and nuts
91501100 Gelatin salad with vegetables
91501110 Gelatin dessert with fruit and whipped topping
91501120 Gelatin dessert with fruit and vegetables
91511010 Gelatin dessert, dietetic, sweetened with low calorie sweetener
91511020 Gelatin dessert, dietetic, with fruit, sweetened with low calorie sweetener
91511030 Gelatin dessert, dietetic, with whipped topping, sweetened with low calorie sweetener
91511050 Gelatin dessert, dietetic, with cream cheese, sweetened with low calorie sweetener
91511060 Gelatin dessert, dietetic, with sour cream, sweetened with low calorie sweetener
91511070 Gelatin dessert, dietetic, with fruit and sour cream, sweetened with low calorie sweetener
91511080 Gelatin dessert, dietetic, with fruit and cream cheese, sweetened with low calorie sweetener
91511090 Gelatin dessert, dietetic, with fruit and vegetable(s), sweetened with low calorie sweetener
91511100 Gelatin salad, dietetic, with vegetables, sweetened with low calorie sweetener
91511110 Gelatin dessert, dietetic, with fruit and whipped topping, sweetened with low calorie sweetener
91512010 Danish dessert pudding
91520100 Yookan (Yokan), a Japanese dessert made with bean paste and sugar
91550100 Coconut cream cake, Puerto Rican style (Bien me sabe, "Tastes good to me")
91550300 Pineapple custard, Puerto Rican style (Flan de pina)
91560100 Haupia (coconut pudding)
91580000 Gelatin, frozen, whipped, on a stick

Mixtures Containing Dairy-Based Puddings, Custards, and Mousses
(Adjusted for a Gelatin Dessert Content of 9.5 to 42.9%)
[2'-FL] = 0.16 to 0.73 g/100 g
14610200 Cheese, cottage cheese, with gelatin dessert
14610210 Cheese, cottage cheese, with gelatin dessert and fruit
14610250 Cheese, cottage cheese, with gelatin dessert and vegetables

<u>Fruit Pie Filling</u>
[2'-FL] = 1.4 g/100 g
61113500 Lemon pie filling
63113030 Cherry pie filling
63113050 Cherry pie filling, low calorie
63203700 Blueberry pie filling

Mixtures Containing Fruit Pie Filling
(Adjusted for a Pie Filling Content of 35.7% to 61.2%)
[2'-FL] = 0.50 to 0.86 g/100 g
53300100 Pie, NFS
53300170 Pie, individual size or tart, NFS
53300180 Pie, fried, NFS
53301000 Pie, apple, two crust
53301070 Pie, apple, individual size or tart
53301080 Pie, apple, fried pie
53301500 Pie, apple, one crust
53301750 Pie, apple, diet
53302000 Pie, apricot, two crust
53302070 Pie, apricot, individual size or tart

53302080	Pie, apricot, fried pie
53303000	Pie, blackberry, two crust
53303070	Pie, blackberry, individual size or tart
53303500	Pie, berry, not blackberry, blueberry, boysenberry, huckleberry, raspberry, or strawberry; two crust
53303510	Pie, berry, not blackberry, blueberry, boysenberry, huckleberry, raspberry, or strawberry; one crust
53303570	Pie, berry, not blackberry, blueberry, boysenberry, huckleberry, raspberry, or strawberry, individual size or tart
53304000	Pie, blueberry, two crust
53304050	Pie, blueberry, one crust
53304070	Pie, blueberry, individual size or tart
53305000	Pie, cherry, two crust
53305010	Pie, cherry, one crust
53305070	Pie, cherry, individual size or tart
53305080	Pie, cherry, fried pie
53305700	Pie, lemon (not cream or meringue)
53305720	Pie, lemon (not cream or meringue), individual size or tart
53305750	Pie, lemon, fried pie
53306000	Pie, mince, two crust
53306070	Pie, mince, individual size or tart
53307000	Pie, peach, two crust
53307050	Pie, peach, one crust
53307070	Pie, peach, individual size or tart
53307080	Pie, peach, fried pie
53307500	Pie, pear, two crust
53307570	Pie, pear, individual size or tart
53308000	Pie, pineapple, two crust
53308070	Pie, pineapple, individual size or tart
53308300	Pie, plum, two crust
53308500	Pie, prune, one crust
53309000	Pie, raisin, two crust
53309070	Pie, raisin, individual size or tart
53310000	Pie, raspberry, one crust
53310050	Pie, raspberry, two crust
53311000	Pie, rhubarb, two crust
53311050	Pie, rhubarb, one crust
53311070	Pie, rhubarb, individual size or tart
53312000	Pie, strawberry, one crust
53313000	Pie, strawberry-rhubarb, two crust
53314000	Pie, strawberry, individual size or tart
53340000	Pie, apple-sour cream
53340500	Pie, cherry, made with cream cheese and sour cream
53341000	Pie, banana cream
53341070	Pie, banana cream, individual size or tart
53345000	Pie, lemon cream
53345070	Pie, lemon cream, individual size or tart
53346500	Pie, pineapple cream
53347000	Pie, pumpkin
53347070	Pie, pumpkin, individual size or tart
53347100	Pie, raspberry cream
53348000	Pie, strawberry cream
53348070	Pie, strawberry cream, individual size or tart
53381000	Pie, lemon meringue
53381070	Pie, lemon meringue, individual size or tart
53410100	Cobbler, apple

53410200 Cobbler, apricot
53410300 Cobbler, berry
53410500 Cobbler, cherry
53410800 Cobbler, peach
53410850 Cobbler, pear
53410860 Cobbler, pineapple
53410880 Cobbler, plum
53410900 Cobbler, rhubarb

"Fruit Prep"
(Adjusted for a Fruit Prep Content of 40% to 67.3%)
[2'-FL] = 1.2 to 2.0 g/100 g
53415100 Crisp, apple, apple dessert
53415200 Fritter, banana
53415220 Fritter, berry
53415300 Crisp, blueberry
53415400 Crisp, cherry
53415500 Crisp, peach
53415600 Crisp, rhubarb
53440000 Strudel, apple
53440300 Strudel, berry
53440500 Strudel, cherry
53440700 Strudel, peach
53440750 Strudel, pineapple
53440800 Strudel, cheese and fruit
53450000 Turnover or dumpling, apple
53450300 Turnover or dumpling, berry
53450500 Turnover or dumpling, cherry
53450800 Turnover or dumpling, lemon
53451000 Turnover or dumpling, peach
53451500 Turnover, guava
53451750 Turnover, pumpkin
53452100 Pastry, fruit-filled
63402010 Banana whip
63402030 Prune whip

(Adjusted for a Fruit Prep Content of <1% to 38.6%)
[2'-FL] = 0.01 to 1.16 g/100 g
53101250 Cake, angel food, with fruit and icing or filling
53102100 Cake or cupcake, applesauce, without icing or filling
53102200 Cake or cupcake, applesauce, with icing or filling
53102600 Cake or cupcake, banana, without icing or filling
53102700 Cake or cupcake, banana, with icing or filling
53104550 Cheesecake with fruit
53113000 Cake, jelly roll
53118500 Cake, torte
53122070 Cake, shortcake, biscuit type, with whipped cream and fruit
53122080 Cake, shortcake, biscuit type, with fruit
53123070 Cake, shortcake, sponge type, with whipped cream and fruit
53123080 Cake, shortcake, sponge type, with fruit
53123500 Cake, shortcake, with whipped topping and fruit, diet
53220000 Cookie, fruit-filled bar
53220010 Cookie, fruit-filled bar, fat free
53220030 Cookie, fig bar

53220040	Cookie, fig bar, fat free
53224250	Cookie, lemon bar
53233010	Cookie, oatmeal, with raisins
53233080	Cookie, oatmeal sandwich, with peanut butter and jelly filling
53237000	Cookie, raisin
53237010	Cookie, raisin sandwich, cream-filled
53241600	Cookie, butter or sugar, with fruit and/or nuts
53415120	Fritter, apple
53430200	Crepe, dessert type, fruit-filled
53453150	Empanada, Mexican turnover, fruit-filled
53453170	Empanada, Mexican turnover, pumpkin
53510100	Danish pastry, with fruit
53521140	Doughnut, jelly
53610170	Coffee cake, crumb or quick-bread type, with fruit
55801010	Funnel cake with sugar and fruit

Grain Products and Pastas

Bars, Including Snack Bars, Meal-Replacement Bars, Breakfast Bars

[2'-FL] = 1.20 g/100 g

53710400	Fiber One Chewy Bar
53710500	Kellogg's Nutri-Grain Cereal Bar
53710502	Kellogg's Nutri-Grain Yogurt Bar
53710504	Kellogg's Nutri-Grain Fruit and Nut Bar
53710600	Milk 'n Cereal bar
53710700	Kellogg's Special K bar
53710800	Kashi GOLEAN Chewy Bars
53710802	Kashi TLC Chewy Granola Bar
53710804	Kashi GOLEAN Crunchy Bars
53710806	Kashi TLC Crunchy Granola Bar
53710900	Nature Valley Chewy Trail Mix Granola Bar
53710902	Nature Valley Chewy Granola Bar with Yogurt Coating
53710904	Nature Valley Sweet and Salty Granola Bar
53710906	Nature Valley Crunchy Granola Bar
53711000	Quaker Chewy Granola Bar
53711002	Quaker Chewy 90 Calorie Granola Bar
53711004	Quaker Chewy 25% Less Sugar Granola Bar
53711006	Quaker Chewy Dipps Granola Bar
53711100	Quaker Granola Bites
53712000	Snack bar, oatmeal
53712100	Granola bar, NFS
53712200	Granola bar, lowfat, NFS
53712210	Granola bar, nonfat
53713000	Granola bar, reduced sugar, NFS
53713100	Granola bar, peanuts, oats, sugar, wheat germ
53714200	Granola bar, chocolate-coated, NFS
53714210	Granola bar, with coconut, chocolate-coated
53714220	Granola bar with nuts, chocolate-coated
53714230	Granola bar, oats, nuts, coated with non-chocolate coating
53714250	Granola bar, coated with non-chocolate coating
53714300	Granola bar, high fiber, coated with non-chocolate yogurt coating
53714400	Granola bar, with rice cereal

53714500	Breakfast bar, NFS
53720100	Balance Original Bar
53720200	Clif Bar
53720210	Clif Kids Organic Zbar
53720300	PowerBar
53720400	Slim Fast Original Meal Bar
53720500	Snickers Marathon Protein bar
53720600	South Beach Living Meal Bar
53720610	South Beach Living High Protein Bar
53720700	Tiger's Milk bar
53720800	Zone Perfect Classic Crunch nutrition bar
53729000	Nutrition bar or meal replacement bar, NFS
53714510	Breakfast bar, date, with yogurt coating
53714520	Breakfast bar, cereal crust with fruit filling, lowfat

Jams and Jellies, Commercial

Jellies and Jams, Fruit Preserves, Fruit Butters

[2'-FL] = 6.0 g/100 g

63307010	Cranberry-orange relish, uncooked
63307100	Cranberry-raspberry sauce
91401000	Jelly, all flavors
91402000	Jam, preserves, all flavors
91403000	Fruit butter, all flavors
91404000	Marmalade, all flavors
91405000	Jelly, dietetic, all flavors, sweetened with artificial sweetener
91405500	Jelly, reduced sugar, all flavors
91406000	Jams, preserves, marmalades, dietetic, all flavors, sweetened with artificial sweetener
91406500	Jams, preserves, marmalades, sweetened with fruit juice concentrates, all flavors
91406600	Jams, preserves, marmalades, low sugar (all flavors)
91407100	Guava paste
91407120	Sweet potato paste
91407150	Bean paste, sweetened

Milk, Whole and Skim

Acidophilus or Fortified Milks, Fluid Milks, Reconstituted Milk Powders

[2'-FL] = 0.12 g/100 g

11100000	Milk, NFS
11111000	Milk, whole
11111100	Milk, low sodium, whole
11111150	Milk, calcium fortified, whole
11111160	Milk, calcium fortified, low fat (1%)
11111170	Milk, calcium fortified, fat free (skim)
11112110	Milk, reduced fat (2%)
11112120	Milk, acidophilus, low fat (1%)
11112130	Milk, acidophilus, reduced fat (2%)
11112210	Milk, low fat (1%)
11113000	Milk, fat free (skim)
11114300	Milk, lactose free, low fat (1%)
11114320	Milk, lactose free, fat free (skim)

