

Nutrition & Biosciences  
Danisco USA, Inc.  
3329 Agriculture Drive  
Madison, WI 53716  
800-255-6837



# 865



May 28, 2019

U.S. Food and Drug Administration  
Center for Food Safety and Applied Nutrition  
Office of Food Additive Safety  
HFS-200  
5100 Paint Branch Parkway  
College Park, MD 20740-3835

Re: GRAS Notice – Exemption Claim for *Lactobacillus acidophilus* NCFM® use in Infant Formula

Dear Office of Food Additive Safety:

Please accept the enclosed documentation in electronic format, submitted as notice of a GRAS exemption claim for the use of the live microbial culture *Lactobacillus acidophilus* NCFM in non-exempt infant formulas and toddler formulas.

For your consideration, we present a comprehensive GRAS assessment, concluding that *L. acidophilus* NCFM is Generally Recognized as Safe for this intended use in accordance with the August 17, 2016 Final Rule (81 FR 54959).

Please note that the GRAS status of this substance has been previously notified by Danisco USA, Inc. (GRN 357) covering its intended use as an ingredient in foods, including certain dairy products, functional beverages, nutritional powders, juices, bars, ready-to-eat breakfast cereals, chewing gum and confections, with an FDA Response of no questions. The enclosed documentation comprises a fresh, comprehensive assessment of safety in infant populations and does not incorporate information from GRN 357. It is therefore being submitted as a new GRAS notice, rather than an amendment.

Please do hesitate to contact me at any time to discuss details or to request supplemental information as needed.

Thank you for your time and consideration.

Sincerely,

A grey rectangular box redacting the signature of Elizabeth McCartney.

Elizabeth McCartney  
DuPont Nutrition & Biosciences  
Phone: 608-395-2661  
Fax: 608-395-2603  
Email: [elizabeth.mccartney@dupont.com](mailto:elizabeth.mccartney@dupont.com)

**FDA USE ONLY**

GRN NUMBER 000865	DATE OF RECEIPT May 29, 2019
ESTIMATED DAILY INTAKE	INTENDED USE FOR INTERNET
NAME FOR INTERNET	
KEYWORDS	

DEPARTMENT OF HEALTH AND HUMAN SERVICES  
Food and Drug Administration

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

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

1. Type of Submission (*Check one*)

New       Amendment to GRN No. \_\_\_\_\_       Supplement to GRN No. \_\_\_\_\_

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

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

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

	Name of Contact Person Elizabeth McCartney	Position or Title Regulatory Affairs Specialist
	Organization ( <i>if applicable</i> ) Danisco USA, Inc. (DuPont Nutrition & Biosciences)	
	Mailing Address ( <i>number and street</i> ) 3329 Agriculture Drive	

City Madison	State or Province Wisconsin	Zip Code/Postal Code 53716	Country United States of America
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Telephone Number 608-395-2661	Fax Number 608-395-2603	E-Mail Address elizabeth.mccartney@dupont.com
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	Name of Contact Person	Position or Title
	Organization ( <i>if applicable</i> )	
	Mailing Address ( <i>number and street</i> )	

City	State or Province	Zip Code/Postal Code	Country
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Telephone Number	Fax Number	E-Mail Address
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## SECTION C – GENERAL ADMINISTRATIVE INFORMATION

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

Lactobacillus acidophilus NCFM®

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

Electronic Submission Gateway  Electronic files on physical media

Paper

If applicable give number and type of physical media

One DVR

3. For paper submissions only:

Number of volumes \_\_\_\_\_

Total number of pages \_\_\_\_\_

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

Yes (Proceed to Item 5)  No (Proceed to Item 6)

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

a) GRAS Notice No. GRN \_\_\_\_\_

b) GRAS Affirmation Petition No. GRP \_\_\_\_\_

c) Food Additive Petition No. FAP \_\_\_\_\_

d) Food Master File No. FMF \_\_\_\_\_

e) Other or Additional (describe or enter information as above) \_\_\_\_\_

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

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

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

Yes (Proceed to Item 8)

No (Proceed to Section D)

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

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

No

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

Yes, a redacted copy of the complete submission

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

No

## SECTION D – INTENDED USE

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

*L. acidophilus* NCFM is intended to be used in non-exempt infant formulas and toddler formulas. It is intended to be added to infant and toddler formulas at a level of  $10^8$  cfu/gm which will ensure at least  $10^6$  cfu/gm throughout the shelf-life of the product. *L. acidophilus* NCFM is intended to act as a probiotic microorganism.

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

(Check one)

Yes  No

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

(Check one)

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



**SECTION E – PARTS 2 -7 OF YOUR GRAS NOTICE**

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

- PART 2 of a GRAS notice: Identity, method of manufacture, specifications, and physical or technical effect (170.230).
- PART 3 of a GRAS notice: Dietary exposure (170.235).
- PART 4 of a GRAS notice: Self-limiting levels of use (170.240).
- PART 5 of a GRAS notice: Experience based on common use in foods before 1958 (170.245).
- PART 6 of a GRAS notice: Narrative (170.250).
- PART 7 of a GRAS notice: List of supporting data and information in your GRAS notice (170.255)

**Other Information**

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

Yes  No

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

Yes  No

**SECTION F – SIGNATURE AND CERTIFICATION STATEMENTS**

1. The undersigned is informing FDA that Elizabeth McCartney  
*(name of notifier)*

has concluded that the intended use(s) of Lactobacillus acidophilus NCFM\*  
*(name of notified substance)*

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

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

Danisco USA, Inc. 3329 Agriculture Drive Madison, WI 53716  
*(address of notifier or other location)*

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

3. Signature of Responsible Official, Agent, or Attorney	Printed Name and Title Elizabeth McCartney - Regulatory Affairs Specialist	Date (mm/dd/yyyy) <u>05/28/2019</u>
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**SECTION G – LIST OF ATTACHMENTS**

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

Attachment Number	Attachment Name	Folder Location (select from menu) (Page Number(s) for paper Copy Only)
1	CoverLetter_L.acidophilusNCFMInfant_2019-05-28.pdf	Administrative
2	GRASNotice_L.acidophilusNCFMInfant_2019-05-28.pdf	Submission

**OMB Statement:** Public reporting burden for this collection of information is estimated to average 170 hours per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to: Department of Health and Human Services, Food and Drug Administration, Office of Chief Information Officer, [PRASStaff@fda.hhs.gov](mailto:PRASStaff@fda.hhs.gov). (Please do NOT return the form to this address). An agency may not conduct or sponsor, and a person is not required to respond to, a collection of information unless it displays a currently valid OMB control number.



# **COMPREHENSIVE GRAS ASSESSMENT**

**of**

***Lactobacillus acidophilus* NCFM**

**Food Usage Conditions for  
General Recognition of Safety**

**for**

**Danisco USA, Inc.**

**4/16/2019**

**LSRO Solutions, LLC  
2286 Dunster Lane  
Rockville, MD 20854-6112  
240-292-9445**

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## II. FOREWORD

Ingredients for use in foods must undergo premarket approval by the U.S. Food and Drug Administration (FDA) as food additives or, alternatively, the ingredients to be incorporated into foods must be determined to be Generally Recognized as Safe (GRAS). In 1997, FDA altered the GRAS determination process by eliminating the formal GRAS petitioning process and replacing the petitioning process with a notification procedure. While outlining the necessary content to be considered in making a GRAS determination, FDA encouraged that such determinations be provided to FDA in the form of a notification. However, notifying FDA of such determinations is strictly voluntary.

At the request of Danisco USA, Inc. (dba DuPont Nutrition and Health) , LSRO Solutions, LLC (“LSRO”) has undertaken an independent safety evaluation of *Lactobacillus acidophilus* NCFM, ATCC SD5221 (“*L. acidophilus* NCFM”) to ascertain whether or not the intended use as described herein can be considered to be GRAS based on scientific procedures as defined by 21 CFR 170.3(o)(20).

Danisco USA, Inc. (“Danisco”) provided background information addressing the safety/toxicity of *L. acidophilus* NCFM; the intended food uses; and compositional details, specifications, and methods of preparation. Danisco was asked to include adverse reports, as well as those that support conclusions of safety. Determining how much *L. acidophilus* NCFM can be safely consumed, i.e., the use levels, is critical in the determination of safe exposure levels for *L. acidophilus* NCFM when consumed as a food ingredient. The composite safety/toxicity studies, in concert with exposure information, ultimately provide the specific scientific foundation for the GRAS evaluation.

The safety/toxicity studies, consumption/exposure information, and other related documentation were augmented with an independent search of the scientific and regulatory literature conducted by LSRO Solutions, LLC, through January, 2019 and summarized in this dossier. A GRAS assessment was developed based on publicly available safety information. Pertinent references are listed in Section VIII.H.

### III. PART 1. SIGNED STATEMENTS AND CERTIFICATIONS

#### Claim of Exclusion From the Requirement for Premarket Approval <sup>1</sup>

Danisco has concluded that the proposed uses of *L. acidophilus* NCFM that meet the specifications described herein are GRAS in accordance with Section 201(s) of the Federal Food, Drug, and Cosmetic Act. This conclusion was made in concert with a panel of experts (GRAS Panel) who are qualified by scientific training and experience. The GRAS determination is based on scientific procedures as described in the following sections. The evaluation accurately reflects the conditions of the intended uses in non-exempt infant formula.

To the best of our knowledge, this determination is a complete, representative, and balanced submission that includes unfavorable information, as well as favorable information, known to us and pertinent to the evaluation of the safety and GRAS status of the uses of this ingredient in food.

**Signed:**



Michael C. Falk, Ph.D.  
LSRO Solutions LLC  
2286 Dunster Lane  
Rockville, MD 20854-6112

**Date:** XXX

(i) Name and Address of Notifier

Danisco USA, Inc.  
Four New Century Parkway  
New Century, KS 66031

As the sponsoring party, Danisco accepts responsibility for the conclusion of GRAS status that has been made for *L. acidophilus* NCFM as described in the subject claim. Consequently, *L. acidophilus* NCFM is exempt from premarket approval requirements for food ingredients.

(ii) Common Name and Identity

*Lactobacillus acidophilus* strain NCFM, ATCC SD5221 ("*L. acidophilus* NCFM")

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<sup>1</sup> Pursuant to 21 CFR 170.35(c(1)); see 81 FR 54960, 17 August 2016:  
Accessible at <https://www.gpo.gov/fdsys/pkg/FR-2016-08-17/pdf/2016-19164.pdf>



### (iii) Conditions of Intended Uses in Food

*L. acidophilus* NCFM is intended to be used in non-exempt infant formulas and toddler formulas. It is intended to be added to infant and toddler formulas at a level of  $10^8$  cfu/gm which will ensure at least  $10^6$  cfu/gm throughout the shelf-life of the product. *L. acidophilus* NCFM is intended to act as a probiotic microorganism.

### (iv) Basis for GRAS Conclusion

Pursuant to 21 CFR 170.30, conclusions of the GRAS status for *L. acidophilus* NCFM, as described herein, have been based on scientific procedures as discussed in the detailed description provided below.

### (v) Availability of Information

The data and information forming the basis for this GRAS determination and the exemption claim asserted herein are available for FDA review and copying during customary business hours at the following address, or will be sent to FDA either in an electronic format that is accessible for FDA evaluation or on paper, upon request to:

Elizabeth McCartney, Regulatory Affairs  
DuPont Nutrition & Biosciences  
3329 Agriculture Drive  
Madison, WI 53716  
Tel: 608-395-8408  
Elizabeth.mccartney@dupont.com

### (vi) Disclosure

No data or information contained in parts 2 through 7 of this GRAS notice are exempt from disclosure under the Freedom of Information Act, 5 U.S.C. 552.

### (vii) Trade secrets

This document does not contain any trade secrets or confidential information that would need to be submitted, as required by §170.270, to the Food Safety Inspection Service (FSIS) of the U. S. Department of Agriculture.

### (viii) Complete submission

We certify that, to the best of our knowledge, this GRAS notice for *L. acidophilus* NCFM in foods is a complete, representative, and balanced submission that includes unfavorable information, as well as favorable information, known to us and pertinent to the evaluation of the safety and GRAS status of the uses of the substance.

## IV. PART 2. IDENTITY, MANUFACTURE, SPECIFICATIONS AND EFFECT

### A. Name of the GRAS Organism

*Lactobacillus acidophilus* strain NCFM preserved in the ATCC database both as ATCC 700396 and in the safe deposit of the ATCC as deposit number SD5221 and also in the German Collection of Microorganisms and Cell Cultures as DSM22091, is a member of the *Lactobacillaceae* family.

Multiple strain designations appear in the literature for *L. acidophilus* NCFM<sup>®</sup> or for single colony isolates of the NCFM<sup>®</sup> parent culture. The designations NCFM<sup>®</sup>, N2, NCK56, NCK45, N2 and RL8K are essentially identical strains. The parent NCFM<sup>®</sup> culture carried the NCSU laboratory designation RL8K and was composed of rough and smooth variants (mixed parent culture). These variants were designated RL8K-R (bile sensitive) and RL8K-S (bile resistant) upon isolation from the RL8K culture (Klaenhammer and Kleeman, 1981). N2 is a smooth, bile-resistant isolate from NCFM<sup>®</sup> selected by scientists at Marschall Products (now Danisco USA Inc.) as a bile-resistant colony, and this pure culture is used as the seed for all commercial production runs of NCFM<sup>®</sup>. The mixed parent culture has not been used commercially or for research studies since 1975. NCK56 is a Klaenhammer laboratory designation for N2, and NCK45 is a Klaenhammer laboratory designation for the parent culture NCFM<sup>®</sup> comprised of rough and smooth colony variants NCFM<sup>®</sup>. These different isolates cannot be differentiated genetically by pulsed field gel electrophoresis (Walker et al., 1996), a technique which provides an electrophoretic pattern of restriction enzyme digested chromosomal DNA. A purified isolate of the RL8K-S culture, designated ATCC700396, was subjected to chromosomal DNA sequencing (Altermann et al., 2005). In some papers involving NCFM<sup>®</sup>, the strain is not identified or NCFM<sup>®</sup> may be present only as one strain in a combination of several lactic acid bacteria. However, the source of the culture used is generally provided, indicating that the strain used was *L. acidophilus* from Marschall, Miles, Rhone-Poulenc, Rhodia Food, or Danisco. These different company names reflect changes in business structure or ownership of the company marketing NCFM<sup>®</sup>, but not in marketing rights. In some cases, commercial cultures provided to a study were coded to keep the identity of specific strains confidential. In studies conducted by Simenhoff and colleagues on small bowel bacterial overgrowth in chronic kidney failure patients, *L. acidophilus* NCFM<sup>®</sup> was abbreviated LBA. Any confusion over use of NCFM<sup>®</sup> in publications included in this document was clarified by personal communications with company representatives and researchers. In the United States, NCFM<sup>®</sup> is a registered trademark of the North Carolina Dairy Foundation.

NCBI Taxonomy ID: 47715

Kingdom: Bacteria

Phylum: *Firmicutes*

Class: *Bacilli*

Order: *Lactobacillales*

Family: *Lactobacillaceae*

Genus: *Lactobacillus*

Species: *acidophilus*

The entire genome of *L. acidophilus* NCFM has been sequenced (Altermann et al., 2005). The annotated sequence is available from GenBank under accession No. CP00033.

## B. Source of the GRAS Organism

*L. acidophilus* NCFM was isolated from the intestinal tract of a healthy human and characterized in the food microbiology laboratories at North Carolina State University (Gilliland et al., 1975).

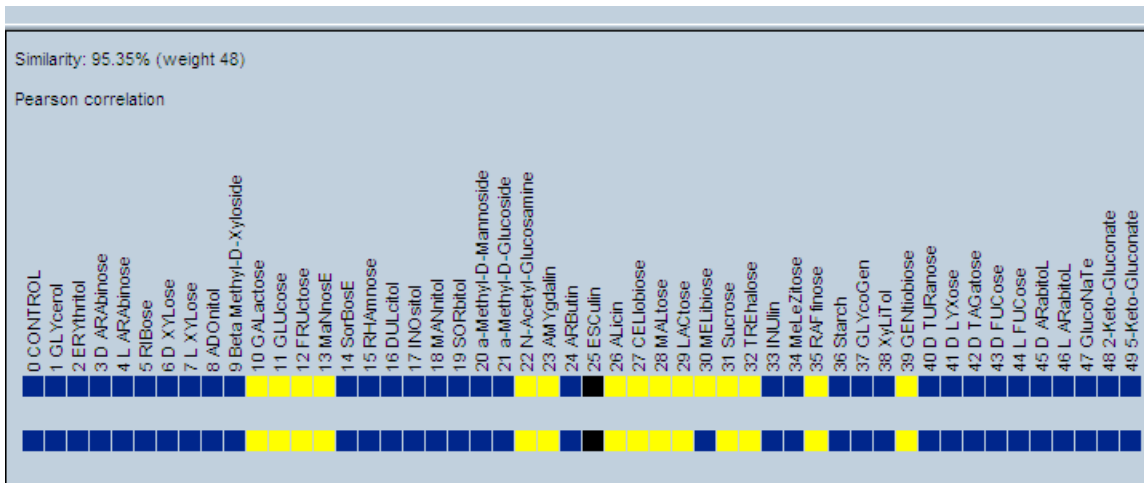
## C. Description of the GRAS Organism

## 1. Genotypic and Phenotypic Strain Identification

*L. acidophilus* NCFM<sup>®</sup> has been shown by several phenotypic (Gilliland and Speck, 1977) and genotypic (Kullen et al., 2000; Sanders et al., 1996) criteria to be a member of the type A1 *L. acidophilus* species. Hybridization with a species-specific oligonucleotide probe (5' TCTTCGATGCATCCACA 3'; 7) using slot blots provided further evidence that NCFM<sup>®</sup> belongs to the type A1 *L. acidophilus* group (Sanders et al., 1996). Sequencing of the 16S ribosomal RNA gene of NCFM<sup>®</sup> and La-14 confirmed its identity as *L. acidophilus* ((Sanders et al., 1996); internal Danisco documentation). Fermentation and growth characteristics of *L. acidophilus* NCFM<sup>®</sup> are similar to *L. acidophilus* La-14 (Figure 1). Fermentation and growth characteristics of NCFM<sup>®</sup> and the neotype strain ATCC 4356 are identical (Table 1). The NCFM<sup>®</sup> and La-14 DNA have a 34.7% GC ratio. Fermentation results in 34% D- and 66% L-lactic acid for both strains.

Employing optical mapping is an OPGEN technology (Madison, WI) to evaluate the restriction profiles, *L. acidophilus* NCFM was found to be >99.9% similar to *L. acidophilus* La-14 (data not shown). This demonstrates that the genomes are highly collinear and nearly identical in size and genetic content that provides a comparative genomic overview using restriction digests of chromosomes. The two *L. acidophilus* genomes have been further investigated using raw sequence information that confirmed that the strains are above 99.9% identical. Genetically, Danisco has been unable to detect difference in gene content. In contrast, Danisco has been able to detect differences in gene sequences, notably regarding a single SNP (single nucleotide polymorphisms) which results in an additional band via the Opgen technology.

**Figure 1 Fermentation characteristics of *L. acidophilus* NCFM compared to *L. acidophilus* La-14**



Adapted from (Danisco USA, 2010)



**Table 1 Growth and fermentation of *L. acidophilus* NCFM compared to neotype *L. acidophilus* strain**

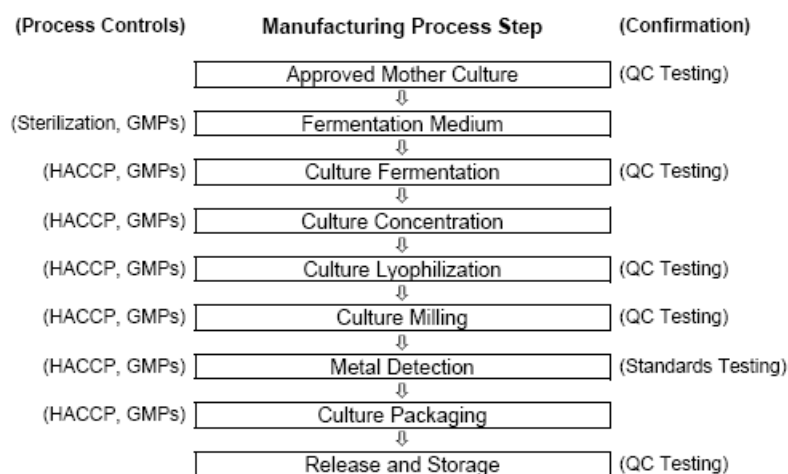
Characteristic	NCFM	<i>L. acidophilus</i> ATCC 4356 (Neotype)
Growth @ 15°C	–	–
Growth @ 45°C	+	+
Ammonia from arginine	–	–
Aesculin	+	+
Cellobiose	+	+
Galactose	+	+
Lactose	+	+
Maltose	+	+
Mannitol	–	–
Rhamnose	–	–
Salicin	+	+
Sorbitol	–	–
Sucrose	+	+
Trehalose	+	+

Adapted from (Sanders et al., 1996) and (Danisco USA, 2010)

#### D. Manufacturing Process

*L. acidophilus* NCFM<sup>®</sup> is manufactured in accordance with the U.S. Food & Drug Administration’s current Good Manufacturing Practices (cGMP) guidelines in an FDA regulated and inspected facility. The manufacturing process is summarized below.

**Figure 2 Manufacturing Flow Diagram**



## Culture

The DuPont manufacturing process for the production of cultures is a batch type fermentation process where a blend of proteins, carbohydrate, and vitamins and minerals are blended with water, sterilized, and then inoculated with the selected bacteria. Each fermentation product has a defined growth medium and fermentation growth conditions (e.g. pH, temperature).

*L. acidophilus* NCFM is manufactured in compliance with the U.S. Food and Drug Administration's current Good Manufacturing Practice guidelines (cGMP) in FDA regulated and inspected facilities. All ingredients utilized are food grade or approved for use by the FDA.

## Master Seeds

The source organism used is *L. acidophilus* NCFM. The cultures are maintained in the culture bank of DuPont as frozen 1mL vials at -80°C. DuPont independently verifies the identity of the culture in each vial. Each seed lot in the culture bank is fully characterized to ensure the identity of the seed strains. From the seed vials, DuPont produces concentrated starter for the industrial fermentation.

DuPont takes great care to ensure the quality of the product because the bacterial fermentation products produced by DuPont are destined to be either directly consumed or used as starter cultures for food fermentations such as yogurt manufacture. These quality control processes begin with the identification, storage and handling of the bacteria seed stocks.

Genus and species designation for each bacterial species have been determined by 16S rRNA testing. For identification on strain level, a specific DNA-fingerprinting technique is applied that ensures identity of the seed stocks. The fingerprinting technique is applied prior to preservation of every strain.

A Master Seed repository is maintained for each of the bacterial strains at the Danisco Global Culture Collection (DGCC) in Niebüll, Germany. The repository is a collection of purified, tested, and qualified Master Seed stocks derived from single strain isolates stored at -180°C in liquid nitrogen to maintain long term cell viability.

The microbiological quality of the Master Seeds is determined by testing for microbiological contamination at the DGCC, including Non-lactic count, *Enterobacteria*, *Enterococcus*, *Listeria*, *Salmonella*, *Micrococcus Staphylococcus*, Aerobic and anaerobic spore formers, and Yeast & Mold.

These identity and purity specifications are absolute acceptance criteria for the Master Seeds. If a Master Seed vial lot fails any of the required tests, the lot is placed on Quality Control (QC) hold to prohibit use and the lot is subsequently destroyed.

## Working Seeds

All Working Seeds are prepared under controlled conditions from Master Seed stock meeting established acceptance criteria (described above) and each new lot of Working Seeds is held in "quarantine" pending QC testing (strain identity and purity as described for the Master Seeds) and release. If the Working Seed vial lot fails any of the required tests, the lot is placed on QC hold and

destroyed. Qualified, tested Working Seed stocks are stored at -80°C until used in production fermentation.

The use of tandem Master and Working seed inventories reduces the risk of genetic drift over time due to excessive sub-culturing of strains and insures the integrity of the strain collection.

All steps in the preparation of Master and Working seed are documented in a specified database, allowing traceability of every seed preparation down to each single batch of raw material used.

### **Fermentation Process**

The fermentation process takes place in the DuPont manufacturing facilities in Madison, WI or Rochester, NY. The fermentation begins by withdrawing one of the working seed vials and scaling-up via a series of fermentations until a commercial size batch is complete. The fermentation starts off in a 100mL vessel and is then incrementally transferred to larger size vessels, which can be as large as a 45,000 L fermentation vessel.

*L. acidophilus* NCFM has an optimum pH and temperature for ideal growth. As each organism produces organic acids during metabolism, an ammonium hydroxide base must be injected into the medium to maintain pH at the proper set point in order to maintain the optimum pH during growth.

The fermentation production process of each is a closed system with no product exposure from seed inoculation to cell harvest. Prior to each fermentation batch, all mixing tanks, heat exchangers, lines, fermenters and centrifuges are cleaned via automated clean-in-place systems. Systems are then either steamed or chemically sanitized prior to product contact.

At the manufacturing facility, there are two methods to measure growth in the fermenter. First, flow meters on the ammonium hydroxide feed lines to the fermenters measure the volume of base used to maintain optimum growth pH of the culture. The base addition rate is proportional to the acid developed in the fermentation, which is proportional to cell growth rates.

Second, the pH in the fermenter is monitored on digital display and on recording charts. By consulting these charts, the growth characteristic of a given fermentation can be determined.

Fermenters are normally cooled to stop the fermentation when the pH and base addition data indicate that the fermentation has entered stationary phase. Cooled fermentate is pumped through continuous flow centrifuges and the bacteria are concentrated. Cryoprotectant (a blend of sugars and inorganic phosphate) is added to cooled concentrate and the mixture is then pelletized by immersion of concentrate droplets in liquid nitrogen. These concentrate pellets are then freeze-dried.

### **Milling Process**

The milling process takes place entirely in the DuPont Madison facility. The freeze-dried pellets are milled according to standard procedures utilizing a Fitzpatrick mill fitted with a mesh screen operating at 2000 rpm. Production batch records contain mill charge (blending speed) and appropriate operator sign-off.

## Blending Process

The blending process is performed in the DuPont plant under 21 CFR 111 cGMPs. Blending can occur by either blending in Marion and/or V-blender mixers, or by utilizing Intermediate Bulk Containers (IBCs). The processes are slightly different, but are used interchangeably depending on available resources.

Milled pellets, along with approved excipients, are added to the blender. All ingredients added to the blender, both milled pellets and excipients, are documented on production batch record containing traceability information and appropriate operator sign off. Milling and ingredient addition is performed in a controlled environment.

The blender is allowed to mix for an established amount of time prior to packaging to ensure homogeneity. Product is dispensed out of blender and through metal detector prior to packaging.

## Packaging

Bulk packaging of the product is carried out in a controlled environment. The HVAC system consists of an air-handling unit with air-cooled direct expansion type condenser including ducted heater for reheating. Pressure relief dampers operate in conjunction with the fresh air intake system maintaining the whole area at a positive pressure to prevent contaminant infiltration to the packaging room. The area design conditions are as follows:

Dry Bulb Temperature	72° F
Relative Humidity	≤ 35% RH

HEPA filter is used in the packaging room as the final filter for particulate removal in these demanding operating conditions.

Quality Systems

## Quality Systems

The DuPont Madison and Rochester plants have fully implemented HACCP plans, Standard Operating Procedures and Quality Control programs to ensure the quality of each product. DuPont Madison has numerous certifications, including ISO FSSC 22000 food safety certification, ISO 9001 Quality Management System certification, and NSF Dietary Supplements cGMP certification. Danisco Rochester maintains ISO 9001 Quality Management System certification and ISO FSSC 22000 Food Safety System certification.

Quality control laboratories are maintained on site. Quality control personnel are qualified by training and experience to test products and to release product based on specifications. In addition, a third-party laboratory with ISO 17025 certification, located in Madison WI, performs QC testing for DuPont under contract. The Quality Control unit utilizes a SAP quality module (Inventory Control System) for the specification, quality control data entry and product release. No product can be released for use without acceptance by the Quality Control unit according to specified acceptance criteria.

Each bacteria fermentation product must meet specifications and must have a confirmation of identity (compared to the Master Seed) by 16S rRNA sequence analysis or RiboPrinter analysis. Microbiological

testing is performed by trained QC microbiologists in the Madison plant laboratory and certified external laboratory using standard methods.

Cleaning verification and quality testing of the process rooms and equipment are under the control of Manufacturing and Quality Assurance, following the established SOPs. Fermentation rooms are isolated from the freeze-drying processes and access is controlled. Materials cannot enter the milling and blending process areas prior to cleaning, sanitation and subsequent cleaning verification via ATP testing. Room access is controlled by appropriate signage, and additional protective gowning must be worn in processing rooms where product is potentially exposed. Operator sign-off for cleaning, sanitation and testing is required on the production batch record. Quality Assurance is responsible for review of completed batch tickets.

Process rooms are segregated from other manufacturing areas with appropriate closures. Room air quality is controlled via HEPA air filtration of incoming air and maintenance of positive pressure in the process rooms relative to adjacent processing areas. HEPA filtration operation is monitored for performance; air quality is monitored monthly by Quality Assurance. Operators may not bring materials into process areas where HEPA filtration and positive pressure is not functioning to specification. Operators sign-off on the production batch record for temperature and humidity and record the temperature and humidity on the batch ticket. Quality Assurance is responsible for review of completed batch tickets.

Rooms and equipment used in manufacturing are approved for production only after cleaning, sanitization and quality inspection. Prior to verification of the process room for production, as specified in the appropriate SOP, the blending room is sprayed from ceiling to floor with 145-160°F water. All large equipment having any product contact surfaces is thoroughly scrubbed / foamed with a neutral detergent cleaner, rinsed with cold water, sanitized with an acid/iodine based sanitizer and re-rinsed with cold water. The floor is sanitized with acid/iodine sanitizer.

Process rooms and equipment are tested by Quality Assurance following cleaning and sanitation for microbial contamination and test results are documented with Quality Assurance sign-off. ATP and Microbiological swabs are taken after cleaning and sanitation. Room and equipment surfaces must be negative by test in order to qualify for use in production. Batch records are maintained as per Standard Operating Procedures and are provided to Quality Assurance for each batch produced. Quality Assurance is responsible for production batch record review

## 1. Product Specifications

**Table 2 Product Specifications for Danisco's *L. acidophilus* NCFM**

Parameter	Specification	Method
<b><u>Appearance</u></b>		
Form <sup>1</sup>	Freeze-dried powder	Visual
Color <sup>1</sup>	White to cream-colored	Visual
Particle size <sup>2</sup>	40 mesh	Fitzmill Screen
Viable cell count <sup>3</sup>	>2.0 x 10 <sup>11</sup> cfu/g	ISO 7889/IDF 117
<b><u>Heavy Metals<sup>4</sup></u></b>		
Arsenic	< 1.0 ppm	AOAC 984.27
Lead	< 0.5 ppm	AOAC 984.27
Cadmium	< 0.2 ppm	AOAC 984.27
Mercury	< 0.05 ppm	EPA 7471
<b><u>Microbial Specifications<sup>5</sup></u></b>		
<i>Enterococci</i> (cfu/g) <sup>3</sup>	≤ 100 cfu/g	SMEDP, 17 <sup>th</sup> ed
Non-lactic Cell Count <sup>3</sup>	≤ 5000 cfu/g	ISO 13559
Coliform (MPN) <sup>3</sup>	< 10 cfu/g	AOAC 966.24
<i>Escherichia coli</i> (MPN) <sup>3</sup>	Negative by test (< 0.3/g)	AOAC 966.24
<i>Salmonella</i> <sup>3</sup>	Negative by test in 40 g	AOAC 2004.03
<i>Listeria monocytogenes</i> <sup>3</sup>	Negative by test in 25 g	AOAC 999.06
<i>Chronobacter sakazakii</i> <sup>6</sup>	Negative by test in 10 g	AOAC 2018.1

<sup>1</sup> Specification provided on Product Description sheet, not listed on Certificate of Analysis or Batch Analysis summary

<sup>2</sup> Internal Specification recorded in Batch Record

<sup>3</sup> Specification reported on Certificate of Analysis

<sup>4</sup> Specification provided on Heavy Metal Statement, not listed on Certificate of Analysis or Batch Analysis summary

<sup>5</sup> Yeast and mold are Internal Specification only. They are tested on bulk intermediate powder, not reported on COA

<sup>6</sup> *Chronobacter sakazakii* is an Internal Specification only.

## 2. Batch Analysis

Certificates of analysis of 4 non-consecutive batches of finished product are included in Appendix A. These indicate that the manufacturing process consistently meets product specifications and is not



contaminated with heavy metals. The heavy metal analysis is not included in the Certificate of Analysis and is evaluated separately as part of a routine surveillance testing program. Danisco certifies that this product complies with the FCC specifications: Arsenic < 1ppm, Lead < 0.5ppm, Mercury < 0.05 ppm, and Cadmium < 0.2 ppm (Appendix A). Molds and yeast are evaluated separately as part of the routine inspection plan.

**Table 3 Analysis of Production Batches of Danisco's *L. acidophilus* NCFM**

Parameter	Specification	Batch				
Viable cell count (cfu/g)	>2.00 x 10 <sup>11</sup>	3.31 x 10 <sup>11</sup>	3.73 x 10 <sup>11</sup>	3.21 x 10 <sup>11</sup>	3.83 x 10 <sup>11</sup>	3.65 x 10 <sup>11</sup>
<i>Enterococci</i> (cfu/g)	≤ 100	< 100	< 100	< 100	< 100	< 100
Non-lactic Cell Count <sup>3</sup>	≤ 5000	< 5000	< 5000	< 5000	< 5000	< 5000
Coliform (MPN)	< 10	10.0	10.0	10.0	10.0	10.0
<i>Escherichia coli</i> (MPN) by test	Negative (< 0.3/g)	Negative	Negative	Negative	Negative	Negative
<i>Staphylococcus</i> (coagulase +) by test	Negative (<10/g)	Negative	Negative	Negative	Negative	Negative
<i>Salmonella</i>	Negative in 40 g	Negative	Negative	Negative	Negative	Negative
<i>Listeria monocytogenes</i>	Negative in 25 g	Negative	Negative	Negative	Negative	Negative

### 3. Stability Testing

Stability testing is done on a regular basis by Danisco Innovation.