11114330	Milk, lactose free, reduced fat (2%)
11114350	Milk, lactose free, whole
11120000	Milk, dry, reconstituted, NS as to fat content
11121100	Milk, dry, reconstituted, whole
11121210	Milk, dry, reconstituted, low fat (1%)
11121300	Milk, dry, reconstituted, fat free (skim)

Dry Milks
(Adjusted for being reconstituted at 24 g powder to 240 mL water)
[2'-FL] = 1.32 g/100 g

11810000	Milk, dry, not reconstituted, NS as to fat content
11811000	Milk, dry, not reconstituted, whole
11812000	Milk, dry, not reconstituted, low fat (1%)
11813000	Milk, dry, not reconstituted, fat free (skim)

Mixtures Containing Milk
(Adjusted for a Milk Content of 50.3% to 87.5%)
[2'-FL] = 0.06 to 0.11 g/100 g

11513400	Chocolate milk, made from syrup, NS as to type of milk
11513500	Chocolate milk, made from syrup with whole milk
11513550	Chocolate milk, made from syrup with reduced fat milk (2%)
11513600	Chocolate milk, made from syrup with low fat milk (1%)
11513700	Chocolate milk, made from syrup with fat free milk (skim)
11513800	Chocolate milk, made from light syrup, NS as to type of milk
11513801	Chocolate milk, made from light syrup with whole milk
11513802	Chocolate milk, made from light syrup with reduced fat milk (2%)
11513803	Chocolate milk, made from light syrup with low fat milk (1%)
11513804	Chocolate milk, made from light syrup with fat free milk (skim)
11513850	Chocolate milk, made from sugar free syrup, NS as to type of milk
11513851	Chocolate milk, made from sugar free syrup with whole milk
11513852	Chocolate milk, made from sugar free syrup with reduced fat milk (2%)
11513853	Chocolate milk, made from sugar free syrup with low fat milk (1%)
11513854	Chocolate milk, made from sugar free syrup with fat free milk (skim)
92101900	Coffee, Latte
92101901	Coffee, Latte, nonfat
92101904	Coffee, Latte, flavored
92101905	Coffee, Latte, nonfat, flavored
92101910	Coffee, Latte, decaffeinated
92101911	Coffee, Latte, decaffeinated, nonfat
92101917	Coffee, Latte, decaffeinated, flavored
92101918	Coffee, Latte, decaffeinated, nonfat, flavored
92101950	Coffee, Cafe Mocha
92101955	Coffee, Cafe Mocha, nonfat
92101965	Coffee, Cafe Mocha, decaffeinated
92101970	Coffee, Cafe Mocha, decaffeinated, nonfat
92102500	Coffee, Iced Latte
92102501	Coffee, Iced Latte, nonfat
92102510	Coffee, Iced Latte, decaffeinated
92102511	Coffee, Iced Latte, decaffeinated, nonfat
92161000	Coffee, Cappuccino
92161001	Coffee, Cappuccino, nonfat
92162000	Coffee, Cappuccino, decaffeinated
92162001	Coffee, Cappuccino, decaffeinated, nonfat

Mixtures Containing Milk
(Adjusted for a Milk Content of 16.1 to 49.9%)
[2'-FL] = 0.02 to 0.06 g/100 g

92101810	Coffee, macchiato
92101820	Coffee, macchiato, sweetened
92101850	Coffee, cafe con leche
92101851	Coffee, cafe con leche, decaffeinated
92101920	Frozen coffee drink
92101921	Frozen coffee drink, nonfat
92101925	Frozen coffee drink, with whipped cream
92101926	Frozen coffee drink, nonfat, with whipped cream
92101930	Frozen coffee drink, decaffeinated
92101931	Frozen coffee drink, decaffeinated, nonfat
92101935	Frozen coffee drink, decaffeinated, with whipped cream
92101936	Frozen coffee drink, decaffeinated, nonfat, with whipped cream
92102000	Frozen mocha coffee drink
92102010	Frozen mocha coffee drink, nonfat
92102030	Frozen mocha coffee drink, with whipped cream
92102040	Frozen mocha coffee drink, nonfat, with whipped cream
92102060	Frozen mocha coffee drink, decaffeinated
92102070	Frozen mocha coffee drink, decaffeinated, nonfat
92102090	Frozen mocha coffee drink, decaffeinated, with whipped cream
92102100	Frozen mocha coffee drink, decaffeinated, nonfat, with whipped cream
92102503	Coffee, Iced Latte, flavored
92102504	Coffee, Iced Latte, nonfat, flavored
92102513	Coffee, Iced Latte, decaffeinated, flavored
92102514	Coffee, Iced Latte, decaffeinated, nonfat, flavored
92102600	Coffee, Iced Cafe Mocha
92102601	Coffee, Iced Cafe Mocha, nonfat
92102610	Coffee, Iced Cafe Mocha, decaffeinated
92102611	Coffee, Iced Cafe Mocha, decaffeinated, nonfat
92306800	Tea, hot, chai, with milk
92610030	Horchata beverage, made with milk
92611100	Atole de avena (oatmeal beverage with milk)
92613010	Atole (corn meal beverage)
92613510	Atole de chocolate / Champurrado (cornmeal beverage with chocolate and milk)

Milk Products

<u>Flavored Milks</u>
[2'-FL] = 0.12 g/100 g

11115000	Buttermilk, fat free (skim)
11115100	Buttermilk, low fat (1%)
11115200	Buttermilk, reduced fat (2%)
11115300	Buttermilk, whole
11115400	Kefir, NS as to fat content
11511000	Chocolate milk, NFS
11511100	Chocolate milk, ready to drink, whole
11511200	Chocolate milk, ready to drink, reduced fat (2%)
11511300	Chocolate milk, ready to drink, fat free (skim)
11511400	Chocolate milk, ready to drink, low fat (1%)
11511550	Chocolate milk, ready to drink, reduced sugar, NS as to milk

11511600	Nesquik, chocolate milk, ready to drink, low fat (1%)
11511610	Nesquik, chocolate milk, ready to drink, fat free (skim)
11511700	Nesquik, chocolate milk, ready to drink, low fat (1%), no sugar added
11512010	Hot chocolate / Cocoa, ready to drink
11512020	Hot chocolate / Cocoa, ready to drink, made with nonfat milk
11512100	Hot chocolate / Cocoa, ready to drink, with whipped cream
11512110	Hot chocolate / Cocoa, ready to drink, made with nonfat milk and whipped cream
11513000	Chocolate milk, made from dry mix, NS as to type of milk
11513100	Chocolate milk, made from dry mix with whole milk
11513150	Chocolate milk, made from dry mix with reduced fat milk (2%)
11513200	Chocolate milk, made from dry mix with low fat milk (1%)
11513300	Chocolate milk, made from dry mix with fat free milk (skim)
11513350	Chocolate milk, made from reduced sugar mix, NS as to type of milk
11513355	Chocolate milk, made from reduced sugar mix with whole milk
11513360	Chocolate milk, made from reduced sugar mix with reduced fat milk (2%)
11513365	Chocolate milk, made from reduced sugar mix with low fat milk (1%)
11513370	Chocolate milk, made from reduced sugar mix with fat free milk (skim)
11513380	Nesquik, chocolate milk, made from dry mix, NS as to type of milk
11513381	Nesquik, chocolate milk, made from dry mix with whole milk
11513382	Nesquik, chocolate milk, made from dry mix with reduced fat milk (2%)
11513383	Nesquik, chocolate milk, made from dry mix with low fat milk (1%)
11513384	Nesquik, chocolate milk, made from dry mix with fat free milk (skim)
11513390	Nesquik, chocolate milk, made from no sugar added dry mix, NS as to type of milk
11513391	Nesquik, chocolate milk, made from no sugar added dry mix with whole milk
11513392	Nesquik, chocolate milk, made from no sugar added dry mix with reduced fat milk (2%)
11513393	Nesquik, chocolate milk, made from no sugar added dry mix with low fat milk (1%)
11513394	Nesquik, chocolate milk, made from no sugar added dry mix with fat free milk (skim)
11514110	Hot chocolate / Cocoa, made with dry mix and whole milk
11514120	Hot chocolate / Cocoa, made with dry mix and reduced fat milk (2%)
11514130	Hot chocolate / Cocoa, made with dry mix and low fat milk (1%)
11514140	Hot chocolate / Cocoa, made with dry mix and fat free milk (skim)
11514320	Hot chocolate / Cocoa, made with no sugar added dry mix and whole milk
11514330	Hot chocolate / Cocoa, made with no sugar added dry mix and reduced fat milk (2%)
11514340	Hot chocolate / Cocoa, made with no sugar added dry mix and low fat milk (1%)
11514350	Hot chocolate / Cocoa, made with no sugar added dry mix and fat free milk (skim)
11519040	Strawberry milk, NFS
11519050	Strawberry milk, whole
11519105	Strawberry milk, reduced fat (2%)
11519200	Strawberry milk, low fat (1%)
11519205	Strawberry milk, fat free (skim)
11525000	Milk, malted, natural flavor, made with milk
11526000	Milk, malted, chocolate, made with milk
11541400	Milk shake with malt
11542100	Milk shake, fast food, chocolate
11542200	Milk shake, fast food, flavors other than chocolate
11543000	Milk shake, bottled, chocolate
11543010	Milk shake, bottled, flavors other than chocolate
11551050	Licuado / Batido (milk fruit drink)
11553100	Fruit smoothie, NFS
11553110	Fruit smoothie, with whole fruit and dairy
11553120	Fruit smoothie, with whole fruit and dairy, added protein
11553130	Fruit smoothie juice drink, with dairy
11560000	Yoo-hoo, chocolate milk drink
78101100	Fruit and vegetable smoothie

78101110 Fruit and vegetable smoothie, added protein
78101120 Fruit and vegetable smoothie, bottled
92171000 Coffee, bottled/canned
92171010 Coffee, bottled/canned, light

Dry Mixtures of Flavored Milks, Cocoa
(Adjusted for Not Being Reconstituted, 28 g powder to 240 mL of water)
[2'-FL] = 1.15 g/100 g
11830100 Hot chocolate / Cocoa, dry mix, not reconstituted
11830115 Hot chocolate / Cocoa, dry mix, no sugar added, not reconstituted
11830150 Cocoa powder, not reconstituted (no dry milk)
11830160 Chocolate beverage powder, dry mix, not reconstituted
11830165 Chocolate beverage powder, reduced sugar, dry mix, not reconstituted
11830260 Milk, malted, dry mix, not reconstituted
11830400 Strawberry beverage powder, dry mix, not reconstituted

Milk-Based Meal Replacement Beverages or Diet beverages
[2'-FL] = 0.12 g/100 g
95101000 Boost, nutritional drink, ready-to-drink
95101010 Boost Plus, nutritional drink, ready-to-drink
95102000 Carnation Instant Breakfast, nutritional drink, regular, ready-to-drink
95103000 Ensure, nutritional shake, ready-to-drink
95103010 Ensure Plus, nutritional shake, ready-to-drink
95104000 Glucerna, nutritional shake, ready-to-drink
95105000 Kellogg's Special K Protein Shake
95106000 Muscle Milk, ready-to-drink
95106010 Muscle Milk, light, ready-to-drink
95110000 Slim Fast Shake, meal replacement, regular, ready-to-drink
95110010 Slim Fast Shake, meal replacement, sugar free, ready-to-drink
95110020 Slim Fast Shake, meal replacement, high protein, ready-to-drink
95120000 Nutritional drink or meal replacement, ready-to-drink, NFS
95120010 Nutritional drink or meal replacement, high protein, ready-to-drink, NFS
95120020 Nutritional drink or meal replacement, high protein, light, ready-to-drink, NFS

Powdered Milk-Based Meal Replacement Beverages
(Adjusted for Not Being Reconstituted, 16 g powder to 240 mL of water or milk)
[2'-FL] = 1.92 g/100 g
95220000 Nutritional drink mix or meal replacement, powder, NFS
95220010 Nutritional drink mix or meal replacement, high protein, powder, NFS

Not Reconstituted Milk-Based Meal Replacement Beverages
(Adjusted for Not Being Reconstituted, 20 g powder to 240 mL of milk)
[2'-FL] = 1.56 g/100 g
95201000 Carnation Instant Breakfast, nutritional drink mix, regular, powder
95201010 Carnation Instant Breakfast, nutritional drink mix, sugar free, powder