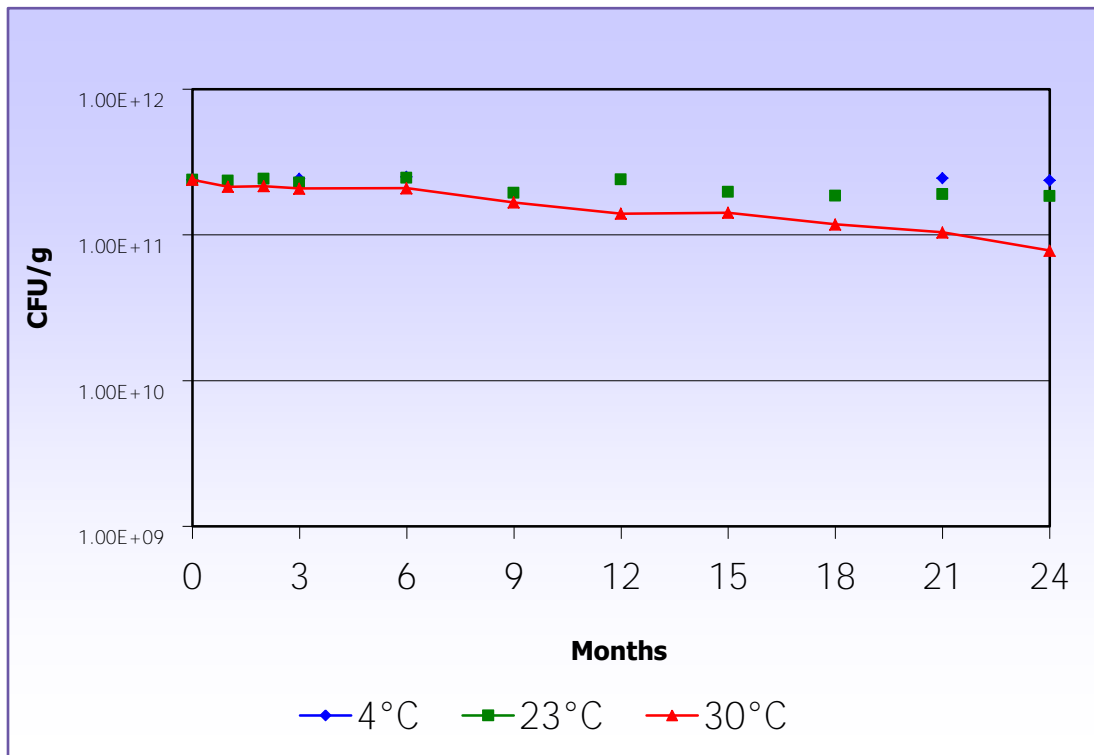
To determine the stability of *L. acidophilus* NCFM® freeze-dried powder, a sample is obtained from our production facility. The product is then packaged into individual foil sachets for each time point and temperature. The foil sachets are made of the same material which is used for packaging at the production facility. The sachets are stored in incubators at fixed temperatures of a walk-in cooler. These units are at ambient humidity.

At each time point, sachets are pulled from each temperature and enumerated according to the Danisco Quality Control Enumeration Method.

The resulting stability graph gives an idea of the survival of *L. acidophilus* NCFM<sup>®</sup> over two years at each respective temperature. The water activity of the freeze-dried powder is typically below 0.1. pH does not generally have an effect on freeze-dried products.

At room temperature, freeze-dried NCFM had 77% recovery after 2 years storage. This is excellent stability for a probiotic powder. At 30°C storage, the same powder had 33% recovery. This is very good stability for a probiotic powder. This type of stability allows the deliverability of a target amount of live culture throughout shelf life of the final food product.

Figure 3 Two year stability study for NCFM



#### 4. Antibiotic Resistance and Virulence

Although there is negligible concern for translocation, toxigenicity, or any adverse nutritional activity from consumption of *L. acidophilus* NCFM<sup>®</sup>, the presence of transferable antibiotic resistance genes must also be assessed. Although the presence of such genes does not in itself comprise a risk (an antibiotic resistant *Lactobacillus* is not a pathogen), there is concern that cultures which carry transferable antibiotic resistance genes may transfer these genes to less innocuous members of the commensal microbiota in vivo. Genomic sequencing did not detect any known antibiotic resistance genes in NCFM<sup>®</sup> (Altermann et al., 2005). The Minimum Inhibitory Concentration (MIC) was assessed by exposing *L. acidophilus* NCFM in triplicate to antibiotics in broth microdilution cultures according to the testing procedure recommended by EFSA (EFSA, 2012). The antibiotic sensitivity is displayed in Table 4. *L. acidophilus* NCFM did not demonstrate resistance at levels exceeding the breakpoints set by EFSA (Morovic et al., 2017)

*Lactobacillus acidophilus* are listed as Biosafety Level 1 organisms by the American Biological Safety Association, indicating that they are unlikely or not associated to cause disease in healthy human adults. (<http://www.absa.org/>).

**Table 4 Antibiotic Resistance Profile**

Antibiotic	Breakpoint	NCFM
	MIC (µg/mL)	
Gentamycin	16	2
Kanamycin	64	32
Streptomycin	16	2
Tetracycline	4	2
Erythromycin	1	0.06
Clindamycin	1	0.5
Choramphenicol	4	4
Ampicillin	1	0.5
Vancomycin	2	<0.25
Virginamycin	4	0.5

In addition to surveying annotations in the RAST subsystems Phages, Prophages, Transposable elements, Plasmids (PPTP), and Virulence, Disease, and Defense (VDD), ATCC SD551 were compared by custom BLAST in Geneious to the following public databases:

- MVIRdb. Microbial Virulence Database; <http://mvirdb.llnl.gov/>; (Zhou et al., 2006).
- DBETHdb. Database of Bacterial ExoToxins for Humans; <http://www.hpppi.icb.res.in/btox/>.
- ARDB. Antibiotic Resistance Genes Database; <https://ardb.cbcb.umd.edu/>(Liu and Pop, 2008)

The nucleotide and protein sequences of ATCC SD551 annotations were compared to all these databases. Results that match at least 35% of sequence identities in an 80 amino acid sliding window were considered suspect and analyzed further by BLASTp in both the NCBI and UniProt for protein function. Searches from NCBI collections were refined based on target, as the searches can broadly incorporate elements that are not related to the query (for example, if “bacteriocin” is in the title of the reference).

Although genomic analysis identified partial sequence homolog to known virulent gene families, further analysis revealed no elements known to be harmful to humans (Morovic et al., 2017).

## 5. Biogenic Amines

PCR-based analysis of the *L. acidophilus* NCFM genome did not detect the *hdc* or *tdc*, the histidine and tyrosine decarboxylase genes, respectively (Morovic et al., 2017). These genes are responsible for producing histamine and tyramine, the most commonly produced biogenic amines by some strains of the genus *Lactobacillus* (Morovic et al., 2017).

## 6. GMO Status

Danisco certifies that *L. acidophilus* NCFM has not been genetically modified. *L. acidophilus* NCFM does not consist of, nor contains, nor is produced from genetically modified organisms according to the definitions of Regulation (EC) 1829/2003 and Regulation (EC) 1830/2003 of the European Parliament and of the Council of 22 September 2003 (Appendix B).

## 7. Allergens

*L. acidophilus* NCFM is produced as a single strain with no added excipients and does not contain allergens as determined by The Food Allergen Labeling and Consumer Protection Act of 2004 (FALCPA) (Public Law 108-282) (U.S. Food and Drug Administration, 2006), including protein derived from milk, eggs, fish, crustacean shellfish, mollusks, tree nuts, wheat, peanuts, soybeans, celery, mustard and sesame seeds (Appendix B). Neither *Lactobacillus* or *L. acidophilus* are known to be food allergens (Castellazzi et al., 2013) and there have been no reported allergenic responses in the *L. acidophilus* NCFM clinical studies.

## V. PART 3. DIETARY EXPOSURE

### A. Current Dietary Exposure

*L. acidophilus* NCFM is in common use in foods as described in Part 5 below. We could not locate information regarding the current dietary exposure to *L. acidophilus* NCFM.

### B. Intended Human Food Uses (Estimated Daily Intake)

Danisco proposes the use of the *L. acidophilus* NCFM in non-exempt infant formulas and toddler formulas at a level of  $1 \times 10^8$  cfu per gm of powdered infant formula powder that is intended for consumption by term infants from the time of birth through 2 years of age. This level of *L. acidophilus* NCFM is intended to ensure a minimum concentration of  $10^6$  cfu/g throughout the 12-18 month shelf life of the infant formula powder. With normal dilution of the infant formula powder in water according to label directions (i.e., 13.5 g/ 100 mL) and assuming an average daily formula intake of 800 milliliters, Danisco estimates that the daily intake of *L. acidophilus* NCFM microorganism would be approximately  $10^9$ - $10^{10}$  cfu per day. The intended use of *L. acidophilus* NCFM does not encompass use by infants or toddlers who might have immune problems. *L. acidophilus* NCFM will serve as a probiotic organism.

## VI. PART 4. SELF-LIMITING LEVELS OF USE

### A. Self-limiting

The intended use of Danisco's *L. acidophilus* NCFM is not self-limiting. The intended uses are limited to those foods that can sustain living *L. acidophilus* NCFM for the shelf-life of the food.

## B. Estimate of Dietary Exposure of Other Substances

All ingredients in the fermentation and processing are of food grade approved for use by the FDA. There are no other dietary exposures of other substances relevant to the determination of GRAS status.

## VII. PART 5. EXPERIENCE BASED ON COMMON USE IN FOODS

### A. Common Use in Foods

*L. acidophilus* is in common use in yogurt, buttermilk, kefir, miso, tempeh and other fermented foods (Bernardeau et al., 2006). *L. acidophilus* NCFM has been used in foods, including certain dairy products, functional beverages, nutritional powders, juices, bars, ready-to-eat breakfast cereals, chewing gum and confections at levels as high as  $5 \times 10^{10}$  cfu/250g serving since 2010 (Danisco USA, 2010). No estimates of cumulative exposure to *L. acidophilus* NCFM could be found.

### B. Summary of Regulatory History

In 2010, Danisco USA Inc. notified the U.S. Food and Drug Administration (FDA) that they concluded *Lactobacillus acidophilus* NCFM was Generally Recognized as Safe (GRAS) for foods that can sustain living *L. acidophilus* NCFM during shelf life including dairy products, functional beverages, nutritional powders, juices, bars, RTE breakfast cereals, chewing gum, and confections. *L. acidophilus* NCFM is intended to be added to these foods at concentrations needed to provide at least  $10^9$  cfu/250g serving throughout the shelf life of the product (Danisco USA, 2010). The initial addition level may be as high as  $5 \times 10^{10}$  cfu/250g serving (i.e.  $2 \times 10^8$  cfu/g) in order to insure at least  $10^9$  cfu/250g serving remains viable over the product shelf life. The FDA responded to this notification that it had no questions (FDA, 2011).

Various other *Lactobacilli* and *Bifidobacteriae* probiotics have been the subject of notifications for intended use in infant formula and have received responses of no questions from the FDA (see Table 5).

**Table 5 GRAS Notifications for Probiotic Use in Infant Formula**

GRN Number	Species	Date
49	<i>Bifidobacterium lactis</i> Bb12	2002
231	<i>Lactobacillus rhamnosus</i> GG	2008
268	<i>Bifidobacterium longum</i> BB536	2009
281	<i>Lactobacillus rhamnosus</i> HN001	2009
410	<i>Lactobacillus reuteri</i> DSM 17938	2012
454	<i>Bifidobacterium breve</i> M-16V	2013
455	<i>Bifidobacterium breve</i> M-16V	2013
531	<i>Lactobacillus fermentum</i> CECT 5716	2015
758	<i>Lactobacillus helveticus</i> R0052 <i>Bifidobacterium longum</i> subsp <i>infantis</i> R0033 <i>Bifidobacterium bifidum</i> R0071	2018

In 2005, the Scientific Committee recommended to the European Food Safety Authority (EFSA) a generic approach to assess the safety of microorganisms used in food or feed and the production of food/feed additives (EFSA, 2007). This system was intended to be similar to the Generally Recognized as Safe (GRAS) definition used in the U.S. but modified to account for the regulatory practices in Europe. The system is referred to as Qualified Presumption of Safety (QPS). The Scientific Committee recommended policies and practices for the routine assessment of microorganisms based on taxonomy, familiarity, pathogenicity, and end use. If a microorganism is approved as QPS, it would not require further regulatory review prior to introduction into the food supply. Lactic acid bacteria (including *Lactobacillus* species) were among the microorganisms recommended to be reviewed in this initial document.

*Lactobacillus* species were reviewed under the QPS system in 2007, 2008, 2009, 2010, 2011, 2013, and 2017 (EFSA, 2007, 2008, 2009, 2010, 2011, 2012, 2013, 2017). *L. acidophilus* was among the taxonomic units included in the initial QPS review of lactobacilli. In the initial review, the Scientific Committee concluded that the weight of evidence available for these species was sufficient and provided as least the same degree of confidence as a case-by-case assessment (EFSA, 2007). The Scientific Committee reviewed the available evidence regarding the involvement of lactobacilli in human disease. Reviewing and summarizing the occasional reports of *Lactobacillus* bacteremia, the Scientific Committee concluded Lactobacillemia occurred primarily in immunocompromised or those suffering from severe underlying illness and that the *Lactobacillus* species described herein can be considered non-pathogenic to humans. They emphasized the long history of safe use in the food chain and reported no safety concerns.

Each subsequent QPS review (EFSA, 2007 2008, 2009, 2010, 2011, 2013, 2017) evaluated the totality of the scientific information each year and reaffirmed the QPS status of *L. acidophilus*.

*L. acidophilus* appears on the inventory of microorganisms with a documented history of use in human food that was compiled by the International Dairy Federation (IDF) in collaboration with the European Food and Feed Cultures Association (Morgensen et al., 2002). The inventory lists microbial strains used by the food industry that have a long history of use in food without reported adverse effects. In 2012, the IDF Bulletin 455 updated the inventory and once again included *L. acidophilus* as part of its inventory of microbial food cultures (MFC) safe for use in fermented food products (Bourdichon et al., 2012).

An expert consultation by the Food and Agriculture Organization/World Health Organization concluded that “adequate scientific evidence exists to indicate that there is potential for the derivation of health benefits from consuming food containing probiotics” and that “there is good evidence that specific strains of probiotics are safe for human use and able to confer some health benefits on the host, but such benefits cannot be extrapolated to other strains without experimentation” (FAO/WHO, 2001). Various species of the genera Lactobacillae including *L. rhamnosus* GG and *L. acidophilus* were specifically addressed in this review.

An earlier expert consultation by the Food and Agriculture Organization/World Health Organization conducted a toxicological evaluation of a number of food additives (FAO/WHO, 1974). Without explicit justification, the report stated “some evidence that the neonate has difficulties in utilizing the D isomer of lactic acid; it was considered, therefore, that neither this nor the racemate should be used in foods for infants less than three months old. Metabolic studies on the utilization of D (-)-lactic acid in infants are needed.”



The CODEX Standard for Infant Formula for children under 12 months (DOCEX STAN 72-1981) contains the restriction under "Optional Ingredients" - "Only L (+) lactic acid-producing cultures may be used". This also applies to the use of cultures as acidity regulators. This restriction is also applied in the Codex Standard for Processed Cereal-based Foods for Infants and Young Children (CODEX STAN 074-1981). Some reviewers suggested that the committee based this conclusion on reports on the effect of acidified formulas (Droese and Stolley, 1962; Droese and Stolley, 1964; Jacobs and Christian, 1957). The health effect of acidified formulas is discussed in detail below in Section VIII.C.

## VIII. PART 6. NARRATIVE

### A. Animal Studies

The acute toxicity of *L. acidophilus* NCFM was evaluated in female (nulliparous and non-pregnant) Crl:CD (SD) rats (Morovic et al., 2017). *L. acidophilus* NCFM (5000 mg/kg bw,  $1.72 \times 10^{12}$  cfu/kg bw) was administered by gavage in a dose volume of 20 mL/kg to rats fasted approximately 16 h prior to dosing. The test was conducted consistent with US FDA and OECD guidelines.<sup>2</sup> Additionally, *L. acidophilus* NCFM was tested as part of the multi-probiotic mixture HOWARU Restore. HOWARU Restore is composed of *L. acidophilus* NCFM, *Bifidobacterium animalis* subsp. *lactis* BI-04, *Lactobacillus paracasei* Lpc-37, and *B. animalis* subsp. *lactis* Bi-07 in a 1:1:1:1 ratio (5000 mg/kg bw,  $2.64 \times 10^{12}$  cfu/kg bw). The animals were observed for 15 days. There were no incidents of mortality, clinical abnormalities, or body weight losses in any animal for either treatment. There were "no gross findings detected at necropsy that were suggestive of acute toxicity".

Roager et al. (2014) reported on the effect of *L. acidophilus* NCFM on the gastrointestinal metabolome of monocolonized (MC) and germ free (GF) Swiss Webster mice (n=5/group). *L. acidophilus* NCFM treated MC and GF mice increased the deconjugation of bile acids, increased the abundance of  $\alpha$ -tocopherol in the cecum and colon, and impacted carbohydrate metabolism. Safety-related parameters were not reported.

Petersen et al. (2012) reported on the physiological, immunological and genetic effects of oral administration of *L. acidophilus* NCFM or *L. salivarius* Ls-33 in female Balb/c and SCID mice. The bacteria ( $10^9$  cfu) were administered by gavage daily for 5 weeks. Probiotic-fed SCID mice were protected from colitis and had lowered serum levels of inflammatory cytokines. No safety-related parameters were reported.

Wang et al. (2012) studied reported on the effect of daily oral administration of fermented milk supplemented with *L. acidophilus* NCFM ( $6.6 \times 10^7$  cfu/g), *Bifidobacterium lactis* Bi-07 ( $8 \times 10^7$  cfu/g) and isomaltooligosaccharide in pathogen-free, male BALB/c mice (n=10/group) for 13 days. Analysis of the intestinal microbiota revealed increases in bifidobacteria and lactobacilli. The authors report there were "no adverse effects on the mice". In the same publication, pathogen-free, female Kunming mice (n=60/group) were administered 0.8 g/kg bw, 8 g/kg bw or 24 g/kg bw via gavage for 30 days. The mice were found to have significantly increased delayed-type hypersensitivity, plaque-forming cells, and half-hemolysis values. No other safety-related parameters were reported.

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<sup>2</sup> US FDA, 2003, Acute oral toxicity tests. Redbook 2000 IV.C.@; OECD, 2008. Guidelines for testing of chemicals. Section 4. Part 425.

The role of *L. acidophilus* NCFM and other *Lactobacillus* and *Bifidobacterium* genera on modulating intestinal pain and inducing opioid and cannabinoid receptors in mice and rats was reported by (Rousseaux et al., 2007). *L. acidophilus* NCFM ( $10^9$  cfu/day was administered orally to Balb/c mice (n=8/group) and Sprague-Dawley rats (n=10/group) daily for 15 days. *L. acidophilus* NCFM treatment resulted in an antinociceptive effect of the same magnitude as 1 mg morphine/kg bw. This effect was inhibited by peritoneal administration of a CB-2 antagonist but not by opioid receptor antagonists. No safety-related parameters were reported.

The safety of the *L. acidophilus* NCFM<sup>®</sup> was reported in a colitis mouse model using Trinitrobenzenesulphonic acid (TNBS) to induce colitis. In healthy female Balb/c mice (n=5/group), gavage administration of *L. acidophilus* NCFM<sup>®</sup> did not show any potential adverse effect on mouse activity, weight and colon inflammation. In TNBS-treated mice, no significant improvement was reported in the group fed *L. acidophilus* NCFM<sup>®</sup>. High doses ( $10^{10}$  cfu) of *L. acidophilus* NCFM<sup>®</sup> led to no translocation of the organism or abnormal translocation of the intestinal microflora (Daniel et al., 2006).

The capacity of probiotic bacteria to colonize and infect two types of gnotobiotic, immunocompromised mice: bg/bg-nu/nu/+ (produce thymus-matured T-cells, euthymic) and bg/bg-nu/nu (athymic) was reported by (Wagner et al., 1997) This beige nude mouse model has defects in phagocytic cells and NK cell activity and lacks a functional thymus. Mice, (male and female, adult and neonatal, n=7-12/group) were inoculated with one of the following strains: *L. acidophilus* NCFM<sup>®</sup>, *L. reuteri*, *L. rhamnosus* GG or *Bifidobacterium animalis* by swabbing oral cavity and anal area with culture of  $10^8$  cfu/ml to create monoassociated mice (germ-free animals, colonized with only one strain). This resulted in colonization of the stomach, small and large intestines in the parental generation and in subsequent generations of mice colonized via exposure to monoassociated mothers and feces. Results of this study included

- no morbidity or mortality in adult mice;
- no adverse effects on growth parameters;
- no gross or histopathological findings, including no abscesses in the stomach or small intestine;
- translocation of *L. acidophilus* NCFM<sup>®</sup> to other tissues was reported (also LGG and *B. animalis*), but there was no evidence of inflammation or other pathologic findings in tissue sections of translocated mice;
- no deaths in gnotobiotic, immunocompromised mice; and
- evidence of induction of immunoglobulins, IgM and IgG.

It is concluded from the above, that NCFM<sup>®</sup> had no adverse effects in growth, survival, activity and weight of immunocompromised mice. Translocation was not reported at doses as high as  $10^8$  cfu/day..

## B. Clinical Studies

Clinical studies using *L. acidophilus* NCFM are summarized below and tabulated in Appendix C. Sixty one publications reporting the results of 55 clinical trials using *L. acidophilus* NCFM were identified in the literature search. The vast majority of studies were randomized, blinded, placebo-control trials (32), the remaining studies were either open-label (either controlled or uncontrolled) and published prior to 1990.

A total of 2,476 subjects were treated in these studies and the total number of treatment days was 5,340,732. In the longest of these studies, the treatment duration was 182 days. Doses ranged from  $10^6 - 4 \times 10^{11}$  cfu/day but the dose in most studies clustered around  $1 - 2 \times 10^{10}$  cfu/day. Stratified by health status, 28 studies were conducted on healthy subjects and 21 studies conducted on subjects compromised by such factors as atopic dermatitis, small bowel bacterial overgrowth, cancer, lactose intolerance, Crohn's disease, irritable bowel syndrome, or HIV. Stratified by age, studies on elderly, adults, children, and infants were the subject of 4, 47, 8, and 1, respectively (some studies did not report the age of the subjects). In one study, breastfeeding women were treated and the effects on their children were monitored. One study was a case study reporting on two subjects. Other than the case studies, the studies either reported no treatment-related adverse events, described the NCFM treatment as well tolerated, or did not report any safety-related endpoints. When adverse events were noted, they were generally confined to gastrointestinal issues, were equally distributed between treatment and control groups, were generally considered mild and reversible, and were not considered related to NCFM treatment.

Four studies reported in detail on safety-related parameters and are described below.

Four publications describe the outcome of probiotic supplementation on respiratory and gastrointestinal illness in physically active, healthy men and women (Cox et al., 2014; West et al., 2014a; West et al., 2014b; West et al., 2016). In a randomized, double-blinded, placebo-controlled trial, 465 participants were assigned to one of three groups: Group 1 received  $2 \times 10^9$  cfu *Bifidobacterium animalis* subsp. *lactis* B1-04, Group 2 received  $5 \times 10^9$  cfu each of *Lactobacillus acidophilus* NCFM and *Bifidobacterium animalis* subsp. *lactis* Bi-07, and Group 3 received a placebo daily for 150 days. Respiratory and gastrointestinal symptoms were evaluated. Both the probiotic-treated groups had delayed time to onset of upper respiratory illness. One participant withdrew from Group 2 for headaches and three participants withdrew for uncomfortable gastrointestinal symptoms (1 from Group 3 and 2 from Group 2). Sub-cohorts of the original study group were evaluated for innate immune system markers (West et al., 2014a), circulating T (Treg) cells (West et al., 2016), and hematology and clinical chemistry parameters (Cox et al., 2014). The treatments were reported as well-tolerated and no clinically relevant adverse events were reported in any study.

Cox et al. (2014) reported no treatment-related differences in 125 participants from the intervention trial, 39 participants from Group 1, 41 participants from Group 2, and 45 participants from Group 3. There were no differences among the groups in hematology (white cell count, neutrophils, lymphocytes, monocytes, eosinophils); electrolytes (sodium, potassium chloride, magnesium, phosphate); liver function (alanine transaminase, aspartate transaminase, alkaline phosphatase, lactate dehydrogenase, total bilirubin), kidney function (carbon dioxide, uric acid, Tprotein, albumin); and metabolic markers (total cholesterol, triglycerides, insulin, thyroid-stimulating hormone, and C-reactive protein). There were small, but significant changes in calcium (decrease in the post-intervention Group 3) and urea (decrease in the post-intervention Group 2). These changes were within established assay-specific laboratory reference ranges and frequently observed in both supplement and placebo groups, and therefore, were not considered to be a result of the probiotic supplementation.

The effect of a multi-probiotic combination on antibiotic associated diarrhea (AAD) and *Clostridium difficile*-associated diarrhea (CDAD) risk was evaluated in a randomized, triple-blind, placebo-controlled, parallel-arm study in 503 adult in-patients requiring antibiotic therapy (Ouweland et al., 2014). The probiotic mixture contained *L. acidophilus* NCFM, *L. paracasei* Lpc-37, *B. lactis* Bi-07, and *B. lactis* BI-04

in a 1:1:1:1 ratio. Subjects were randomized into three groups: high dose received  $1.7 \times 10^{10}$  cfu (n=186), low dose received  $4.17 \times 10^9$  cfu (n=168), or placebo (n=167) daily for up to 7 days after the final dose of antibiotic. The primary endpoint was the incidence of AAD and secondary endpoints were incidence of CDAD, diarrhea duration, stools per day, bloody stools, fever, abdominal cramping, and bloating. The incidence of AAD, incidence of fever, abdominal pain and bloating were decreased in a dose-responsive manner. The number of liquid stools and average duration of diarrhea was decreased in the high-dose group compared to control. The high dose group had fewer drop outs than the low dose and the placebo groups. The adverse event rate in the high dose, low dose and placebo groups were 4.2%, 4.2%, and 7.2%, respectively. Adverse events included allergy to seafood, arrhythmia, fever, headache, left upper arm fracture, runny nose, and vomiting. One serious adverse event was reported; a 38-year-old male with a history of coronary heart disease died of a myocardial infarction. None of these adverse events were judged by the investigators to be related to the study product.

The effect of *L. acidophilus* NCFM on irritable bowel syndrome (IBS) symptoms and quality of life was reported in a randomized, triple blinded, placebo-controlled trial in 391 adult subjects with IBS (Lyra et al., 2016). Subjects were randomized into groups receiving either  $10^{10}$  cfu (n=131),  $10^9$  cfu (n=129) or placebo (n=131) daily for 12 weeks. The primary efficacy outcome was lessening of IBS symptoms. All subjects were included in the safety analysis. Adverse events (AEs) were counted by volunteer, and characterized by event, type, treatment, severity, and causality. The AEs were evenly distributed in all groups. The most common treatment-emergent AEs were GI disorders (abdominal discomfort, abdominal distension, abdominal pain, constipation, diarrhea, flatulence), gastroenteritis, and influenza. Mild GI symptoms were reported 9, 7, and 7 subjects in the high dose, low dose, and placebo groups, respectively. AEs leading to drop out occurred in 10, 4, and 3 subjects in the high dose, low dose, and placebo groups, respectively. These AEs were not considered to be treatment-related because they are all common IBS symptoms. Two serious AEs were reported, pneumonia with fever and cough and syncope that resulted in a hospital visit. Neither of these was associated with the probiotic treatment or any trial procedure.

The effect of a probiotic combination treatment on symptoms of bloating was investigated in a randomized, double blind, placebo-controlled trial in 60 adult subjects with functional bowel disorders (FBD) (Ringel-Kulka et al., 2011). Subjects were randomized into two groups, one receiving a probiotic mixture of *L. acidophilus* NCFM and *B. lactis* Bi-07 ( $2 \times 10^{11}$  cfu/day) or placebo for 8 weeks. The primary endpoint was global relief of GI symptoms and secondary endpoints included changes in symptom severity, well being, and quality of life. Safety parameters reported were blood tests, kidney and liver function, blood urea nitrogen, total protein and albumin, TSH, fecal calprotectin, plasma elastase, and markers for inflammatory response and neutrophil degradation. Most common adverse events were cold symptoms, fatigue, abdominal pain, and sinus infection. No adverse events or changes in blood tests were reported]. There were no differences between groups in fecal sample markers and no adverse events were judged by the investigators to be associated with the supplement.

### ***L. acidophilus* in infants and children**

Numerous clinical trials have utilized formula containing *L. acidophilus* strains alone and in combination with other probiotic species and reported no instances of lactic acidosis, no adverse events, and found the treatment generally well tolerated. Many of the studies were conducted in preterm or VLBW infants, infants that could be considered at risk for bacterial overgrowth, immature digestive tracts, and

utilized strains that are known to produce D-lactic acid. Below are summaries of a selective but representative set of these studies.

In a randomized, double-blinded, placebo-controlled parallel arm trial, (Reuman et al., 1986) compared bacterial colonization in 30 premature infants fed infant formula containing either  $5 \times 10^{10}$  cfu *L. acidophilus* (strain unspecified), control formula or untreated control from birth until time of discharge (mean 59 days). There were no differences between the groups in mortality or morbidity. There was no impact on facultative gram-negative enteric bacterial colonization among the groups. Lactic acid was not measured and no signs of acidosis were reported.

In a prospective, matched control trial, (Hoyos, 1999) evaluated the effect of a mixture of *L. acidophilus* ATCC 4356 and *B. longum subsp. infantis* in newborn infants. All infants admitted to the hospital (n=1282) received  $2.5 \times 10^8$  cfu each from day of admittance until discharge (mean 8 days) and the rate of necrotizing enterocolitis (NEC) was compared to historic controls (n=1237). There were no complications attributed to the probiotics. The incidence of NEC and NEC-associated fatalities were reduced in the probiotic-treated group compared to the control group. Lactic acid was not measured and no signs of acidosis were reported.

In a randomized, double-blind, placebo-controlled, parallel arm pilot study, (Lin et al., 2005) reported the effect of a mixture of *L. acidophilus* ATCC 4356 and *B. longum subsp. infantis* in VLBW infants. Infants were randomized into two groups, one receiving breast milk alone (n=187) and one receiving breast milk containing the  $2.5 \times 10^8$  cfu each of the probiotic mixture (n=180) until discharge (mean 46.7 days). The incidence of death, NEC, or sepsis was lower in the probiotic group. None of the blood cultures grew *Lactobacillus* or *Bifidobacterium* species. Lactic acid was not measured and no signs of acidosis were reported.

Lee et al. (2007) fed  $10^8$  cfu/day *L. acidophilus* ATCC 4356 mixed in either breast milk or formula to preterm infants in a randomized, placebo-controlled trial. Infants were randomized into two groups, n=27 treated with *L. acidophilus* and n=46 treated with placebo for the duration of hospitalization (39 days and 41 days, respectively). There was a trend towards increase in sepsis in the control group. Feeding tolerance was increased in the *L. acidophilus* group assessed by absence of abdominal distension, residual more than 50% of previous feeding at the time of next feeding, vomiting, or loose mucoid stool. There were no differences in the incidence of hyaline membrane diseases, intraventricular hemorrhage, cholestatic jaundice, persistent ductus arteriosus, or the usage of indomethacin.

In a randomized, double-blind, placebo-controlled, parallel arm trial, Lin et al. (2008) reported on the effect of a mixture of *L. acidophilus* ATCC 4356 and *B. longum subsp. infantis* in VLBW infants. Infants were randomized into two groups, one receiving breast milk alone (n=217) and one receiving breast milk containing the probiotic mixture (n=217) ( $2.5 \times 10^8$  cfu each) twice daily for 6 weeks. The incidence of death, NEC, or sepsis was lower in the probiotic group. No adverse effects (sepsis, flatulence, or diarrhea) were reported. Lactic acid was not measured and no signs of acidosis were reported.

The effect of prebiotic/probiotic combinations on preterm infants in a randomized, double-blinded, placebo-controlled parallel arm trial was reported by (Underwood et al., 2009). Infants were divided into three groups, one group received a mixture of  $1 \times 10^{10}$  cfu *L. rhamnosus* GG plus fructo-oligosaccharide (n=30), the other group received a mixture of *L. acidophilus* DS, *B. longum*, *B. bifidum*,

and *B. infantis* ( $1 \times 10^{10}$  cfu each) (n=31) or placebo (n=29) daily for 28 days. There were no differences in growth among the groups and no differences in colonization rate for lactobacilli between the probiotic treated groups. There were no changes in fecal lactic acid or total short chain fatty acid. No adverse reactions were noted. Neither serum nor urinary lactic acid was measured and no signs of acidosis were reported.

The effect of enteral administration of probiotics in very low birth weight, preterm infants in a prospective, randomized, double-blind, control trial was reported by Samanta et al. (2009). Infants received either breast milk (n=95) or  $2.5 \times 10^9$  cfu each of *B. infantis*, *B. bifidum*, *B. longum*, and *L. acidophilus* (strain unspecified) (n=91) until discharge. The number of days required to reach full enteral feeding was reduced in the probiotic group, indicating better feeding tolerance. The duration of hospital stay, incidence of NEC, incidence of culture-proven sepsis, and death rate were all lower in the probiotic group compared to the control group. Lactic acid was not measured and no signs of acidosis were reported.

Esaiassen et al. (2018) reported on the effect of a mixture of *L. acidophilus* ATCC 4356 and *B. longum subsp. infantis* in newborn extremely preterm infants in a prospective observation study. The study included three groups, extremely preterm infants (n=31) receiving  $2.5 \times 10^8$  cfu each of *L. acidophilus* ATCC 4356 and *B. longum subsp. infantis*, very preterm infants (n=35) receiving control formula, and healthy, full-term infants (n=10) receiving control formula. The mean duration of treatment was 46 days. Infants were followed for 4 months. The probiotic treated group had higher median relative abundance of Bifidobacterium than the other two groups. Lactobacillus was detected in small amounts in all groups but the relative abundance increased up to 4 months. The authors concluded that probiotic supplementation “may induce colonization resistance and alleviate harmful effects of antibiotics on the gut microbiota”. Lactic acid was not measured and no signs of acidosis were reported.