Not Reconstituted Milk-Based Meal Replacement Beverages
(Adjusted for not being reconstituted, 26 g powder to 227 mL of water)
[2'-FL] = 1.20 g/100 g
95202010 Muscle Milk, light, powder
95210000 Slim Fast Shake Mix, powder
95210010 Slim Fast Shake Mix, sugar free, powder
95210020 Slim Fast Shake Mix, high protein, powder

Not Reconstituted Milk-Based Meal Replacement Beverages
(Adjusted for not being reconstituted, 70 g powder to 454 mL of water)
[2'-FL] = 0.90 g/100 g

95202000 Muscle Milk, regular, powder

Yogurt
[2'-FL] = 0.53 g/100 g

11410000 Yogurt, NS as to type of milk or flavor
11411010 Yogurt, plain, NS as to type of milk
11411100 Yogurt, plain, whole milk
11411200 Yogurt, plain, low fat milk
11411300 Yogurt, plain, nonfat milk
11411400 Yogurt, Greek, plain, whole milk
11411410 Yogurt, Greek, plain, low fat
11411420 Yogurt, Greek, plain, nonfat milk
11420000 Yogurt, vanilla, NS as to type of milk
11421000 Yogurt, vanilla, whole milk
11422000 Yogurt, vanilla, low fat milk
11422100 Yogurt, vanilla, low fat milk, light
11423000 Yogurt, vanilla, nonfat milk
11424000 Yogurt, vanilla, nonfat milk, light
11424500 Yogurt, Greek, vanilla, whole milk
11424510 Yogurt, Greek, vanilla, low fat
11424520 Yogurt, Greek, vanilla, nonfat
11425000 Yogurt, chocolate, NS as to type of milk
11426000 Yogurt, chocolate, whole milk
11427000 Yogurt, chocolate, nonfat milk
11428000 Yogurt, Greek, chocolate, nonfat
11430000 Yogurt, fruit, NS as to type of milk
11431000 Yogurt, fruit, whole milk
11432000 Yogurt, fruit, low fat milk
11432500 Yogurt, fruit, low fat milk, light
11433000 Yogurt, fruit, nonfat milk
11433500 Yogurt, fruit, nonfat milk, light
11434000 Yogurt, Greek, fruit, whole milk
11434010 Yogurt, Greek, fruit, low fat
11434020 Yogurt, Greek, fruit, nonfat

Mixtures Containing Yogurt
(Adjusted for a Yogurt Content of 34.6% to 93.2%)
[2'-FL] = 0.18 to 0.49 g/100 g

11446000 Fruit and low fat yogurt parfait
83115000 Yogurt dressing

Processed Fruits and Fruit Juices

Fruit Drinks
[2'-FL] = 0.12 g/100 g

64134015 Fruit smoothie, with whole fruit (no dairy)
64134020 Fruit smoothie, with whole fruit (no dairy), added protein
64134030 Fruit smoothie juice drink (no dairy)
64134100 Fruit smoothie, light

64134200	Fruit smoothie, bottled
64200100	Fruit nectar, NFS
64201010	Apricot nectar
64201500	Banana nectar
64202010	Cantaloupe nectar
64203020	Guava nectar
64204010	Mango nectar
64205010	Peach nectar
64210010	Papaya nectar
64213010	Passion fruit nectar
64215010	Pear nectar
64221010	Soursop (Guanabana) nectar
92307500	Iced Tea / Lemonade juice drink
92307510	Iced Tea / Lemonade juice drink, light
92307520	Iced Tea / Lemonade juice drink, diet
92432000	Fruit juice drink, citrus, carbonated
92433000	Fruit juice drink, noncitrus, carbonated
92510610	Fruit juice drink
92510650	Tamarind drink (Refresco de tamarindo)
92510720	Fruit punch, made with fruit juice and soda
92510730	Fruit punch, made with soda, fruit juice, and sherbet or ice cream
92510955	Lemonade, fruit juice drink
92510960	Lemonade, fruit flavored drink
92511015	Fruit flavored drink
92511250	Fruit juice beverage, 40-50% juice, citrus
92512090	Pina Colada, nonalcoholic
92512110	Margarita mix, nonalcoholic
92513000	Fruit flavored smoothie drink, frozen (no dairy)
92513010	Fruit flavored smoothie drink, frozen, light (no dairy)
92530410	Fruit flavored drink, with high vitamin C
92530510	Cranberry juice drink, with high vitamin C
92530610	Fruit juice drink, with high vitamin C
92531030	Sunny D
92541010	Fruit flavored drink, powdered, reconstituted
92542000	Fruit flavored drink, with high vitamin C, powdered, reconstituted
92550030	Fruit juice drink, with high vitamin C, light
92550035	Fruit juice drink, light
92550040	Fruit juice drink, diet
92550110	Cranberry juice drink, with high vitamin C, light
92550200	Grape juice drink, light
92550350	Orange juice beverage, 40-50% juice, light
92550360	Apple juice beverage, 40-50% juice, light
92550370	Lemonade, fruit juice drink, light
92550380	Pomegranate juice beverage, 40-50% juice, light
92550610	Fruit flavored drink, with high vitamin C, diet
92550620	Fruit flavored drink, diet
92552000	Fruit flavored drink, with high vitamin C, powdered, reconstituted, diet
92552010	Fruit flavored drink, powdered, reconstituted, diet
92552020	Sunny D, reduced sugar
92552030	Capri Sun, fruit juice drink
92582100	Fruit juice drink, with high vitamin C, plus added calcium
92582110	Sunny D, added calcium

Frozen Fruit Drinks

(Adjusted for Not Being Reconstituted, 1 Cup Juice Mix to 3 Cups Water)
[2'-FL] = 0.48 g/100 g

92511000 Lemonade, frozen concentrate, not reconstituted

Concentrated Fruit Drinks
(Adjusted for Not Being Reconstituted, 55 mL of Frozen Concentrate to Produce a 240 mL Beverage)
[2'-FL] = 0.64 g/100 g

92512040 Frozen daiquiri mix, frozen concentrate, not reconstituted
92512050 Frozen daiquiri mix, from frozen concentrate, reconstituted

Powdered Fruit Drinks
(Adjusted for Not Being Reconstituted, 16 g Powder to 240 mL of Water)
[2'-FL] = 1.92 g/100 g

92900100 Fruit flavored drink, with high vitamin C, powdered, not reconstituted
92900110 Fruit flavored drink, powdered, not reconstituted
92900200 Fruit flavored drink, powdered, not reconstituted, diet

Mixtures Containing Fruit Drinks
(Adjusted for a Fruit Drink Content of 50% to 74.7%)
[2'-FL] = 0.06 to 0.09 g/100 g

92530950 Vegetable and fruit juice drink, with high vitamin C
92550400 Vegetable and fruit juice drink, with high vitamin C, diet
92550405 Vegetable and fruit juice drink, with high vitamin C, light
93301213 Vodka and lemonade

Fruit Juices
[2'-FL] = 0.12 g/100 g

61201020 Grapefruit juice, 100%, NS as to form
61201220 Grapefruit juice, 100%, canned, bottled or in a carton
61201225 Grapefruit juice, 100%, with calcium added
61201620 Grapefruit juice,100%, frozen, reconstituted
61204000 Lemon juice, 100%, NS as to form
61204200 Lemon juice, 100%, canned or bottled
61207000 Lime juice, 100%, NS as to form
61207200 Lime juice, 100%, canned or bottled
61210000 Orange juice, 100%, NFS
61210220 Orange juice, 100%, canned, bottled or in a carton
61210250 Orange juice, 100%, with calcium added, canned, bottled or in a carton
61210620 Orange juice, 100%, frozen, reconstituted
61210820 Orange juice, 100%, with calcium added, frozen, reconstituted
61213220 Tangerine juice, 100%
61213800 Fruit juice blend, citrus, 100% juice
61213900 Fruit juice blend, citrus, 100% juice, with calcium added
64100100 Fruit juice, NFS
64100110 Fruit juice blend, 100% juice
64100200 Cranberry juice blend, 100% juice
64100220 Cranberry juice blend, 100% juice, with calcium added
64101010 Apple cider
64104010 Apple juice, 100%
64104030 Apple juice, 100%, with calcium added
64104600 Blackberry juice, 100%
64105400 Cranberry juice, 100%, not a blend
64116020 Grape juice, 100%

64116060 Grape juice, 100%, with calcium added
64120010 Papaya juice, 100%
64121000 Passion fruit juice, 100%
64124020 Pineapple juice, 100%
64126000 Pomegranate juice, 100%
64132010 Prune juice, 100%
64132500 Strawberry juice, 100%
64133100 Watermelon juice, 100%

Frozen Fruit Juices
(Adjusted for Not Being Reconstituted, 1 Cup Juice Mix to 3 Cups Water)
[2'-FL] = 0.48 g/100 g
61210720 Orange juice, 100%, frozen, not reconstituted

Mixtures Containing Fruit Juices
(Adjusted for a Fruit Juice Content of 3.6% to 75.3%)
[2'-FL] = <0.01 to 0.09 g/100 g
78101000 Vegetable and fruit juice, 100% juice, with high vitamin C
93301032 Cape Cod
93301040 Daiquiri
93301075 Greyhound
93301085 Kamikaze
93301111 Martini, flavored
93301115 Mimosa
93301132 Orange Blossom
93301139 Salty Dog
93301140 Screwdriver
93301141 Seabreeze
93301200 Pina Colada
93301230 Sloe gin fizz
93301270 Fruit punch, alcoholic
93301275 Champagne punch
93301280 Singapore Sling
93301310 Mai Tai
93301320 Tequila Sunrise
93301330 Gin Rickey
93301370 Fuzzy Navel
93301600 Gin fizz
93302100 Zombie

Sweet Sauces, Toppings, and Syrups

<u>Syrups Used to Flavor Milk Beverages</u>
(Adjusted for a Syrup Content of 12.5 to 13.3%)
[2'-FL] = 0.09 g/100 g
11513400 Chocolate milk, made from syrup, NS as to type of milk
11513500 Chocolate milk, made from syrup with whole milk
11513550 Chocolate milk, made from syrup with reduced fat milk (2%)
11513600 Chocolate milk, made from syrup with low fat milk (1%)
11513700 Chocolate milk, made from syrup with fat free milk (skim)
11513750 Chocolate milk, made from syrup with non-dairy milk
11513800 Chocolate milk, made from light syrup, NS as to type of milk
11513801 Chocolate milk, made from light syrup with whole milk

11513802	Chocolate milk, made from light syrup with reduced fat milk (2%)
11513803	Chocolate milk, made from light syrup with low fat milk (1%)
11513804	Chocolate milk, made from light syrup with fat free milk (skim)
11513805	Chocolate milk, made from light syrup with non-dairy milk
11513850	Chocolate milk, made from sugar free syrup, NS as to type of milk
11513851	Chocolate milk, made from sugar free syrup with whole milk
11513852	Chocolate milk, made from sugar free syrup with reduced fat milk (2%)
11513853	Chocolate milk, made from sugar free syrup with low fat milk (1%)
11513854	Chocolate milk, made from sugar free syrup with fat free milk (skim)
11513855	Chocolate milk, made from sugar free syrup with non-dairy milk
91301130	Fruit flavored syrup used for milk beverages