Hong Chau et al. (2018) reported on the effect *L. acidophilus* La-14 on the treatment of acute watery diarrhea in a randomized, double-blinded, placebo-controlled parallel arm trial. Children (mean age 15 months) were randomized into two study groups, one receiving two daily doses of  $2 \times 10^8$  cfu *L. acidophilus* La-14 (n=143) and one receiving placebo (n=147) for 5 days. No adverse events were reported in either group. Lactic acid was not measured and no signs of acidosis were reported.

## C. Other Safety Information

### Putative Lactic Acid-related Syndromes

#### D-Lactic Acidosis

Lukasik et al. (2018) identified five randomized controlled clinical trials covering 544 healthy infants, some case reports, and experimental studies and found no clinically relevant adverse effects of D-lactic acid-producing probiotics and fermented infant formulas in healthy children. The clinical trials are summarized in Table 6. The authors concluded that “probiotics and fermented formulas did not cause D-lactic acidosis in healthy children.” Because blood lactate concentrations were not measured in those clinical trials, the authors could not rule out the possibility of subclinical D-lactate accumulation.



**Table 6 Lactic Acid Producing Probiotics in Infants**

Reference	Population	Intervention	Outcomes	Results
(Connolly et al., 2005)	24 healthy infants	<i>L. reuteri</i> ATCC 55730 1 x 10 <sup>8</sup> cfu/day for 12 months	Blood D-lactic acid levels, clinical symptoms	No differences in D-lactic blood levels, no symptoms of acidosis observed. No adverse events.
(Haschke-Becher et al., 2008)	71 healthy 16-week-old infants	Infant formula with <i>L. johnsonii</i> LA 1 (0.8 – 1.1 x 10 <sup>10</sup> cfu/day for 4 weeks	Urinary D-lactic and L-lactic, weight gain, length gain	No difference between groups in urinary D-lactic. Both formula fed groups higher D-lactic than breastfed infants. D-lactic acid levels with normal range.
(Papagaroufalis et al., 2014)	88 healthy term infants <72 hours of age	Infant formula with <i>L. reuteri</i> DSM 17938 1.2 x 10 <sup>6</sup> cfu/mL (approx. 1.2 x 10 <sup>8</sup> cfu/day) for 28 days	Urinary D-lactic, ratio of D-lactic to L-lactic, blood acid, pH, anthropometry, tolerance, sleep patterns, duration of crying, adverse events	Higher urinary D-lactic at day 7, 14, and 112 compared to control. D-lactic levels within normal range in all groups. No other differences between groups concerning D-lactic-related outcomes
(Lee et al., 2007)	140 healthy, term 14-day-old infants	<i>L. reuteri</i> DSM 17938 1 x 10 <sup>8</sup> cfu/day for 6 months	Urinary D-lactic and L-lactic, anthropometry, digestive tolerance, stool bacterial counts, adverse events	No differences in urinary lactic concentrations between groups, all values within normal range
(Manzano et al., 2017)	221 healthy, term, 3-12 month old infants	<i>B. longum</i> Subsp infantis R0033 or <i>L. helveticus</i> R0052 or <i>B. bifidum</i> R0071 3 x 10 <sup>9</sup> cfu/day for 8 weeks	Urinary D-lactic, anthropometry, adverse events	Urinary D-lactic levels below quantification limit of the method. No differences in anthropometric measures or adverse events.

Numerous lactic acid producing probiotic bacteria have been concluded to be GRAS and have received letters of no objection by the FDA for use in food. Although the ratio of D- to L-lactic acid produced by the individual strains that were the subject of these GRAS notifications has not been published or referred to in the GRAS notification, lactate production has been reported for the species to which they belong by (Pot et al., 2014) and (de Vries and Stouthamer, 1968) (Table 7). It should be noted that the relative proportion of D- to L-lactic acid production may be strain specific and is likely to depend on the culture conditions. Lactic acidosis was not addressed in any of the GRAS notifications for any of these probiotics, either for intended use as an ingredient in food or intended for use in infant formula.

**Table 7 GRAS Notices for Probiotic Species That Produce D-Lactic Acid**

Species/strain	GRN	D/L Production
<i>L. rhamnosus GG</i>	GRN 231	DL
<i>L. reuteri DSM 17938</i>	GRN 254	DL
<i>B. longum BB536</i>	GRN 268	DL
<i>L. rhamnosus HN001</i>	GRN 288 & GRN 281	DL
<i>L. acidophilus NCFM</i>	GRN 357	DL
<i>L. sakei</i>	GRN 378	DL
<i>L. reuteri NCIMB 30242</i>	GRN 440 & GRN 409	DL
<i>L. acidophilus La-14</i>	GRN 502	DL
<i>L. fermentum CECT 5716</i>	GRN 531	DL
<i>L. plantarum 299v</i>	GRN 685	DL
<i>L. plantarum Lp-115</i>	GRN 722	DL
<i>L. helveticus R0052</i>	GRN 758	DL
<i>L. casei subsp. paracasei Lpc-38</i>	GRN 736	DL

### Acidified formula

Feeding formula acidified with 0.23 g racemic lactic acid/100 mL formula to 80 full-term infants did not result in clinical symptoms of acidosis (Jacobs and Christian, 1957). Blood lactate levels were not analyzed in this study. Other studies contradicted these findings. Feeding acidified milk or acidified formula containing a 1% racemic mixture of D- and L-lactic for 7 – 10 days to 36 premature infants resulted in decreased weight gain, increased urinary excretion of lactate, and metabolic acidosis (blood pH dropped from 7.39 to 7.25) compared to controls (Goldman et al., 1961). (Droese and Stolley, 1962) reported that 30 % of newborn infants in their first three months fed formula containing 0.34g to 0.5 g lactic acid/ 100 g milk formula for 10 days had clinical evidence of metabolic acidosis. (Droese and Stolley, 1964) reported that feeding formula with 80% L-lactic acid and 20% D-lactic acid resulted body weight loss, diarrhea, decreased blood pH, and increased urinary excretion of D-lactic compared to control. One infant exhibited symptoms of acidosis. Blood lactic acid levels were not measured in any of these studies, none of the studies were randomized or placebo-controlled. It is not clear if any of these formulas contained live bacteria. A systematic review and meta-analysis of fermented infant formulas containing significant amounts of lactic acid but not containing any live bacteria found no

negative health effects (Szajewska et al., 2015). None of the five included trials reported blood or urinary lactate concentrations or the content of D- or L-lactate in the formulas.

### **Case Study Reviews**

Several reviews summarized multiple case reports of lactic acidosis in adults and children (Fabian et al., 2017; Ku et al., 2006; Uribarri et al., 1998). In all, over 50 cases were reported with significant overlap among the reviews. In all cases but one, the subjects had short bowel syndrome due to either surgical resection, or intestinal bypass surgery. (Uribarri et al., 1998) reviewed 29 case reports of lactic acidosis. They found the predominant underlying condition for lactic acidosis in these case reports was short bowel syndrome due to either surgical resection of the intestine (17 cases) or intestinal bypass surgery for treatment of obesity (10 cases). The remaining cases were due to malabsorption secondary to exocrine pancreatic insufficiency or misplacement of a feeding tube. In those studies where metabolism was measured, there was a “substantial” rate of metabolism of D-lactate. Sources of D-lactate in these case reports include a wide variety of fruits and vegetables and lactated Ringer solution and peritoneal dialysate, which contain racemic mixtures of both isomers. (Uribarri et al., 1998) concluded that the presences of abnormal bacterial flora in the colon as well as impaired metabolism of D-lactate are almost prerequisites for development of the syndrome. (Ku et al., 2006) presented a case report of a boy with lactic acidosis secondary to bowel resection and feeding of a probiotic mixture containing *L. acidophilus* and *Bifidobacterium* spp. They also reviewed 21 cases of children under the age of 19 years from 15 reports. All the cases had short bowel syndrome. Serum D-lactate was reported in only ten patients and only eight had elevated D-lactate levels. (Fabian et al., 2017) reported on a case of lactic acidosis secondary to bowel resection and also provided a general review of the literature drawing many of the same conclusions as Uribarri and Ku.

### **Summary of Lactic Acidosis**

Early concerns about the potential for the intake of D-lactic acid and/or D-lactic acid-producing bacteria are primarily based on studies using acidified formula. More recently, several clinical trials found the use of D-lactic acid producing bacteria in infant formula did not cause lactic acidosis. Lactic acidosis has been strongly linked to individuals with short bowel syndrome or in rare cases, impaired absorption due to pancreatic insufficiency. Numerous probiotic bacteria have been concluded to be GRAS for use in conventional food and infant formula. The probiotic bacteria that were the subject of these notifications are from species known to produce D-lactic acid, although the D-lactic acid production capability of the specific strains was not considered in these reviews.

### **Chronic Fatigue Syndrome**

Vitetta et al. (2017) addressed the benefits and potential adverse consequences of D-lactate producing probiotic bacteria. This review addressed the hypothesis proposed by (Sheedy et al., 2009) that lactic acid producing bacteria including *Enterococcus* sp. and *Streptococcus* sp. are linked to the neurocognitive and mitochondrial dysfunction experienced in Chronic Fatigue Syndrome (CFS). Vitetta disagreed because (1) Sheedy did not take into account *Lactobacillaceae*, the predominant commensals that produce D- and DL-lactic acid; (2) the genera that Sheedy measured are considered to be L-lactic producers, and (3) Sheedy did not measure blood lactate levels. Further clinical studies conducted with children demonstrate that administering probiotic bacteria that produce D-lactate are safe and do not cause long-term increases in blood D-lactate. Osteoarthritis patients without the CFS symptomology present with stool bacterial counts similar to those reported by Sheedy.

## Brain fogginess (BF), Lactic acidosis, and Small Intestinal Bacterial Overgrowth (SIBO)

The putative interaction between probiotics, SIBO, and D-lactic acidosis was reported in a prospective observational study of 38 adult patients with gas and bloating (Rao et al., 2018a). Subjects were divided into two groups, with BF (n=30) and without BF (n=8). Brain fogginess was defined as mental confusion, cloudiness, impaired judgment, poor short-term memory and difficulty with concentration. Lactic acidosis was assessed by a novel method measuring blood L-lactic acid and urinary D-lactic acid levels after an oral carbohydrate challenge and concurrent glucose breath test. All patients in the BF group were taking probiotics containing lactobacillus species, bifidobacterium species or *Streptococcus thermophilus*. D-lactic acidosis was reported in 23 of 30 patients of the BF group along with concurrent L-lactic acidosis in 9 patients. D-lactic acidosis was reported in 2 of 8 patients in the non-BF group and concurrent L-lactic acidosis in one patient. The prevalence of SIBO was higher in the BF group. After discontinuation of probiotics and a course of antibiotics in the SIBO patients, BF resolved and gastrointestinal symptoms improved.

In a response to (Rao et al., 2018a), (Sachdeva et al., 2018) took issue with the subjective measure of BF, the low incidence of SIBO (46.7%) and positive glucose breath test (36.7%) as well as the irreproducibility of the breath test results. Only one of 30 subjects with BF had elevated baseline urinary D-lactic acid levels and only 9 of 30 had elevated peak serum L-lactic acid levels. Sachdeva reported that other anomalies in the frequency of lactic acidosis, proportion of patients with negative breath test, and culture positive SIBO were difficult to reconcile with the Rao's conclusions. BF has been associated with a variety of syndromes (CFS, fibromyalgia, postural tachycardia) and may be triggered by factors such as lack of sleep, drugs, toxins, alcohol, metabolic and hormonal factors. These were not ruled out in the patient selection process. Rao and Yu (2018) responded to these comments and noted that BF is a feature in only a select group of SIBO patients in whom the small bowel is colonized with D-lactic acid producing organisms and further asserted that the combined diagnostic methodology employed to detect SIBO, glucose breath test and duodenal aspirates, is more sensitive than either test alone and explains the apparent discrepancies elaborated by Sachdeva.

In a separate response to Rao et al. (2018a), Quigley et al. (2018) took issue with the assertion that the probiotics consumed by the patients in the study produced D-lactic acid, that Rao et al. had erroneously equated all probiotics, that bacteria involved with SIBO can produce other metabolites which could lead to neuropsychiatric symptoms, that the patients in the study were being treated with other therapies during the study, and that this was not a randomized, double-blind study. Given the nature of the symptoms being addressed, the heterogeneity of the patient population, the novel techniques employed, the study conclusions must be considered preliminary. Rao et al. (2018b) recognized that probiotics represent one plausible factor among others in BF and responded to these comments with plans to conduct better controlled studies to identify the underlying connections. Rao et al (2018b) took issue with Quigley's assertions about the production of D-lactic acid by the bacterial genera in the study.

### D. Summary of Safety Information

#### 1. Summary of Safety Information on *L. acidophilus* NCFM

The key information supporting the safe use of *L.acidophilus* NCFM is found in the clinical literature. The 61 articles based on 55 clinical trials reporting on treatment with *L.acidophilus* NCFM included randomized double-blind, placebo-controlled clinical trials, crossover studies, observational cohorts, and open-label studies and included 2,476 treated subjects and 5,340,732 treatment days. The treatment duration ranged up to 180 days and the doses of *L.acidophilus* NCFM ranged from  $10^5$  –  $10^{12}$  cfu/day. These studies either reported no treatment-related adverse events, the *L. acidophilus* NCFM treatment as described as well tolerated, or there were no reported safety-related endpoints. When adverse events were reported, they were generally confined to gastrointestinal issues, were equally distributed between treatment and control groups, were generally considered mild and reversible, and were not considered related to *L.acidophilus* NCFM treatment.

None of the nine clinical trials utilizing various *L. acidophilus* strains in infants and children reported any treatment-related adverse events.

Previous GRAS notifications (GRN 231, GRN 281) have concluded that Lactobacilli as probiotics in infant and toddler formula are GRAS at levels of  $10^8$  cfu/gm of powdered infant formula; levels that will ensure  $10^5$  cfu/gm throughout the shelf life of the formula. Based on these levels and an average consumption of infant formula of 800 ml/day, the estimated intake of probiotics will be  $10^9$  –  $10^{10}$  cfu/day. These intakes are consistent with the levels of *L. acidophilus* reported in various clinical trials in infant and adult subjects.

An unpublished acute toxicity study in rats reported an LD<sub>50</sub> of greater than 5000 mg/kg bw, the highest dose tested. In another acute toxicity study, *L. acidophilus* NCFM was evaluated alone and as part of a multi-component probiotic mixture reported a LD<sub>50</sub> greater than 5000 mg probiotic mixture/kg bw, the highest dose tested. *L. acidophilus* NCFM was evaluated in various animal models. None of these animal model studies were designed to evaluate toxicity, although some safety-related parameters were reported. None of these studies reported *L. acidophilus*-related morbidity or mortality or adverse effects.

Based on evidence utilizing acidified formulas, the FAO/WHO raised concerns about lactic acidosis in infant formula. Subsequently, CODEX issued a standard restricting the use of probiotics to those producing only L-lactic acid. However, more recent studies have demonstrated that the use of D-lactic producing probiotics in infant formula does not cause lactic acidosis and have confirmed that lactic acidosis is a problem only in individuals with short bowel syndrome and not in healthy infants. Reports linking D-lactic producing bacteria to chronic fatigue syndrome and brain fogginess are based on preliminary, poorly controlled experiments that have not been reproduced by other investigators. Various concerns about study design undermine the authors' preliminary conclusions.

An unpublished acute toxicity study reported an LD<sub>50</sub> of greater than 5000 mg/kg bw, the highest dose tested. An acute toxicity study of *L. acidophilus* NCFM as part of a multi-component probiotic mixture reported a LD<sub>50</sub> greater than 5000 mg probiotic mixture/kg bw, the highest dose tested. *L. acidophilus* NCFM was evaluated in various animal models. None of these studies were designed to evaluate toxicity, although some safety-related parameters were reported. None of these studies reported *L. acidophilus*-related morbidity or mortality or adverse effects.

## 2. Safety of Lactic Acid Bacteria and Lactobacillus Species

Lactic acid bacteria (LAB) and lactobacilli have a long history of safe use in foods (Bernardeau et al., 2008; Salminen et al., 1998). Lactobacilli are intrinsically resistant to some antibiotics. Because this antibiotic resistance is not transferable and LAB are sensitive to many antibiotics in common clinical use, they present no safety concern. Lactobacillemia induced by food, particularly fermented dairy products, is extremely rare and only occurs in predisposed patients (Bernardeau et al., 2008). Lactobacilli are found wherever substances rich in carbohydrates are available (Bernardeau et al., 2008).

The Food and Agriculture Organization and World Health Organization expert consultation reported that “lactobacilli have a long history of use as probiotics without established risk to humans, and this remains the best proof of their safety....no pathogenic or virulence properties have been found for lactobacilli” (FAO/WHO, 2002). The safety of probiotic bacteria was recently reviewed (Sanders et al., 2007). Any probiotic strain, including members of the *genera Lactococcus, Lactobacillus, and Bifidobacterium* is considered safe, as long as the strain is devoid of any transferable antibiotic resistance genes.

Infections in humans by these genera are extremely rare. There have been 180 reported cases of lactobacillemia and 69 reported cases of infective endocarditis attributed to lactobacilli during the past 30 years. In most cases of endocarditis, dental surgery occurred in the days or weeks preceding the disease. These infections resulted from native sources of these genera and not from consumption of probiotics products. Only two cases of *Lactobacillus* infection were linked with probiotic consumption. Increasing consumption of probiotic lactobacilli has not led to an increase in such opportunistic infections in consumers. The risk of infection by these genera is in the “negligible” range, considering that exposure to them is universal and persistent, not only through probiotic products but also as common colonizers of the human body (the digestive tract and oral and vaginal cavities). This lack of pathogenicity extends across all age groups (including preterm infants and pregnant women). Caution is recommended for immunocompromised and critically ill patients such as those suffering from acute pancreatitis, bone marrow transplant or recently operated patients and/or those given parenteral nutrition (Sanders et al., 2007).

In a comprehensive evidence-based review and meta-analysis of the literature regarding the safety of probiotics, 622 peer-reviewed research articles were evaluated (Hempel et al., 2011). Of these, 235 studies reported only nonspecific safety statements such as “well tolerated” but did not indicate specific adverse events or what kinds of events were monitored. The remaining 387 studies predominantly investigated *Lactobacillus*, alone or in combination with other genera, most often *Bifidobacterium*. These studies were pooled to evaluate the relative risks (RR) of use of probiotics, active or lyophilized, single ingredients or in combination, in all delivery vehicles when used to improve health. The following key relative risk results germane to the current report are listed along with 95% confidence intervals (CI), p value, and the number of randomized clinical trials (RCT) included in the pool.

- There was **no evidence of increased risk from interventions with probiotics** compared to control groups
  - a. based on the number of participants with adverse events (RR 0.98, CI: 0.93 – 1.04, p=0.537, 121 RCT)
  - b. based on the number of adverse-event incidences (RR 1.00, CI: 0.93 – 1.07, p=0.999, 208 RCT)



- c. **“None of the case series, controlled clinical trials, or parallel and crossover RCT reported an infection caused by the administered probiotics”** though few reported that they monitored for this
- There was no indication participants using probiotic organisms experienced more:
  - a. Gastrointestinal events  
(RR 1.03, CI: 0.89 – 1.18, p=0.693, 126 RCT)
  - b. Infections  
(RR 1.00, CI: 0.87 – 1.16, p=0.967, 65 RCT)
  - c. Or other adverse events  
(RR 1.01, CI: 0.91 – 1.12, p=0.923, 131 RCT)
- Stratified by probiotic genus there was **no indication that participants using *Lactobacillus* experienced an increased risk.**  
(RR 0.98, CI: 0.87 – 1.11, p=0.785)
- **Stratified by age there was no indication of increased risk of adverse events for children, adults, or elderly.**
- Although case studies have reported serious adverse events in health compromised, not generally healthy participants, **subgroup analyses of RCT did not show an increased risk of adverse events in either:**
  - a. **Medium health-compromised participants**  
(RR 1.03, CI: 0.94 – 1.13, p=0.491)
  - b. **Critically ill patients**  
(RR 0.79, CI: 0.51 – 1.22, p=0.286)
- There was **no indication that consumption of probiotics lead to hospital admission or lengthened hospitalization.** Most of these studies were based on *Lactobacillus* interventions.  
(RR 1.06, CI: 0.97 – 1.16, p=0.201, 66 RCT)
- There was no indication that consumption of probiotics increased the risk of adverse events in individuals concomitantly taking:
  - a. Antibiotics  
(RR 1.07, CI: 0.94 – 1.23, p=0.271)
  - b. Corticosteroids  
(RR 1.04, CI: 0.88 – 1.22, p=0.650)

The strength of these conclusions is somewhat mitigated by the inconsistency between the results of RCT and case studies, the lack of systematic reporting of adverse events, and poor documentation in the studies evaluated. The authors concluded the RCT-based evidence does not indicate an increased risk of adverse events. “The available evidence in RCTs does not indicate an increased risk; however, rare adverse events are difficult to assess and despite the substantial number of publications, the current literature is not well equipped to answer questions on the safety of probiotic interventions with confidence.”

Whelan and Myers (2010) conducted a systematic review of the safety of probiotics in patients receiving nutritional support. The review included 76 case reports, randomized controlled trials, and nonrandomized trials. In these studies 4131 patients received probiotics and 3643 patients were in a relevant comparator group. The risk factors included central venous catheters and disorders associated with increased bacterial translocation. The authors reported “most trials showed either no effect or a positive effect on outcomes related to safety (eg, mortality and infections). Only 3 trials showed

increased complications, which were largely noninfectious in nature and in specific patient groups (eg, transplant and pancreatitis).”

## E. GRAS Criteria

FDA defines “safe” or “safety” as it applies to food ingredients as:

“...reasonable certainty in the minds of competent scientists that the substance is not harmful under the intended conditions of use. It is impossible in the present state of scientific knowledge to establish with complete certainty the absolute harmlessness of the use of any substance.”<sup>3</sup>

Amplification is provided in that the determination of safety is to include probable consumption of the substance in question, the cumulative effect of the substance, and appropriate safety factors. It is FDA’s operational definition of safety that serves as the framework against which this evaluation is provided.

Furthermore, in discussing GRAS criteria, FDA notes that:

“...General recognition of safety requires common knowledge about the substance throughout the scientific community knowledgeable about the safety of substances directly or indirectly added to food.”

“General recognition of safety through experience based on common use in food prior to January 1, 1958, shall be based solely on food use of the substance prior to January 1, 1958, and shall ordinarily be based upon generally available data and information.”<sup>4</sup>

Practically speaking, the standard for GRAS has become “reasonable certainty of no harm under the intended conditions of use.” FDA discusses in more detail what is meant by the requirement of general knowledge and acceptance of pertinent information within the scientific community, i.e., the so-called “common knowledge element,” in terms of the two following elements:<sup>5</sup>

- Data and information relied upon to establish safety must be generally available; this is most commonly established by utilizing published, peer-reviewed scientific journals; and
- There must be a basis to conclude that there is consensus (but not unanimity) among qualified scientists about the safety of the substance for its intended use, and this is established by relying upon secondary scientific literature such as published review articles, textbooks, or compendia, or by obtaining opinions of expert panels or opinions from authoritative bodies, such as the National Academy of Sciences.

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<sup>3</sup> See 21 CFR 170.3(j).

<sup>4</sup> See 21 CFR 170.30(a).

<sup>5</sup> See 62 FR 18938 (17 April 1997) Accessible at <http://www.gpo.gov/fdsys/pkg/FR-1997-04-17/pdf/97-9706.pdf> Accessed June 25, 2014.

The apparent imprecision of the terms “appreciable,” “at the time,” and “reasonable certainty” demonstrates that the FDA recognizes the impossibility of providing absolute safety in this or any other area (Lu, 1988; Renwick, 1990; Rulis and Levitt, 2009).

## F. Common Knowledge Elements for GRAS Conclusions

### 1. Public Availability of Scientific Information

The key evidence in this determination has been published in a peer review journal. Various other safety assessments, risk assessments, animal and human studies have all been published in peer reviewed journals or made publicly available on government websites.

## G. GRAS Panel Conclusions

The GRAS Panel individually and collectively critically evaluated the materials summarized above. The GRAS Panel evaluated the safety of *L. acidophilus* NCFM using a decision tree analysis developed by (Pariza et al., 2015). Based on their critical evaluation of the information on the safety of *L. acidophilus* NCFM summarized above, they unanimously concluded that Danisco’s *L. acidophilus* NCFM, manufactured consistent with cGMP and meeting food grade specifications, is Generally Recognized As Safe (GRAS) based on scientific procedures for addition to infant formula and toddler formula, at a level of  $1 \times 10^8$  cfu per gm of powdered infant formula powder. This level of *L. acidophilus* NCFM is intended to ensure a minimum concentration of  $10^6$  cfu/gm throughout the 12-18 month shelf life of the infant formula powder. It is the GRAS Panel’s opinion that other qualified and competent scientists reviewing the same publicly available information would reach the same conclusions. See Appendix D GRAS Panel Statement.

## H. Conclusion of GRAS Status

Danisco has concluded that its *L. acidophilus* NCFM is GRAS for use in infant formula and toddler formulas at a level of  $1 \times 10^8$  cfu/gm of powdered infant formula powder that is intended for consumption by term infants from the time of birth through 2 years of age. This level of *L. acidophilus* NCFM is intended to ensure a minimum concentration of  $10^6$  cfu/gm throughout the 12-18 month shelf life of the infant formula powder formula. This GRAS status conclusion is based on key data from human clinical trials available in the public domain pertaining to the safety of *L. acidophilus* NCFM, as discussed herein, and on consensus among a panel of experts (the GRAS Panel) who are qualified by scientific training and experience to evaluate the safety of food ingredients. The GRAS Panel convened by Danisco independently and critically evaluated all data and information presented herein, and concluded that Danisco’s *L. acidophilus* NCFM is GRAS based on scientific procedures for use in infant formulas and toddler formulas.

## IX. PART 7. LIST OF SUPPORTING DATA AND INFORMATION

### A. List of Abbreviations

AV	acid value
Bw	body weight
CFSAN	Center for Food Safety and Nutrition
CGMPs	Current Good Manufacturing Practices
CO <sub>2</sub>	carbon dioxide
EFSA	European Food Safety Authority
EHCF	extensively hydrolyzed casein formula
ELBW	extremely low birth weight
EU	European Union
F <sub>0</sub>	parental generation
FAO	Food and Agriculture Organization
FCC	Food Chemicals Codex
FDA	Food and Drug Administration
FSNZ	Food Safety New Zealand
GI	gastrointestinal
GMO	genetically modified organism
GOS	galacto-oligosaccharide
GRAS	Generally Recognized As Safe
GRASP	GRAS Petition
GRN	GRAS notification
HAACP	Hazard Analysis and Critical Control Points
HD	high dose
HDL	high-density lipoprotein
IBS	irritable bowel syndrome
IDL	intermediate-density lipoprotein
IOM	Institute of Medicine
ISO	International Standardization Organization
LD	low dose
LD <sub>50</sub>	median lethal dose
LDH	lactate dehydrogenase
LDL	low-density lipoprotein
MD	middle dose
meq	milliequivalents
NDA	Dietetic Products, Nutrition and Allergies
NDIN	New Dietary Ingredient Notification
NICU	neonatal intensive care unit
NOAEL	No Observed Adverse Effect Level
NR	not reported
OECD	Organization for Economic Cooperation and Development
PCE	polychromatic erythrocyte
ppb	parts per billion
ppm	parts per million
PV	peroxide value

RBC	red blood cell
VLBW	very low birth weight
vLDL	very low density lipoprotein
WBC	white blood cell
WHO	World Health Organization

## B. References

Altermann, E., Russell, W.M., Azcarate-Peril, M.A., Barrangou, R., Buck, B.L., McAuliffe, O., Souther, N., Dobson, A., Duong, T., Callanan, M., Lick, S., Hamrick, A., Cano, R., Klaenhammer, T.R., 2005. Complete genome sequence of the probiotic lactic acid bacterium *Lactobacillus acidophilus* NCFM. *Proc Natl Acad Sci U S A* 102, 3906-3912.

Bernardeau, M., Guguen, M., Vernoux, J.P., 2006. Beneficial lactobacilli in food and feed: long-term use, biodiversity and proposals for specific and realistic safety assessments. *FEMS Microbiology Reviews* 30, 487-513.

Bernardeau, M., Vernoux, J.P., Henri-Dubernet, S., Gueguen, M., 2008. Safety assessment of dairy microorganisms: the *Lactobacillus* genus. *Int J Food Microbiol* 126, 278-285.

Bourdichon, F., Berger, B., Casaregola, S., Farrokh, C., Frisvad, J.C., Gerds, M.L., Hammes, W.P., Harnett, J., Huys, G., Laulund, S., Ouwehand, A.C., Powell, I.B., Prajapati, J.B., Seto, Y., Ter Schure, E., Van Boven, A., Vankerchhoven, V., Zgoda, A., Bech Hansen, E., 2012. Safety demonstration of microbial food cultures (MFC) in fermented food products. *Bulletin of the International Dairy Federation* 455, 1-62.

Castellazzi, A.M., Valsecchi, C., Caimmi, S., Licari, A., Marseglia, A., Leoni, M.C., Caimmi, D., Miraglia del Giudice, M., Leonardi, S., La Rosa, M., Marseglia, G.L., 2013. Probiotics and food allergy. *Ital J Pediatr* 39, 47.

Connolly, E., Abrahamsson, T., Björkstén, B., 2005. Safety of D (-)-lactic acid producing bacteria in the human infant. *Journal of pediatric gastroenterology and nutrition* 41, 489-492.

Cox, A.J., West, N.P., Horn, P.L., Lehtinen, M.J., Koerbin, G., Pyne, D.B., Lahtinen, S.J., Fricker, P.A., Cripps, A.W., 2014. Effects of probiotic supplementation over 5 months on routine haematology and clinical chemistry measures in healthy active adults. *Eur J Clin Nutr* 68, 1255-1257.

Daniel, C., Poiret, S., Goudercourt, D., Dennin, V., Leyer, G., Pot, B., 2006. Selecting lactic acid bacteria for their safety and functionality by use of a mouse colitis model. *Appl Environ Microbiol* 72, 5799-5805.

Danisco USA, I., 2010. *Lactobacillus acidophilus* NCRM GRN 357, in: CFSAN (Ed.).

de Vries, W., Stouthamer, A.H., 1968. Fermentation of glucose, lactose, galactose, mannitol, and xylose by bifidobacteria. *J Bacteriol* 96, 472-478.

Droese, W., Stolley, H., 1962. [Is the use of lactic acid in the nutrition of the young, healthy infant still justified?]. *Arch Kinderheilkd* 166, 9-16.

Droese, W., Stolley, H., 1964. [The effect of lactic acid (D / L), citric acid and protein on the organic acids in the urine of healthy infants in the 1st quarter of life], Ernährung der Fröhgeborenen. Karger Publishers, Basel, pp. 63-72.

EFSA, 2007. Introduction of a Qualified Presumption of Safety (QPS) approach for assessment of selected microorganisms referred to EFSA. Opinion of the Scientific Committee (Question No EFSA-Q-2005-293). The EFSA J 587, 1-16.

EFSA, 2008. Scientific Opinion The maintenance of the list of QPS microorganisms intentionally added to food or feed. Scientific Opinion of the Panel on Biological Hazards (Question No EFSA-Q-2008-006). EFSA J 923, 1-48.

EFSA, 2009. Scientific Opinion on the maintenance of the list of QPS microorganisms intentionally added to food or feed (2009 update). EFSA J 7, 1431.

EFSA, 2010. Scientific Opinion on the maintenance of the list of QPS biological agents intentionally added to food and feed (2010 update). EFSA on Biological Hazards (BIOHAZ). EFSA J 8, 1944.

EFSA, 2011. Scientific Opinion on the maintenance of the list of QPS biological agents intentionally added to food or feed (2011 update). Scientific Opinion of the Panel on Biological Hazards (Question No EFSA-Q-2011-00070). EFSA J 9, 2497.

EFSA, 2012. Guidance on the assessment of bacterial susceptibility to antimicrobials of human and veterinary importance. Scientific Opinion of the EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP). EFSA J 10, 2740.

EFSA, 2013. Scientific Opinion on the maintenance of the list of QPS biological agents intentionally added to food and feed (2013 update) (Question No EFSA-Q-2013-00019). EFSA Journal 11, 3449.

EFSA, 2017. Scientific Opinion on the maintenance of the list of QPS biological agents intentionally added to food and feed (2015 update) (Question No EFSA-Q-2013-00019). EFSA Journal 15, 4663.

Esaiassen, E., Hjerde, E., Cavanagh, J.P., Pedersen, T., Andresen, J.H., Rettedal, S.I., Stoen, R., Nakstad, B., Willassen, N.P., Klingenberg, C., 2018. Effects of Probiotic Supplementation on the Gut Microbiota and Antibiotic Resistome Development in Preterm Infants. Front Pediatr 6, 347.

Fabian, E., Kramer, L., Siebert, F., Hogenauer, C., Raggam, R.B., Wenzl, H., Krejs, G.J., 2017. D-lactic acidosis - case report and review of the literature. Z Gastroenterol 55, 75-82.

FAO/WHO, 1974. Toxicological evaluation of certain food additives with a review of general principles and of specifications, Seventeenth Report of the Joint FAO-WHO Expert Committee on Food Additives, pp. 1-40.

FAO/WHO, 2001. Expert consultation on evaluation of health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria. Prevention 5, 1-10.

FAO/WHO, 2002. Guidelines for the Evaluation of Probiotics in Food. Report of a Joint FAO/WHO Working Group on Drafting Guidelines for the Evaluation of Probiotics in Food, Probiotics in Food. Health and Nutritional Properties and Guidelines for Evaluation, Geneva.

- FDA, U.S., 2011. Agency response letter GRAS Notice No. GRN000357.
- Gilliland, S.E., Speck, M.L., 1977. Use of the Minitek system for characterizing lactobacilli. *Appl Environ Microbiol* 33, 1289-1292.
- Gilliland, S.E., Speck, M.L., Morgan, C.G., 1975. Detection of *Lactobacillus acidophilus* in feces of humans, pigs, and chickens. *Appl Microbiol* 30, 541-545.
- Goldman, H.I., Karelitz, S., Seifter, E., Acs, H., Schell, N.B., 1961. Acidosis in premature infants due to lactic acid. *Pediatrics* 27, 921-930.
- Haschke-Becher, E., Brunser, O., Cruchet, S., Gotteland, M., Haschke, F., Bachmann, C., 2008. Urinary D-lactate excretion in infants receiving *Lactobacillus johnsonii* with formula. *Ann Nutr Metab* 53, 240-244.
- Hempel, S., Newbery, S., Ruelaz, A., Wang, Z., Miles, J.N.V., Suttorp, M.J., Johnsen, B., Shanman, R., Slusser, W., Fu, N., Smith, A., Roth, E., Polak, J., Motala, A., Perry, T., Shekelle, P.G., 2011. Safety of Probiotics to Reduce Risk and Prevent or Treat Disease. Evidence Report/Technology Assessment No. 200. (Prepared by the Southern California Evidence-based Practice Center under Contract No. 290-2007-10062-I.), Rockville, MD.
- Hong Chau, T.T., Minh Chau, N.N., Hoang Le, N.T., Chung The, H., Voong Vinh, P., Nguyen To, N.T., Ngoc, N.M., Tuan, H.M., Chau Ngoc, T.L., Kolader, M.E., Farrar, J.J., Wolbers, M., Thwaites, G.E., Baker, S., Oxford-Vietnam Probiotics Study, G., 2018. A Double-blind, Randomized, Placebo-controlled Trial of *Lactobacillus acidophilus* for the Treatment of Acute Watery Diarrhea in Vietnamese Children. *Pediatr Infect Dis J* 37, 35-42.
- Hoyos, A.B., 1999. Reduced incidence of necrotizing enterocolitis associated with enteral administration of *Lactobacillus acidophilus* and *Bifidobacterium infantis* to neonates in an intensive care unit. *Int J Infect Dis* 3, 197-202.
- Jacobs, H.M., Christian, J.R., 1957. Observations on full-term newborn infants receiving an acidified milk formula. *J Lancet* 77, 157-159.
- Klaenhammer, T.R., Kleeman, E.G., 1981. Growth Characteristics, Bile Sensitivity, and Freeze Damage in Colonial Variants of *Lactobacillus acidophilus*. *Appl Environ Microbiol* 41, 1461-1467.
- Ku, W., Lau, D., Huen, K., 2006. Probiotics Provoked D-lactic Acidosis in Short Bowel Syndrome: Case Report and Literature Review. *HK J Paediatr (new series)* 11, 246-254.
- Kullen, M.J., Sanozky-Dawes, R.B., Crowell, D.C., Klaenhammer, T.R., 2000. Use of the DNA sequence of variable regions of the 16S rRNA gene for rapid and accurate identification of bacteria in the *Lactobacillus acidophilus* complex. *J Appl Microbiol* 89, 511-516.
- Lee, S.J., Cho, S.J., Park, E.A., 2007. Effects of probiotics on enteric flora and feeding tolerance in preterm infants. *Neonatology* 91, 174-179.
- Lin, H.C., Hsu, C.H., Chen, H.L., Chung, M.Y., Hsu, J.F., Lien, R.I., Tsao, L.Y., Chen, C.H., Su, B.H., 2008. Oral probiotics prevent necrotizing enterocolitis in very low birth weight preterm infants: a multicenter, randomized, controlled trial. *Pediatrics* 122, 693-700.

Lin, H.C., Su, B.H., Chen, A.C., Lin, T.W., Tsai, C.H., Yeh, T.F., Oh, W., 2005. Oral probiotics reduce the incidence and severity of necrotizing enterocolitis in very low birth weight infants. *Pediatrics* 115, 1-4.

Liu, B., Pop, M., 2008. ARDB—antibiotic resistance genes database. *Nucleic acids research* 37, D443-D447.

Lu, F.C., 1988. Acceptable daily intake: inception, evolution, and application. *Regul Toxicol Pharmacol* 8, 45-60.

Lukasik, J., Salminen, S., Szajewska, H., 2018. Rapid review shows that probiotics and fermented infant formulas do not cause d-lactic acidosis in healthy children. *Acta Paediatr* 107, 1322-1326.

Lyra, A., Hillila, M., Huttunen, T., Mannikko, S., Taalikka, M., Tennila, J., Tarpila, A., Lahtinen, S., Ouwehand, A.C., Veijola, L., 2016. Irritable bowel syndrome symptom severity improves equally with probiotic and placebo. *World J Gastroenterol* 22, 10631-10642.

Manzano, S., De Andres, J., Castro, I., Rodriguez, J.M., Jimenez, E., Espinosa-Martos, I., 2017. Safety and tolerance of three probiotic strains in healthy infants: a multi-centre randomized, double-blind, placebo-controlled trial. *Benef Microbes* 8, 569-578.

Morgensen, G., Salminen, S., O'Brien, J., Holzapfel, A., Shortt, C., Fonden, R., Miller, G.D., Donohue, D., Playne, M., Crittenden, R., Salvadori, B., Zink, R., 2002. Inventory of microorganisms with a documented history of use in food, *Health Benefits and Safety Evaluation of Certain Food Components.*, 377 ed.

Morovic, W., Roper, J.M., Smith, A.B., Mukerji, P., Stahl, B., Rae, J.C., Ouwehand, A.C., 2017. Safety evaluation of HOWARU® Restore (Lactobacillus acidophilus NCFM, Lactobacillus paracasei Lpc-37, Bifidobacterium animalis subsp. lactis BI-04 and B. lactis Bi-07) for antibiotic resistance, genomic risk factors, and acute toxicity. *Food and Chemical Toxicology* 110, 316-324.

Ouwehand, A.C., DongLian, C., Weijian, X., Stewart, M., Ni, J., Stewart, T., Miller, L.E., 2014. Probiotics reduce symptoms of antibiotic use in a hospital setting: a randomized dose response study. *Vaccine* 32, 458-463.

Papagaroufalis, K., Fotiou, A., Egli, D., Tran, L.A., Steenhout, P., 2014. A Randomized Double Blind Controlled Safety Trial Evaluating d-Lactic Acid Production in Healthy Infants Fed a Lactobacillus reuteri-containing Formula. *Nutr Metab Insights* 7, 19-27.

Pariza, M.W., Gillies, K.O., Kraak-Ripple, S.F., Leyer, G., Smith, A.B., 2015. Determining the safety of microbial cultures for consumption by humans and animals. *Regul Toxicol Pharmacol* 73, 164-171.

Petersen, E.R., Claesson, M.H., Schmidt, E.G., Jensen, S.S., Ravn, P., Olsen, J., Ouwehand, A.C., Kristensen, N.N., 2012. Consumption of probiotics increases the effect of regulatory T cells in transfer colitis. *Inflammatory bowel diseases* 18, 131-142.

Pot, B., Felis, G.E., De Bruyne, K., Tsakalidou, E., Papadimitriou, K., Leisner, J., Vandamme, P., 2014. The genus Lactobacillus, in: W.H. Holzapfel, B.J.B. Wood (Eds.), *Lactic acid bacteria: biodiversity and taxonomy*. John Wiley & Sons.



Quigley, E.M.M., Pot, B., Sanders, M.E., 2018. 'Brain Fogginess' and D-Lactic Acidosis: Probiotics Are Not the Cause. *Clin Transl Gastroenterol* 9, 187.

Rao, S.S.C., Rehman, A., Yu, S., Andino, N.M., 2018a. Brain fogginess, gas and bloating: a link between SIBO, probiotics and metabolic acidosis. *Clin Transl Gastroenterol* 9, 162.

Rao, S.S.C., Yu, S., 2018. Response to Sachdeva et al: Brain Fogginess and SIBO Is Not a Mirage. *Clin Transl Gastroenterol* 9, 194.

Rao, S.S.C., Yu, S., Tetangco, E.P., Yan, Y., 2018b. Probiotics can Cause D-Lactic Acidosis and Brain Fogginess: Reply to Quigley et al. *Clin Transl Gastroenterol* 9, 207.

Renwick, A.G., 1990. Acceptable daily intake and the regulation of intense sweeteners. *Food Addit Contam* 7, 463-475.

Reuman, P.D., Duckworth, D.H., Smith, K.L., Kagan, R., Bucciarelli, R.L., Ayoub, E.M., 1986. Lack of effect of *Lactobacillus* on gastrointestinal bacterial colonization in premature infants. *Pediatr Infect Dis* 5, 663-668.

Ringel-Kulka, T., Palsson, O.S., Maier, D., Carroll, I., Galanko, J.A., Leyer, G., Ringel, Y., 2011. Probiotic bacteria *Lactobacillus acidophilus* NCFM and *Bifidobacterium lactis* Bi-07 versus placebo for the symptoms of bloating in patients with functional bowel disorders: a double-blind study. *J Clin Gastroenterol* 45, 518-525.

Roager, H.M., Sulek, K., Skov, K., Frandsen, H.L., Smedsgaard, J., Wilcks, A., Skov, T.H., Villas-Boas, S.G., Licht, T.R., 2014. *Lactobacillus acidophilus* NCFM affects vitamin E acetate metabolism and intestinal bile acid signature in monocolonized mice. *Gut Microbes* 5, 296-303.

Rousseaux, C., Thuru, X., Gelot, A., Barnich, N., Neut, C., Dubuquoy, L., Dubuquoy, C., Merour, E., Geboes, K., Chamaillard, M., Ouwehand, A., Leyer, G., Carcano, D., Colombel, J.F., Ardid, D., Desreumaux, P., 2007. *Lactobacillus acidophilus* modulates intestinal pain and induces opioid and cannabinoid receptors. *Nat Med* 13, 35-37.

Rulis, A.M., Levitt, J.A., 2009. FDA'S food ingredient approval process: Safety assurance based on scientific assessment. *Regul Toxicol Pharmacol* 53, 20-31.

Sachdeva, S., Puri, A.S., Kumar, A., Dalal, A., Dahale, A.S., 2018. Brain Fogginess and SIBO: A Link or Just a Mirage? *Clin Transl Gastroenterol* 9, 184.

Salminen, S., von, W.A., Morelli, L., Marteau, P., Brassart, D., De Vos, W.M., Fonden, R., Saxelin, M., Collins, K., Mogensen, G., Birkeland, S.E., Mattila-Sandholm, T., 1998. Demonstration of safety of probiotics -- a review. *Int J Food Microbiol* 44, 93-106.

Samanta, M., Sarkar, M., Ghosh, P., Ghosh, J., Sinha, M., Chatterjee, S., 2009. Prophylactic probiotics for prevention of necrotizing enterocolitis in very low birth weight newborns. *J Trop Pediatr* 55, 128-131.

Sanders, M.E., Gibson, G., Gill, H.S., Guarner, F., 2007. Probiotics: Their Potential to Impact Human Health, pp. 1-20.

- Sanders, M.E., Walker, D.C., Walker, K.M., Aoyama, K., Klaenhammer, T.R., 1996. Performance of commercial cultures in fluid milk applications. *J Dairy Sci* 79, 943-955.
- Sheedy, J.R., Wettenhall, R.E., Scanlon, D., Gooley, P.R., Lewis, D.P., McGregor, N., Stapleton, D.I., Butt, H.L., KL, D.E.M., 2009. Increased d-lactic Acid intestinal bacteria in patients with chronic fatigue syndrome. *In Vivo* 23, 621-628.
- Szajewska, H., Skorka, A., Piescik-Lech, M., 2015. Fermented infant formulas without live bacteria: a systematic review. *Eur J Pediatr* 174, 1413-1420.
- Underwood, M.A., Salzman, N.H., Bennett, S.H., Barman, M., Mills, D.A., Marcobal, A., Tancredi, D.J., Bevins, C.L., Sherman, M.P., 2009. A randomized placebo-controlled comparison of 2 prebiotic/probiotic combinations in preterm infants: impact on weight gain, intestinal microbiota, and fecal short-chain fatty acids. *J Pediatr Gastroenterol Nutr* 48, 216-225.
- Uribarri, J., Oh, M.S., Carroll, H.J., 1998. D-lactic acidosis. A review of clinical presentation, biochemical features, and pathophysiologic mechanisms. *Medicine (Baltimore)* 77, 73-82.
- Vitetta, L., Coulson, S., Thomsen, M., Nguyen, T., Hall, S., 2017. Probiotics, D-Lactic acidosis, oxidative stress and strain specificity. *Gut Microbes* 8, 311-322.
- Wagner, R.D., Warner, T., Roberts, L., Farmer, J., Balish, E., 1997. Colonization of congenitally immunodeficient mice with probiotic bacteria. *Infect Immun* 65, 3345-3351.
- Walker, D.C., Aoyama, K., Klaenhammer, T.R., 1996. Electrotransformation of *Lactobacillus acidophilus* group A1. *FEMS microbiology letters* 138, 233-237.
- Wang, S., Zhu, H., Lu, C., Kang, Z., Luo, Y., Feng, L., Lu, X., 2012. Fermented milk supplemented with probiotics and prebiotics can effectively alter the intestinal microbiota and immunity of host animals. *Journal of Dairy Science* 95, 4813-4822.
- West, N.P., Horn, P.L., Barrett, S., Warren, H.S., Lehtinen, M.J., Koerbin, G., Brun, M., Pyne, D.B., Lahtinen, S.J., Fricker, P.A., 2014a. Supplementation with a single and double strain probiotic on the innate immune system for respiratory illness. *e-SPEN Journal* 9, e178-e184.
- West, N.P., Horn, P.L., Pyne, D.B., Gebiski, V.J., Lahtinen, S.J., Fricker, P.A., Cripps, A.W., 2014b. Probiotic supplementation for respiratory and gastrointestinal illness symptoms in healthy physically active individuals. *Clinical nutrition (Edinburgh, Scotland)* 33, 581-587.
- West, N.P., Horn, P.L., Pyne, D.B., Warren, H.S., Asad, S., Cox, A.J., Lahtinen, S.J., Lehtinen, M.J., Fricker, P.A., Cripps, A.W., Fazekas de St Groth, B., 2016. Probiotic supplementation has little effect on peripheral blood regulatory T cells. *J Allergy Clin Immunol* 138, 1749-1752 e1747.
- Whelan, K., Myers, C.E., 2010. Safety of probiotics in patients receiving nutritional support: a systematic review of case reports, randomized controlled trials, and nonrandomized trials. *Am J Clin Nutr* 91, 687-703.

Zhou, C.E., Smith, J., Lam, M., Zemla, A., Dyer, M.D., Slezak, T., 2006. MvirDB—a microbial database of protein toxins, virulence factors and antibiotic resistance genes for bio-defence applications. *Nucleic acids research* 35, D391-D394.

## I. Appendices

## **Appendix A: Manufactured Batch Analysis**

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**Certificate of Analysis**

Date: 11 Mar 2019

Our ref. no.: 0

Your ref.

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Material:	1223579	HOWARU Dophilus 200B - 1 KG
Batch No.:		Best before date: 15 Jan 2021
Quantity:	0.000	Production date: 16 Jan 2019

---

Test	Result	Specification	Unit	Reference
Viable Cell Count	3.65E+11	> 2.00E+11	/g	ISO 7889/IDF 117
Enterococcus	< 100	< 100	/g	SMEDP
Non Lactics	< 5000	< 5000	/g	ISO 13559
Coliforms	< 10.0	< 10.0	/g	AOAC
E. coli, neg. by test (<0.3/g)	Negative	Negative		AOAC
Staph. aureus, neg. by test (<10/g)	Negative	Negative		AOAC
Salmonella, negative in 40 g	Negative	Negative		AOAC
Listeria, negative in 25 g	Negative	Negative		AOAC

**Comments**

Exceeds 200 billion CFU/gm of freeze-dried Lb. acidophilus.

AOAC references above reflect the current edition of AOAC.

Each probiotic intermediate is confirmed to the genus/species level (or sub-species level where applicable) based on DNA fingerprinting analysis prior to milling and blending.

Refer to the Product Description (PD) for other non-batch related information. This product batch has been manufactured and released in compliance with FSSC 22000.

**Certificate of Analysis**

Date: 11 Mar 2019

Our ref. no.: 0

Your ref.

---

Material:	1223579	HOWARU Dophilus 200B - 1 KG
Batch No.:		Best before date: 15 Jan 2021
Quantity:	0.000	Production date: 16 Jan 2019

---

The material property data reported in this COA are representative values obtained in laboratory tests conducted on product sample taken from the batch produced. DuPont certifies that the values reported are the results of the tests conducted. DuPont makes no warranties regarding those values or the product in this COA, including as to fitness for a particular purpose or merchantability of goods produced from this supplied product or the supplied product itself, except for warranties expressly stated in the DuPont Product Description (PD) or otherwise agreed in writing by DuPont. All other warranties are specifically excluded. DuPont's standard terms and conditions apply to the sale of the supplied product unless otherwise agreed in writing by DuPont.

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**Phil Ihrke**

Quality Control Department

**Certificate of Analysis**

Date: 11 Mar 2019

Our ref. no.: 0

Your ref.

---

Material:	1223579	HOWARU Dophilus 200B - 1 KG
Batch No.:		Best before date: 29 Oct 2020
Quantity:	0.000	Production date: 30 Oct 2018

---

Test	Result	Specification	Unit	Reference
Viable Cell Count	3.31E+11	> 2.00E+11	/g	ISO 7889/IDF 117
Enterococcus	< 100	< 100	/g	SMEDP
Non Lactics	< 5000	< 5000	/g	ISO 13559
Coliforms	< 10.0	< 10.0	/g	AOAC
E. coli, neg. by test (<0.3/g)	Negative	Negative		AOAC
Staph. aureus, neg. by test (<10/g)	Negative	Negative		AOAC
Salmonella, negative in 40 g	Negative	Negative		AOAC
Listeria, negative in 25 g	Negative	Negative		AOAC

**Comments**

Exceeds 200 billion CFU/gm of freeze-dried Lb. acidophilus.

AOAC references above reflect the current edition of AOAC.

Each probiotic intermediate is confirmed to the genus/species level (or sub-species level where applicable) based on DNA fingerprinting analysis prior to milling and blending.

Refer to the Product Description (PD) for other non-batch related information. This product batch has been manufactured and released in compliance with FSSC 22000.

**Certificate of Analysis**

Date: 11 Mar 2019

Our ref. no.: 0

Your ref.

---

Material:	1223579	HOWARU Dophilus 200B - 1 KG
Batch No.:	[REDACTED]	Best before date: 29 Oct 2020
Quantity:	0.000	Production date: 30 Oct 2018

---

The material property data reported in this COA are representative values obtained in laboratory tests conducted on product sample taken from the batch produced. DuPont certifies that the values reported are the results of the tests conducted. DuPont makes no warranties regarding those values or the product in this COA, including as to fitness for a particular purpose or merchantability of goods produced from this supplied product or the supplied product itself, except for warranties expressly stated in the DuPont Product Description (PD) or otherwise agreed in writing by DuPont. All other warranties are specifically excluded. DuPont's standard terms and conditions apply to the sale of the supplied product unless otherwise agreed in writing by DuPont.

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**Phil Ihrke**

Quality Control Department



**Certificate of Analysis**

Date: 11 Mar 2019

Our ref. no.: 0

Your ref.

---

Material:	1223579	HOWARU Dophilus 200B - 1 KG
Batch No.:		Best before date: 05 Sep 2020
Quantity:	0.000	Production date: 06 Sep 2018

---

Test	Result	Specification	Unit	Reference
Viable Cell Count	3.73E+11	> 2.00E+11	/g	ISO 7889/IDF 117
Enterococcus	< 100	< 100	/g	SMEDP
Non Lactics	< 5000	< 5000	/g	ISO 13559
Coliforms	< 10.0	< 10.0	/g	AOAC
E. coli, neg. by test (<0.3/g)	Negative	Negative		AOAC
Staph. aureus, neg. by test (<10/g)	Negative	Negative		AOAC
Salmonella, negative in 40 g	Negative	Negative		AOAC
Listeria, negative in 25 g	Negative	Negative		AOAC

**Comments**

Exceeds 200 billion CFU/gm of freeze-dried Lb. acidophilus.

AOAC references above reflect the current edition of AOAC.

Each probiotic intermediate is confirmed to the genus/species level (or sub-species level where applicable) based on DNA fingerprinting analysis prior to milling and blending.

Refer to the Product Description (PD) for other non-batch related information. This product batch has been manufactured and released in compliance with FSSC 22000.

**Certificate of Analysis**

Date: 11 Mar 2019

Our ref. no.: 0

Your ref.

---

Material:	1223579	HOWARU Dophilus 200B - 1 KG
Batch No.:		Best before date: 05 Sep 2020
Quantity:	0.000	Production date: 06 Sep 2018

---

The material property data reported in this COA are representative values obtained in laboratory tests conducted on product sample taken from the batch produced. DuPont certifies that the values reported are the results of the tests conducted. DuPont makes no warranties regarding those values or the product in this COA, including as to fitness for a particular purpose or merchantability of goods produced from this supplied product or the supplied product itself, except for warranties expressly stated in the DuPont Product Description (PD) or otherwise agreed in writing by DuPont. All other warranties are specifically excluded. DuPont's standard terms and conditions apply to the sale of the supplied product unless otherwise agreed in writing by DuPont.

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**Phil Ihrke**

Quality Control Department

**Certificate of Analysis**

Date: 11 Mar 2019

Our ref. no.: 0

Your ref.

---

Material:	1223579	HOWARU Dophilus 200B - 1 KG
Batch No.:		Best before date: 28 Jun 2020
Quantity:	0.000	Production date: 29 Jun 2018

---

Test	Result	Specification	Unit	Reference
Viable Cell Count	3.83E+11	> 2.00E+11	/g	ISO 7889/IDF 117
Enterococcus	< 100	< 100	/g	SMEDP
Non Lactics	< 5000	< 5000	/g	ISO 13559
Coliforms	< 10.0	< 10.0	/g	AOAC
E. coli, neg. by test (<0.3/g)	Negative	Negative		AOAC
Staph. aureus, neg. by test (<10/g)	Negative	Negative		AOAC
Salmonella, negative in 40 g	Negative	Negative		AOAC
Listeria, negative in 25 g	Negative	Negative		AOAC

**Comments**

Exceeds 200 billion CFU/gm of freeze-dried Lb. acidophilus.

AOAC references above reflect the current edition of AOAC.

Each probiotic intermediate is confirmed to the genus/species level (or sub-species level where applicable) based on DNA fingerprinting analysis prior to milling and blending.

Refer to the Product Description (PD) for other non-batch related information. This product batch has been manufactured and released in compliance with FSSC 22000.

**Certificate of Analysis**

Date: 11 Mar 2019

Our ref. no.: 0

Your ref.

---

Material:	1223579	HOWARU Dophilus 200B - 1 KG
Batch No.:		Best before date: 28 Jun 2020
Quantity:	0.000	Production date: 29 Jun 2018

---

The material property data reported in this COA are representative values obtained in laboratory tests conducted on product sample taken from the batch produced. DuPont certifies that the values reported are the results of the tests conducted. DuPont makes no warranties regarding those values or the product in this COA, including as to fitness for a particular purpose or merchantability of goods produced from this supplied product or the supplied product itself, except for warranties expressly stated in the DuPont Product Description (PD) or otherwise agreed in writing by DuPont. All other warranties are specifically excluded. DuPont's standard terms and conditions apply to the sale of the supplied product unless otherwise agreed in writing by DuPont.

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**Phil Ihrke**

Quality Control Department

**Certificate of Analysis**

Date: 11 Mar 2019

Our ref. no.: 0

Your ref.

---

Material:	1223579	HOWARU Dophilus 200B - 1 KG
Batch No.:		Best before date: 26 Apr 2020
Quantity:	0.000	Production date: 27 Apr 2018

---

Test	Result	Specification	Unit	Reference
Viable Cell Count	3.21E+11	> 2.00E+11	/g	ISO 7889/IDF 117
Enterococcus	< 100	< 100	/g	SMEDP
Non Lactics	< 5000	< 5000	/g	ISO 13559
Coliforms	< 10.0	< 10.0	/g	AOAC
E. coli, neg. by test (<0.3/g)	Negative	Negative		AOAC
Staph. aureus, neg. by test (<10/g)	Negative	Negative		AOAC
Salmonella, negative in 40 g	Negative	Negative		AOAC
Listeria, negative in 25 g	Negative	Negative		AOAC

**Comments**

Exceeds 200 billion CFU/gm of freeze-dried Lb. acidophilus.

AOAC references above reflect the current edition of AOAC.

Each probiotic intermediate is confirmed to the genus/species level (or sub-species level where applicable) based on DNA fingerprinting analysis prior to milling and blending.

Refer to the Product Description (PD) for other non-batch related information. This product batch has been manufactured and released in compliance with FSSC 22000.

**Certificate of Analysis**

Date: 11 Mar 2019

Our ref. no.: 0

Your ref.

---

Material:	1223579	HOWARU Dophilus 200B - 1 KG
Batch No.:		Best before date: 26 Apr 2020
Quantity:	0.000	Production date: 27 Apr 2018

---

The material property data reported in this COA are representative values obtained in laboratory tests conducted on product sample taken from the batch produced. DuPont certifies that the values reported are the results of the tests conducted. DuPont makes no warranties regarding those values or the product in this COA, including as to fitness for a particular purpose or merchantability of goods produced from this supplied product or the supplied product itself, except for warranties expressly stated in the DuPont Product Description (PD) or otherwise agreed in writing by DuPont. All other warranties are specifically excluded. DuPont's standard terms and conditions apply to the sale of the supplied product unless otherwise agreed in writing by DuPont.

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**Phil Ihrke**

Quality Control Department

Date: March 11, 2019

Product: 1286076 HOWARU Dophilus PN 200B - 1 KG

To Whom It May Concern,

Please be informed that DuPont Nutrition and Health performs annual surveillance testing for heavy metals on samples of finished products. This analysis is not batch release criteria.

Based on historical data, the above listed product complies with the following specification:

- Arsenic <1 ppm
- Lead <0.5 ppm
- Mercury <0.05 ppm
- Cadmium <0.2 ppm

This information is given in respect of DuPont's policy of openness and transparency with its customers.

Sincerely,

A grey rectangular box redacting the signature of Sarah Pace.

Sarah Pace  
Quality & Food Safety Coordinator  
DuPont - Nutrition & Health

Date: March 11, 2019

Product: 1223579 HOWARU Dophilus 200B - 1 KG

To Whom It May Concern,

Please be informed that Yeast and Mold is not an external specification on the Product Description or Certificate of Analysis for the above listed product. Yeast and Mold is an internal parameter on the intermediate product and is monitored for every batch to ensure the safety of the finished product. We do not disclose the specification or analytical results for this parameter.

This information is given in respect of DuPont's policy of openness and transparency with its customers.

Sincerely,

A grey rectangular box redacting the signature of Sarah Pace.

Sarah Pace  
Quality & Food Safety Coordinator  
DuPont - Nutrition & Health



## **Appendix B: Genetically Modified Organism and Allergen Affirmation**

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Date: March 11, 2019

Product: 1286076 HOWARU Dophilus PN 200B - 1 KG

To Whom It May Concern,

DuPont certifies that the above listed product has not been genetically modified. This product does not consist of, nor contains, nor is produced from genetically modified organisms according to the definitions of Regulation (EC) 1829/2003 and Regulation (EC) 1830/2003 of the European Parliament and of the Council of 22 September 2003.

This information is given in respect of DuPont's policy of openness and transparency with its customers.

Sincerely,

A grey rectangular box redacting the signature of Sarah Pace.

Sarah Pace  
Quality & Food Safety Coordinator  
DuPont - Nutrition & Health

Date: March 11, 2019

Product: 1286076 HOWARU Dophilus PN 200B - 1 KG

To Whom It May Concern,

DuPont certifies that the above listed product does not contain allergens as determined by The Food Allergen Labeling and Consumer Protection Act of 2004 (FALCPA) (Public Law 108-282) (U.S. Food and Drug Administration, 2006), including protein derived from milk, eggs, fish, crustacean shellfish, mollusks, tree nuts, wheat, peanuts, soybean, celery, mustard, and sesame seeds.

This information is given in respect of DuPont's policy of openness and transparency with its customers.

Sincerely,

A grey rectangular box redacting the signature of Sarah Pace.

Sarah Pace  
Quality & Food Safety Coordinator  
DuPont - Nutrition & Health

## **Appendix C: Clinical Studies**

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## Clinical trials investigating *Lactobacillus acidophilus* NCFM.

Human Study	Number of subjects consuming NCFM/ total number of subject & design	Supplementation daily dose and length	Adverse events
<b>Gastrointestinal Ecology</b>			
(Forssten et al., 2014)	40/80 all healthy volunteers received amoxicillin or clavulanate RPCTB parallel arm	$12.5 \times 10^9$ CFU of NCFM (ATCC 700396) and $12.5 \times 10^9$ CFU of Bi-07(ATCC SD5220) combined for 14 days	No between group differences in number of participants reporting any adverse event. One subject in the probiotic group withdrew during the antibiotic supplementation due to upset stomach. No serious adverse events were reported during the study.
(van Zanten et al., 2014)	9/18 healthy adults RDBPC crossover	$1.9 \times 10^9$ CFU of NCFM + 5 g cellobiose for 3 weeks	The treatment was well tolerated. Self-reported GI symptoms did not differ between groups.
(Ouwehand et al., 2014b)	20/40 healthy males RDBPC parallel arm	$2 \times 10^9$ CFU, 4 weeks	Not stated.
(Bjorklund et al., 2012)	24/51 healthy elderly (over 80 yrs) subjects with regular use of NSAIDs RDBPC parallel arm	$2 \times 10^{10}$ CFU of NCFM + 10 g lactitol for 2 weeks	Not stated.
(Larsen et al., 2011)	17 /50 children (7 – 24 mo) with atopic dermatitis RDBPC parallel arm	$10^{10}$ CFU of NCFM for 8 weeks	Not stated.
(Engelbrektsen et al., 2009)	25/51 healthy subjects administered amoxicillin or clavulanate RPC parallel arm (blinding was not reported)	$5 \times 10^9$ CFU of NCFM in a multispecies probiotic supplement containing five strains for 20 days	No significant difference in adverse events between the placebo and probiotic groups. The recorded adverse events included < 7 day lasting diarrhea, vaginal yeast infection and abdominal cramping. None of the subjects discontinued due to adverse events. The adverse effects may also be due to the antibiotic challenge given in the study. The treatment was well tolerated.
(Engelbrektsen et al., 2006)	32/64 healthy subjects RDBPC parallel arm  20/40 healthy individuals administered amoxicillin or clavulanate RDBPC parallel arm	$2 \times 10^7$ - $2 \times 10^8$ CFU of NCFM in a multispecies probiotic supplement containing four strains +/- Frutafit® for nine weeks twice a day  $5 \times 10^9$ CFU of NCFM in a multispecies probiotic supplement containing five strains for 21 days	Not stated.  Not stated.
(Sui et al., 2002)	10/10 healthy subjects Open label	$10^{10}$ CFU of NCFM for 2 weeks	Not stated.
(Varcoe et al., 2002)	10/10 healthy adults RSB comparison parallel arm	$10^{10}$ CFU of NCFM separately in milk and water for 10 - 14 days with 10 -14 day wash-out periods in between	Not stated.
(Gilliland et al., 1978)	21/29 PC parallel arm (3 trials)	$5 \times 10^6$ , $2 \times 10^7$ or $8 \times 10^8$ CFU of NCFM for 23 or 51 days	Not stated.
<b>Small Bowel Bacterial Overgrowth</b>			
(Dunn et al., 1998)	13/29 Patients with end stage kidney disease	$2 \times 10^9$ CFU or <i>L. acidophilus</i> BG2F04 67 days	No adverse events were reported.

Human Study	Number of subjects consuming NCFM/ total number of subject & design	Supplementation daily dose and length	Adverse events
(Simenhoff and Dunn, 1996)	Open label, PC parallel arm 24/24 Hemodialysis patients Open label, PC parallel arm	10 <sup>10</sup> CFU for at least 1 month	Not stated.
(Simenhoff et al., 1996) [Strain LBA = NCFM]	8/13 Patients with end stage kidney disease	10 <sup>10</sup> CFU for 30 – 182 days	No one experienced any side effects.
<b>Synbiotic</b>			
(Gelardi et al., 2017)	52/93 RDBPC parallel arm	NCFM, B. Lactis and FOS(Pollagen(R)) for 4 weeks	Not stated
(Irwin et al., 2017)	20/38 RDBPC parallel arm	Synbiotic containing NCFM 12.5 × 10 <sup>9</sup> CFU and Bi07 12.5 × 10 <sup>9</sup> CFU daily with or without Larch Gum for 8 weeks	9 participants reported gastrointestinal symptoms throughout the intervention, (n=4 in placebo, n=2 in prebiotic, n=1 in probiotic, n = 2 in synbiotic group). The most frequently reported GI symptoms were bloating, diarrhea, gas/flatulence, stomach pain/cramps, constipation, and nausea. No serious adverse events were registered during the study.
(Magro et al., 2014)	26/47 RDBPC parallel arm	10 <sup>9</sup> CFU of NCFM and HN019 with polydextrose (Litesse) for 14 days	Not stated.
(Waitzberg et al., 2013)	50/100 Healthy, constipated adult women RDBPC parallel arm	6 g FOS and 10 <sup>8</sup> -10 <sup>9</sup> CFU of strains Lpc-37, HN001, NCFM and HN019 daily for 30 days	No differences between groups in abdominal symptoms (pain, bloating, flatulence).
(Wang et al., 2012)	50/100	Fermented milk supplemented with NCFM 3,2 X 10 <sup>10</sup> CFU, Bi07 3,8 X 10 <sup>10</sup> CFU and isomaltooligosaccharide for 14 days	Not stated
(Ouwehand et al., 2009b)	24/47 elderly (over 65 y) subjects using NSAIDs regularly on controlled normal diet RDBPC parallel arm	2 x 10 <sup>9</sup> CFU with lactitol for 2 weeks	No significant difference between groups in side-effects or aberrations of intestinal function
(Schrezenmeir et al., 2004)	46/129 acutely ill children (1 – 6 yrs) RDBPC parallel arm	Nutritional supplement Pediasure® Protect with SmartChoice™ contains 3.5 g/L of FOS and 1 × 10 <sup>9</sup> CFU/g of <i>Lactobacillus acidophilus</i> and <i>Bifidobacterium</i> species.	Treatment was well tolerated, no changes from baseline for stool consistency or frequency, diarrhea, constipation, or vomiting. All GI symptoms were considered mild. Adverse events were considered unrelated to treatment.
(Fisberg et al., 2002)	310/626 Children , 1-6 years RDBPC parallel arm	~10 <sup>10</sup> CFU in a nutritional supplement containing FOS and NCFM plus <i>Bifidobacterium</i> ssp. for 4 months.	Incidence of adverse events was very low. None of the serious adverse events were considered treatment-related.
(Swanson et al., 2002)	32/62 healthy adults RDBPC parallel arm	10 <sup>9</sup> CFU with sucrose (15 subjects) or 10 <sup>9</sup> CFU with FOS (17 subjects) for 4 weeks	Not stated. Daily bowel function forms revealed no side-effects.
<b>Immune System Enhancement</b>			
(Ibrahim et al., 2010; Lahtinen et al., 2012)	31/31 elderly (> 70 yr) subjects DBPC crossover	10 <sup>9</sup> CFU of NCFM and <i>L. rhamnosus</i> HN001 for 4 weeks	Not stated.
(Paineau et al., 2008)	9/83 RDBPC parallel arm	2 x 10 <sup>10</sup> CFU for 3 weeks	Not stated.
<b>Allergy Treatment</b>			
(Perrin et al., 2014)	31/31 adults with allergic rhinitis	10 <sup>10</sup> CFU each of NCFM + <i>B. lactis</i> for 4 weeks	No formulation-related adverse events.