Non-Exempt Infant and Follow-On Formula

Infant Formula

[2'-FL] = 0.24 g/100 g

11710000	Infant formula, NFS
11710050	Similac Expert Care Alimentum, infant formula, NS as to form
11710051	Similac Expert Care Alimentum, infant formula, ready-to-feed
11710053	Similac Expert Care Alimentum, infant formula, prepared from powder, made with water, NFS
11710054	Similac Expert Care Alimentum, infant formula, prepared from powder, made with tap water
11710055	Similac Expert Care Alimentum, infant formula, prepared from powder, made with plain bottled water
11710056	Similac Expert Care Alimentum, infant formula, prepared from powder, made with baby water
11710350	Similac Advance, infant formula, NS as to form
11710351	Similac Advance, infant formula, ready-to-feed
11710352	Similac Advance, infant formula, prepared from liquid concentrate, made with water, NFS
11710353	Similac Advance, infant formula, prepared from powder, made with water, NFS
11710354	Similac Advance, infant formula, prepared from liquid concentrate, made with tap water
11710355	Similac Advance, infant formula, prepared from liquid concentrate, made with plain bottled water
11710356	Similac Advance, infant formula, prepared from liquid concentrate, made with baby water
11710357	Similac Advance, infant formula, prepared from powder, made with tap water
11710358	Similac Advance, infant formula, prepared from powder, made with plain bottled water
11710359	Similac Advance, infant formula, prepared from powder, made with baby water
11710360	Similac Advance Organic, infant formula, NS as to form
11710361	Similac Advance Organic, infant formula, ready-to-feed
11710363	Similac Advance Organic, infant formula, prepared from powder, made with water, NFS
11710367	Similac Advance Organic, infant formula, prepared from powder, made with tap water
11710368	Similac Advance Organic, infant formula, prepared from powder, made with plain bottled water
11710369	Similac Advance Organic, infant formula, prepared from powder, made with baby water
11710370	Similac Sensitive, infant formula, NS as to form
11710371	Similac Sensitive, infant formula, ready-to-feed
11710372	Similac Sensitive, infant formula, prepared from liquid concentrate, made with water, NFS
11710373	Similac Sensitive, infant formula, prepared from powder, made with water, NFS
11710374	Similac Sensitive, infant formula, prepared from liquid concentrate, made with tap water
11710375	Similac Sensitive, infant formula, prepared from liquid concentrate, made with plain bottled water
11710376	Similac Sensitive, infant formula, prepared from liquid concentrate, made with baby water
11710377	Similac Sensitive, infant formula, prepared from powder, made with tap water
11710378	Similac Sensitive, infant formula, prepared from powder, made with plain bottled water
11710379	Similac Sensitive, infant formula, prepared from powder, made with baby water
11710380	Similac Sensitive for Spit-Up, infant formula, NS as to form
11710381	Similac Sensitive for Spit-Up, infant formula, ready-to-feed

11710383	Similac Sensitive for Spit-Up, infant formula, prepared from powder, made with water, NFS
11710387	Similac Sensitive for Spit-Up, infant formula, prepared from powder, made with tap water
11710388	Similac Sensitive for Spit-Up, infant formula, prepared from powder, made with plain bottled water
11710389	Similac Sensitive for Spit-Up, infant formula, prepared from powder, made with baby water
11710620	Enfamil PREMIUM Newborn, infant formula, NS as to form
11710621	Enfamil PREMIUM Newborn, infant formula, ready-to-feed
11710626	Enfamil PREMIUM Newborn, infant formula, prepared from powder, made with water, NFS
11710627	Enfamil PREMIUM Newborn, infant formula, prepared from powder, made with tap water
11710628	Enfamil PREMIUM Newborn, infant formula, prepared from powder, made with plain bottled water
11710629	Enfamil PREMIUM Newborn, infant formula, prepared from powder, made with baby water
11710630	Enfamil PREMIUM Infant, infant formula, NS as to form
11710631	Enfamil PREMIUM Infant, infant formula, ready-to-feed
11710632	Enfamil PREMIUM Infant, infant formula, prepared from liquid concentrate, made with water, NFS
11710633	Enfamil PREMIUM Infant, infant formula, prepared from liquid concentrate, made with tap water
11710634	Enfamil PREMIUM Infant, infant formula, prepared from liquid concentrate, made with plain bottled water
11710635	Enfamil PREMIUM Infant, infant formula, prepared from liquid concentrate, made with baby water
11710636	Enfamil PREMIUM Infant, infant formula, prepared from powder, made with water, NFS
11710637	Enfamil PREMIUM Infant, infant formula, prepared from powder, made with tap water
11710638	Enfamil PREMIUM Infant, infant formula, prepared from powder, made with plain bottled water
11710639	Enfamil PREMIUM Infant, infant formula, prepared from powder, made with baby water
11710640	Enfamil PREMIUM LIPIL, infant formula, NS as to form
11710642	Enfamil PREMIUM LIPIL, infant formula, prepared from liquid concentrate, made with water, NFS
11710643	Enfamil PREMIUM LIPIL, infant formula, prepared from powder, made with water, NFS
11710644	Enfamil PREMIUM LIPIL, infant formula, prepared from liquid concentrate, made with tap water
11710645	Enfamil PREMIUM LIPIL, infant formula, prepared from liquid concentrate, made with plain bottled water
11710646	Enfamil PREMIUM LIPIL, infant formula, prepared from liquid concentrate, made with baby water
11710647	Enfamil PREMIUM LIPIL, infant formula, prepared from powder, made with tap water
11710648	Enfamil PREMIUM LIPIL, infant formula, prepared from powder, made with plain bottled water
11710649	Enfamil PREMIUM LIPIL, infant formula, prepared from powder, made with baby water
11710650	Enfamil LIPIL, infant formula, NS as to form
11710651	Enfamil LIPIL, infant formula, ready-to-feed
11710652	Enfamil LIPIL, infant formula, prepared from liquid concentrate, made with water, NFS
11710653	Enfamil LIPIL, infant formula, prepared from powder, made with water, NFS
11710654	Enfamil LIPIL, infant formula, prepared from liquid concentrate, made with tap water
11710655	Enfamil LIPIL, infant formula, prepared from liquid concentrate, made with plain bottled water
11710656	Enfamil LIPIL, infant formula, prepared from liquid concentrate, made with baby water
11710657	Enfamil LIPIL, infant formula, prepared from powder, made with tap water
11710658	Enfamil LIPIL, infant formula, prepared from powder, made with plain bottled water
11710659	Enfamil LIPIL, infant formula, prepared from powder, made with baby water
11710660	Enfamil A.R. Lipil, infant formula, NS as to form
11710661	Enfamil A.R. Lipil, infant formula, ready-to-feed
11710663	Enfamil A.R. LIPIL, infant formula, prepared from powder, made with water, NFS
11710664	Enfamil A.R. LIPIL, infant formula, prepared from powder, made with tap water
11710670	Enfamil Gentlease LIPIL, infant formula, NS as to form
11710671	Enfamil Gentlease LIPIL, infant formula, ready-to-feed
11710673	Enfamil Gentlease LIPIL, infant formula, prepared from powder, made with water, NFS
11710677	Enfamil Gentlease LIPIL, infant formula, prepared from powder, made with tap water
11710678	Enfamil Gentlease LIPIL, infant formula, prepared from powder, made with plain bottled water
11710679	Enfamil Gentlease LIPIL, infant formula, prepared from powder, made with baby water
11710910	Gerber Good Start Gentle Plus, infant formula, NS as to form
11710911	Gerber Good Start Gentle Plus, infant formula, ready-to-feed

11710912 Gerber Good Start Gentle Plus, infant formula, prepared from liquid concentrate, made with water, NFS
11710913 Gerber Good Start Gentle Plus, infant formula, prepared from powder, made with water, NFS
11710914 Gerber Good Start Gentle Plus, infant formula, prepared from liquid concentrate, made with tap water
11710915 Gerber Good Start Gentle Plus, infant formula, prepared from liquid concentrate, made with plain bottled water
11710916 Gerber Good Start Gentle Plus, infant formula, prepared from liquid concentrate, made with baby water
11710917 Gerber Good Start Gentle Plus, infant formula, prepared from powder, made with tap water
11710918 Gerber Good Start Gentle Plus, infant formula, prepared from powder, made with plain bottled water
11710919 Gerber Good Start Gentle Plus, infant formula, prepared from powder, made with baby water
11710920 Gerber Good Start Protect Plus, infant formula, NS as to form
11710923 Gerber Good Start Protect Plus, infant formula, prepared from powder, made with water, NFS
11710927 Gerber Good Start Protect Plus, infant formula, prepared from powder, made with tap water
11710928 Gerber Good Start Protect Plus, infant formula, prepared from powder, made with plain bottled water
11710929 Gerber Good Start Protect Plus, infant formula, prepared from powder, made with baby water
11710960 America's Store Brand, infant formula, NS as to form
11710961 America's Store Brand, infant formula, prepared from liquid concentrate, made with water, NFS
11710962 America's Store Brand, infant formula, prepared from powder, made with water, NFS
11710963 America's Store Brand, infant formula, ready-to-feed
11710964 America's Store Brand, infant formula, prepared from liquid concentrate, made with tap water
11710965 America's Store Brand, infant formula, prepared from liquid concentrate, made with plain bottled water
11710966 America's Store Brand, infant formula, prepared from liquid concentrate, made with baby water
11710967 America's Store Brand, infant formula, prepared from powder, made with tap water
11710968 America's Store Brand, infant formula, prepared from powder, made with plain bottled water
11710969 America's Store Brand, infant formula, prepared from powder, made with baby water

Follow-On Formula

[2'-FL] = 0.24 g/100 g

11710480 Similac Go and Grow, infant formula, NS as to form
11710481 Similac Go and Grow, infant formula, prepared from powder, made with water, NFS
11710482 Similac Go and Grow, infant formula, prepared from powder, made with tap water
11710483 Similac Go and Grow, infant formula, prepared from powder, made with plain bottled water
11710484 Similac Go and Grow, infant formula, prepared from powder, made with baby water

Meal Replacement Products

[2'-FL] = 0.2 g/100 g

11710800 Pediasure, infant formula, NS as to form
11710801 Pediasure, infant formula, ready-to-feed
11710805 Pediasure Fiber, infant formula, NS as to form
11710806 Pediasure Fiber, infant formula, ready-to-feed

Baby Foods

Growing Up (Toddler) Milks

[2'-FL] = 0.2 g/100 g

11710680 Enfamil Enfagrow PREMIUM Next Step LIPIL, infant formula, NS as to form
11710681 Enfamil Enfagrow PREMIUM Next Step LIPIL, infant formula, ready-to-feed

11710683	Enfamil Enfagrow PREMIUM Next Step LIPIL, infant formula, prepared from powder, made with water, NFS
11710687	Enfamil Enfagrow PREMIUM Next Step LIPIL, infant formula, prepared from powder, made with tap water
11710688	Enfamil Enfagrow PREMIUM Next Step LIPIL, infant formula, prepared from powder, made with plain bottled water
11710689	Enfamil Enfagrow PREMIUM Next Step LIPIL, infant formula, prepared from powder, made with baby water
11710690	Enfamil Gentlease Next Step LIPIL, infant formula, NS as to form
11710693	Enfamil Gentlease Next Step LIPIL, infant formula, prepared from powder, made with water, NFS
11710697	Enfamil Gentlease Next Step LIPIL, infant formula, prepared from powder, made with tap water
11710698	Enfamil Gentlease Next Step LIPIL, infant formula, prepared from powder, made with plain bottled water
11710699	Enfamil Gentlease Next Step LIPIL, infant formula, prepared from powder, made with baby water
11710930	Gerber Good Start 2 Gentle Plus, infant formula, NS as to form
11710933	Gerber Good Start 2 Gentle Plus, infant formula, prepared from powder, made with water, NFS
11710937	Gerber Good Start 2 Gentle Plus, infant formula, prepared from powder, made with tap water
11710938	Gerber Good Start 2 Gentle Plus, infant formula, prepared from powder, made with plain bottled water
11710939	Gerber Good Start 2 Gentle Plus, infant formula, prepared from powder, made with baby water
11710940	Gerber Good Start 2 Protect Plus, infant formula, NS as to form
11710943	Gerber Good Start 2 Protect Plus, infant formula, prepared from powder, made with water, NFS
11710947	Gerber Good Start 2 Protect Plus, infant formula, prepared from powder, made with tap water
11710948	Gerber Good Start 2 Protect Plus, infant formula, prepared from powder, made with plain bottled water
11710949	Gerber Good Start 2 Protect Plus, infant formula, prepared from powder, made with baby water

Ready-to-Eat, Ready-to-Serve, Hot Cereals

[2'-FL] = 1.09 g/100 g

57820000	Cereal, baby food, jarred, NFS
57820100	Rice cereal, baby food, jarred, NFS
57822000	Mixed cereal with applesauce and bananas, baby food, jarred
57823000	Oatmeal with applesauce and bananas, baby food, jarred
57824000	Rice cereal with applesauce and bananas, baby food, jarred
57824500	Rice cereal with mixed fruit, baby food, jarred