Human Study	Number of subjects consuming NCFM/ total number of subject & design	Supplementation daily dose and length	Adverse events
(Gobel et al., 2010; Larsen et al., 2011)	RDB cross-over 17/50 children (7 – 24 mo) with atopic dermatitis RDBPC parallel arm	10 <sup>10</sup> CFU NCFM or B. lactis Bi-07 for 8 weeks	Not stated.
(Ouweland et al., 2009a)	24/47 children (mean age 9 yr) with birch pollen allergy RDBPC parallel arm	Two species probiotic 5 X 10 <sup>9</sup> CFU of combination NCFM and BI04 for 4 months	Not stated.
<b>Anti-carcinogenic Activity</b>			
(Goldin and Gorbach, 1984)	10/21 adults devoid of bowel disturbances Open label cross over	10 <sup>9</sup> CFU in milk for 30 days	Not stated.
(Goldin, 1984)	7/7 Open label	>10 <sup>10</sup> CFU	Not stated.
(Goldin et al., 1980)	7/31 young adult subjects (20 to 30 y) with different diets Open label cross over	10 <sup>9</sup> CFU in milk for 30 days	Not stated.
<b>Cholesterol Lowering</b>			
(Thompson et al., 1982)	12/68 healthy adults Parallel comparison Randomization & blinding not reported	10 <sup>10</sup> CFU for 3 weeks	Well tolerated Not stated.
<b>Insulin sensitivity</b>			
(Andreasen et al., 2010)	24/48 Type II diabetic male adults RDBPC parallel arm	10 <sup>10</sup> CFU in capsules for 4 weeks	Not stated.
<b>Improved Lactose Digestion</b>			
(Mustapha et al., 1997) [Strain LA-1 = NCFM]	11/11 lactose healthy, lactose maldigesters RDBPC comparison trial	~4 x 10 <sup>11</sup> CFU for 1 day	Not stated.
(Montes et al., 1995)	20/20 children with lactose maldigestion 5 – 16 yr RSB Comparison trial	10 <sup>10</sup> CFU for 1 day	Lactose intolerance symptoms abated with NCFM. Not stated.
(Lin et al., 1991)	10/10 healthy, adult lactose maldigesters RDBPC crossover	4 X 10 <sup>10</sup> CFU for 1 day	Lactose intolerance symptoms abated with NCFM. Not stated.
(McDonough et al., 1987)	14/14 lactase intolerant adults Open label comparison trial	2,5 X 10 <sup>10</sup> CFU	Not stated.
(Savaiano et al., 1984)	9/9 lactase deficient adults Open label comparison trial	5 X 10 <sup>9</sup> CFU	Not stated.
(Kim and Gilliland, 1983)	23/29 lactose-intolerant adults devoid of other gastrointestinal disturbances Open label PC dose response	~10 <sup>9</sup> - 10 <sup>10</sup> CFU in milk for 6 days	Daily recording of lactose intolerance symptoms revealed no effect due to supplement.
(Newcomer et al., 1983)	89/89 including 61/61 lactase-sufficient IBS subjects; 18/18 lactase deficient subjects; 10/10 healthy subjects RDB Cross over trial	~1-4 x 10 <sup>9</sup> CFU in milk for 1 (lactase-deficient group) or 2 (lactase-sufficient IBS group and healthy subjects) 2 weeks	No intestinal symptoms among healthy subjects. No difference between treatment and control in GI symptoms or lactose intolerance in IBS and lactose intolerant groups.
(Payne et al., 1981)	11/11 with history of lactose malabsorption Unblinded cross over	>10 <sup>10</sup> CFU for 8 days	Mild diarrhea but due to experimental design cannot be attributed to L. acidophilus.
<b>General health</b>			
(Benor et al., 2014)	49 mothers of 25/58 VLBW infants RDBPC parallel arm	Maternal supplementation with 2 x 10 <sup>10</sup> CFU NCFM + B. lactis from postpartum day 1-3	Treatment may decrease the incidence of NEC in VLBW breastfed infants.” The

Human Study	Number of subjects consuming NCFM/ total number of subject & design	Supplementation daily dose and length	Adverse events
		mean duration 13 days	mothers did not report any adverse effects of the treatment." "There were no adverse maternal or infantile effects.
(West et al., 2014a; West et al., 2014b) (Cox et al., 2014; West et al., 2016)	155/465 physically active healthy adults 47/144 physically active healthy adults 45/150 physically active healthy adults 32/93 physically active healthy adults RDBPC parallel arm	NCFM and Bi-07 at $5 \times 10^9$ CFU each or placebo for 150 days	Supplements well tolerated; no clinically adverse events reported.
(Leyer et al., 2009)	222/326 healthy children of 3-5 years of age RDBPC parallel arm	110 subjects received NCFM at $5 \times 10^9$ CFU for 6 months and 112 subjects received NCFM in combination with <i>B. lactis</i> Bi-07 containing $5 \times 10^9$ CFU of each strain	No notable adverse events associated were attributed to study probiotic strains.
<b>Antibiotic associated diarrhea</b>			
(Ouwehand et al., 2014a)	Hospitalized, antibiotic-treated adults high dose 168/503 low dose 168/503 RTBPC parallel arm	NCFM + Bi-04 + Bi-07 + Lpc-37 (HOWARU Restore) $4,17 \times 10^9$ CFU or $1,7 \times 10^{10}$ CFU, administered daily up to 7 days after the final antibiotic dose	No differences in adverse event rate among the groups. No adverse events associated with the supplement.
<b>Gastrointestinal Functionality</b>			
(D'Souza et al., 2017)	133/259 after colonoscopy RDBPC parallel arm	NCFM + Bi-07 at $1.25 \times 10^{10}$ CFU each or placebo for up to 14 days	AE not stated. Bloating and pain measured as outcomes more prevalent in the placebo group.
(Lyra et al., 2016)	129/391 with IBS (low dose) 131/391 with IBS (high dose) RTBPC parallel arm	NCFM $10^9$ CFU (low dose) or NCFM $10^{10}$ CFU (high dose) For 12 weeks	The most common AE were GI disorders (abdominal discomfort, distension, pain or constipation, diarrhea, flatulence), gastroenteritis, and influenza. AEs were evenly distributed in all groups. Two serious AEs (pneumonia, syncope) were unrelated to treatment.
(Ludidi et al., 2014)	21/40 adults with IBS with visceral hypersensitivity RDBPC parallel arm	106 CFU each of NCFM, <i>B. lactis</i> W52, <i>L. casei</i> W56, <i>L. salivarius</i> W57, <i>L. lactis</i> W58 for 6 weeks	Not stated.
(Ringel-Kulka et al., 2014)	Subjects with mild to moderate abdominal pain NCFM; 10/20 NCFM + Bi-07; 10/20 RDB comparison trial	NCFM or NCFM + Bi-07 $2 \times 10^{10}$ CFU for 3 weeks	Not stated. No difference in clinical outcomes between the groups.
(Ringel-Kulka et al., 2011)	31/60 RDBPC parallel	NCFM + Bi-07, $2 \times 10^{11}$ CFU for 8 weeks	Safety parameters reported were blood tests, kidney and liver function, blood urea nitrogen, total protein and albumin, TSH, fecal calprotectin, plasma elastase, and markers for inflammatory response and neutrophil degradation. Most common adverse events were cold symptoms, fatigue, abdominal pain, and sinus infection. No adverse events or changes in blood tests were recorded. No differences between groups in fecal sample markers. No adverse events



Human Study	Number of subjects consuming NCFM/ total number of subject & design	Supplementation daily dose and length	Adverse events
(Faber et al., 2005)	2 IBS subjects, case report	NCFM + Bi-07; 4 X 10 <sup>10</sup> CFU for 8-9 weeks	associated with the supplement. IBS symptoms abated. Adverse effects not stated.
(Heiser et al., 2004)	21/28 HIV positive subjects RDBPC parallel arm	NCFM, bifidobacterium, fiber and glutamine >10 <sup>11</sup> CFU for 12 weeks	The protocol was well tolerated without significant adverse events. There were no adverse events.
(Faber, 2003)	44/44 with (24) or without (20) antibiotics	NCFM 10 <sup>30</sup> CFU + <i>B. infantis</i> 10 <sup>10</sup> CFU for 4 weeks	Not stated.
<b>Oral health</b>			
(Miyazima et al., 2017)	19/60 denture wearers harboring Candida RDBPC parallel arm	NCFM or <i>L. rhamnosus</i> Lr-32 for 8 weeks	Not stated.
<b>Intestinal microbiota</b>			
(Hibberd et al., 2017)	8/15 adults with colon cancer RDBPC parallel arm	7 x 10 <sup>9</sup> CFU NCFM + 1.4 x 10 <sup>10</sup> B. lactis Bi-04 for 31±28 days	Not stated.
<b>Antimicrobial activity</b>			
(Barker et al., 2017; De Wolfe et al., 2018)	16/31 adults experiencing an initial episode of mild to moderate <i>Clostridial difficile</i> infection (CDI) RDBPC parallel arm	1.7 x 10 <sup>10</sup> CFU containing NCFM, <i>L. paracasei</i> Lpc-37, <i>B. lactis</i> Bi-07 and <i>B. lactis</i> BI-04 for 4 weeks	No difference between groups in total number of adverse events. All participants experienced at least one adverse event; GI disorders were most common.

#### References

- Andreasen, A.S., Larsen, N., Pedersen-Skovsgaard, T., Berg, R.M., Moller, K., Svendsen, K.D., Jakobsen, M., Pedersen, B.K., 2010. Effects of Lactobacillus acidophilus NCFM on insulin sensitivity and the systemic inflammatory response in human subjects. *Br J Nutr* 104, 1831-1838.
- Barker, A.K., Duster, M., Valentine, S., Hess, T., Archbald-Pannone, L., Guerrant, R., Safdar, N., 2017. A randomized controlled trial of probiotics for Clostridium difficile infection in adults (PICO). *J Antimicrob Chemother* 72, 3177-3180.
- Benor, S., Marom, R., Ben Tov, A., Armoni Domany, K., Zaidenberg-Israeli, G., Dollberg, S., 2014. Probiotic supplementation in mothers of very low birth weight infants. *Am J Perinatol* 31, 497-504.
- Bjorklund, M., Ouwehand, A.C., Forssten, S.D., Nikkila, J., Tiihonen, K., Rautonen, N., Lahtinen, S.J., 2012. Gut microbiota of healthy elderly NSAID users is selectively modified with the administration of Lactobacillus acidophilus NCFM and lactitol. *Age (Dordr)* 34, 987-999.
- Cox, A.J., West, N.P., Horn, P.L., Lehtinen, M.J., Koerbin, G., Pyne, D.B., Lahtinen, S.J., Fricker, P.A., Cripps, A.W., 2014. Effects of probiotic supplementation over 5 months on routine haematology and clinical chemistry measures in healthy active adults. *Eur J Clin Nutr* 68, 1255-1257.
- D'Souza, B., Slack, T., Wong, S.W., Lam, F., Muhlmann, M., Koestenbauer, J., Dark, J., Newstead, G., 2017. Randomized controlled trial of probiotics after colonoscopy. *ANZ J Surg* 87, E65-E69.
- De Wolfe, T.J., Eggers, S., Barker, A.K., Kates, A.E., Dill-McFarland, K.A., Suen, G., Safdar, N., 2018. Oral probiotic combination of Lactobacillus and Bifidobacterium alters the gastrointestinal microbiota during antibiotic treatment for Clostridium difficile infection. *PLoS One* 13, e0204253.

- Dunn, S.R., Simenhoff, M.L., Ahmed, K.E., Gaughan, W.J., Eltayeb, B.O., Fitzpatrick, M.E.D., Emery, S.M., Ayres, J.W., Holt, K.E., 1998. Effect of oral administration of freeze-dried *Lactobacillus acidophilus* on small bowel bacterial overgrowth in patients with end stage kidney disease: reducing uremic toxins and improving nutrition. *International dairy journal* 8, 545-553.
- Engelbrektsen, A., Korzenik, J.R., Pittler, A., Sanders, M.E., Klaenhammer, T.R., Leyer, G., Kitts, C.L., 2009. Probiotics to minimize the disruption of faecal microbiota in healthy subjects undergoing antibiotic therapy. *J Med Microbiol* 58, 663-670.
- Engelbrektsen, A.L., Korzenik, J.R., Sanders, M.E., Clement, B.G., Leyer, G., Klaenhammer, T.R., Kitts, C.L., 2006. Analysis of treatment effects on the microbial ecology of the human intestine. *FEMS Microbiol Ecol* 57, 239-250.
- Faber, S., Rigden, S., Lukaczer, D., 2005. The use of probiotics in the treatment of irritable bowel syndrome: two case reports. *Altern Ther Health Med* 11, 60-62.
- Faber, S.M., 2003. Are probiotics useful in irritable bowel syndrome? *J Clin Gastroenterol* 37, 93-94.
- Fisberg, M., Maulen-Radovan, I., Tormo, R., Carrascoso, M., Giner, C., Martin, F., Belinchón, P., Costa, C., Pérez, M., Caro, J., 2002. Effect of oral nutritional supplementation with or without synbiotics on sickness and catch-up growth in preschool children. *Int Pediatr* 17, 216-222.
- Forssten, S., Evans, M., Wilson, D., Ouwehand, A.C., 2014. Influence of a probiotic mixture on antibiotic induced microbiota disturbances. *World J Gastroenterol* 20, 11878-11885.
- Gelardi, M., De Luca, C., Taliente, S., Fiorella, M.L., Quaranta, N., Russo, C., Ciofalo, A., Macchi, A., Mancini, M., Rosso, P., Seccia, V., Guagnini, F., Ciprandi, G., 2017. Adjuvant treatment with a symbiotic in patients with inflammatory non-allergic rhinitis. *J Biol Regul Homeost Agents* 31, 201-206.
- Gilliland, S., Speck, M., Nauyok, G., Giesbrecht, F., 1978. Influence of consuming nonfermented milk containing *Lactobacillus acidophilus* on fecal flora of healthy males. *Journal of Dairy Science* 61, 1-10.
- Gobel, R., Larsen, N., Molgaard, C., Jakobsen, M., Michaelsen, K.F., 2010. Probiotics to young children with atopic dermatitis: A randomized placebo-controlled trial. *International Journal of Probiotics and Prebiotics* 5, 53-60.
- Goldin, B.R., 1984. The effect of oral administration of *Lactobacillus* and antibiotics on intestinal bacterial activity and chemical induction of large bowel tumors. *Developments in Industrial Microbiology* 25, 139-150.
- Goldin, B.R., Gorbach, S.L., 1984. The effect of milk and *lactobacillus* feeding on human intestinal bacterial enzyme activity. *Am J Clin Nutr* 39, 756-761.

- Goldin, B.R., Swenson, L., Dwyer, J., Sexton, M., Gorbach, S.L., 1980. Effect of diet and *Lactobacillus acidophilus* supplements on human fecal bacterial enzymes. *J. Natl. Cancer Inst* 64, 255-261.
- Heiser, C.R., Ernst, J.A., Barrett, J.T., French, N., Schutz, M., Dube, M.P., 2004. Probiotics, soluble fiber, and L-Glutamine (GLN) reduce nelfinavir (NFV)- or lopinavir/ritonavir (LPV/r)-related diarrhea. *J Int Assoc Physicians AIDS Care (Chic)* 3, 121-129.
- Hibberd, A.A., Lyra, A., Ouwehand, A.C., Rolny, P., Lindegren, H., Cedgard, L., Wettergren, Y., 2017. Intestinal microbiota is altered in patients with colon cancer and modified by probiotic intervention. *BMJ Open Gastroenterol* 4, e000145.
- Ibrahim, F., Ruvio, S., Granlund, L., Salminen, S., Viitanen, M., Ouwehand, A.C., 2010. Probiotics and immunosenescence: cheese as a carrier. *FEMS Immunol Med Microbiol* 59, 53-59.
- Irwin, C., Khalesi, S., Cox, A.J., Grant, G., Davey, A.K., Bulmer, A.C., Desbrow, B., 2017. Effect of 8-weeks prebiotics/probiotics supplementation on alcohol metabolism and blood biomarkers of healthy adults: a pilot study. *European Journal of Nutrition*, 1-12.
- Kim, H.S., Gilliland, S.E., 1983. *Lactobacillus acidophilus* as a dietary adjunct for milk to aid lactose digestion in humans. *J Dairy Sci* 66, 959-966.
- Lahtinen, S.J., Forssten, S., Aakko, J., Granlund, L., Rautonen, N., Salminen, S., Viitanen, M., Ouwehand, A.C., 2012. Probiotic cheese containing *Lactobacillus rhamnosus* HN001 and *Lactobacillus acidophilus* NCFM(R) modifies subpopulations of fecal lactobacilli and *Clostridium difficile* in the elderly. *Age (Dordr)* 34, 133-143.
- Larsen, N., Vogensen, F.K., Gobel, R., Michaelsen, K.F., Abu Al-Soud, W., Sorensen, S.J., Hansen, L.H., Jakobsen, M., 2011. Predominant genera of fecal microbiota in children with atopic dermatitis are not altered by intake of probiotic bacteria *Lactobacillus acidophilus* NCFM and *Bifidobacterium animalis* subsp. *lactis* Bi-07. *FEMS Microbiol Ecol* 75, 482-496.
- Leyer, G.J., Li, S., Mubasher, M.E., Reifer, C., Ouwehand, A.C., 2009. Probiotic effects on cold and influenza-like symptom incidence and duration in children. *Pediatrics* 124, e172-179.
- Lin, M.Y., Savaiano, D., Harlander, S., 1991. Influence of nonfermented dairy products containing bacterial starter cultures on lactose maldigestion in humans. *J Dairy Sci* 74, 87-95.
- Ludidi, S., Jonkers, D.M., Koning, C.J., Kruimel, J.W., Mulder, L., van der Vaart, I.B., Conchillo, J.M., Masclee, A.A., 2014. Randomized clinical trial on the effect of a multispecies probiotic on visceroperception in hypersensitive IBS patients. *Neurogastroenterol Motil* 26, 705-714.
- Lyra, A., Hillila, M., Huttunen, T., Mannikko, S., Taalikka, M., Tennila, J., Tarpila, A., Lahtinen, S., Ouwehand, A.C., Veijola, L., 2016. Irritable bowel syndrome symptom severity improves equally with probiotic and placebo. *World J Gastroenterol* 22, 10631-10642.

- Magro, D.O., de Oliveira, L.M., Bernasconi, I., Ruela Mde, S., Credidio, L., Barcelos, I.K., Leal, R.F., Ayrizono Mde, L., Fagundes, J.J., Teixeira Lde, B., Ouwehand, A.C., Coy, C.S., 2014. Effect of yogurt containing polydextrose, *Lactobacillus acidophilus* NCFM and *Bifidobacterium lactis* HN019: a randomized, double-blind, controlled study in chronic constipation. *Nutr J* 13, 75.
- McDonough, F.E., Hitchins, A.D., Wong, N.P., Wells, P., Bodwell, C.E., 1987. Modification of sweet acidophilus milk to improve utilization by lactose-intolerant persons. *Am J Clin Nutr* 45, 570-574.
- Miyazima, T.Y., Ishikawa, K.H., Mayer, M., Saad, S., Nakamae, A., 2017. Cheese supplemented with probiotics reduced the *Candida* levels in denture wearers-RCT. *Oral Dis* 23, 919-925.
- Montes, R.G., Bayless, T.M., Saavedra, J.M., Perman, J.A., 1995. Effect of milks inoculated with *Lactobacillus acidophilus* or a yogurt starter culture in lactose-maldigesting children. *J Dairy Sci* 78, 1657-1664.
- Mustapha, A., Jiang, T., Savaiano, D.A., 1997. Improvement of lactose digestion by humans following ingestion of unfermented acidophilus milk: influence of bile sensitivity, lactose transport, and acid tolerance of *Lactobacillus acidophilus*. *J Dairy Sci* 80, 1537-1545.
- Newcomer, A.D., Park, H.S., O'Brien, P.C., McGill, D.B., 1983. Response of patients with irritable bowel syndrome and lactase deficiency using unfermented acidophilus milk. *Am J Clin Nutr* 38, 257-263.
- Ouwehand, A.C., DongLian, C., Weijian, X., Stewart, M., Ni, J., Stewart, T., Miller, L.E., 2014a. Probiotics reduce symptoms of antibiotic use in a hospital setting: a randomized dose response study. *Vaccine* 32, 458-463.
- Ouwehand, A.C., Nermes, M., Collado, M.C., Rautonen, N., Salminen, S., Isolauri, E., 2009a. Specific probiotics alleviate allergic rhinitis during the birch pollen season. *World J Gastroenterol* 15, 3261-3268.
- Ouwehand, A.C., ten Bruggencate, S.J., Schonewille, A.J., Alhoniemi, E., Forssten, S.D., Bovee-Oudenhoven, I.M., 2014b. *Lactobacillus acidophilus* supplementation in human subjects and their resistance to enterotoxigenic *Escherichia coli* infection. *Br J Nutr* 111, 465-473.
- Ouwehand, A.C., Tiihonen, K., Saarinen, M., Putaala, H., Rautonen, N., 2009b. Influence of a combination of *Lactobacillus acidophilus* NCFM and lactitol on healthy elderly: intestinal and immune parameters. *Br J Nutr* 101, 367-375.
- Paineau, D., Carcano, D., Leyer, G., Darquy, S., Alyanakian, M.A., Simoneau, G., Bergmann, J.F., Brassart, D., Bornet, F., Ouwehand, A.C., 2008. Effects of seven potential probiotic strains on specific immune responses in healthy adults: a double-blind, randomized, controlled trial. *FEMS Immunol Med Microbiol* 53, 107-113.
- Payne, D.L., Welsh, J.D., Manion, C.V., Tsegaye, A., Herd, L.D., 1981. Effectiveness of milk products in dietary management of lactose malabsorption. *Am J Clin Nutr* 34, 2711-2715.

Perrin, Y., Nutten, S., Audran, R., Berger, B., Bibiloni, R., Wassenberg, J., Barbier, N., Aubert, V., Moulin, J., Singh, A., Magliola, C., Mercenier, A., Spertini, F., 2014. Comparison of two oral probiotic preparations in a randomized crossover trial highlights a potentially beneficial effect of *Lactobacillus paracasei* NCC2461 in patients with allergic rhinitis. *Clin Transl Allergy* 4, 1.

Ringel-Kulka, T., Goldsmith, J.R., Carroll, I.M., Barros, S.P., Palsson, O., Jobin, C., Ringel, Y., 2014. *Lactobacillus acidophilus* NCFM affects colonic mucosal opioid receptor expression in patients with functional abdominal pain - a randomised clinical study. *Aliment Pharmacol Ther* 40, 200-207.

Ringel-Kulka, T., Palsson, O.S., Maier, D., Carroll, I., Galanko, J.A., Leyer, G., Ringel, Y., 2011. Probiotic bacteria *Lactobacillus acidophilus* NCFM and *Bifidobacterium lactis* Bi-07 versus placebo for the symptoms of bloating in patients with functional bowel disorders: a double-blind study. *J Clin Gastroenterol* 45, 518-525.

Savaiano, D.A., AbouElAnouar, A., Smith, D.E., Levitt, M.D., 1984. Lactose malabsorption from yogurt, pasteurized yogurt, sweet acidophilus milk, and cultured milk in lactase-deficient individuals. *Am J Clin Nutr* 40, 1219-1223.

Schrezenmeir, J., Heller, K., McCue, M., Llamas, C., Lam, W., Burow, H., Kindling-Rohracker, M., Fischer, W., Sengespeik, H.C., Comer, G.M., Alarcon, P., 2004. Benefits of oral supplementation with and without synbiotics in young children with acute bacterial infections. *Infl Clin Pediatr (Phila)* 43, 239-249.

Simenhoff, M.L., Dunn, S.R., 1996. Altered gut flora in uremia. *Journal of Renal Nutrition* 6, 68-74.

Simenhoff, M.L., Dunn, S.R., Zollner, G.P., Fitzpatrick, M.E., Emery, S.M., Sandine, W.E., Ayres, J.W., 1996. Biomodulation of the toxic and nutritional effects of small bowel bacterial overgrowth in end-stage kidney disease using freeze-dried *Lactobacillus acidophilus*. *Miner Electrolyte Metab* 22, 92-96.

Sui, J., Leighton, S., Busta, F., Brady, L., 2002. 16S ribosomal DNA analysis of the faecal lactobacilli composition of human subjects consuming a probiotic strain *Lactobacillus acidophilus* NCFM. *J Appl Microbiol* 93, 907-912.

Swanson, K.S., Grieshop, C.M., Flickinger, E.A., Bauer, L.L., Wolf, B.W., Chow, J., Garleb, K.A., Williams, J.A., Fahey, G.C., Jr., 2002. Fructooligosaccharides and *Lactobacillus acidophilus* modify bowel function and protein catabolites excreted by healthy humans. *J Nutr* 132, 3042-3050.

Thompson, L.U., Jenkins, D.J., Amer, M.A., Reichert, R., Jenkins, A., Kamulsky, J., 1982. The effect of fermented and unfermented milks on serum cholesterol. *Am J Clin Nutr* 36, 1106-1111.

van Zanten, G.C., Krych, L., Roytio, H., Forssten, S., Lahtinen, S.J., Al-Soud, W.A., Sorensen, S., Svensson, B., Jespersen, L., Jakobsen, M., 2014. Synbiotic *Lactobacillus acidophilus* NCFM and cellobiose does not affect human gut bacterial diversity but increases abundance of lactobacilli, bifidobacteria and branched-chain fatty acids: a randomized, double-blinded cross-over trial. *FEMS Microbiol Ecol* 90, 225-236.

- Varcoe, J., Zook, C., Sui, J., Leighton, S., Busta, F., Brady, L., 2002. Variable response to exogenous *Lactobacillus acidophilus* NCFM consumed in different delivery vehicles. *J Appl Microbiol* 93, 900-906.
- Waitzberg, D.L., Logullo, L.C., Bittencourt, A.F., Torrinhas, R.S., Shiroma, G.M., Paulino, N.P., Teixeira-da-Silva, M.L., 2013. Effect of synbiotic in constipated adult women—a randomized, double-blind, placebo-controlled study of clinical response. *Clinical nutrition* 32, 27-33.
- Wang, S., Zhu, H., Lu, C., Kang, Z., Luo, Y., Feng, L., Lu, X., 2012. Fermented milk supplemented with probiotics and prebiotics can effectively alter the intestinal microbiota and immunity of host animals. *Journal of Dairy Science* 95, 4813-4822.
- West, N.P., Horn, P.L., Barrett, S., Warren, H.S., Lehtinen, M.J., Koerbin, G., Brun, M., Pyne, D.B., Lahtinen, S.J., Fricker, P.A., 2014a. Supplementation with a single and double strain probiotic on the innate immune system for respiratory illness. *e-SPEN Journal* 9, e178-e184.
- West, N.P., Horn, P.L., Pyne, D.B., Gebiski, V.J., Lahtinen, S.J., Fricker, P.A., Cripps, A.W., 2014b. Probiotic supplementation for respiratory and gastrointestinal illness symptoms in healthy physically active individuals. *Clinical nutrition (Edinburgh, Scotland)* 33, 581-587.
- West, N.P., Horn, P.L., Pyne, D.B., Warren, H.S., Asad, S., Cox, A.J., Lahtinen, S.J., Lehtinen, M.J., Fricker, P.A., Cripps, A.W., Fazekas de St Groth, B., 2016. Probiotic supplementation has little effect on peripheral blood regulatory T cells. *J Allergy Clin Immunol* 138, 1749-1752 e1747.

## **Appendix D: GRAS Panel Statement**

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# **GRAS Panel Report on the Generally Recognized as Safe (GRAS) Status of the Proposed Uses of *Lactobacillus acidophilus* NCFM**

## **Introduction**

Danisco USA Inc. (dba DuPont Nutrition and Health) convened a panel of independent scientists (the “GRAS Panel”), qualified by their scientific training and relevant national and international experience to evaluate the safety of food ingredients, to conduct a critical and comprehensive evaluation of the available pertinent data and information on *Lactobacillus acidophilus* NCFM and to conclude whether the proposed uses in non-exempt infant formula and toddler formula would be Generally Recognized as Safe (GRAS) based on scientific procedures. The GRAS Panel consisted of the below-signed qualified scientific experts: Michael W. Pariza, Ph.D. (University of Wisconsin), William C. MacLean, M.D., CM, FAAP (Ohio State University), Joseph F. Borzelleca, Ph.D. (Virginia Commonwealth University School of Medicine), and Douglas L. Archer, Ph.D. (University of Florida). Michael C. Falk, Ph.D. (LSRO Solutions LLC) served as technical advisor to the GRAS Panel.

The GRAS Panel, independently and collectively, critically evaluated a comprehensive package of scientific information and data compiled from the literature. The information was presented in a dossier provided by LSRO Solutions LLC (“Comprehensive GRAS Assessment of *Lactobacillus acidophilus* NCFM: Food Usage Conditions for General Recognition of Safety”; April 16, 2019). To the best of our knowledge, this is a complete, representative, and balanced submission that includes unfavorable information, as well as favorable information, known to us and pertinent to the evaluation of the safety and GRAS status of the use of this ingredient in food.

## **Summary and Basis for GRAS**

The GRAS Panel based its conclusions on the following information.

*L. acidophilus* NCFM is intended to be added to non-exempt infant formula and toddler formula consistent with cGMP needed to provide  $10^8$  cfu/gm (approximately  $10^9 - 10^{10}$  cfu/day). This level of *L. acidophilus* NCFM will ensure a level of at least  $10^6$  cfu/gm throughout the shelf life of the product. *L. acidophilus* NCFM is intended to serve as a probiotic microorganism. It will not proliferate in the foods and beverages to which it is added but will decline over the shelf-life of the formula.

*L. acidophilus* is in common use in yogurt, buttermilk, kefir, miso, tempeh and other fermented foods (Bernardeau et al., 2006). *L. acidophilus* NCFM has been used in foods, including certain dairy products, functional beverages, nutritional powders, juices, bars, ready-to-eat breakfast cereals, chewing gum and confections at levels as high as  $5 \times 10^{10}$  cfu/250g serving since 2010.

Danisco USA, Inc. notified the U.S. Food and Drug Administration (FDA) that they concluded *L. acidophilus* NCFM was Generally Recognized as Safe (GRAS) for use in dairy products, functional beverages, nutritional powders, juices, bars, RTE breakfast cereals, chewing gum, and confections (Danisco, 2010, GRN000357). It was intended to be added to these foods at concentrations needed to provide at least  $10^9$  cfu/250g serving throughout the shelf life of the



product. In their submission, Danisco USA concluded that *L. acidophilus* NCFM is non-pathogenic, non-toxicogenic, and not known to produce exotoxin. They found no evidence of a safety hazard and no adverse impact. The FDA responded to this notification that it had no questions (CFSAN, 2010).

Previous GRAS notifications ( e.g. GRN0000231, GRN 0000281) concluded that lactic acid producing probiotic species are GRAS for use in infant formula and that D-lactic acid producing probiotic species are GRAS for use in conventional foods.

*L. acidophilus* has been included in the list of microorganisms found to have a Qualified Presumption of Safety (QPS) by the European Food Safety Authority (EFSA). *L. acidophilus* was included on this list continuously from 2007 through 2017.

*L. acidophilus* NCFM was isolated from the intestinal tract of a healthy human, is well characterized, and has been deposited in the American Type Culture Collection.

Analysis of *L. acidophilus* NCFM confirmed the absence of transferable antibiotic resistance elements, the absence of virulence factors, infectivity elements, and toxins, the uniqueness of the strain, and the identity of the strain to the *L. acidophilus* species.

The *L. acidophilus* NCFM strain is susceptible to various common antibiotics, does not show unusual adherence capability, adverse metabolic activity or infectivity, has demonstrated survivability in the gastrointestinal tract, ability to bind to pathogenic bacteria and prevent the adherence of biogenic bacteria to intestinal mucus, and provides probiotic benefits to the host.

*L. acidophilus* NCFM is produced using standard, well-documented fermentation techniques under current GMP manufacturing conditions using approved food grade materials. The strain is produced reproducibly and meets standard food grade specifications.

The safety of *L. acidophilus* NCFM was evaluated in an acute toxicity study in mice. No treatment related deaths or signs of toxicity were reported after oral administration of  $1.72 \times 10^{12}$  cfu/kg bw, the highest dose tested. The LD50 is greater than  $1.72 \times 10^{12}$  cfu/kg bw under these conditions.