Yogurt and Juice Beverages, Identified as "Baby" Drinks

[2'-FL] = 1.0 g/100 g

67202000	Apple juice, baby food
67202010	Apple juice, with added calcium, baby food
67203000	Apple-fruit juice blend, baby food
67203200	Apple-banana juice, baby food
67203400	Apple-cherry juice, baby food
67203500	Apple-grape juice, baby food
67203600	Apple-peach juice, baby food
67203700	Apple-prune juice, baby food
67203800	Grape juice, baby food
67204000	Mixed fruit juice, not citrus, baby food
67204100	Mixed fruit juice, not citrus, with added calcium, baby food
67205000	Orange juice, baby food
67211000	Orange-apple-banana juice, baby food
67212000	Pear juice, baby food
67230000	Apple-sweet potato juice, baby food
67230500	Orange-carrot juice, baby food

67250100 Banana juice with lowfat yogurt, baby food
67250150 Mixed fruit juice with lowfat yogurt, baby food
67260000 Fruit juice and water drink, with high vitamin C and added calcium, baby food

Desserts, "Junior Type"
[2'-FL] = 1.09 g/100 g

13310000 Custard pudding, flavor other than chocolate, baby food, NS as to strained or junior
13311000 Custard pudding, baby food, flavor other than chocolate, strained
13312000 Custard pudding, baby food, flavor other than chocolate, junior
67100100 Fruit, baby food, NFS
67100110 Fruit bar, with added vitamin C, baby food, toddler
67100200 Tropical fruit medley, baby food, strained
67100300 Apples, baby food, toddler
67101000 Apple-raspberry, baby food, NS as to strained or junior
67101020 Apple-raspberry, baby food, junior
67102000 Applesauce, baby food, NS as to strained or junior
67102020 Applesauce, baby food, junior
67104000 Applesauce and apricots, baby food, NS as to strained or junior
67104020 Applesauce and apricots, baby food, junior
67104030 Applesauce with bananas, baby food, NS as to strained or junior
67104060 Applesauce with bananas, baby food, junior
67104080 Applesauce with cherries, baby food, junior
67104090 Applesauce with cherries, baby food, NS as to strained or junior
67108000 Peaches, baby food, NS as to strained or junior
67108020 Peaches, baby food, junior
67109000 Pears, baby food, NS as to strained or junior
67109020 Pears, baby food, junior
67113000 Apples and pears, baby food, NS as to strained or junior
67113020 Apples and pears, baby food, junior
67114000 Pears and pineapple, baby food, NS as to strained or junior
67114020 Pears and pineapple, baby food, junior
67304000 Plums, baby food, NS as to strained or junior
67304020 Plums, baby food, junior
67307000 Apricots, baby food, NS as to strained or junior
67307020 Apricots, baby food, junior
67308000 Bananas, baby food, NS as to strained or junior
67308020 Bananas, baby food, junior
67309000 Bananas and pineapple, baby food, NS as to strained or junior
67309020 Bananas and pineapple, baby food, junior
67309030 Bananas and strawberry, baby food, junior
67404000 Fruit dessert, baby food, NS as to strained or junior
67404020 Fruit dessert, baby food, junior
67404050 Fruit Supreme dessert, baby food
67404550 Cherry cobbler, baby food, junior
67405000 Peach cobbler, baby food, NS as to strained or junior
67405020 Peach cobbler, baby food, junior
67412000 Dutch apple dessert, baby food, NS as to strained or junior
67412020 Dutch apple dessert, baby food, junior
67414100 Mango dessert, baby food
67415000 Tutti-fruitti pudding, baby food, NS as to strained or junior
67415020 Tutti-fruitti pudding, baby food, junior
67430000 Fruit flavored snack, baby food
67430500 Yogurt and fruit snack, baby food

Baby Crackers, Pretzels, Cookies, and Snack Items

[2'-FL] = 5.7 g/100 g

53801000	Cereal bar with fruit filling, baby food
53803050	Cookie, fruit, baby food
53803100	Cookie, baby food
53803250	Cookie, teething, baby
53803300	Cookie, rice, baby
54350000	Crackers, baby food
54350010	Gerber Finger Foods, Puffs, baby food
54350020	Finger Foods, Puffs, baby food
54360000	Crunchy snacks, corn based, baby food
54408100	Pretzel, baby food
57830100	Gerber Graduates Finger Snacks Cereal, baby food

TRISKELION
RESEARCH FOR BETTER LIVING

V20880/01

14-day oral (diet) dose-range finding study in male rats with 2'-fucosyllactose

DATE	24 November 2016
AUTHOR(S)	A.E. Wallinga, PhD
SPONSOR	Friesland Campina Innovation Bronland 20 6708 WH Wageningen
TRISKELION PROJECT NUMBER	10197 (formerly 093.26005/01)
TRISKELION STUDY CODE	20880/01
GUIDELINE	-
STATUS	Final
NUMBER OF PAGES	38

Report approval

I, the undersigned, hereby declare that this report constitutes a complete and accurate representation of the study and its results. GLP was not claimed for this range-finding study.

Study director

(b) (6)

A.E. Wallinga, PhD

24-nov-2016

Date

Contents

Abbreviations

AWB	Animal Welfare Body
OECD	Organization for Economic Co-operation and Development
SPF	Specific pathogen free

1 General

1.1 Study Sponsor

Sponsor: Friesland Campina Innovation
Bronland 20
6708 WH Wageningen
The Netherland

Monitor: D. Delsing, PhD
Phone: +31 (0)6 5359 8111
E-mail: dianne.delsing@frieslandcampina.com

1.2 Test facility

Triskelion B.V. www.triskelion.nl
Postal address: P.O. Box 844
3700 AV Zeist
The Netherlands
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3704 HE Zeist
The Netherlands
Phone: +31 88 866 2800

1.3 Responsible Personnel

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Phone: +31 88 866 6767
Scientific contributor(s): M.V.W. Wijnands, PhD, DVM
Pathology

1.4 Time schedule

Arrival of the animals : 7 September 2016
Start of the treatment (day 0) : 22 September 2016
Necropsy : 06 October 2016

2 Introduction

2.1 Objective

The objective of this study was to provide information on palatability, the major toxic effects, indicate target organs and select dose levels for a subsequent 13-week oral toxicity study in rats (OECD 408). For this purpose the test substance was administered to male rats for 14 days. The test substance was incorporated at constant concentrations in the diet and fed to the rats during 14 days.

2.2 Animal welfare

The welfare of the animals was maintained in accordance with the general principles governing the use of animals in experiments of the European Communities (Directive 2010/63/EU) and Dutch legislation (The revised Experiments on Animals Act, 2014). This included licensing of the project by the Central Committee on Animal Experimentation (project license 3660) and approval of the study by the Triskelion Animal Welfare Body (AWB number TRIS-185).

3 Materials and methods

3.1 Test substance

Name[1]	: 2'-Fucosyllactose
Chemical name[1]	: 2'-FL
Molecular formula[1]	: $C_{18}H_{32}O_{15}$
CAS Reg No.[1]	: 41263-94-9
Batch number[1]	: MRS02
Appearance	: White powder
Purity[1]	: 94%
Storage conditions[1]	: 2 – 10 °C, protected from light
Quantity	: 20 kg
Date of receipt	: 19 July 2016
Expiry date[1]	: 15 July 2018
Supplier	: Sponsor
Triskelion dispense number	: 160161

[1] Information provided by the sponsor

The certificate of analysis pertaining to the batch of test substance used during this dose range finding study provided by the sponsor is given in Annex 1.

3.2 Administration of the test substance

The test substance was administered to the animals via the diet. Food was provided *ad libitum*. The dietary route of administration was chosen because this is an anticipated route of human exposure.

3.3 Experimental design, groups and dose levels

The 14-day dose range finding study comprised four groups of 4 males each, viz. one control group kept on control diet and three test groups receiving different levels of 2'-Fucosyllactose added to this diet. The test substance was added to the diet as indicated in the table below:

Group	Color code	Dietary level % 2'-Fucosyllactose	Number of males
1 Control	white	0%	4
2 Low-dose	blue	3%	4
3 Mid-dose	green	6%	4
4 High-dose	red	10%	4

For selection of dietary levels for the dose range finding study the intention of the sponsor was taken into account to dose at least 5x the maximum intake as assessed by EFSA (670 mg/kg/day for human infants aged 4-6 months). This is equivalent to about 3500 mg/kg bw/day.

3.4 Test system

The study was conducted with albino rats. The rat were used because this species is considered suitable for this type of study, and is usually required by regulatory agencies. Male Wistar outbred rats (Crl:WI(Han)) were obtained from a colony maintained under SPF-conditions at Charles River Deutschland, Sulzfeld, Germany. The Han rat strain was used because it is routinely used at the test facility for this type of studies.
The rats were about 6-7 weeks old at the commencement of the treatment period. The body weight variation at initiation of treatment did not exceed ±20% of the mean weight. Mean body weight at the start of treatment on day 0 was 174 grams.

3.5 Animal allocation

Seventeen male rats arrived on 7 September 2016 and were taken in their unopened shipping containers to a quarantine room (animal room 5.1.21) and were checked for overt signs of ill health and anomalies. During the quarantine period, serological investigation of the microbiological status was conducted in blood samples taken from 1 randomly selected animal. On 12 September 2016, the results of the serological examinations were received an indicated an acceptable microbiological status. The animals were subsequently released for experimental use, and the quarantine room was cleared for use as experimental room on the same day. The duration of the acclimatization period to the laboratory conditions prior exposure (period between arrival and start of the exposure) was 15 days.
One day before start of the treatment the male rats they were allocated to the various groups by computer randomization proportionally to body weight. Cross reference listings showing animal, group and cage numbers are given in Annex 2. The surplus animal (1 male) was kept in reserve to serve as sentinel. This animal was not used in the study.

3.6 Identification

The study was identified as Triskelion study 20880/01. Before allocation, the individual rats were identified by a transient mark on their tail. After allocation, the rats were identified by a unique animal identification number using subcutaneous transponders.
Each cage was provided with a card showing the color code, the animal identification numbers, the cage number, the group code and the study code.

3.7 Animal husbandry

3.7.1 Animal room

From their arrival the rats were housed under conventional conditions. No other test system was housed in the same room during the study. Lighting was artificial with a sequence of 12 hours light and 12 hours dark. The room was ventilated with about 10 air changes per hour and was maintained at a temperature of 20-24°C. The relative humidity was maintained between 45-65%.

3.7.2 Caging

The animals were kept in macrolon cages (2 rats/cage, separated by sex) with wood shavings (Lignocel) as bedding material, and strips of paper (Enviro-dri) and a wooden block as environmental enrichment. The cages and bedding were changed weekly.

3.7.3 Food and drinking water

Food was provided *ad libitum* from the arrival of the animals until the end of the study. The animals received a cereal-based (closed formula) rodent diet (VRF1 (FG)) from a commercial supplier (SDS Special Diets Services, Whitham, England). The animals of the test groups were kept on experimental diets prepared by mixing this diet with the appropriate amounts of test substance. At 20 September 2016, before the start of the study, one batch of diet was prepared for each dosing group at the selected concentrations. These batches were subdivided in daily portions, and were stored in a freezer (≤-18 °C). Every three or four days, a portion was taken from the freezer to replace the food in the feeders in the animal room.

Each batch of VRF1 (FG)) diet is analyzed by the supplier for nutrients and contaminants. The certificate of analysis pertaining to the batch used (batch number 2372) is included in Annex 3. The food was provided as a powder in stainless steel cans, covered by a perforated stainless steel plate to prevent spillage. The food in the feeders was replaced with fresh portions twice weekly and filled up as needed.

Drinking water was provided *ad libitum* from the arrival of the animals until the end of the study. Each cage was supplied with domestic mains tap-water suitable for human consumption (quality guidelines according to Dutch legislation based on EC Council Directive 98/83/EC). The water was given in polypropylene bottles, which were cleaned weekly and filled as needed. Results of the routine physical, chemical and microbial examination of the drinking water as conducted by the supplier are made available to the test facility. In addition, the supplier periodically (twice per year) analyses water samples taken on the premises of the test facility for a limited number of variables. The results of the most recent analyses are included in Annex 4.

3.8 Observations, analyses and measurements

3.8.1 General clinical observations

Each animal was observed daily in the morning hours by cage-side observations and, if necessary, handled to detect signs of toxicity. All cages were checked again in the afternoon for dead or moribund animals to minimize loss of animals from the study. All abnormalities, signs of ill health or reactions to treatment were recorded (a listing of clinical signs is provided in Annex 5).

3.8.2 Body weight

The body weight of each animal was recorded once during the acclimatization period, at initiation of treatment, and twice per week thereafter (of which the last time was on the day of scheduled necropsy).