*L. acidophilus* NCFM was evaluated in several repeated dose studies in mice and rats. Although none of the repeated dose studies were designed as safety studies, a number of studies were designed to increase the susceptibility to potential adverse effects and also reported safety/toxicology endpoints. Daily doses as high as  $10^{10}$  cfu/kg bw/day were administered for study durations up to 5 weeks in these studies. In most cases *L. acidophilus* NCFM protected against the various challenges (colitis, intestinal pain, delayed-type hypersensitivity) and did not result in *L. acidophilus* NCFM-induced safety-related effects.

*L. acidophilus* NCFM was evaluated in 61 human studies including 2,476 subjects and 5,340,732 treatment days. The treatment duration was from 5 to 182 days and the doses of *L. acidophilus* NCFM ranged from  $10^6$  to  $4 \times 10^{11}$  cfu/day (median dose  $2 \times 10^{10}$  cfu/day). Twenty eight studies were conducted with healthy subjects and in 21 studies the subjects were described as compromised by such factors as atopic dermatitis, small bowel bacterial overgrowth, cancer, lactose intolerance, Crohn's disease, irritable bowel syndrome, or HIV. Stratified by age, studies on elderly, adults, children, and infants were the subject of 4, 47, 8, and 1, respectively (some

studies did not report the age of the subjects). The studies either reported no treatment-related adverse events, described the NCFM treatment as well tolerated, or did not report any safety-related endpoints. When adverse events were noted, they were generally confined to gastrointestinal issues, were equally distributed between treatment and control groups, were generally considered mild and reversible, and were not considered related to treatment with *L. acidophilus* NCFM. The GRAS Panel considered these published clinical studies pivotal in supporting the safety of *L. acidophilus* NCFM.

None of the nine clinical trials that evaluated various *L. acidophilus* strains in infants and children reported any treatment-related adverse events.

Based on evidence utilizing acidified formulas, the FAO/WHO raised concerns about lactic acidosis in infant formula. Subsequently, CODEX issued a standard restricting the use of probiotics to those producing only L-lactic acid. More recent studies demonstrated that the use of D-lactic producing probiotics in infant formula did not cause lactic acidosis and confirmed that lactic acidosis is a problem only in individuals with short bowel syndrome and not in healthy infants. Reports linking D-lactic producing bacteria to chronic fatigue syndrome and brain foginess are based on poorly-designed, preliminary, poorly controlled studies that have not been confirmed.

Systematic reviews of the safety of lactic acid bacteria and *Lactobacillus* species used as probiotics concluded that these microbes are safe as long as they are devoid of any transferable antibiotic resistance genes.

The safety of *L. acidophilus* NCFM was further evaluated using the decision tree analysis of Pariza *et al.* (2015). Based on the outcome of the decision tree for determining the safety of microbial cultures for consumption by humans and animals (Table 1), including strain characterization and genome sequencing, screening for undesirable attributes and metabolites, and experimental evidence of safety by appropriately designed safety evaluation studies, it was concluded that *L. acidophilus* NCFM is not pathogenic and not toxigenic and is “deemed to be safe for use in the manufacture of food, probiotics, and dietary supplements for human consumption.”

## **Common Knowledge Elements of GRAS Determinations**

The first common knowledge element for a GRAS determination is that data and information relied upon to establish safety must be generally available; this is most commonly established by utilizing published, peer-reviewed scientific journals for the safety assessment. The human clinical studies that provided key evidence on which this GRAS determination was based were published in the peer-reviewed scientific literature.

The second common knowledge element required for a GRAS determination is consensus among qualified scientists that the safety of the proposed uses of the substance has been demonstrated. The GRAS Panel agrees there are adequate data in the scientific literature to conclude that *L. acidophilus* NCFM is a common component of food sources for man and


animals and that the weight of the available evidence demonstrates that the proposed uses are safe without any evidence of adverse effects.

*L. acidophilus* NCFM is GRAS for use in conventional foods in the United States and is in common use in food preparation in the United States.

## Conclusion

We, the undersigned members of the GRAS Panel, are qualified by scientific education and experience to evaluate the safety of the addition of probiotic bacteria to conventional foods. We have individually and collectively critically evaluated the materials on the safety of *L. acidophilus* NCFM summarized above, and we unanimously conclude that Danisco's *L. acidophilus* NCFM, manufactured consistent with cGMP and meeting food grade specifications, is Generally Recognized As Safe (GRAS) based on scientific procedures for addition to non-exempt infant formula and toddler formula at a level of  $10^8$  cfu/gm (approximately  $10^9 - 10^{10}$  cfu/day). This level of *L. acidophilus* NCFM will ensure a minimum concentration of  $10^6$  cfu/gm throughout the shelf life of the product.

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


  
\_\_\_\_\_  
Michael W. Pariza, Ph.D. (Chair)  
Emeritus Director Food Research Institute  
Professor Emeritus Department of Food Sciences  
University of Wisconsin

24 April 2019

Date

\_\_\_\_\_  
William C. MacLean, Jr. MD, CM, FAAP  
Clinical Professor of Pediatrics  
Ohio State University

Date


  
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Joseph F. Borzelleca, Ph.D.  
Professor Emeritus  
Pharmacology and Toxicology  
School of Medicine  
Virginia Commonwealth University

22 April 2019

Date

\_\_\_\_\_  
Douglas Archer, Ph.D.  
Professor  
Food Science and Human Nutrition  
University of Florida

Date

  
\_\_\_\_\_  
Michael C. Falk, Ph.D.  
LSRO Solutions LLC

29 April 2019

Date


## Conclusion

We, the undersigned members of the GRAS Panel, are qualified by scientific education and experience to evaluate the safety of the addition of probiotic bacteria to conventional foods. We have individually and collectively critically evaluated the materials on the safety of *L. acidophilus* NCFM summarized above, and we unanimously conclude that Danisco's *L. acidophilus* NCFM, manufactured consistent with cGMP and meeting food grade specifications, is Generally Recognized As Safe (GRAS) based on scientific procedures for addition to non-exempt infant formula and toddler formula at a level of  $10^8$  cfu/gm (approximately  $10^9 - 10^{10}$  cfu/day). This level of *L. acidophilus* NCFM will ensure a minimum concentration of  $10^6$  cfu/gm throughout the shelf life of the product.

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


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Michael W. Pariza, Ph.D. (Chair) Date  
Emeritus Director Food Research Institute  
Professor Emeritus Department of Food Sciences  
University of Wisconsin

 4/22/19  
\_\_\_\_\_  
William C. MacLean, Jr. MD, CM, FAAP Date  
Clinical Professor of Pediatrics  
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Joseph F. Borzelleca, Ph.D. Date  
Professor Emeritus  
Pharmacology and Toxicology  
School of Medicine  
Virginia Commonwealth University

 4/26/19  
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Douglas Archer, Ph.D. Date  
Professor  
Food Science and Human Nutrition  
University of Florida

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Michael C. Falk, Ph.D. Date  
LSRO Solutions LLC  
Advisor to the GRAS Panel

Table 1: Decision Tree Analysis for Determining the Safety of Microbial Cultures for Consumption

1. Has the <b>strain</b> <sup>i</sup> been characterized for the purpose of assigning an unambiguous genus and species name using currently accepted methodology? <sup>ii</sup> (If YES, go to 2. If NO, the strain must be characterized and unambiguously identified before proceeding).	YES
2. Has the <b>strain</b> genome been sequenced? (If YES, go to 3. If NO, the genome must be sequenced before proceeding to 3.) <sup>iii</sup>	YES
3. Is the <b>strain</b> genome free of genetic elements <sup>iv</sup> encoding virulence factors <sup>v</sup> and/or toxins <sup>v</sup> associated with pathogenicity? <sup>vi</sup> (If YES, go to 4. If NO, go to 15.)	YES
4. Is the <b>strain</b> genome free of functional and transferable antibiotic resistance gene DNA? <sup>vii</sup> (If YES, go to 5. If NO, go to 15.)	YES
5. Does the <b>strain</b> produce antimicrobial substances? <sup>viii</sup> (If NO, go to 6. If YES, go to 15.)	NO
6. Has the <b>strain</b> been genetically modified using rDNA techniques? (If YES, go to 7. If NO, go to 8.)	NO
7. Do the expressed product(s) that are encoded by the introduced DNA have a history of safe use in food? <sup>ix</sup> (If YES, go to 8. If NO, the expressed product(s) must be shown to be safe before proceeding to 8.) <sup>x</sup>	NA
8. Was the <b>strain</b> isolated from a food that has a history of safe consumption for which the <b>species</b> , to which the strain belongs, is a substantial <sup>xi</sup> and characterizing <sup>xii</sup> component (not simply an 'incidental isolate')? (If YES, go to 9. If NO, go to 13.) <sup>xiii</sup>	NO
9. Has the <b>species</b> , to which the strain belongs, undergone a comprehensive peer-reviewed safety evaluation and been affirmed to be safe for food use by an authoritative group of qualified scientific experts? <sup>xiv</sup> (If YES, go to 10. If NO, go to 13.)	YES
10. Do scientific findings published since completion of the comprehensive peer-reviewed safety evaluation cited in question 9a continue to support the conclusion that the <b>species</b> , to which the strain belongs, is safe for use in food? (If YES, go to 11. If NO, go to 13.)	YES
11. Will the intended use of the <b>strain</b> expand exposure to the <b>species</b> beyond the group(s) that typically consume the species in "traditional" food(s) in which it is typically found (for example, will a strain that was isolated from a fermented food typically consumed by healthy adults be used in food intended for an 'at risk' group)? (If NO, go to 12. If YES, go to 13.)	YES

12. Will the intended use of the <b>strain</b> expand intake of the <b>species</b> (for example, increasing the number of foods beyond the traditional foods in which the species typically found, or using the strain as a probiotic rather than as a fermented food starter culture, which may significantly increase the single dose and/or chronic exposure)? (If NO, go to 14. If YES, go to 13.)	NA
13. Does the <b>strain</b> induce undesirable physiological effects in appropriately designed safety evaluation studies? <sup>xv</sup> If yes, go to 15. If no, go to 14.)	NO
<b>14. The strain is deemed to be safe for use in the manufacture of food, probiotics, and dietary supplements for human consumption.</b>	YES
15. The strain is NOT APPROPRIATE for human or animal consumption. <sup>xvi</sup>	

<sup>i</sup> A strain is a “population of organisms that descends from a single organism or pure culture isolate.” P. 392, Prescott, Harley and Klein, 1996, Microbiology, Wiley. We recognize that the genotype and/or phenotype of a strain may change slightly when carried in culture, but such changes are irrelevant to safety considerations because there is no known mechanism or precedent for isolated strains in culture to begin spontaneously expressing pathogenic traits, unless that potential was already present in the genome at the time of isolation.

<sup>ii</sup> Whole Genome Sequencing provides distinct advantages for identification and characterization of microorganisms. In-depth analysis, including functional and comparative genomic studies, is afforded by sequencing the whole genome. This technology can provide a wealth of information that can be used for identification and characterization, including evidence of genetic evolution for adaptation of a species to a nutrient-rich environment, such as dairy products or the gastrointestinal tract (Pfeiler, EA, Klaenhammer, TR. 2007. The genomics of lactic acid bacteria. TRENDS in Microbiol, 15(12); 546-553). Less comprehensive molecular analysis, such as RAPD, FISH, and MLST, may also provide adequate information for identification, but the characterization ability is often times limited within a bacterial species (Gosiewski, T, Chmielarczyk, A, Strus M, Brzychczy-Wloch M, Heczko PB. 2012. The application of genetics methods to differentiation of three *Lactobacillus* species of human origin. Ann Microbiol 62:1437-1445).

<sup>iii</sup> The genomic sequence provides the tools to mine the genome for a number of functions, uncovering information spanning from safety to host-cell interactions (Callanan, M. 2005. Mining the Probiotic Genome: Advanced Strategies, Enhanced Benefits, Perceived Obstacles. Current Pharmaceutical Design, 11: 25-36). From a regulatory perspective, the ability to show percentage/regions of similarity and differentiation between a new strain of interest in comparison with a *type strain*, or an accepted strain with history of safe use, is beneficial (U.S. FDA; July 2011. Draft Guidance for Industry: Dietary Supplements: New Dietary Ingredient Notifications and Related Issues). The genome sequence is analogous to a chemical specification for a food ingredient, that is, it defines precisely what is being evaluated and permits a genetic assessment of pathogenic and toxigenic potential. Isolates from a type-strain culture collection, or a strain collection held by a commercial culture manufacturer, may be considered to have the same safety characteristics as, and to be *substantially equivalent* to, the original source pure culture, so in these cases the requirement for genome sequencing may be satisfied by sequencing the genome of the original source pure culture.

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<sup>iv</sup> The term "genetic elements" refers to gene sequences encoded in the chromosome or extra-chromosomal DNA.

<sup>v</sup> Known genetic element sequences for virulence factors and protein toxins are searchable, e.g. the MvirDb database of microbial virulence factors (<http://mvirdb.llnl.gov>) [ref Nucl. Acids Res.(2007) 35 (suppl 1): D391-D394.doi: 10.1093/nar/gkl791].

<sup>vi</sup> In considering the issue of "pathogenicity" and the potential to produce an infection, it is important to distinguish between *true pathogens* (i.e., microbes that possess virulence factors and are therefore capable of crossing or evading non-compromised host barriers) versus *opportunistic pathogens* (i.e., microbes that do not possess the required virulence factors to produce an infection in a non-compromised host). Typically this can be accomplished via genome analysis for known virulence factors coupled with a comprehensive search of the peer-reviewed scientific literature for infectious potential.

<sup>vii</sup> A functional antibiotic resistance gene results in an antibiotic resistance phenotype.

<sup>viii</sup> In this context, the term 'antimicrobial substances' refers to antibiotics that are used in medical or veterinary applications, for example substances that are positive in the JECFA test (FAO. 1981. *FAO Food and Nutrition Paper: 25th Session of the Joint FAO/WHO Expert Committee on Food Additives*, Appendix A, pp. 317–318, FAO/WHO, Geneva, Switzerland.)

<sup>ix</sup> The use of the terms "food" and "feed" includes supplements, which are in most jurisdictions considered to be a subset of the general categories.

<sup>x</sup> Demonstration of the safety of the expressed product may be accomplished by testing, e.g. toxicological testing as required by various regulatory bodies such as the US FDA Redbook 2000 (<http://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/IngredientsAdditive sGRASPackaging/ucm2006826.htm>) or by establishing a substantial equivalence of the test article to a substance with a safe history of use in food, or, in the case of animal feed additives, establishing a substantial equivalence of the test article to a substance with a history of safe use in target animal feeds.

<sup>xi</sup> Food fermentations, e.g. Cheddar cheese or yogurt, commonly result in "substantial" microbial food culture populations of  $10^6$ - $10^8$  colony forming units per gram of the food. Significance should be judged relative to the fermented food, i.e. numbers of different organisms in a microbial population may change during the course of the life of the fermented food, e.g. Lactobacilli counts in Cheddar cheese are routinely low in the initial stages of cheese maturation, but begin to increase in numbers while the Lactococci, responsible for initial acid production, count decreases as the cheese ripens and pH decrease. [Spatial and temporal distribution of non-starter lactic acid bacteria in Cheddar cheese. N.A. Fitzsimons, T.M. Cogan, S. Condon, T. Beresford. *Journal of Applied Microbiology* 90(4): 600–608, 2001; Kosikowski, F. V., and V. V. Mistry. *Cheese and Fermented Milk Foods*. 1997. 3rd Ed. F. V. Kosikowski, L. L. C. Westport, CT.]

<sup>xii</sup> A species is a "characterizing" component of a food if it has a measurable impact on flavor, texture, stability or preservation properties that are characteristic of the food, e.g. typical color and flavor of "blue" cheeses derived from *Penicillium roqueforti*; or surface texture, flavor and odor of Limburger cheese resulting from *Brevibacterium linens* growth on the surface. The color and flavor of "blue" cheese and the aroma, flavor and texture of Limburger cheese are characteristic of the food and the microbial cultures that are responsible for these traits are characterizing components.



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<sup>xiii</sup> A strain that was isolated from a type-strain or a commercial culture, with a history of safe use in food fermentations, is deemed to have satisfied this requirement and may proceed to 9a.

<sup>xiv</sup> For example, the Qualified Presumption of Safety list (<http://www.efsa.europa.eu/en/topics/topic/qps.htm>) prepared and periodically updated by the European Food Safety Authority is the output from a systematic safety review of the included microorganisms by qualified experts.

<sup>xv</sup> Experimental evidence of safety is required. Such evidence may include, but is not necessarily limited to, studies in appropriate animal models, and clinical trials in humans.

<sup>xvi</sup> In some cases, the strain may be shown to be appropriate by test and re-application of the decision tree, e.g., where an undesirable genetic element has been removed from a strain's genome.

AB-LIFE<sup>®</sup> has been marketed as a food supplement in various European countries since 2012.



# GRAS Notice Amendment GRN 000865 Danisco USA, Inc.

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January 20, 2020

Stephanie Hice, PhD  
Center for Food Safety and Applied Nutrition  
Office of Food Additive Safety  
U.S. Food and Drug Administration

Re: Response to questions regarding GRAS Notice No. 000865

Dear Dr. Hice:

Below please find DuPont Nutrition and Biosciences' (hereafter referred to as DuPont) response to questions received on January 6, 2020 regarding GRAS Notice No. 000865 for *Lactobacillus acidophilus* ATCC SD5221 (also referred to as NCFM).

Question 1: *The notice does not mention the type of formula the notifier intends to add L. acidophilus ATCC SD5221 to. Please indicate the intended source of the protein base (e.g., milk, soy, whey, etc.) of infant formula.*

Response: The intended sources of the protein base for non-exempt infant formula include cow's milk and soybean.

Question 2: *Please note that, while the United States does not have a definition for "toddler formula", the Agency recognizes it as formula intended for infants 12+ months of age. However, there are some formulas labeled as "toddler formulas" but are intended for infants 9-18 months of age. These products must follow the infant formula regulations as the intended population includes infants less than 12 months of age.*

Response: DuPont would like to clarify that in this notification "toddler formula" refers to formulas intended for young children 12 months and older.

Question 3: *Please note that the term "probiotic" is neither a regulatory term, nor a scientific term, and its use in the notice appears to have context as a marketing term denoting or connoting beneficial effects. The Agency's evaluation of GRAS notices focuses exclusively on the safety of the ingredient in food, and not about purported beneficial effects of the substance.*

Response: DuPont understands that the Agency's evaluation of our GRAS notice will focus exclusively on the safety of this ingredient in the intended food applications, and understands that FDA does not review or support beneficial effects.

Question 4: *On page 4, the notifier states that the intended use of L. acidophilus ATCC SD5221 is GRAS based on scientific procedures as defined by 21 CFR 170.3(o)(20). The appropriate citation is 21 CFR 170.30(b). For the administrative record, please make a statement that corrects this reference.*

Response: DuPont would like to correct the statement on page 4 as follows: “the intended use of *L. acidophilus* ATCC SD5221 is GRAS based on scientific procedures as defined by 21 CFR 170.30(b).”

Question 5: *On page 6, the notifier states that the intended use of L. acidophilus ATCC SD5221 is GRAS based on scientific procedures as defined by 21 CFR 170.30. The appropriate citation is 21 CFR 170.30(b). For the administrative record, please make a statement that corrects this reference.*

Response: DuPont would like to correct the statement on page 6 as follows: “the intended use of *L. acidophilus* ATCC SD5221 is GRAS based on scientific procedures as defined by 21 CFR 170.30(b).”

Question 6: *On page 8, the notifier includes a citation corresponding to “internal Danisco documentation”, however, this reference is not provided in the notice.*

Response: The reference for the “internal Danisco documentation” on page 8 should be Morovic, W. 2014, *Taxonomy of Lactobacillus acidophilus NCFM Microbial Products*, Internal DuPont Nutrition and Health report.

Question 7: *The notifier states that approved excipients are added to the blender during manufacturing (page 12), however, on page 18 the notifier states that L. acidophilus SD5221 is produced with no added excipients. Please clarify whether excipients are used during production of L. acidophilus ATCC SD5221.*

Response: DuPont would like to clarify that no excipients are used during the production of *L. acidophilus* ATCC SD5221. This sentence on page 12 should be deleted.

Question 8: *Please state whether any of the raw materials used in the fermentation are major allergens or derived from major allergens.*

Response: The raw materials used in fermentation are neither major allergens nor derived from major allergens. Please see revised allergen affirmation statement which replaces the statement in Appendix B.

Question 9: *The notifier should indicate that all analytical methods used to analyze the batches for conformance with the stated specifications have been validated for that particular purpose.*

Response: We have utilized official methods (AOAC, ISO, USP, etc.) for analysis of *L. acidophilus* ATCC SD5221 to demonstrate conformance with the stated specifications. The use of these validated methods precludes the need for any additional method validation by DuPont.

Question 10: *Please provide the analysis of 3 non-consecutive batches of heavy metals to demonstrate conformance with the stated specifications.*

Response: Heavy metals are tested annually as part of our surveillance program. DuPont's risk assessment established heavy metals as a low risk hazard (based on maintaining raw material supplier specifications for heavy metals, sourcing from low risk countries, review of industry recalls for material/supplier, etc.). Therefore, NCFM is included in our annual monitoring program.

Our annual surveillance program for monitoring heavy metals and pesticides has been in place since 2005. The strain *L. acidophilus* NCFM is tested annually to confirm compliance. Results of analysis from 3 non-consecutive lots of this strain have been provided.

Question 11: *For the administrative record, please provide the full citation for "SMEDP, 17th ed".*

Response: The complete citation for "SMEDP, 17<sup>th</sup> ed." is Wehr, M and Frank, JF. 2004. Standard Methods for the Examination of Dairy Products, 17<sup>th</sup> Edition.

Question 12: *The notifier states that the method used to detect mercury is EPA 7471 (page 14), which corresponds to detection of mercury in solid or semisolid wastes. Please clarify if this method is appropriate and fit for purpose.*

Response: On page 14, Table 2: Product Specifications for Danisco's *L. acidophilus* NCFM, DuPont would like to correct the method used to detect mercury and the other 3 heavy metals. The method should be AOAC 2013.06. See below for revised Table 2.

Question 13: *The notifier states that the method used to detect Listeria monocytogenes is AOAC 999.06 (page 14), which corresponds to detection of Listeria spp. in dairy products, vegetables, seafood, raw meat and poultry, processed meat and poultry. Please clarify if this method is appropriate and fit for purpose.*

Response: On page 14, Table 2, Dupont would like to make the following corrections. The applicable test is for *Listeria* spp. (not *Listeria monocytogenes*) and the method utilized is AOAC 2004.06, which is appropriate for the intended purpose. See revised table 2 Product Specifications for Danisco's *L. acidophilus* NCFM, included below.

Question 14: *The notifier states that the method used to detect coliforms and Escherichia coli is AOAC 966.24 (page 14), which corresponds to detection of coliforms and E. coli in nuts and nut products/tree nut meats. Please clarify if this method is appropriate and fit for purpose.*

Response: The FDA Bacteriological Analytical Manual (BAM) Chapter 4: Enumeration of *Escherichia coli* and *Coliform* bacteria is used by the FDA to test these organisms in all food products. This chapter list methods approved for use including the MPN method which is the AOAC method 966.24. The sample preparation and dilutions come from FDA BAM Chapter 4.

Additionally, FDA Regional testing laboratories reference the AOAC 966.24 in their laboratory analysis for all food products. Therefore, this method is appropriate and fit for purpose.

Question 15: *The provided specification for E. coli is <0.3/gram. Please provide the unit of measurement for this specification.*

Response: DuPont would like to clarify that on page 14, Table 2 Product Specifications for Danisco's *L. acidophilus* NCFM, the specification for *E. coli* should be <0.3 CFU/g. Correction is included in revised Table 2 below.

Question 16: *The notifier provides a specification for Salmonella serovars, listed as negative by test in 40 grams. Please provide a limit for Salmonella serovars that is in compliance with 21 CFR 106.55.*

Response: DuPont would like to clarify that for *Salmonella serovars* our sampling size of 40 g is larger than the requirement of absence in 25 g in 21 CFR 106.55. This is a more rigorous standard. By demonstrating absence in a larger sample, we have confirmed compliance with the requirement of absence of 25 g as per the CFR.

Question 17: *References to "Chronobacter sakazakii" on page 14 should read Cronobacter sakazakii. Please make a statement that corrects this reference.*

Response: On page 14, DuPont would like to correct the references to "*Chronobacter sakazakii*" to *Cronobacter sakazakii*.

Question 18: *A specification was provided for C. sakazakii, however, the certificates of analysis for the batch analyses (pages A-2 through A-11) do not provided data for C. sakazakii. Please provide the analysis of 3 non-consecutive batches of L. acidophilus ATCC SD5221 to demonstrate conformance with the stated C. sakazakii specification.*

Response: Please see new attachment with certificate of analysis of 3 non-consecutive batches of *L. acidophilus* ATCC SD5221 which includes data for *C. sakazakii* (referred to as *Enterobact. sakazakii*,) and demonstrate conformance with the stated specification for *C. sakazakii*. Please note additionally, the method for *C. sakazakii* in Table 2 Product Specifications for Danisco's *L. acidophilus* NCFM (p. 14) should be ISO 22964, and is revised in Table 2 below.

Question 19: *The provided specification for coliforms is <10 CFU/gram (page 14), however, in Table 3, the results from each batch analysis is 10 CFU/gram (page 15). Please clarify whether the provided specification for coliforms is <10 CFU/g.*

Response: The specification for coliforms on page 14 (Table 2) should be  $\leq 10$  CFU/gram. See revised Table 2 Product Specifications for Danisco's *L. acidophilus* NCFM below.

Question 20: *Staphylococcus (coagulase +)* is included in Table 3, batch analyses (page 15), however, it is not included in Table 2, product specifications (page 14).

Response: Please see revised Table 2, Product Specifications, which now includes specifications and method of analysis for *Staphylococcus (coagulase +)*.

Question 21: *The notifier assesses antibiotic resistance of L. acidophilus ATCC SD5221, and concludes that L. acidophilus ATCC SD5221 "... did not demonstrate resistance at levels exceeding the breakpoints set by EFSA"* (page 16), however, according to the data presented in the antibiotic resistance profile on page 17, the minimum inhibitory concentration for chloramphenicol is equivalent to the breakpoint set by EFSA. Please discuss this result.

Response: DuPont would like to clarify that a minimum inhibitory concentration equivalent to breakpoint is considered susceptible, according to the guidance in the 2012 opinion of the EFSA FEEDAP panel as cited in notification (EFSA, 2012). (Guidance on the assessment of bacterial susceptibility to antimicrobials of human and veterinary importance. Scientific Opinion of the EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP). EFSA J 10, 2740). The text on page 16 should read "did not demonstrate resistance at levels *equivalent to or exceeding the breakpoints set by EFSA*" (new text is italicized).

Question 22: *References to "choramphenicol" on page 17 should read chloramphenicol. Please make a statement that corrects this reference.*

Response: DuPont would like to correct the spelling on page 17, the correct spelling for "choramphenicol" should be chloramphenicol.

Question 23: *References to "virginamycin" on page 17 should read virginiamycin. Please make a statement that corrects this reference.*

Response: DuPont would like to correct the spelling on page 17, the correct spelling of "virginamycin" on page 17 should be virginiamycin.

Question 24: *On page 17 the notifier references "ATCC SD551"; please clarify what "ATCC SD551" is in reference to.*

Response: DuPont would like to correct the reference to "ATCC SD551". It should be ATCC SD 5221.

Question 25: *Please provide a reference for the estimated daily intake of infant formula listed as 800 mL (page 18).*

Response: We have identified 2 references to support the estimated daily intake of infant formula of 800 mL on page 18.

- Raiten, D. J., Talbot, J. M. & Waters, J. H. (1998) Assessment of nutrient requirements for infant formulas. Prepared by the Life Sciences Research Office, 9650 Rockville Pike, Bethesda MD. J Nutr 128: 11S.
- National Research Council. Subcommittee on the Tenth Edition of the RDAs. Food and Nutrition Board (1989) Recommended Dietary Allowances, 10th. National Academy Press, Washington, D.C.

Question 26: *The notifier states that the intended use of L. acidophilus ATCC SD5221 is GRAS based on scientific procedures (21 CFR 170.30(b)), however, includes a discussion in Part 5, Experience Based on Common Use in Foods (page 19). Please note, that the information provided in Part 5 does not meet the regulatory definition of “Common Use in Foods” as defined by 21 CFR Part 170.245. We note, that the provided discussion should be incorporated into Part 6, Narrative, as defined by 21 CFR Part 170.250*


Response: DuPont would like to clarify that the information in Part 5 should be incorporated into the beginning of Part 6, Narrative. Part 5 should read *The GRAS conclusion is based on scientific procedures and not on common use in food before 1958.*

Question 27: *Please clarify whether L. acidophilus ATCC SD5221 is intended to be used in foods under the jurisdiction of the U.S. Department of Agriculture (USDA).*

Response: DuPont clarifies that the scope of this notification for ATCC SD5221 is for use in non-exempt infant formulas and toddler formulas, not for foods under the jurisdiction of USDA.

Please let me know if there are any additional questions about DuPont’s GRAS notification GRN 865.

Kind regards,



Elizabeth McCartney  
Regulatory Affairs Specialist  
DuPont Nutrition and Biosciences



Table 2: Product Specifications for Danisco's *L. acidophilus* NCFM

Parameter	Specification	Method
<b><u>Appearance</u></b>		
Form <sup>1</sup>	Freeze-dried powder	Visual
Color <sup>1</sup>	White to cream-colored	Visual
Particle size <sup>2</sup>	40 mesh	Fitzmill Screen
Viable cell count <sup>3</sup>	>2.0 x 10 <sup>11</sup> CFU/g	ISO 7889/IDF 117
<b><u>Heavy Metals<sup>4</sup></u></b>		
Arsenic	< 1.0 ppm	AOAC 2013.06
Lead	< 0.5 ppm	AOAC 2013.06
Cadmium	< 0.2 ppm	AOAC 2013.06
Mercury	< 0.05 ppm	AOAC 2013.06
<b><u>Microbial Specifications</u></b>		
<i>Enterococci</i> (CFU/g) <sup>3</sup>	≤ 100 CFU/g	SMEDP, 17 <sup>th</sup> ed
Non-lactic Cell Count <sup>3</sup>	≤ 5000 CFU/g	ISO 13559
Coliform (MPN) <sup>3</sup>	≤ 10 CFU/g	AOAC 966.24
<i>Escherichia coli</i> (MPN) <sup>3</sup>	Negative by test (<0.3CFU/g)	AOAC 966.24
<i>Staphylococcus</i> (coagulase +) by test	Negative by test (<10 CFU/g)	AOAC 975.55
<i>Salmonella</i> <sup>3</sup>	Negative by test in 40 g	AOAC 2004.03
<i>Listeria spp</i> <sup>3</sup>	Negative by test in 25 g	AOAC 2004.06
<i>Cronobacter sakazakii</i>	Negative by test in 10 g	ISO 22964

<sup>1</sup> Specification provided on Product Description sheet, not listed on Certificate of Analysis or Batch Analysis summary

<sup>2</sup> Internal Specification recorded in Batch Record

<sup>3</sup> Specification reported on Certificate of Analysis

<sup>4</sup> Specification provided on Heavy Metal Statement, not listed on Certificate of Analysis. Annual heavy metal surveillance testing of 3 non-consecutive lots are provided.

DuPont Nutrition and Biosciences  
3322 - 3329 Agriculture Drive  
Madison, WI 53716



Date: January 9, 2020

Product: 1286076 HOWARU Dophilus PN 200B - 1 KG

To Whom It May Concern,

DuPont certifies that the above listed product including raw materials used in the fermentation process does not contain allergens as determined by The Food Allergen Labeling and Consumer Protection Act of 2004 (FALCPA) (Public Law 108-282) (U.S. Food and Drug Administration, 2006), including protein derived from milk, eggs, fish, crustacean shellfish, mollusks, tree nuts, wheat, peanuts, soybean, celery, mustard, and sesame seeds.

This information is given in respect of DuPont's policy of openness and transparency with its customers.

Sincerely,



Sarah Pace  
Quality & Food Safety Coordinator  
DuPont - Nutrition and Biosciences

**Person in charge** John M. Reuther  
**Client Support** Jeremy BlauchDanisco, a DuPont Company  
ATTN: Michele Fojut  
3322 Agriculture Drive  
Madison, WI 53716

Reporting Date 11/28/2017



AR-17-QA-071030-01

**REPORT OF ANALYSIS****Sample Code** 468-2017-1018B100**Sample Description** PROBIOTIC  
**Client Sample Code** 1103062519  
**Sample Reference** NCFM (LACTOBACILLUS ACIDOPHILUS)**Reception Date** 10/18/2017  
**Reception Temperature** 25 (Celsius)  
**Sample Condition** Acceptable  
**Purchase Order** 4501382942**Test Results****Result****QA24X - Aflatoxin M1 (HPLC)****Completion Date:** 10/24/2017 **Method:** ISO 14501

\* Aflatoxin M1

&lt;0.05 µg/kg

**QA101 - Aflatoxin B1 B2 G1 G2 (LC-MSMS)****Completion Date:** 10/25/2017 **Method:** AOAC 999.07 Modified

Aflatoxin B1

&lt;1.0 µg/kg

Aflatoxin B2

&lt;1.0 µg/kg

Aflatoxin G1

&lt;1.0 µg/kg

Aflatoxin G2

&lt;1.0 µg/kg

Aflatoxins total

&lt;4.0 µg/kg

**QA00L - Citrinin (LC-MSMS)****Completion Date:** 10/20/2017 **Method:** VICAM CitriTest LC-MS/MS

\* Citrinin

&lt;15 µg/kg

**QA034 - Fumonisin (IAC-LC-MSMS)****Completion Date:** 10/25/2017 **Method:** JAOAC, 92 (2), 496.

Fumonisin (B1+B2+B3)

&lt;30 µg/kg

Fumonisin B1

&lt;10 µg/kg

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**Eurofins Sample Code: 468-2017-1018B100**
**Client Sample Code: 1103062519**
**REPORT OF ANALYSIS**


AR-17-QA-071030-01

**Test Results**

 Fumonisin B2  
 Fumonisin B3

**Result**

 <10 µg/kg  
 <10 µg/kg

**QA404 - Ochratoxin A (LC-MSMS)**
**Completion Date:** 10/25/2017 **Method:** AOAC 999.07 Modified  
 Ochratoxin A

&lt;2 µg/kg

**QA091 - Tricothecenes**
**Completion Date:** 10/25/2017 **Method:**

- \* Diacetoxyscirpenol (DAS)
- \* HT-2 Toxin
- \* Neosolaniol
- \* T-2 Toxin

 <50 µg/kg  
 <20 µg/kg  
 <50 µg/kg  
 <20.0 µg/kg

**QA21P - Mycotoxins (DON, T-2, ZON, NIV, LC-MSMS)**
**Completion Date:** 10/25/2017 **Method:** Internal method LC-MS/MS

- \* Deoxynivalenol (Vomitoxin)
- \* Zearalenone (ZON)
- \* T-2 Toxin
- \* HT-2 Toxin
- \* Nivalenol (NIV)

 <20 µg/kg  
 <10 µg/kg  
 <10 µg/kg  
 <10 µg/kg  
 <20 µg/kg

**QA106 - Aluminum (ICP-AES)**
**Completion Date:** 10/27/2017 **Method:** J.AOAC 92, 1484-1518 (2009)

- \* Aluminum (Al)

1.0 mg/kg

**QA172 - Antimony (ICP-MS)**
**Completion Date:** 10/27/2017 **Method:** AOAC 2013.06

- \* Antimony (Sb)

0.03 mg/kg

**QA133 - Arsenic (ICP-MS)**
**Completion Date:** 10/27/2017 **Method:** AOAC 2013.06

- Arsenic (As)

0.08 mg/kg

**QA03T - Barium (ICP-AES)**
**Completion Date:** 10/27/2017 **Method:** J.AOAC 92, 1484-1518 (2009)

- \* Barium (Ba)

0.26 mg/kg

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Eurofins Sample Code: 468-2017-1018B100

Client Sample Code: 1103062519


**REPORT OF ANALYSIS**
**Test Results**
**Result**

<b>QA00Y - Boron (ICP-AES)</b>		
Completion Date: 10/27/2017	Method: J.AOAC 92, 1484-1518 (2009)	
* Boron (B)		1.5 mg/kg
<b>QA205 - Cadmium (ICP-MS)</b>		
Completion Date: 10/27/2017	Method: AOAC 2013.06	
Cadmium (Cd)		<0.01 mg/kg
<b>QA00G - Chromium (ICP-AES)</b>		
Completion Date: 10/27/2017	Method: AOAC 2011.14	
Chromium (Cr)		0.6 mg/kg
<b>QA008 - Cobalt (ICP-AES)</b>		
Completion Date: 10/27/2017	Method: AOAC 2011.14	
* Cobalt (Co)		0.43 mg/kg
<b>QA007 - Copper (ICP-AES)</b>		
Completion Date: 10/27/2017	Method: AOAC 2011.14	
* Copper (Cu)		1.2 mg/kg
<b>QA417 - Lead (ICP-MS)</b>		
Completion Date: 10/27/2017	Method: AOAC 2013.06	
Lead (Pb)		<0.02 mg/kg
<b>QA003 - Manganese (ICP-AES)</b>		
Completion Date: 10/27/2017	Method: AOAC 2011.14	
* Manganese (Mn)		43.20 mg/kg
<b>QD610 - Mercury (ICP-MS)</b>		
Completion Date: 10/27/2017	Method: AOAC 2013.06	
Mercury (Hg)		<0.010 mg/kg
<b>QA10W - Strontium (ICP-MS)</b>		
Completion Date: 10/30/2017	Method: AOAC 2013.06	
* Strontium (Sr)		1.8 mg/kg

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**Test Results**
**Result**
**QA374 - Molybdenum in Food ICP-AES**

Completion Date: 10/27/2017 Method: AOAC 2011.14

\* Molybdenum (Mo)

0.63 mg/kg

**QA00A - Nickel (ICP-AES)**

Completion Date: 10/27/2017 Method: J.AOAC 92, 1484-1518 (2009)

\* Nickel

0.19 mg/kg

**QA005 - Selenium (ICP-MS)**

Completion Date: 10/27/2017 Method: AOAC 2013.06

\* Selenium (Se)

0.03 mg/kg

**QD424 - Tin in Food ICP-MS**

Completion Date: 10/27/2017 Method: AOAC 2013.06

\* Tin

0.02 mg/kg

**QA006 - Zinc (ICP-AES)**

Completion Date: 10/27/2017 Method: AOAC 2011.14

\* Zinc (Zn)

64.4 mg/kg

**QA0KS - Chlorate and Perchlorate (LC-MS/MS)**

Completion Date: 10/24/2017 Method: FDA method

Chlorate

1.11 mg/kg

Perchlorate

&lt;0.01 mg/kg

**QA245 - 1,4-Dioxane**

Completion Date: 10/24/2017 Method: USP/NF 467

\* 1,4-Dioxane

&lt;5.0 µg/g

**QA04D - Residual Solvents (GC-MS)**

Completion Date: 10/26/2017 Method: EPA 5021

\* 1,1,1-Trichloroethane

&lt;0.50 mg/kg

\* 1,1,2-Trichloroethane

&lt;0.50 mg/kg

\* 1,1-Dichloroethene

&lt;0.50 mg/kg

\* 1,2-Dichloroethane

&lt;0.50 mg/kg

\* 1,2-Dimethoxyethane

&lt;1.0 mg/kg

\* 1-Butanol

&lt;5.0 mg/kg

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**Eurofins Sample Code: 468-2017-1018B100**
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**REPORT OF ANALYSIS**


AR-17-QA-071030-01

**Test Results**

- \* 2-Butanon (Methylethylketon)
- \* Acetone
- \* Benzene
- \* Butyl acetate
- \* Carbon tetrachloride
- \* Chlorobenzene
- \* Chloroform
- \* Cyclohexane
- \* Dichloromethane
- \* Ethanol
- \* Ethyl acetate
- \* Heptane
- \* Hexane (sum of normal, iso, and 3-methyl pentane)
- \* Isopropanol
- \* MBK (2-Hexanone)
- \* Methanol
- \* Methyl-tert-butylether (MTBE)
- \* Tetralin
- \* Toluene
- \* Trichloroethylene
- \* Xylenes (sum)
- \* Sample extraction solvent

**Result**

- <0.20 mg/kg
- <5.0 mg/kg
- <0.10 mg/kg
- <0.50 mg/kg
- <0.50 mg/kg
- <0.50 mg/kg
- <0.10 mg/kg
- <2.0 mg/kg
- <0.50 mg/kg
- <1.0 mg/kg
- <1.0 mg/kg
- <1.0 mg/kg
- <0.50 mg/kg
- <5.0 mg/kg
- <1.0 mg/kg
- <5.0 mg/kg
- <0.10 mg/kg
- <5.0 mg/kg
- <0.50 mg/kg
- <1.0 mg/kg
- <1.0 mg/kg

Matrix Modifying Solution

**QA711 - Cyhexatin/Azocyclotin (LC-MSMS)**
**Completion Date:** 10/25/2017 **Method:** EN 15662:2008; modified

- \* Cyhexatin/Azocyclotin (Sum) <0.02 mg/kg

**QA749 - Diquat**
**Completion Date:** 10/25/2017 **Method:** Internal LC-MS/MS

- \* Diquat <0.01 mg/kg

**QA602 - EBDCs (Dithiocarbamates) (CS2 method, GC-MS)**
**Completion Date:** 10/24/2017 **Method:** J. Agric. Food Chem. Vol. 49 pp 2152, 2001

- \* Total Dithiocarbamates, as CS2 <0.01 mg/kg

**QA24Y - Haloxyfop, total (Food, LC-MSMS)**
**Completion Date:** 10/25/2017 **Method:** Internal method

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**Test Results**
**Result**

\* Haloxyfop

&lt;0.003 mg/kg

**QA429 - Paraquat**
**Completion Date:** 10/25/2017 **Method:** Method published by the CRL (24.04.2005)

\* Paraquat

&lt;0.01 mg/kg

**QA622 - Dithiocarbamates (incl. Propineb) GC-MS)**
**Completion Date:** 10/24/2017 **Method:** J. Agric. Food Chem. Vol. 49 pp 2152, 2001

\* Dithiocarbamates (as CS2)

&lt;0.006 mg/kg

**QA01P - Pesticides Quechers GC-MSMS**
**Completion Date:** 10/26/2017 **Method:** AOAC 2007.01

Screened pesticides

Not detected

**QA01R - Pesticides Quechers-LC-MS/MS**
**Completion Date:** 10/26/2017 **Method:** AOAC 2007.01

Screened pesticides

Not detected

**QA083 - Pesticides - Glyphosate Compounds (LC-MSMS)**
**Completion Date:** 10/25/2017 **Method:** J. Agric. Food Chem. 2004, 52, 4057-4063

Glufosinate

&lt;0.01 mg/kg

Glyphosate

&lt;0.01 mg/kg

**QA107 - Pesticides - Acid Herbicides LC-MSMS**
**Completion Date:** 10/25/2017 **Method:** Internal method

\* 2,4,5-T

&lt;0.01 mg/kg

\* 2,4-D

&lt;0.01 mg/kg

\* 2,4-DB

&lt;0.01 mg/kg

\* Acifluorfen

&lt;0.01 mg/kg

\* Aminopyralid

&lt;0.01 mg/kg

\* Bentazone

&lt;0.01 mg/kg

\* Bispyribac

&lt;0.01 mg/kg

\* Bromoxynil

&lt;0.01 mg/kg

\* Clopyralid

&lt;0.01 mg/kg

\* Dicamba

&lt;0.01 mg/kg

\* Diflufenzopyr

&lt;0.01 mg/kg

\* Fluazifop

&lt;0.01 mg/kg

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**Eurofins Sample Code: 468-2017-1018B100**
**Client Sample Code: 1103062519**


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## REPORT OF ANALYSIS

### Test Results

- \* Flucarbazone
- \* Fluroxypyr
- \* Fomesafen
- \* Haloxyfop
- \* Imazamox
- \* Imazapyr
- \* Imazaquin
- \* Imazethapyr
- \* MCPA
- \* Picloram
- \* Quinclorac
- \* Triclopyr
- \* MCPB

### Result

- <0.01 mg/kg
- <0.01 mg/kg
- <0.01 mg/kg
- <0.003 mg/kg
- <0.01 mg/kg
- <0.01 mg/kg
- <0.01 mg/kg
- <0.01 mg/kg
- <0.01 mg/kg
- <0.01 mg/kg
- <0.01 mg/kg
- <0.01 mg/kg
- <0.01 mg/kg

### QA0GX - Pesticides - EU Banned (GC-MSMS)

**Completion Date: 10/27/2017 Method: EN 12393**

- |                              |              |
|------------------------------|--------------|
| * Aldrin                     | <0.003 mg/kg |
| * Dieldrin                   | <0.003 mg/kg |
| * Aldrin/ Dieldrin (Sum)     | <0.003 mg/kg |
| * Endrin                     | <0.003 mg/kg |
| * HCB (Hexachlorbenzene)     | <0.003 mg/kg |
| * Heptachlor                 | <0.003 mg/kg |
| * Heptachlor epoxide, trans- | <0.003 mg/kg |
| * Heptachlor (sum)           | <0.003 mg/kg |
| * Nitrofen                   | <0.003 mg/kg |
| * Chlordane (total)          | <0.004 mg/kg |
| * Fenthion                   | <0.003 mg/kg |

### QA0FX - Pesticides - EU Banned (LC-MSMS)

**Completion Date: 10/27/2017 Method: HPLC-MS/MS**

- |                            |              |
|----------------------------|--------------|
| * Cadusaphos               | <0.006 mg/kg |
| * Demeton                  | <0.004 mg/kg |
| * Demeton-S-methyl         | <0.004 mg/kg |
| * Demeton-S-methyl-sulfone | <0.004 mg/kg |
| * Oxydemeton-methyl        | <0.004 mg/kg |
| * Ethoprophos              | <0.008 mg/kg |

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Eurofins Sample Code: 468-2017-1018B100

Client Sample Code: 1103062519



AR-17-QA-071030-01

**REPORT OF ANALYSIS**
**Test Results**

- \* Disulfoton-sulfon
- \* Disulfoton-sulfoxide
- \* Disulfoton (sum)
- \* Disulfoton
- \* Fensulfothion
- \* Fensulfothion-oxon-sulfone
- \* Fensulfothion-sulfone
- \* Fensulfothion (sum)
- \* Fipronil
- \* Fipronil, desulfinyl-
- \* Fipronil (sum)
- \* Omethoate
- \* Terbufos
- \* Terbufos-sulfoxide
- \* Terbufos-sulfone
- \* Terbufos (sum)

**Result**

<0.003 mg/kg  
<0.003 mg/kg  
<0.003 mg/kg  
<0.003 mg/kg  
<0.003 mg/kg  
<0.003 mg/kg  
<0.003 mg/kg  
<0.003 mg/kg  
<0.003 mg/kg  
<0.004 mg/kg  
<0.004 mg/kg  
<0.004 mg/kg  
<0.003 mg/kg  
<0.003 mg/kg  
<0.003 mg/kg  
<0.003 mg/kg

**QA812 - Benzo(a)pyrene (GC-MSMS)**
**Completion Date:** 10/26/2017 **Method:** Internal method

- \* Benzo(a)pyrene <0.50 µg/kg

**QA22D - Polynuclear Aromatic Hydrocarbons (GC-MSMS)**
**Completion Date:** 10/26/2017 **Method:** Internal method

- Benz(a)anthracene <0.50 µg/kg
- Chrysene <0.50 µg/kg
- Benzo(b)fluoranthene <0.50 µg/kg
- Benzo(a)pyrene <0.50 µg/kg
- Sum of all positive identified PAH <2.0 µg/kg

**QA0ND - Glycidyl esters (GC-MSMS)**
**Completion Date:** 11/27/2017 **Method:** AOCS Cd 29b-13

- Glycidol (calculated) <0.08 mg/kg

**QA0NC - Monochloropropanediols (sum of free and esters)**
**Completion Date:** 11/27/2017 **Method:** AOCS Cd 29b-13

- Total 2-MCPD (free and bound) <0.10 mg/kg
- Total 3-MCPD (free and bound) <0.10 mg/kg

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Eurofins Sample Code: 468-2017-1018B100

Client Sample Code: 1103062519

**REPORT OF ANALYSIS**


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**Test Results**
**Result**
**GFF02 - Dioxins and Furans (17 PCDD/F)**
**Completion Date:** 10/25/2017 **Method:** Internal method

* 2,3,7,8-TetraCDD	<0.0112 ng/kg MC12%
* 1,2,3,7,8-PentaCDD	<0.0147 ng/kg MC12%
* 1,2,3,4,7,8-HexaCDD	<0.0223 ng/kg MC12%
* 1,2,3,6,7,8-HexaCDD	<0.0305 ng/kg MC12%
* 1,2,3,7,8,9-HexaCDD	<0.0288 ng/kg MC12%
* 1,2,3,4,6,7,8-HeptaCDD	<0.0470 ng/kg MC12%
* OctaCDD	<0.340 ng/kg MC12%
* 2,3,7,8-TetraCDF	<0.0305 ng/kg MC12%
* 1,2,3,7,8-PentaCDF	<0.0211 ng/kg MC12%
* 2,3,4,7,8-PentaCDF	<0.0329 ng/kg MC12%
* 1,2,3,4,7,8-HexaCDF	<0.0346 ng/kg MC12%
* 1,2,3,6,7,8-HexaCDF	<0.0317 ng/kg MC12%
* 1,2,3,7,8,9-HexaCDF	<0.0235 ng/kg MC12%
* 2,3,4,6,7,8-HexaCDF	<0.0288 ng/kg MC12%
* 1,2,3,4,6,7,8-HeptaCDF	<0.0329 ng/kg MC12%
* 1,2,3,4,7,8,9-HeptaCDF	<0.0229 ng/kg MC12%
* OctaCDF	<0.0704 ng/kg MC12%
* WHO(2005)-PCDD/F TEQ (lower-bound)	ND ng/kg MC12%
ND - not determined since none of the corresponding congeners was above the LOQ	
* WHO(2005)-PCDD/F TEQ (upper-bound)	0.0605 ng/kg MC12%

**S1003 - Propylene thiourea (PTU) and Ethylene thiourea (ETU)**
**Completion Date:** 10/27/2017 **Method:** Internal method

* Ethylene thiourea (ETU)	<0.003 mg/kg
* PTU (Propylene Thiourea)	<0.003 mg/kg

**QA0LY - Fentin (LC/MSMS)**
**Completion Date:** 10/25/2017 **Method:** EN 15662:2008; modified

* Fentin	<0.003 mg/kg
----------	--------------

**JJ0FF - Fusarium toxins big (DON, ZON T2, HT2, NIV, ADON, DAS, FX)**
**Completion Date:** 10/26/2017 **Method:** Internal method

* Deoxynivalenol (Vomitoxin)	<20 µg/kg
* Zearalenone (ZON)	<10 µg/kg

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**Client Sample Code: 1103062519**
**REPORT OF ANALYSIS**


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**Test Results**

- \* T-2 Toxin
- \* HT-2 Toxin
- \* sum T-2 HT-2 toxin
- \* Nivalenol (NIV)
- \* 3-Acetyldeoxynivalenol (3-ADON)
- \* 15-Acetyldeoxynivalenol (15-ADON)
- \* Diacetoxyscirpenol (DAS)
- \* Fusarenone X (FX)

**Result**

<10 µg/kg  
 <10 µg/kg  
 <20 µg/kg  
 <20 µg/kg  
 <20 µg/kg  
 <20 µg/kg  
 <10 µg/kg  
 <20 µg/kg

**QA0NE - MCPD+Glycidol Extraction (Non-oil samples)**

**Completion Date:** 11/27/2017 **Method:** AOCS Cd 29b-13  
 Sample Preparation

DONE

**GFF07 - polychlorinated biphenyls (12 WHO PCB + 6 ICES PCB)**

**Completion Date:** 10/25/2017 **Method:** Internal method

* PCB 77	<3.52 ng/kg MC12%
* PCB 81	<0.159 ng/kg MC12%
* PCB 105	<2.29 ng/kg MC12%
* PCB 114	<0.311 ng/kg MC12%
* PCB 118	<8.22 ng/kg MC12%
* PCB 123	<0.235 ng/kg MC12%
* PCB 126	<0.147 ng/kg MC12%
* PCB 156	<1.29 ng/kg MC12%
* PCB 157	<0.241 ng/kg MC12%
* PCB 167	<0.646 ng/kg MC12%
* PCB 169	<0.704 ng/kg MC12%
* PCB 189	<0.235 ng/kg MC12%
* WHO(2005)-PCB TEQ (lower-bound)	ND ng/kg MC12%
ND - not determined since none of the corresponding congeners was above the LOQ	
* WHO(2005)-PCB TEQ (upper-bound)	0.0366 ng/kg MC12%
* PCB 28	<0.0587 µg/kg MC12%
* PCB 52	<0.0587 µg/kg MC12%
* PCB 101	<0.0587 µg/kg MC12%
* PCB 138	<0.0587 µg/kg MC12%
* PCB 153	<0.0587 µg/kg MC12%
* PCB 180	<0.0587 µg/kg MC12%

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Eurofins Sample Code: 468-2017-1018B100

Client Sample Code: 1103062519

**REPORT OF ANALYSIS**

AR-17-QA-071030-01

**Test Results**

- \* WHO(2005)-PCDD/F+PCB TEQ (lower-bound)  
ND - not determined since none of the corresponding congeners was above the LOQ
- \* WHO(2005)-PCDD/F+PCB TEQ (upper-bound)

**Result**ND ng/kg MC12%  
0.0972 ng/kg MC12%*\*This is not covered by our current A2LA accreditation.*Respectfully Submitted,  
Eurofins Central Analytical LaboratoriesResults shown in this report relate solely to the item  
submitted for analysis.  
Uncertainty can be obtained upon request.

Cheryl D. Stephenson, Ph.D., Laboratory Director

TESTING CERT  
#2993-01

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**Person in charge** John M. Reuther  
**Client Support** Jeremy Blauch

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Reporting Date 11/23/2018



AR-18-QA-080014-01

## REPORT OF ANALYSIS

**Sample Code** 468-2018-1106B142

**Sample Description** PROBIOTIC  
**Client Sample Code** M85110B, 1103297193  
**Sample Reference** NCFM (LACTOBACIULLUS ACIDOPHILUS)

**Reception Date** 11/06/2018  
**Reception Temperature** 25 (Celsius)  
**Sample Condition** Acceptable  
**Purchase Order** 4501382942

### Test Results

### Result

**QA24X - Aflatoxin M1 (HPLC)**

**Completion Date:** 11/13/2018 **Method:** ISO 14501  
 Aflatoxin M1

<0.05 µg/kg

**QA20B - Aflatoxin Profile (HPLC)**

**Completion Date:** 11/14/2018 **Method:** AOAC 2000.16

Aflatoxin B1

<0.10 µg/kg

Aflatoxin B2

<0.10 µg/kg

Aflatoxin G1

<0.10 µg/kg

Aflatoxin G2

<0.10 µg/kg

Sum of Aflatoxins B1,B2,G1,G2

<0.40 µg/kg

**QA00L - Citrinin (LC-MSMS)**

**Completion Date:** 11/12/2018 **Method:** VICAM CitriTest LC-MS/MS

\* Citrinin

<15 µg/kg

**QA034 - Fumonisin (IAC-LC-MSMS)**

**Completion Date:** 11/09/2018 **Method:** JAOAC, 92 (2), 496.

Fumonisin (B1+B2+B3)

<30 µg/kg

Fumonisin B1

<10 µg/kg

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Eurofins Sample Code: 468-2018-1106B142

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Test Results	Result
Fumonisin B2	<10 µg/kg
Fumonisin B3	<10 µg/kg
<b>QA404 - Ochratoxin A (LC-MSMS)</b>	
Completion Date: 11/12/2018 Method: AOAC 999.07 Modified	
Ochratoxin A	<5 µg/kg
<b>QA106 - Aluminum (ICP-AES)</b>	
Completion Date: 11/14/2018 Method: J.AOAC 92, 1484-1518 (2009)	
* Aluminum (Al)	0.4 mg/kg
<b>QA172 - Antimony (ICP-MS)</b>	
Completion Date: 11/14/2018 Method: AOAC 2013.06	
* Antimony (Sb)	<0.01 mg/kg
<b>QA03T - Barium (ICP-AES)</b>	
Completion Date: 11/14/2018 Method: J.AOAC 92, 1484-1518 (2009)	
* Barium (Ba)	0.18 mg/kg
<b>QA00Y - Boron (ICP-AES)</b>	
Completion Date: 11/14/2018 Method: J.AOAC 92, 1484-1518 (2009)	
* Boron (B)	<0.5 mg/kg
<b>QA008 - Cobalt (ICP-AES)</b>	
Completion Date: 11/14/2018 Method: AOAC 2011.14	
* Cobalt (Co)	0.47 mg/kg
<b>QA007 - Copper (ICP-AES)</b>	
Completion Date: 11/14/2018 Method: AOAC 2011.14	
* Copper (Cu)	0.4 mg/kg
<b>QA003 - Manganese (ICP-AES)</b>	
Completion Date: 11/14/2018 Method: AOAC 2011.14	
* Manganese (Mn)	26.80 mg/kg
<b>QA18V - Chromium (ICP-MS)</b>	
Completion Date: 11/15/2018 Method: AOAC 2013.06	

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Test Results	Result
* Chromium (Cr)	0.04 mg/kg
<b>QA10W - Strontium (ICP-MS)</b> Completion Date: 11/14/2018 Method: AOAC 2013.06	
* Strontium (Sr)	1.4 mg/kg
<b>QA374 - Molybdenum in Food ICP-AES</b> Completion Date: 11/14/2018 Method: AOAC 2011.14	
* Molybdenum (Mo)	0.27 mg/kg
<b>QA00A - Nickel (ICP-AES)</b> Completion Date: 11/14/2018 Method: J.AOAC 92, 1484-1518 (2009)	
* Nickel	0.34 mg/kg
<b>QA005 - Selenium (ICP-MS)</b> Completion Date: 11/14/2018 Method: AOAC 2013.06	
* Selenium (Se)	0.02 mg/kg
<b>QD424 - Tin in Food ICP-MS</b> Completion Date: 11/15/2018 Method: AOAC 2013.06	
* Tin	<0.02 mg/kg
<b>QA006 - Zinc (ICP-AES)</b> Completion Date: 11/14/2018 Method: AOAC 2011.14	
* Zinc (Zn)	40.1 mg/kg
<b>QA20Q - Heavy Metals As-Cd-Pb-Hg (Low LOQ, ICP-MS)</b> Completion Date: 11/14/2018 Method: AOAC 2013.06	
Arsenic (As)	63 µg/kg
Cadmium (Cd)	2.8 µg/kg
Lead (Pb)	<4.0 µg/kg
Mercury (Hg)	<2.0 µg/kg
<b>QA12A - Radioactivity (Cesium-134/137, Iodine-131)</b> Completion Date: 11/07/2018 Method: FDA WEAC.RN.Method3	
* Activity in Cesium 134	<1.0 Bq/kg
* Activity in Cesium 137	<1.0 Bq/kg

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Eurofins Sample Code: 468-2018-1106B142

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**Test Results**
**Result**

* Activity in Iodine 131	<2.0 Bq/kg
<b>QA012 - Nitrate (Ion Chromatography)</b>	
<b>Completion Date:</b> 11/19/2018 <b>Method:</b> Internal Method based on EN 12014-2	
* Nitrate (as NO <sub>3</sub> )	<10 mg/kg
<b>QA396 - Nitrite (Colorimetric)</b>	
<b>Completion Date:</b> 11/14/2018 <b>Method:</b> AOAC 968.07	
* Nitrite (as N)	<1.0 mg N/kg
<b>QA0KS - Chlorate and Perchlorate (LC-MS/MS)</b>	
<b>Completion Date:</b> 11/12/2018 <b>Method:</b> FDA method	
Chlorate	0.74 mg/kg
Perchlorate	<0.01 mg/kg
<b>QA245 - 1,4-Dioxane</b>	
<b>Completion Date:</b> 11/13/2018 <b>Method:</b> USP/NF 467	
* 1,4-Dioxane	<5.0 µg/g
<b>QA315 - Hexane, residual (GC)</b>	
<b>Completion Date:</b> 11/13/2018 <b>Method:</b> USP/NF 467	
* n-Hexane	<5.0 µg/g
<b>QA04D - Residual Solvents (GC-MS)</b>	
<b>Completion Date:</b> 11/14/2018 <b>Method:</b> EPA 5021	
* 1,1,1-Trichloroethane	<0.50 mg/kg
* 1,1,2-Trichloroethane	<0.50 mg/kg
* 1,1-Dichloroethene	<0.50 mg/kg
* 1,2-Dichloroethane	<0.50 mg/kg
* 1,2-Dimethoxyethane	<1.0 mg/kg
* 1-Butanol	<5.0 mg/kg
* 2-Butanon (Methylethylketon)	<0.20 mg/kg
* Acetone	<5.0 mg/kg
* Benzene	<0.10 mg/kg
* Butyl acetate	<0.50 mg/kg
* Carbon tetrachloride	<0.50 mg/kg
* Chlorobenzene	<0.50 mg/kg

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**Test Results**

	<b>Result</b>
* Chloroform	<0.10 mg/kg
* Cyclohexane	<2.0 mg/kg
* Dichloromethane	<0.50 mg/kg
* Ethanol	<1.0 mg/kg
* Ethyl acetate	<1.0 mg/kg
* Heptane	<1.0 mg/kg
* Hexane (sum of normal, iso, and 3-methyl pentane)	<0.50 mg/kg
* Isopropanol	<5.0 mg/kg
* MBK (2-Hexanone)	<1.0 mg/kg
* Methanol	<5.0 mg/kg
* Methyl-tert-butylether (MTBE)	<0.10 mg/kg
* Tetralin	<5.0 mg/kg
* Toluene	<0.50 mg/kg
* Trichloroethylene	<1.0 mg/kg
* Xylenes (sum)	<1.0 mg/kg
* Sample extraction solvent	Matrix Modifying Solution

**QA711 - Cyhexatin/Azocyclotin (LC-MSMS)**
**Completion Date:** 11/13/2018 **Method:** EN 15662:2008; modified

\* Cyhexatin/Azocyclotin (Sum) <0.02 mg/kg
**QA602 - EBDCs (Dithiocarbamates) (CS2 method, GC-MS)**
**Completion Date:** 11/14/2018 **Method:** J. Agric. Food Chem. Vol. 49 pp 2152, 2001

\* Total Dithiocarbamates, as CS2 <0.01 mg/kg
**QA24Y - Haloxyfop, total (Food, LC-MSMS)**
**Completion Date:** 11/08/2018 **Method:** Internal method

\* Haloxyfop <0.003 mg/kg
**QA737 - Methyl Bromide (Headspace GC-MS)**
**Completion Date:** 11/09/2018 **Method:** EPA 5021

\* Methyl bromide <0.01 mg/kg
**QA622 - Dithiocarbamates (incl. Propineb) GC-MS)**
**Completion Date:** 11/14/2018 **Method:** J. Agric. Food Chem. Vol. 49 pp 2152, 2001

\* Dithiocarbamates (as CS2) <0.006 mg/kg

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Eurofins Sample Code: 468-2018-1106B142

Client Sample Code: M85110B, 1103297193

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**Test Results**
**Result**
**QA01P - Pesticides Quechers GC-MSMS**

Completion Date: 11/14/2018 Method: AOAC 2007.01

Screened pesticides

Not detected

**QA01R - Pesticides Quechers-LC-MS/MS**

Completion Date: 11/13/2018 Method: AOAC 2007.01

Screened pesticides

Not detected

**QA083 - Pesticides - Glyphosate Compounds (LC-MSMS)**

Completion Date: 11/09/2018 Method: J. Agric. Food Chem. 2004, 52, 4057-4063

Glufosinate

&lt;0.01 mg/kg

Glyphosate

&lt;0.01 mg/kg

**QA107 - Pesticides - Acid Herbicides LC-MSMS**

Completion Date: 11/08/2018 Method: Internal method

\* 2,4,5-T

&lt;0.01 mg/kg

\* 2,4-D

&lt;0.01 mg/kg

\* 2,4-DB

&lt;0.01 mg/kg

\* Acifluorfen

&lt;0.01 mg/kg

\* Aminopyralid

&lt;0.01 mg/kg

\* Bentazone

&lt;0.01 mg/kg

\* Bispyribac

&lt;0.01 mg/kg

\* Bromoxynil

&lt;0.01 mg/kg

\* Clopyralid

&lt;0.01 mg/kg

\* Dicamba

&lt;0.01 mg/kg

\* Diflufenzopyr

&lt;0.01 mg/kg

\* Fluazifop

&lt;0.01 mg/kg

\* Flucarbazone

&lt;0.01 mg/kg

\* Fluroxypyr

&lt;0.01 mg/kg

\* Fomesafen

&lt;0.01 mg/kg

\* Haloxyfop

&lt;0.003 mg/kg

\* Imazamox

&lt;0.01 mg/kg

\* Imazapyr

&lt;0.01 mg/kg

\* Imazaquin

&lt;0.01 mg/kg

\* Imazethapyr

&lt;0.01 mg/kg

\* MCPA

&lt;0.01 mg/kg

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Eurofins Sample Code: 468-2018-1106B142

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**Test Results**

- \* Picloram
- \* Quinclorac
- \* Triclopyr
- \* MCPB

**Result**

<0.01 mg/kg  
 <0.01 mg/kg  
 <0.01 mg/kg  
 <0.01 mg/kg

**QA0GX - Pesticides - EU Banned (GC-MSMS)**

Completion Date: 11/14/2018 Method: EN 12393

- \* Aldrin
- \* Dieldrin
- \* Aldrin/ Dieldrin (Sum)
- \* Endrin
- \* HCB (Hexachlorbenzene)
- \* Heptachlor
- \* Heptachlor epoxide, trans-
- \* Heptachlor (sum)
- \* Nitrofen
- \* Chlordane (total)

<0.003 mg/kg  
 <0.003 mg/kg  
 <0.003 mg/kg  
 <0.003 mg/kg  
 <0.003 mg/kg  
 <0.003 mg/kg  
 <0.003 mg/kg  
 <0.003 mg/kg  
 <0.003 mg/kg  
 <0.004 mg/kg

**QA0FX - Pesticides - EU Banned (LC-MSMS)**

Completion Date: 11/13/2018 Method: HPLC-MS/MS

- \* Cadusaphos
- \* Demeton
- \* Demeton-S-methyl
- \* Demeton-S-methyl-sulfone
- \* Oxydemeton-methyl
- \* Ethoprophos
- \* Disulfoton-sulfon
- \* Disulfoton-sulfoxide
- \* Disulfoton (sum)
- \* Disulfoton
- \* Fensulfothion
- \* Fensulfothion-oxon-sulfone
- \* Fensulfothion-sulfone
- \* Fensulfothion (sum)
- \* Fipronil
- \* Fipronil, desulfinyl-

<0.006 mg/kg  
 <0.004 mg/kg  
 <0.004 mg/kg  
 <0.004 mg/kg  
 <0.004 mg/kg  
 <0.008 mg/kg  
 <0.003 mg/kg  
 <0.003 mg/kg  
 <0.003 mg/kg  
 <0.003 mg/kg  
 <0.003 mg/kg  
 <0.003 mg/kg  
 <0.003 mg/kg  
 <0.003 mg/kg  
 <0.003 mg/kg  
 <0.004 mg/kg  
 <0.004 mg/kg

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Eurofins Sample Code: 468-2018-1106B142

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### Test Results

	<b>Result</b>
* Fipronil (sum)	<0.004 mg/kg
* Omethoate	<0.003 mg/kg
* Terbufos	<0.003 mg/kg
* Terbufos-sulfoxide	<0.003 mg/kg
* Terbufos-sulfone	<0.003 mg/kg
* Terbufos (sum)	<0.003 mg/kg

### QA036 - Pesticides - Quaternary Ammonium Cmpds (LC-MSMS)

Completion Date: 11/12/2018 Method: Method published by the CRL (24.04.2005)