3.8.3 Food consumption

Food consumption was measured per cage (2 rats per cage) by weighing the feeders. The consumption was measured over successive three- or four-day periods during the treatment period.

3.8.4 Intake of the test substance

The intake of the test substance per kg body weight per day was calculated from the nominal dietary concentration, the food consumption and the body weight using the body weight and food consumption measured at day 4, 7, 10 and 14.

3.8.5 Necropsy and pathology

On 6 October after 14 days of dosing, all animals were sacrificed. The animals were killed by exsanguination from the abdominal aorta under CO_2/O_2 anesthesia and then examined grossly for pathological changes. The kidneys, liver and caecum (full and empty) were weighed and relative organ weights (g/kg body weight) were calculated from the absolute organ weight and the terminal body weight.

3.9 Statistical analysis of the results

The statistical procedures for analysis of data are described below.

Body weight data collected after initiation of treatment: 'AnCova & Dunnett's Test' with automatic data transformation. Day 0 body weight data were used as covariate in the analysis of the post-treatment data unless removed during data pre-processing. The 'AnCova & Dunnett's Test' is an automatic decision tree consisting of:

(1) Data pre-processing tests. These tests start with transformation 'None'. First, suitability of the covariate was checked (criteria: sufficient cases, at least 2; variability of covariate non-zero; covariate effects sufficiently parallel over the groups, significance level parallelism test 0.01). Next, normality of data distribution (Shapiro-Wilks test; significance level 0.05) and homogeneity of variances (Levene test; significance level 0.05) were checked. If any of these three checks failed they were repeated using Log transformation.
If checks on log-transformed, covariate-adjusted data failed, the covariate was removed and the normality and homogeneity checks were repeated. If these checks passed on transformations 'None' or 'Log', data were analyzed without covariate. If they failed, data were rank-transformed and the covariate was reinstated.

(2) A group test assessing whether or not group means were all equal (one-way analysis of covariance [Ancova], or one-way analysis of variance [Anova] if the covariate is removed). If the group test showed no significant non-homogeneity of group means ($p \geq 0.05$), group summary tables do not show whether or not a covariate was used in the analysis.

(3) Post-hoc analysis. If the group test showed significant ($p < 0.05$) non-homogeneity of group means, pairwise comparisons with the control group were conducted by Dunnett's multiple comparison test (significance levels 0.01 and 0.05).

Pre-treatment body weight and organ weight, data: 'Generalized Anova/Ancova Test' with automatic data transformation method. This test is an automatic decision tree consisting of:

(1) Data pre-processing tests. First, normality of data distribution (Shapiro-Wilks test) and homogeneity of variances (Levene test) were checked (initial transformation 'None'). If any of these checks failed ($p < 0.05$) they were repeated using Log transformation. If checks on log-transformed data failed, data were rank-transformed.

(2) A group test assessing whether or not group means were all equal (parametric for untransformed or log-transformed data: one-way analysis of variance [Anova]; non-parametric for rank transformed data: Kruskal-Wallis test).

(3) Post-hoc analysis. If the group test showed significant ($p < 0.05$) non-homogeneity of group means, pairwise comparisons with the control group were conducted by Dunnett's multiple comparison test (parametric after Anova, non-parametric after Kruskal-Wallis; significance levels 0.01 and 0.05).

Food consumption: Dunnett's multiple comparison test.

Because numerous variables were subjected to statistical analysis, the overall false positive rate (Type I errors) was greater than suggested by a probability level of 0.05. Therefore, the final interpretation of results was based not only on statistical analysis but also on other considerations such as dose-response relationships and whether the results were significant in the light of other biological and pathological findings.

In the summary tables, the decision tree used is indicated in the column heading by the letter 'c' for the AnCova & Dunnett's Test or the letter 'g' for the Generalized Anova/Ancova Test. Where an element of the decision tree is different for a new parameter or time point on the same page (e.g. subsequent time points have a different transformation due to automatic transformations), the letter is followed by a number (e.g. g, g1, g2, etc.). The results of the decision trees are explained in footnotes below the summary tables. Statistical significances resulting from the pairwise comparisons with the control group (post-hoc analysis) are marked against the appropriate treatment group by * ($p < 0.05$) or ** ($p<0.01$).

Arithmetic means and standard deviations are given in the tables of continuous and semi-continuous data.

4 Results

Mean data are reported in tables, individual data are given in appendices.

4.1 Clinical signs (Table 1; Appendix 1)

None of the rats died during the study. There were no clinical signs.

4.2 Body weights (Table 2; Appendix 2)

There were no statistically significant differences in body weights.

4.3 Food consumption (Table 4; Appendix 3)

Food consumption was not affected by the test substance.

4.4 Intake of the test substance (Table 4)

The overall mean intake of the test substance in the low-, mid- and high-dose group was 2.67, 5.08 and 7.99 g/kg body weight/day.

4.5 Organ weights (Table 5 and 6; Appendix 4 and 5)

The relative liver weight was statistically significantly decreased in males of the mid- and high dose group. The absolute weight of this organ was decreased in the high-dose group only.

The absolute and relative weights of the filled and empty caecum were increased in the mid- and high-dose group. The changes were statistically significant, except for the empty caecum weight in the mid-dose group.

No changes were observed in the absolute and relative weights of the kidneys.

4.6 Macroscopic examination

At necropsy, no macroscopic changes were observed. No table was presented.

5 Discussion and conclusion

In this dose range finding study, 2'-Fucosyllactose was administered to male rats at dietary levels of 0%, 3%, 6% and 10% for 14 consecutive days. This resulted in intake of 0, 2.67, 5.08, 7.99 g/kg bw/day.

The test substance was well tolerated and did not affect general condition, growth or food intake. Decreases were noted in liver weight in the mid-dose and high-dose groups. Decrease in liver weight is generally not considered toxicologically significant. The increase in cecal weights in the mid-dose and high-dose groups are considered a physiological adaptation to the administration of large amounts of the test substance, known to occur upon consumption of poorly digestible, fermentable sugars, rather than a toxic effect (WHO 1987).

Based on the above findings, the following dietary levels are proposed for a subsequent 13-week oral (diet) toxicity study with 2'-fucosyllactose: 0%, 3%, 6% and 10%.

6 Documentation and retention of records

The following study specific materials will be archived for 5 years:
- Raw data (or true copies if unstable)
- Original study plan and final report

General raw data will be retained for at least 25 years, after which they may be destroyed without further notice. These may include, but are not necessarily limited to:
- Facility-based documents
- System calibration and quality control data
- General registrations potentially used for more than one study

After reporting of the main study tissue specimens will be discarded.

All materials will be retained in the archives of TNO, Utrechtseweg 48, 3704 HE Zeist, The Netherlands. The archiving period for starts on the cover date of the final report.

7 References

World Health Organisation (1987). Principles for the safety assessment of food additives and contaminants in food. *Envir. Health Criteria* 70, 39-59.

Tables

20880/01 - 14-day oral (diet) dose-range finding study in male rats with 2'-fucosyllactose

Table 1: Clinical observations

Observation Type: All Types	Male			
From Day 0 (Start Date) to 14 (Start Date)	0 g/kg	30 g/kg	60 g/kg	100 g/kg
DEAD Killed scheduled	4	4	4	4

Values = Number of Animals Affected

20880/01 - 14-day oral (diet) dose-range finding study in male rats with 2'-fucosyllactose

Table 2: Body weight

Day(s) Relative to Start Date

Sex: Male		Body weight		Bodyweights			
		Bodywt day -x (g) [g]	Bodywt day 0 (g) [g1]	Bodywt (g) [c]	Bodywt (g) [c]	Bodywt (g) [c]	Bodywt (g) [c]
		-1	0	4	7	11	14
0 g/kg	Mean	171.10	174.40	199.20	217.88	238.83	255.58
	SD	9.68	10.13	11.21	12.12	12.89	14.62
	N	4	4	4	4	4	4
30 g/kg	Mean	174.93	176.08	205.13	224.55	247.40	264.18
	SD	6.77	4.48	8.34	9.62	10.28	11.83
	N	4	4	4	4	4	4
60 g/kg	Mean	173.08	176.18	202.28	219.10	240.08	257.30
	SD	8.70	8.48	8.95	9.23	9.88	9.38
	N	4	4	4	4	4	4
100 g/kg	Mean	175.08	177.25	203.28	220.28	240.03	256.60
	SD	10.13	10.32	10.39	10.02	12.78	13.37
	N	4	4	4	4	4	4

[g] - Anova & Dunnett
[g1] - Anova
[c] - Ancova/Anova & Dunnett {Covariate: Bodywt day 0}: ** = p < 0.01

20880/01 - 14-day oral (diet) dose-range finding study in male rats with 2'-fucosyllactose

Table 3: Food consumption

Day numbers relative to Start Date

Group	Sex		From: 0	4	7	11
			To: 4	7	11	14
1	m	Mean	17.869	19.217	19.681	19.558
		S.D.	1.847	0.542	1.140	1.308
		N	2	2	2	2
2	m	Mean	19.331	19.750	20.638	21.225
		S.D.	0.362	0.754	0.265	1.120
		N	2	2	2	2
3	m	Mean	17.669	19.042	19.125	19.692
		S.D.	0.203	0.695	0.194	0.224
		N	2	2	2	2
4	m	Mean	16.519	17.467	18.069	19.458
		S.D.	1.494	0.283	1.352	0.059
		N	2	2	2	2

Statistics Test: Dunnett Test: * p < 0.05; ** p < 0.01

Food consumption was meassured per cage (5 animals per cage) over the periods indicated and expressed as g/animal/day

Group 1 - 0 g/kg Group 2 - 30 g/kg Group 3 - 60 g/kg Group 4 - 100 g/kg

20880/01 - 14-day oral (diet) dose-range finding study in male rats with 2'-fucosyllactose

Table 4. Test substance intake

Group No.	Group description	Dietary concentration 2'-Fucosyllactose	Calculated actual concentration 2'-Fucosyllactose (mg/ kg body weight)		
			Week 1	Week 2	Overall mean
1	Control	0	0.00	0.00	0.00
2	Low-dose	3%	2.90	2.50	2.67
3	Mid-dose	6%	5.53	4.75	5.08
4	High-dose	10%	8.49	7.62	7.99

20880/01 - 14-day oral (diet) dose-range finding study in male rats with 2'-fucosyllactose

Table 5: Absolute organ weights

Day(s): 14 Relative to Start Date

Sex: Male		Terminal body wgt (g) [g]	Kidneys (g) [g]	Liver (g) [g]	Caecum full (g) [g1]	Caecum empty (g) [g]
0 g/kg	Mean	255.58	1.975	10.465	6.4208	1.2115
	SD	14.62	0.255	0.473	0.6505	0.2041
	N	4	4	4	4	4
30 g/kg	Mean	264.18	2.018	10.533	7.0143	1.2498
	SD	11.83	0.142	0.406	0.8154	0.0645
	N	4	4	4	4	4
60 g/kg	Mean	257.30	1.840	9.593	9.9680*	1.4793
	SD	9.38	0.062	0.312	2.8277	0.1825
	N	4	4	4	3	4
100 g/kg	Mean	256.60	1.815	9.295*	9.9970*	1.5585*
	SD	13.37	0.190	0.700	1.4500	0.1559
	N	4	4	4	4	4

[g] - Anova & Dunnett: * = p < 0.05
[g1] - Anova & Dunnett(Log): * = p < 0.05

20880/01 - 14-day oral (diet) dose-range finding study in male rats with 2'-fucosyllactose

Table 6: Relative organ weights

Day(s): 14 Relative to Start Date

Sex: Male		Terminal body wgt (g) [g]	Kidneys rel.wgt (g/kg body wgt) [g]	Liver rel.wgt (g/kg body wgt) [g]	Caecum-F rel.wgt (g/kg body wgt) [g1]	Caecum-E rel.wgt (g/kg body wgt) [g]
0 g/kg	Mean	255.58	7.705	41.01	25.10	4.72
	SD	14.62	0.556	2.17	1.74	0.56
	N	4	4	4	4	4
30 g/kg	Mean	264.18	7.638	39.89	26.59	4.73
	SD	11.83	0.434	0.96	3.30	0.11
	N	4	4	4	4	4
60 g/kg	Mean	257.30	7.153	37.29*	38.72**	5.74*
	SD	9.38	0.133	0.29	9.68	0.53
	N	4	4	4	3	4
100 g/kg	Mean	256.60	7.060	36.22**	38.87**	6.07**
	SD	13.37	0.430	1.75	4.43	0.50
	N	4	4	4	4	4

[g] - Anova & Dunnett: * = p < 0.05; ** = p < 0.01
[g1] - Kruskal-Wallis & Dunnett on Ranks: ** = p < 0.01

Appendices

20880/01 - 14-day oral (diet) dose-range finding study in male rats with 2'-fucosyllactose

Appendix 1: Clinical observations

0 g/kg Sex: Male	Observation Type: All Types	From Day 0 (Start Date) to 14 (Start Date)
2	DEAD Killed scheduled	14
4	DEAD Killed scheduled	14
30	DEAD Killed scheduled	14
32	DEAD Killed scheduled	14

30 g/kg Sex: Male	Observation Type: All Types	From Day 0 (Start Date) to 14 (Start Date)
6	DEAD Killed scheduled	14
8	DEAD Killed scheduled	14
18	DEAD Killed scheduled	14
20	DEAD Killed scheduled	14

60 g/kg Sex: Male	Observation Type: All Types	From Day 0 (Start Date) to 14 (Start Date)
10	DEAD Killed scheduled	14
12	DEAD Killed scheduled	14
22	DEAD Killed scheduled	14
24	DEAD Killed scheduled	14

100 g/kg Sex: Male	Observation Type: All Types	From Day 0 (Start Date) to 14 (Start Date)
14	DEAD Killed scheduled	14
16	DEAD Killed scheduled	14
26	DEAD Killed scheduled	14
28	DEAD Killed scheduled	14

Values=Clin Obs Range

20880/01 - 14-day oral (diet) dose-range finding study in male rats with 2'-fucosyllactose

Appendix 2: Body weight

Sex: Male Day(s) Relative to Start Date

0 g/kg						
	Bodywt day -x (g)	Bodywt (g)	Bodywt (g)	Bodywt (g)	Bodywt (g)	Bodywt (g)
	-1	0	4	7	11	14
2	173.4	174.7	198.6	217.3	239.3	253.9
4	158.7	162.1	186.1	205.0	227.4	242.7
30	182.1	186.9	213.5	234.2	256.7	276.4
32	170.2	173.9	198.6	215.0	231.9	249.3
Mean	171.10	174.40	199.20	217.88	238.83	255.58
SD	9.68	10.13	11.21	12.12	12.89	14.62
N	4	4	4	4	4	4

30 g/kg						
	Bodywt day -x (g)	Bodywt (g)	Bodywt (g)	Bodywt (g)	Bodywt (g)	Bodywt (g)
	-1	0	4	7	11	14
6	167.8	169.8	196.1	215.1	239.5	256.0
8	173.4	175.9	202.3	220.1	241.2	255.8
18	174.4	179.3	206.1	225.5	246.8	263.9
20	184.1	179.3	216.0	237.5	262.1	281.0
Mean	174.93	176.08	205.13	224.55	247.40	264.18
SD	6.77	4.48	8.34	9.62	10.28	11.83
N	4	4	4	4	4	4

20880/01 - 14-day oral (diet) dose-range finding study in male rats with 2'-fucosyllactose

Appendix 2: Body weight

Sex: Male Day(s) Relative to Start Date

60 g/kg						
	Bodywt day -x (g)	Bodywt (g)	Bodywt (g)	Bodywt (g)	Bodywt (g)	Bodywt (g)
	-1	0	4	7	11	14
10	181.8	183.0	209.0	225.6	244.5	261.5
12	161.2	163.8	189.1	205.5	227.1	246.4
22	176.2	178.5	204.9	221.4	238.5	253.4
24	173.1	179.4	206.1	223.9	250.2	267.9
Mean	173.08	176.18	202.28	219.10	240.08	257.30
SD	8.70	8.48	8.95	9.23	9.88	9.38
N	4	4	4	4	4	4

100 g/kg						
	Bodywt day -x (g)	Bodywt (g)	Bodywt (g)	Bodywt (g)	Bodywt (g)	Bodywt (g)
	-1	0	4	7	11	14
14	186.6	187.5	213.7	224.8	243.3	256.0
16	178.9	183.9	209.0	230.4	253.4	271.2
26	162.8	165.1	190.1	207.0	222.7	239.0
28	172.0	172.5	200.3	218.9	240.7	260.2
Mean	175.08	177.25	203.28	220.28	240.03	256.60
SD	10.13	10.32	10.39	10.02	12.78	13.37
N	4	4	4	4	4	4

20880/01 - 14-day oral (diet) dose-range finding study in male rats with 2'-fucosyllactose

Appendix 3: Food consumption

Day numbers relative to Start Date

Group	Sex	Cage	No In Cage	From: / To:	0 / 4	4 / 7	7 / 11	11 / 14
1	m	2	2		16.56	18.83	18.88	18.63
		16	2		19.18	19.60	20.49	20.48
				Mean	17.869	19.217	19.681	19.558
				S.D.	1.847	0.542	1.140	1.308
				N	2	2	2	2
2	m	4	2		19.08	19.22	20.45	20.43
		10	2		19.59	20.28	20.83	22.02
				Mean	19.331	19.750	20.638	21.225
				S.D.	0.362	0.754	0.265	1.120
				N	2	2	2	2
3	m	6	2		17.81	18.55	19.26	19.53
		12	2		17.53	19.53	18.99	19.85
				Mean	17.669	19.042	19.125	19.692
				S.D.	0.203	0.695	0.194	0.224
				N	2	2	2	2
4	m	8	2		17.58	17.67	19.03	19.42
		14	2		15.46	17.27	17.11	19.50
				Mean	16.519	17.467	18.069	19.458
				S.D.	1.494	0.283	1.352	0.059
				N	2	2	2	2

Food consumption was meassured per cage (5 animals per cage) over the periods indicated and expressed as g/animal/day

Group 1 - 0 g/kg Group 2 - 30 g/kg Group 3 - 60 g/kg Group 4 - 100 g/kg

20880/01 - 14-day oral (diet) dose-range finding study in male rats with 2'-fucosyllactose

Appendix 4: Absolute organ weights

Sex: Male Day(s) Relative to Start Date

0 g/kg	Terminal body wgt (g)	Kidneys (g)	Liver (g)	Caecum full (g)	Caecum empty (g)
	14	14	14	14	14
2	253.9	2.01	11.05	6.381	1.271
4	242.7	1.75	9.92	6.424	0.958
30	276.4	2.32	10.57	7.235	1.447
32	249.3	1.82	10.32	5.643	1.170
Mean	255.58	1.975	10.465	6.4208	1.2115
SD	14.62	0.255	0.473	0.6505	0.2041
N	4	4	4	4	4

30 g/kg	Terminal body wgt (g)	Kidneys (g)	Liver (g)	Caecum full (g)	Caecum empty (g)
	14	14	14	14	14
6	256.0	2.05	10.16	6.250	1.173
8	255.8	1.98	10.56	7.042	1.228
18	263.9	1.85	10.32	8.137	1.274
20	281.0	2.19	11.09	6.628	1.324
Mean	264.18	2.018	10.533	7.0143	1.2498
SD	11.83	0.142	0.406	0.8154	0.0645
N	4	4	4	4	4

20880/01 - 14-day oral (diet) dose-range finding study in male rats with 2'-fucosyllactose

Appendix 4: Absolute organ weights

Sex: Male Day(s) Relative to Start Date

60 g/kg	Terminal body wgt (g)	Kidneys (g)	Liver (g)	Caecum full (g)	Caecum empty (g)
	14	14	14	14	14
10	261.5	1.82	9.81	1.432 E	1.453
12	246.4	1.79	9.25	6.831	1.379
22	253.4	1.82	9.41	10.752	1.341
24	267.9	1.93	9.90	12.321	1.744
Mean	257.30	1.840	9.593	9.9680	1.4793
SD	9.38	0.062	0.312	2.8277	0.1825
N	4	4	4	3	4

100 g/kg	Terminal body wgt (g)	Kidneys (g)	Liver (g)	Caecum full (g)	Caecum empty (g)
	14	14	14	14	14
14	256.0	1.90	8.91	11.332	1.587
16	271.2	1.97	9.64	10.956	1.521
26	239.0	1.54	8.54	8.152	1.375
28	260.2	1.85	10.09	9.548	1.751
Mean	256.60	1.815	9.295	9.9970	1.5585
SD	13.37	0.190	0.700	1.4500	0.1559
N	4	4	4	4	4

E = Excluded due to weighing error; the weight of the full caecum was not measured

20880/01 - 14-day oral (diet) dose-range finding study in male rats with 2'-fucosyllactose

Appendix 5: Relative organ weights

Sex: Male Day(s) Relative to Start Date

0 g/kg	Terminal body wgt (g)	Kidneys rel.wgt (g/kg body wgt)	Liver rel.wgt (g/kg body wgt)	Caecum-F rel.wgt (g/kg body wgt)	Caecum-E rel.wgt (g/kg body wgt)
	14	14	14	14	14
2	253.9	7.92	43.5	25.1	5.0
4	242.7	7.21	40.9	26.5	3.9
30	276.4	8.39	38.2	26.2	5.2
32	249.3	7.30	41.4	22.6	4.7
Mean	255.58	7.705	41.01	25.10	4.72
SD	14.62	0.556	2.17	1.74	0.56
N	4	4	4	4	4

30 g/kg	Terminal body wgt (g)	Kidneys rel.wgt (g/kg body wgt)	Liver rel.wgt (g/kg body wgt)	Caecum-F rel.wgt (g/kg body wgt)	Caecum-E rel.wgt (g/kg body wgt)
	14	14	14	14	14
6	256.0	8.01	39.7	24.4	4.6
8	255.8	7.74	41.3	27.5	4.8
18	263.9	7.01	39.1	30.8	4.8
20	281.0	7.79	39.5	23.6	4.7
Mean	264.18	7.638	39.89	26.59	4.73
SD	11.83	0.434	0.96	3.30	0.11
N	4	4	4	4	4

20880/01 - 14-day oral (diet) dose-range finding study in male rats with 2'-fucosyllactose

Appendix 5: Relative organ weights

Sex: Male Day(s) Relative to Start Date

60 g/kg	Terminal body wgt (g)	Kidneys rel.wgt (g/kg body wgt)	Liver rel.wgt (g/kg body wgt)	Caecum-F rel.wgt (g/kg body wgt)	Caecum-E rel.wgt (g/kg body wgt)
	14	14	14	14	14
10	261.5	6.96	37.5	5.5 E	5.6
12	246.4	7.26	37.5	27.7	5.6
22	253.4	7.18	37.1	42.4	5.3
24	267.9	7.20	37.0	46.0	6.5
Mean	257.30	7.153	37.29	38.72	5.74
SD	9.38	0.133	0.29	9.68	0.53
N	4	4	4	3	4

100 g/kg	Terminal body wgt (g)	Kidneys rel.wgt (g/kg body wgt)	Liver rel.wgt (g/kg body wgt)	Caecum-F rel.wgt (g/kg body wgt)	Caecum-E rel.wgt (g/kg body wgt)
	14	14	14	14	14
14	256.0	7.42	34.8	44.3	6.2
16	271.2	7.26	35.5	40.4	5.6
26	239.0	6.44	35.7	34.1	5.8
28	260.2	7.11	38.8	36.7	6.7
Mean	256.60	7.060	36.22	38.87	6.07
SD	13.37	0.430	1.75	4.43	0.50
N	4	4	4	4	4

E = Excluded due to weighing error; the weight of the full caecum was not measured

Annexes

Annex 1: CoA test substance

Product	**: Vivinal FL**
Product code	: NA (developmental product)
Batchnumber	: MRS02
Date of production	: 02-07-2016
Contact person	: Jan-Willem Boots (R&D)

Description : Human milk oligosaccharide

Typical analysis : Dry matter 97%, moisture 3%, 2'-Fucosyllactose 94%, lactose 1%, glucose 1%, fucose 1%

Chemical/ physical:	**Specification**	**Results**	**Method of analysis**
Total moisture	max. 5%	3%	ISO 760 (modified), Karl Fischer
2'-Fucosyllactose	min. 90%	>94%	FC-method using HPAEC-PAD
3-Fucosyllactose	max. 3%	<1%	FC-method using HPAEC-PAD
Difucosyllactose	max. 3%	<1%	FC-method using HPAEC-PAD
Fucose	max. 2%	<1%	FC-method using HPAEC-PAD
Lactose	max. 2%	<1%	FC-method using HPAEC-PAD
Glucose	max. 2%	<1%	FC-method using HPAEC-PAD
Protein	max. 0.01%	0.002%	Bradford
Sulphated ash	max. 0.2%	0.06%	NEN 6810 (modified)
Nitrite	max. 1 mg/kg	<0.1	ISO 14673-2/IDF 189-2
Nitrate	max. 50 mg/kg	0.2	ISO 14673-2/IDF 189-2
pH (10%)	3.0 - 7.5	3.9	FC-method using NEN 3775

Microbiological:

Aerobic mesophilic count	max. 3000 cfu/g	<1000	FC-method equivalent to ISO 4833
Enterobacteriaceae	absent in 1 g	<1	FC-method, BPW 18h 37°C, SD, VRBG 18-24h 37°C
E. coli	absent in 1 g	<1	FC-method, LMX 25h, Coli ID 24h
Yeasts	max. 30 cfu/g	<1	FC-method equivalent to ISO 6611
Moulds	max. 30 cfu/g	<1	FC-method equivalent to ISO 6611
Presumptive Bacillus cereus	max. 100 cfu/g	<1	FC-method equivalent to ISO 7932
Staphylococcus aureus	absent in 1 g	<1	FC-method, G&C 42h 37°C, PCR
Sulphite reducing clostridia spores	max. 30 cfu/g	<1	FC-method using IJFM 27 (1995) 185-200 Weenk
Clostridium perfringens	absent in 1 g	neg	FC-method, RPM 20h 46°C, confirmation
Salmonella	absent in 1 g	neg	FC-method equivalent to ISO 6579
Cronobacter spp.	absent in 1 g	neg	FC-method equivalent to ISO/TS 22964

Wageningen, 15-07-2016

(b) (6)

Jan-Willem Boots

i/o Dianne Delsing

(b) (6)

PSB-en: (b) (6)

02 AUG. 2016

Dispense nr.: 160161

Annex 2: Cross reference list

20880/01 - 14-day oral (diet) dose-range finding study in male rats with 2'-fucosyllactose

Animal Number	Group	Cage	Sex
2	Control	2	M
4	Control	2	M
6	Low-dose	4	M
8	Low-dose	4	M
10	Mid-dose	6	M
12	Mid-dose	6	M
14	High-dose	8	M
16	High-dose	8	M
18	Control	10	M
20	Control	10	M
22	Low-dose	12	M
24	Low-dose	12	M
26	Mid-dose	14	M
28	Mid-dose	14	M
30	High-dose	16	M
32	High-dose	16	M

Annex 3: Analysis of the diet

Quality Control Certificate of Analysis

Product:	**VRF1 (P) VRF1 (FG)**
Premix Batch Numbers:	**18329**

Batch Number:	**2372**
Date of Manufacture:	**11.04.2016**
Expiry Date	**10.01.2017**

NUTRIENTS	Unit	Result	Tolerance Limits		Analysis Error (actual) or (%)	Limit of Quantification
			Min	Max		
Moisture	%	9.4	9.0	11.5	2.0	0.1 g/100g
Crude Fat (A)	%	5.2	3.8	6.2	16.4	0.1 g/100g
Crude Protein	%	19.4	17.4	20.4	1.9	0.1 g/100g
Crude Fibre	%	4.4	2.8	5.2	14.0	0.1 g/100g
Ash	%	5.8	4.5	7.0	2.7	0.1 g/100g
NFE (by difference)	%	55.8	48.0	60.0	n/a	n/a
Calcium	mg/kg	10500	8000	12000	6.5	5 mg/kg
Phosphorus	mg/kg	6010	4000	8300	7.5	2 mg/kg
Sodium	mg/kg	2750	2500	3500	8	10 mg/kg
Potassium	mg/kg	8770	5700	9700	6.9	50 mg/kg
Copper	mg/kg	20	13	25	18	0.6 mg/kg
Manganese	mg/kg	127	85	185	2.1	0.6 mg/kg
Vitamin A	iu/kg	31600	20000	55000	15	700 iu/kg
Vitamin E	mg/kg	112	80	150	10	1 mg/kg

CONTAMINANTS

Nitrogen Derivative Quality	Unit	Result	Tolerance Limits		Analysis Error (actual) or (%)	Limit of Detection
			Min	Max		
Nitrate	mg/kg	74	sum of NO_3 and NO_2 <500		13.8	5.0 mg/kg
Nitrite	mg/kg	Not detected	sum of NO_3 and NO_3 <500		25.0	6.0 mg/kg
Heavy Metal Quality						
Arsenic	mg/kg	0.10	-	1.00	7.7	0.002 mg/kg
Cadmium	mg/kg	0.14		0.25	17.8	0.001 mg/kg
Lead	mg/kg	0.11	-	1.50	17.4	0.005 mg/kg
Mercury	mg/kg	Not Detected	-	0.10	20.0	0.001 mg/kg

Annex 3: Analysis of the diet

Mycotoxin Quality

B1 Aflatoxin	µg/kg	<0.2	-	-	-	0.2 µg/kg
B2 Aflatoxin	µg/kg	<0.2	-	-	-	0.2 µg/kg
G1 Aflatoxin	µg/kg	<0.2	-	-	-	0.2 µg/kg
G2 Aflatoxin	µg/kg	<0.2	-	-	-	0.2 µg/kg
Total Aflatoxins (by HPLC)	µg/kg	<0.8	-	5.0	25.0	0.8 µg/kg each of B1, B2, G1, G2

Microbiological Quality

Entero Bacteriaceae	cfu/g	<5	-	5.0	-	5 cfu/g
Escherichia Coli	cfu/g	None Detected	-	None Detected	-	5.0 cfu/g
Fungal Units	cfu/g	40	-	1000	-	10.0 cfu/g
Salmonellae Species	cfu/g	None Detected	-	None Detected	-	Absent in 25g
Total Viable Organisms	cfu/g	<10	-	100000	-	10.0 cfu/g

Miscellaneous Quality

Antibiotic Activity M. luteus S. aureus B. subtilis		Non Detected	-	None	-	-
Selenium	µg/kg	266	-	600	13.8	20.0 µg/kg

Pesticides	Unit	Result	Tolerance Limits		Analysis Error (actual) or (%)	Limit of Detection
			Min	Max		
Total P.C.B.	µg/kg	Not Detected	-	<50.0	-	10 µg/kg
Toal D.D.T.	µg/kg	Not Detected	-	sum<50.0	-	1.0 µg/kg
Dieldrin	µg/kg	Not Detected	-	<20.0	-	1.0 µg/kg
Lindane	µg/kg	Not Detected	-	<100	-	1.0 µg/kg
Heptachlor	µg/kg	Not Detected	-	sum<10.0	-	1.0 µg/kg
Malathion	µg/kg	Not Detected	-	<5000	-	20.0 µg/kg

Notes:
The results are in line with expected values.

SDS AUTHORISATION		
Signed	(b) (6)	(b) (6)
Dated	06/06/2016	06/06/2016
Name	Myriam Lunn	Penny Buttling
Position	Quality Services Manager	Senior Nutritionist

Annex 4: Analysis of drinking water

Results of periodical analyses in drinking water collected on the premises of the test facility.

Drinking water was sampled and analysed by the local waterworks (Vitens). The samples were collected on 19 May 2016 (08:40 hr) in room number 05.1.13 at TNO Triskelion, Utrechtseweg 48, Zeist.
The results presented in the table below were reported by Vitens on 25 May 2016

Parameter	Unit	Result
Temperature in situ	°C	18,2
Odour (semi-quantitative)[1,2]		2
Taste (semi-quantitative)[1,2]		1
pH		8.05
Electrical conductivity (20°C)	mS/m	24,2
Turbidity	FTU	<0.1
Oxygen	mg/l O_2	11.1
Nitrite	mg/l NO_2	<0.01
Nitrate	mg/l NO_3	7.80
Ammonia	mg/l NH_4	<0.03
Cadmium	µg/l	<0.10
Lead	µg/l	<0.5
Copper	µg/l	79,9
Iron	mg/l	<0.01
Manganese	mg/l	<0.005
Total Organic Carbon (Non Purgeable Organic Carbon)	mg/l C	<0.5
Coli bacteria (37°C)	#/100 ml	<1
Escherichia coli (37°C)	#/100 ml	<1
Aeromonas bacteria (30°C)	#/100 ml	<1
Plate count (22°C)	#/ml	1

[1] Remark: The expiration date for the determination of odour and taste was exceeded. This may have increased the inaccuracy of the measurement.
[2] This observation was evaluated by Vitens as 'No abnormal change'.

Conclusion:
The above parameters meet the requirements of the Dutch Drinking Water Act.

Annex 5: Listing of clinical signs

The clinical signs listed below are derived from the lexicon which is part of the computer programme used for the recording of clinical observations

RESPIRATION
Sniffing
Grunting
Increased rate
Decreased rate
Irregular
Dyspnea
Shallow
Sneezing
Mouth breathing

GENERAL
Thin
Emaciated
Obese
Weakened
Unconscious
Pale
Red
Jaundice
Cyanosis
Warm
Cold
Dehydrated
Increased muscle tension

MOUTH
Malocclusion of incisors
Lower incisors light color
Lower incisors white
Upper incisors light color
Hemorrhagic discharge
Salivation
Stomatitis
Wart-like lesion(s)
Encrustation(s)
Chewing movement

ABDOMEN
Distension
Tense/firm
Blue/grey
Nodule(s)
Umbilical hernia

FAECES
Increased defecation
Decreased defecation
Hard
Soft
Diarrhea
Pale
Hemorrhagic
Black

BEHAVIOUR
Muscle weakness
Lethargic
Hunched posture
Excessive scratching
Hyperactive
Hypoactive
Aggressive
Stereotypy
Tremors
Convulsions
Ataxia
Circling movements
Vomiting
Vocalization
Chattering
Excessive grooming
Prone position
Myoclonic jerks

SKIN/FUR
Alopecic area(s)
Sparsely haired area(s)
Piloerection
Soiled fur
Depigmented fur
Edema
Abscess(es)
Pimple(s)
Subcutaneous nodule(s)
Erythema
Scaly
Hematoma
Hematoma iatrogenic
Encrustation(s)
Wound(s)
Shaving wound(s)
Scar tissue
Sc. color inj. site
Color ventral of inj. site
Red iatrogenic
Scaly iatrogenic

INJECTION SITE
Small nodule
Small red sc nodule
Redness
Swollen
Warm
Shaving wound/encrustation
Hematoma sc
Red nodule with white core
Red sc nodule with wound

HEAD
Tilted
Local/general swelling
Trimmed whiskers
Erythema between ears

NOSE
Encrustation(s)
Wound
Hemorrhagic discharge
Discharge-other than red
Crooked
Swollen
Itching
Skin protrusion

EYES
Discharge
Encrustation(s)
Blepharospasm
Blepharitis
Redness conjunctivae
Microphthalmia
Macrophthalmia
Exophthalmus
Dark red
Pale
Corneal opacity/keratitis
Cataract
Panophthalmitis
Complete degeneration
Protruding nictitant

EARS
Encrustration(s)
Wound(s)
Ear canal greased
Ear canal hemorrhagic
Hematoma iatrogenic
Necrotizing ear pinna
Ear pinna (partly) gone
Nodule
Swollen
Erythema

PENIS
Prolapse
Purulent discharge
Hemorrhagic discharge
Swollen preputium

PERINEUM
Soiled with urine
Soiled with feces
Soiled with blood
Erythema
Vaginal blood
Vaginal pus
Vaginal occlusion
Membrane present
Prolapsus ani -et recti
Vulva red
Vulva swollen
Vulva nodule

EXTREMITIES (LEG(S))
Encrustation(s)
Wound(s)
Swollen leg
Broken leg
Leg(s) gone
Stiffness
Muscle weakness
Lameness
Hard skin
Pododermatitis
Swollen toe(s)
Toe(s) gone
Nail(s) gone
Popliteal lymph node enlarged

TAIL
Ringtail
Kink
(Partially) discolored
Encrustation(s)
Wound(s)
Scaly
Local thickening
Tip of tail missing
Short and thick

TESTES
Cryptorchidism
Small
Large
Firm
Soft

URETHRA
Urethritis

URINE
Hematuria

Abbreviations: inj. site = injection site sc = subcutaneous