* Diquat	<0.01 mg/kg
* Paraquat	<0.01 mg/kg
* Chlormequat	<0.01 mg/kg
* Mepiquat	<0.01 mg/kg
* Difenzoquat	<0.01 mg/kg

### QA016 - Acrylamide (LC-MSMS, FDA method)

Completion Date: 11/13/2018 Method: FDA method

Acrylamide	<2.0 µg/kg
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### QA812 - Benzo(a)pyrene (GC-MSMS)

Completion Date: 11/12/2018 Method: Internal

* Benzo(a)pyrene	<0.50 µg/kg
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### QA22D - Polynuclear Aromatic Hydrocarbons (GC-MSMS)

Completion Date: 11/12/2018 Method: Internal method

Benz(a)anthracene	<0.50 µg/kg
Chrysene	<0.50 µg/kg
Benzo(b)fluoranthene	<0.50 µg/kg
Benzo(a)pyrene	<0.50 µg/kg
Sum of all positive identified PAH	<2.0 µg/kg

### QA0ND - Glycidyl esters (GC-MSMS)

Completion Date: 11/23/2018 Method: AOCS Cd 29b-13

Glycidol (calculated)	<0.08 mg/kg
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### QA0NC - Monochloropropanediols (sum of free and esters)

Completion Date: 11/23/2018 Method: AOCS Cd 29b-13

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**Eurofins Sample Code: 468-2018-1106B142**
**Client Sample Code: M85110B, 1103297193**

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**Test Results**

 Total 2-MCPD (free and bound)  
 Total 3-MCPD (free and bound)

**Result**

 <0.10 mg/kg  
 <0.10 mg/kg

**QA0R6 - Dichloropropanol**
**Completion Date:** 11/14/2018 **Method:**

- \* 1,3-Dichloro-2-propanol
- \* 2,3-Dichloro-1-propanol

 <0.10 mg/kg  
 <0.10 mg/kg

**GFF03 - Dioxins and Furans (17 PCDD/F)**
**Completion Date:** 11/15/2018 **Method:** Internal

- \* 2,3,7,8-TetraCDD
- \* 1,2,3,7,8-PentaCDD
- \* 1,2,3,4,7,8-HexaCDD
- \* 1,2,3,6,7,8-HexaCDD
- \* 1,2,3,7,8,9-HexaCDD
- \* 1,2,3,4,6,7,8-HeptaCDD
- \* OctaCDD
- \* 2,3,7,8-TetraCDF
- \* 1,2,3,7,8-PentaCDF
- \* 2,3,4,7,8-PentaCDF
- \* 1,2,3,4,7,8-HexaCDF
- \* 1,2,3,6,7,8-HexaCDF
- \* 1,2,3,7,8,9-HexaCDF
- \* 2,3,4,6,7,8-HexaCDF
- \* 1,2,3,4,6,7,8-HeptaCDF
- \* 1,2,3,4,7,8,9-HeptaCDF
- \* OctaCDF
- \* WHO(2005)-PCDD/F TEQ (lower-bound)
- \* WHO(2005)-PCDD/F TEQ (medium-bound)
- \* WHO(2005)-PCDD/F TEQ (upper-bound)

 <0.0107 ng/kg MC12%  
 <0.0140 ng/kg MC12%  
 <0.0213 ng/kg MC12%  
 <0.0292 ng/kg MC12%  
 <0.0275 ng/kg MC12%  
 <0.0449 ng/kg MC12%  
 <0.325 ng/kg MC12%  
 <0.0292 ng/kg MC12%  
 <0.0202 ng/kg MC12%  
 <0.0314 ng/kg MC12%  
 <0.0331 ng/kg MC12%  
 <0.0303 ng/kg MC12%  
 <0.0224 ng/kg MC12%  
 <0.0275 ng/kg MC12%  
 <0.0314 ng/kg MC12%  
 <0.0219 ng/kg MC12%  
 <0.0673 ng/kg MC12%  
 ND  
 0.0289 ng/kg MC12%  
 0.0578 ng/kg MC12%

**GFDRY - Dry Residue**
**Completion Date:** 11/15/2018 **Method:** Internal

- \* dry residue

99.0 %

**S1003 - Propylene thiourea (PTU) and Ethylene thiourea (ETU)**
**Completion Date:** 11/14/2018 **Method:** Internal Method, P-14.065-5

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Eurofins Sample Code: 468-2018-1106B142

Client Sample Code: M85110B, 1103297193

**REPORT OF ANALYSIS**


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**Test Results**

- \* Ethylene thiourea (ETU)
- \* PTU (Propylene Thiourea)

**Result**

<0.003 mg/kg  
<0.003 mg/kg

**QA0LY - Fentin (LC/MSMS)**
**Completion Date:** 11/13/2018 **Method:** EN 15662:2008; modified

- \* Fentin

&lt;0.003 mg/kg

**QA26E - Fusarium Toxins full (LC-MSMS)**
**Completion Date:** 11/14/2018 **Method:** Romer Mycosep LC-MSMS

- 15-Acetyldeoxynivalenol (15-ADON)
- 3-Acetyldeoxynivalenol (3-ADON)
- Deoxynivalenol (Vomitoxin)
- Diacetoxyscirpenol (DAS)
- Fusarenone X
- HT-2 Toxin
- Neosolaniol
- Nivalenol (NIV)
- T-2 Toxin
- Zearalenone

<20 µg/kg  
<20 µg/kg  
<10 µg/kg  
<50 µg/kg  
<20 µg/kg  
<10 µg/kg  
<50 µg/kg  
<10 µg/kg  
<1.0 µg/kg  
<5.0 µg/kg

**ZVM01 - Mineral oil MOSH, MOAH, high fatty food**
**Completion Date:** 11/13/2018 **Method:** Own method (procedure conform NEN-EN 16995)

- \* MOSH/POSH (saturated) C10-16
- \* MOSH/POSH (saturated) C17-20
- \* MOSH/POSH (saturated) C20-25
- \* MOSH/POSH (saturated) C25-35
- \* MOSH/POSH (saturated) C35-50
- \* MOSH/POSH C20-35 (sum, calculated)
- \* MOSH/POSH C10-50 (sum, calculated)
- \* MOAH (aromatic) C10-16
- \* MOAH (aromatic) C16-20
- \* MOAH (aromatic) C20-25
- \* MOAH (aromatic) C25-35
- \* MOAH (aromatic) C35-50
- \* MOAH C16-35 (sum, calculated)
- \* MOAH C10-50 (sum, calculated)

<1 mg/kg  
<1 mg/kg  
<1 mg/kg  
<1 mg/kg  
<1 mg/kg  
<2 mg/kg  
<6 mg/kg  
<0.15 mg/kg  
<0.15 mg/kg  
<0.15 mg/kg  
<0.15 mg/kg  
<0.15 mg/kg  
<0.5 mg/kg  
<1 mg/kg

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Eurofins Sample Code: 468-2018-1106B142

Client Sample Code: M85110B, 1103297193

**REPORT OF ANALYSIS**


AR-18-QA-080014-01

**Test Results**
**Result**
**GFF08 - polychlorinated biphenyls (12 WHO PCB + 6 ICES PCB)**

Completion Date: 11/15/2018 Method: Internal

* PCB 77	<1.01 ng/kg MC12%
* PCB 81	<0.151 ng/kg MC12%
* PCB 105	<2.19 ng/kg MC12%
* PCB 114	<0.297 ng/kg MC12%
* PCB 118	<7.85 ng/kg MC12%
* PCB 123	<0.224 ng/kg MC12%
* PCB 126	<0.140 ng/kg MC12%
* PCB 156	<1.23 ng/kg MC12%
* PCB 157	<0.230 ng/kg MC12%
* PCB 167	<0.617 ng/kg MC12%
* PCB 169	<0.673 ng/kg MC12%
* PCB 189	<0.224 ng/kg MC12%
* WHO(2005)-PCB TEQ (lower-bound)	ND
* WHO(2005)-PCB TEQ (medium-bound)	0.0174 ng/kg MC12%
* WHO(2005)-PCB TEQ (upper-bound)	0.0347 ng/kg MC12%
* PCB 28	<0.0561 µg/kg MC12%
* PCB 52	<0.0561 µg/kg MC12%
* PCB 101	<0.0561 µg/kg MC12%
* PCB 138	<0.0561 µg/kg MC12%
* PCB 153	<0.0561 µg/kg MC12%
* PCB 180	<0.0561 µg/kg MC12%
* Total 6 ndl-PCB (lower-bound)	ND
* Total 6 ndl-PCB (medium-bound)	0.168 µg/kg MC12%
* Total 6 ndl-PCB (upper-bound)	0.336 µg/kg MC12%

**QA0NV - Pesticides - Organotin (LC-MSMS)**

Completion Date: 11/13/2018 Method: Internal LC-MS/MS

* Cyhexatin/Azocyclotin (Sum)	<0.01 mg/kg
* Fenbutatin oxide	<0.01 mg/kg
* Fentin compounds calc. as Triphenyltin	<0.01 mg/kg

**QA0PK - Pesticides Quechers (Supplemental)-GC-MS/MS**

Completion Date: 11/14/2018 Method: AOAC 2007.01

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Eurofins Sample Code: 468-2018-1106B142

Client Sample Code: M85110B, 1103297193

## REPORT OF ANALYSIS



AR-18-QA-080014-01

### Test Results

- \* Chinomethionate
- \* Diclofop-methyl
- \* Fluridone
- \* Formothion
- \* Pyraclofos
- \* Pyraflufen-ethyl
- \* Silafluofen

### Result

- <0.01 mg/kg
- <0.01 mg/kg
- <0.01 mg/kg
- <0.01 mg/kg
- <0.01 mg/kg
- <0.01 mg/kg
- <0.01 mg/kg

### QA0PJ - Pesticides Quechers (Supplemental)-LC-MS/MS

Completion Date: 11/14/2018 Method: AOAC 2007.01

- \* Azamethipos
- \* Bensulfuron methyl
- \* Benzovindiflupyr
- \* Chlorfluazuron
- \* Chlorimuron-Ethyl
- \* Dinotefuran
- \* Emamectin
- \* Ethiprole
- \* Fluazinam
- \* Indaziflam
- \* Isopyrazam
- \* Mesosulfuron-methyl
- \* Metrafenone
- \* Nicosulfuron
- \* Phoxim
- \* Saflufenacil
- \* Triasulfuron
- \* Tribenuron-methyl
- \* Zoxamide

- <0.010 mg/kg
- <0.01 mg/kg
- <0.010 mg/kg
- <0.02 mg/kg
- <0.010 mg/kg
- <0.01 mg/kg
- <0.01 mg/kg
- <0.01 mg/kg
- <0.01 mg/kg
- <0.01 mg/kg
- <0.01 mg/kg
- <0.010 mg/kg
- <0.01 mg/kg
- <0.010 mg/kg
- <0.01 mg/kg
- <0.010 mg/kg
- <0.010 mg/kg
- <0.010 mg/kg

### QA0LN - Phthalate Compounds (GC-MSMS)

Completion Date: 11/14/2018 Method: Internal method

- \* Benzyl butyl phthalate (BBP)
- \* Dibutyl phthalate (DBP)
- \* Dibutyladipate
- \* Diethyl hexyl phthalate (DEHP)

- <0.05 mg/kg
- <0.05 mg/kg
- <0.05 mg/kg
- <0.05 mg/kg

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Eurofins Sample Code: 468-2018-1106B142

Client Sample Code: M85110B, 1103297193

**REPORT OF ANALYSIS**

AR-18-QA-080014-01

**Test Results**

- \* Diethyladipate
- \* Diethylhexyl adipate (DEHA)
- \* Diethyl phthalate (DEP)
- \* Di-isobutyl phthalate (DiBP)
- \* Di-isobutyladipate
- \* Diisodecylphthalate (DIDP)
- \* Diisoheptylphthalate (DiHP)
- \* Diisononylphthalate (DINP)
- \* Dimethyl phthalate (DMP)
- \* Di-n-octylphthalate (DNOP)
- \* Dinonyl phthalate (DNP)
- \* Dipentylphthalate (DPP)
- \* Di-n-hexyl phthalate

**Result**

<0.05 mg/kg  
<0.05 mg/kg  
<0.05 mg/kg  
<0.05 mg/kg  
<0.05 mg/kg  
<0.05 mg/kg  
<0.05 mg/kg  
<0.05 mg/kg  
<0.05 mg/kg  
<0.05 mg/kg  
<0.05 mg/kg  
<0.05 mg/kg  
<0.05 mg/kg

**GFTE1 - TEQ-Totals WHO-PCDD/F and PCB****Completion Date:** 11/15/2018 **Method:** Internal

- \* WHO(2005)-PCDD/F+PCB TEQ (lower-bound)
- \* WHO(2005)-PCDD/F+PCB TEQ (medium-bound)
- \* WHO(2005)-PCDD/F+PCB TEQ (upper-bound)

ND  
0.0463 ng/kg MC12%  
0.0926 ng/kg MC12%

*\*This is not covered by our current A2LA accreditation.*

Respectfully Submitted,  
Eurofins Central Analytical Laboratories

Results shown in this report relate solely to the item  
submitted for analysis.  
Uncertainty can be obtained upon request.

Victoria Siegel, Analytical Service Manager



TESTING CERT  
#2993-01

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**Person in charge**  
**Client Support**

John M. Reuther  
Jeremy Blauch

Danisco USA Inc.  
ATTN: Michele Fojut  
3322 Agriculture Dr  
Madison, WI 53716

Reporting Date 05/28/2019



AR-19-QA-038591-01

**REPORT OF ANALYSIS**
**Sample Code** 468-2019-05150228

**Sample Description** PROBIOTIC  
**Client Sample Code** PO8821059 1103478955  
**Sample Reference** NCFM (LACTOBACILLUS ACIDOPHILUS)

**Reception Date** 05/15/2019  
**Reception Temperature** 25 (Celsius)  
**Sample Condition** Acceptable  
**Purchase Order** 4501382942

**Test Results**
**Result**
**QA24X - Aflatoxin M1 (HPLC)**
**Completion Date:** 05/20/2019 **Method:** ISO 14501  
Aflatoxin M1

&lt;0.05 µg/kg

**QA20B - Aflatoxin Profile (HPLC)**
**Completion Date:** 05/23/2019 **Method:** AOAC 2000.16

Aflatoxin B1

&lt;0.10 µg/kg

Aflatoxin B2

&lt;0.10 µg/kg

Aflatoxin G1

&lt;0.10 µg/kg

Aflatoxin G2

&lt;0.10 µg/kg

Sum of Aflatoxins B1,B2,G1,G2

&lt;0.40 µg/kg

**QA00L - Citrinin (LC-MSMS)**
**Completion Date:** 05/22/2019 **Method:** VICAM CitriTest LC-MS/MS

\* Citrinin

&lt;15 µg/kg

**QA034 - Fumonisin (IAC-LC-MSMS)**
**Completion Date:** 05/20/2019 **Method:** JAOAC, 92 (2), 496.

Fumonisin (B1+B2+B3)

&lt;30 µg/kg

Fumonisin B1

&lt;10 µg/kg

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Eurofins Sample Code: 468-2019-05150228

Client Sample Code: PO8821059 1103478955

## REPORT OF ANALYSIS



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Test Results	Result
Fumonisin B2	<10 µg/kg
Fumonisin B3	<10 µg/kg
<b>QA404 - Ochratoxin A (LC-MSMS)</b>	
Completion Date: 05/23/2019 Method: AOAC 999.07 Modified	
Ochratoxin A	<5.0 µg/kg
Adjusted LOQ for this matrix.	
<b>QA106 - Aluminum (ICP-AES)</b>	
Completion Date: 05/23/2019 Method: J.AOAC 92, 1484-1518 (2009)	
* Aluminum (Al)	1.5 mg/kg
<b>QA172 - Antimony (ICP-MS)</b>	
Completion Date: 05/23/2019 Method: AOAC 2013.06	
* Antimony (Sb)	0.01 mg/kg
<b>QA03T - Barium (ICP-AES)</b>	
Completion Date: 05/23/2019 Method: J.AOAC 92, 1484-1518 (2009)	
* Barium (Ba)	0.23 mg/kg
<b>QA00Y - Boron (ICP-AES)</b>	
Completion Date: 05/23/2019 Method: J.AOAC 92, 1484-1518 (2009)	
* Boron (B)	0.7 mg/kg
<b>QA008 - Cobalt (ICP-AES)</b>	
Completion Date: 05/23/2019 Method: AOAC 2011.14	
* Cobalt (Co)	0.23 mg/kg
<b>QA007 - Copper (ICP-AES)</b>	
Completion Date: 05/23/2019 Method: AOAC 2011.14	
* Copper (Cu)	<0.2 mg/kg
<b>QA003 - Manganese (ICP-AES)</b>	
Completion Date: 05/23/2019 Method: AOAC 2011.14	
* Manganese (Mn)	32.55 mg/kg
<b>QA18V - Chromium (ICP-MS)</b>	

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Eurofins Sample Code: 468-2019-05150228

Client Sample Code: PO8821059 1103478955

**REPORT OF ANALYSIS**


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Test Results	Result
<b>Completion Date:</b> 05/22/2019 <b>Method:</b> AOAC 2013.06 * Chromium (Cr)	0.09 mg/kg
<b>QA10W - Strontium (ICP-MS)</b> <b>Completion Date:</b> 05/22/2019 <b>Method:</b> AOAC 2013.06 * Strontium (Sr)	1.5 mg/kg
<b>QA374 - Molybdenum in Food ICP-AES</b> <b>Completion Date:</b> 05/23/2019 <b>Method:</b> AOAC 2011.14 * Molybdenum (Mo)	0.12 mg/kg
<b>QA00A - Nickel (ICP-AES)</b> <b>Completion Date:</b> 05/23/2019 <b>Method:</b> J.AOAC 92, 1484-1518 (2009) * Nickel	<0.10 mg/kg
<b>QA005 - Selenium (ICP-MS)</b> <b>Completion Date:</b> 05/22/2019 <b>Method:</b> AOAC 2013.06 * Selenium (Se)	0.03 mg/kg
<b>QD424 - Tin in Food ICP-MS</b> <b>Completion Date:</b> 05/23/2019 <b>Method:</b> AOAC 2013.06 * Tin	<0.02 mg/kg
<b>QA006 - Zinc (ICP-AES)</b> <b>Completion Date:</b> 05/22/2019 <b>Method:</b> AOAC 2011.14 * Zinc (Zn)	42.7 mg/kg
<b>QA20Q - Heavy Metals As-Cd-Pb-Hg (Low LOQ, ICP-MS)</b> <b>Completion Date:</b> 05/22/2019 <b>Method:</b> AOAC 2013.06	
Arsenic (As)	40 µg/kg
Cadmium (Cd)	<2.0 µg/kg
Lead (Pb)	7.9 µg/kg
Mercury (Hg)	<2.0 µg/kg
<b>QA12A - Radioactivity (Cesium-134/137, Iodine-131)</b> <b>Completion Date:</b> 05/23/2019 <b>Method:</b> FDA WEAC.RN.Method3 * Activity in Cesium 134	<1.0 Bq/kg

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Eurofins Sample Code: 468-2019-05150228

Client Sample Code: PO8821059 1103478955

**REPORT OF ANALYSIS**


AR-19-QA-038591-01

<b>Test Results</b>	<b>Result</b>
* Activity in Cesium 137	<1.0 Bq/kg
* Activity in Iodine 131	<2.0 Bq/kg
<b>QA012 - Nitrate (Ion Chromatography)</b>	
<b>Completion Date:</b> 05/20/2019 <b>Method:</b> Internal Method based on EN 12014-2	
* Nitrate (as NO <sub>3</sub> )	33 mg/kg
<b>QA396 - Nitrite (Colorimetric)</b>	
<b>Completion Date:</b> 05/20/2019 <b>Method:</b> AOAC 968.07	
* Nitrite (as N)	<1.0 mg N/kg
<b>QA0KS - Chlorate and Perchlorate (LC-MS/MS)</b>	
<b>Completion Date:</b> 05/24/2019 <b>Method:</b> FDA method	
Chlorate	1.88 mg/kg
Perchlorate	<0.01 mg/kg
<b>QA245 - 1,4-Dioxane</b>	
<b>Completion Date:</b> 05/17/2019 <b>Method:</b> USP/NF 467	
* 1,4-Dioxane	<5.0 µg/g
<b>QA315 - Hexane, residual (GC)</b>	
<b>Completion Date:</b> 05/23/2019 <b>Method:</b> USP/NF 467	
* n-Hexane	<5.0 µg/g
<b>QA04D - Residual Solvents (GC-MS)</b>	
<b>Completion Date:</b> 05/20/2019 <b>Method:</b> EPA 5021	
* 1,1,1-Trichloroethane	<0.50 mg/kg
* 1,1,2-Trichloroethane	<0.50 mg/kg
* 1,1-Dichloroethene	<0.50 mg/kg
* 1,2-Dichloroethane	<0.50 mg/kg
* 1,2-Dimethoxyethane	<1.0 mg/kg
* 1-Butanol	<5.0 mg/kg
* 2-Butanon (Methylethylketon)	<0.20 mg/kg
* Acetone	<5.0 mg/kg
* Benzene	<0.10 mg/kg
* Butyl acetate	<0.50 mg/kg
* Carbon tetrachloride	<0.50 mg/kg

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Eurofins Sample Code: 468-2019-05150228

Client Sample Code: PO8821059 1103478955

## REPORT OF ANALYSIS



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### Test Results

* Chlorobenzene	<0.50 mg/kg
* Chloroform	<0.10 mg/kg
* Cyclohexane	<2.0 mg/kg
* Dichloromethane	<0.50 mg/kg
* Ethanol	<1.0 mg/kg
* Ethyl acetate	<1.0 mg/kg
* Heptane	<1.0 mg/kg
* Hexane (sum of normal, iso, and 3-methyl pentane)	<0.50 mg/kg
* Isopropanol	<5.0 mg/kg
* MBK (2-Hexanone)	<1.0 mg/kg
* Methanol	<5.0 mg/kg
* Methyl-tert-butylether (MTBE)	<0.10 mg/kg
* Tetralin	<5.0 mg/kg
* Toluene	<0.50 mg/kg
* Trichloroethylene	<1.0 mg/kg
* Xylenes (sum)	<1.0 mg/kg
* Sample extraction solvent	Matrix Modifying Solution

### Result

#### QA711 - Cyhexatin/Azocyclotin (LC-MSMS)

Completion Date: 05/23/2019 Method: EN 15662:2008; modified

\* Cyhexatin/Azocyclotin (Sum) <0.02 mg/kg

#### QA602 - EBDCs (Dithiocarbamates) (CS2 method, GC-MS)

Completion Date: 05/22/2019 Method: J. Agric. Food Chem. Vol. 49 pp 2152, 2001

\* Total Dithiocarbamates, as CS2 <0.01 mg/kg

#### QA24Y - Haloxyfop, total (Food, LC-MSMS)

Completion Date: 05/22/2019 Method: Internal method

\* Haloxyfop <0.003 mg/kg

#### QA737 - Methyl Bromide (Headspace GC-MS)

Completion Date: 05/21/2019 Method: EPA 5021

\* Methyl bromide <20 mg/kg

Adjusted LOQ for this matrix.

#### QA622 - Dithiocarbamates (incl. Propineb) GC-MS

Completion Date: 05/22/2019 Method: J. Agric. Food Chem. Vol. 49 pp 2152, 2001

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Eurofins Sample Code: 468-2019-05150228

Client Sample Code: PO8821059 1103478955

**REPORT OF ANALYSIS**


AR-19-QA-038591-01

**Test Results**
**Result**

\* Dithiocarbamates (as CS2)

&lt;0.006 mg/kg

**QA01P - Pesticides Quechers GC-MSMS**

Completion Date: 05/21/2019 Method: AOAC 2007.01

Screened pesticides

Not detected

**QA01R - Pesticides Quechers-LC-MS/MS**

Completion Date: 05/23/2019 Method: AOAC 2007.01

Screened pesticides

Not detected

**QA083 - Pesticides - Glyphosate Compounds (LC-MSMS)**

Completion Date: 05/22/2019 Method: J. Agric. Food Chem. 2004, 52, 4057-4063

Glufosinate

&lt;0.10 mg/kg

Glyphosate

&lt;0.10 mg/kg

**QA107 - Pesticides - Acid Herbicides LC-MSMS**

Completion Date: 05/22/2019 Method: Internal method

\* 2,4,5-T

&lt;0.01 mg/kg

\* 2,4-D

&lt;0.01 mg/kg

\* 2,4-DB

&lt;0.01 mg/kg

\* Acifluorfen

&lt;0.01 mg/kg

\* Aminopyralid

&lt;0.01 mg/kg

\* Asulam

&lt;0.01 mg/kg

\* Bentazone

&lt;0.01 mg/kg

\* Bispyribac

&lt;0.01 mg/kg

\* Bromoxynil

&lt;0.01 mg/kg

\* Clopyralid

&lt;0.01 mg/kg

\* Dicamba

&lt;0.01 mg/kg

\* Diflufenzopyr

&lt;0.01 mg/kg

\* Fluazifop

&lt;0.01 mg/kg

\* Flucarbazone

&lt;0.01 mg/kg

\* Fluroxypyr

&lt;0.01 mg/kg

\* Fomesafen

&lt;0.01 mg/kg

\* Haloxyfop

&lt;0.003 mg/kg

\* Imazamox

&lt;0.01 mg/kg

\* Imazapyr

&lt;0.01 mg/kg

\* Imazaquin

&lt;0.01 mg/kg

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Eurofins Sample Code: 468-2019-05150228

Client Sample Code: PO8821059 1103478955

## REPORT OF ANALYSIS



AR-19-QA-038591-01

### Test Results

- \* Imazethapyr
- \* MCPA
- \* MCPB
- \* Mesotrione
- \* Picloram
- \* Quinclorac
- \* Triclopyr

### Result

<0.01 mg/kg  
 <0.01 mg/kg  
 <0.01 mg/kg  
 <0.01 mg/kg  
 <0.01 mg/kg  
 <0.01 mg/kg  
 <0.01 mg/kg

### QA0GX - Pesticides - EU Banned (GC-MSMS)

Completion Date: 05/24/2019 Method: EN 12393

- \* Aldrin
- \* Dieldrin
- \* Aldrin/ Dieldrin (Sum)
- \* Endrin
- \* HCB (Hexachlorbenzene)
- \* Heptachlor
- \* Heptachlor epoxide, trans-
- \* Heptachlor (sum)
- \* Nitrofen
- \* Chlordane (total)

<0.003 mg/kg  
 <0.003 mg/kg  
 <0.003 mg/kg  
 <0.003 mg/kg  
 <0.003 mg/kg  
 <0.003 mg/kg  
 <0.003 mg/kg  
 <0.003 mg/kg  
 <0.003 mg/kg  
 <0.004 mg/kg

### QA0FX - Pesticides - EU Banned (LC-MSMS)

Completion Date: 05/28/2019 Method: HPLC-MS/MS

- \* Cadusaphos
- \* Demeton
- \* Demeton-S-methyl
- \* Demeton-S-methyl-sulfone
- \* Oxydemeton-methyl
- \* Ethoprophos
- \* Disulfoton-sulfon
- \* Disulfoton-sulfoxide
- \* Disulfoton (sum)
- \* Disulfoton
- \* Fensulfothion
- \* Fensulfothion-oxon-sulfone
- \* Fensulfothion-sulfone

<0.006 mg/kg  
 <0.004 mg/kg  
 <0.004 mg/kg  
 <0.004 mg/kg  
 <0.004 mg/kg  
 <0.008 mg/kg  
 <0.003 mg/kg  
 <0.003 mg/kg  
 <0.003 mg/kg  
 <0.003 mg/kg  
 <0.003 mg/kg  
 <0.003 mg/kg  
 <0.003 mg/kg

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Eurofins Sample Code: 468-2019-05150228

Client Sample Code: PO8821059 1103478955

**REPORT OF ANALYSIS**


AR-19-QA-038591-01

**Test Results**

- \* Fensulfothion (sum)
- \* Fipronil
- \* Fipronil, desulfinyl-
- \* Fipronil (sum)
- \* Omethoate
- \* Terbufos
- \* Terbufos-sulfoxide
- \* Terbufos-sulfone
- \* Terbufos (sum)

**Result**

- <0.003 mg/kg
- <0.004 mg/kg
- <0.004 mg/kg
- <0.004 mg/kg
- <0.003 mg/kg
- <0.003 mg/kg
- <0.003 mg/kg
- <0.003 mg/kg
- <0.003 mg/kg

**QA036 - Pesticides - Quaternary Ammonium Cmpds (LC-MSMS)**
**Completion Date:** 05/23/2019 **Method:** Method published by the CRL (24.04.2005)

- \* Diquat <0.01 mg/kg
- \* Paraquat <0.01 mg/kg
- \* Chlormequat <0.01 mg/kg
- \* Mepiquat <0.01 mg/kg
- \* Difenzoquat <0.01 mg/kg

**QA016 - Acrylamide (LC-MSMS, FDA method)**
**Completion Date:** 05/23/2019 **Method:** FDA method

- Acrylamide <2.0 µg/kg

**QA812 - Benzo(a)pyrene (GC-MSMS)**
**Completion Date:** 05/22/2019 **Method:** Internal

- \* Benzo(a)pyrene <0.50 µg/kg

**QA22D - Polynuclear Aromatic Hydrocarbons (GC-MSMS)**
**Completion Date:** 05/20/2019 **Method:** Internal method

- Benz(a)anthracene <0.50 µg/kg
- Chrysene <0.50 µg/kg
- Benzo(b)fluoranthene <0.50 µg/kg
- Benzo(a)pyrene <0.50 µg/kg
- Sum of all positive identified PAH <2.0 µg/kg

**QA0ND - Glycidyl esters (GC-MSMS)**
**Completion Date:** 05/23/2019 **Method:** AOCS Cd 29b-13

- Glycidol (calculated) <0.08 mg/kg

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Eurofins Sample Code: 468-2019-05150228

Client Sample Code: PO8821059 1103478955



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**REPORT OF ANALYSIS**
**Test Results**
**Result**
**QA0NC - Monochloropropanediols (sum of free and esters)**
**Completion Date:** 05/23/2019 **Method:** AOCS Cd 29b-13

 Total 2-MCPD (free and bound) <0.10 mg/kg  
 Total 3-MCPD (free and bound) <0.10 mg/kg

**QA0SD - Aluminum (ICP-MS)**
**Completion Date:** 05/23/2019 **Method:** AOAC 2013.06

\* Aluminium 1.4 mg/kg

**QA0R6 - Dichloropropanols**
**Completion Date:** 05/21/2019 **Method:**

 \* 1,3-Dichlor-2-propanol <0.10 mg/kg  
 \* 2,3-Dichloro-1-propanol <0.10 mg/kg

**GFF03 - Dioxins and Furans (17 PCDD/F)**
**Completion Date:** 05/20/2019 **Method:** Internal

* 2,3,7,8-TetraCDD	<0.0114 ng/kg MC12%
* 1,2,3,7,8-PentaCDD	<0.0150 ng/kg MC12%
* 1,2,3,4,7,8-HexaCDD	<0.0228 ng/kg MC12%
* 1,2,3,6,7,8-HexaCDD	<0.0312 ng/kg MC12%
* 1,2,3,7,8,9-HexaCDD	<0.0294 ng/kg MC12%
* 1,2,3,4,6,7,8-HeptaCDD	<0.0479 ng/kg MC12%
* OctaCDD	<0.348 ng/kg MC12%
* 2,3,7,8-TetraCDF	<0.0312 ng/kg MC12%
* 1,2,3,7,8-PentaCDF	<0.0216 ng/kg MC12%
* 2,3,4,7,8-PentaCDF	<0.0336 ng/kg MC12%
* 1,2,3,4,7,8-HexaCDF	<0.0354 ng/kg MC12%
* 1,2,3,6,7,8-HexaCDF	<0.0324 ng/kg MC12%
* 1,2,3,7,8,9-HexaCDF	<0.0240 ng/kg MC12%
* 2,3,4,6,7,8-HexaCDF	<0.0294 ng/kg MC12%
* 1,2,3,4,6,7,8-HeptaCDF	<0.0336 ng/kg MC12%
* 1,2,3,4,7,8,9-HeptaCDF	<0.0234 ng/kg MC12%
* OctaCDF	<0.0719 ng/kg MC12%
* WHO(2005)-PCDD/F TEQ (lower-bound)	Not Detected
* WHO(2005)-PCDD/F TEQ (medium-bound)	0.0309 ng/kg MC12%
* WHO(2005)-PCDD/F TEQ (upper-bound)	0.0618 ng/kg MC12%

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Eurofins Sample Code: 468-2019-05150228

Client Sample Code: PO8821059 1103478955

**REPORT OF ANALYSIS**


AR-19-QA-038591-01

**Test Results**
**Result**
**GFDRY - Dry Residue**
**Completion Date:** 05/20/2019 **Method:** Internal

\* dry residue

89.5 %

**ZP003 - Propylene thiourea (PTU) and Ethylene thiourea (ETU)**
**Completion Date:** 05/22/2019 **Method:** Internal Method P-14.190

\* Ethylene thiourea (ETU)

\* PTU (Propylene Thiourea)

0.007 mg/kg

&lt;0.003 mg/kg

**QA0LY - Fentin (LC/MSMS)**
**Completion Date:** 05/23/2019 **Method:** EN 15662:2008; modified

\* Fentin

&lt;0.003 mg/kg

**QA26E - Fusarium Toxins full (LC-MSMS)**
**Completion Date:** 05/23/2019 **Method:** Romer Mycosep LC-MSMS

15-Acetyldeoxynivalenol (15-ADON)

&lt;20 µg/kg

3-Acetyldeoxynivalenol (3-ADON)

&lt;20 µg/kg

Deoxynivalenol (Vomitoxin)

&lt;10 µg/kg

Diacetoxyscirpenol (DAS)

&lt;50 µg/kg

Fusarenone X

&lt;20 µg/kg

HT-2 Toxin

&lt;10 µg/kg

Neosolaniol

&lt;50 µg/kg

Nivalenol (NIV)

&lt;10 µg/kg

T-2 Toxin

&lt;1.0 µg/kg

Zearalenone

&lt;5.0 µg/kg

**ZVM01 - Mineral oil MOSH, MOAH, high fatty food**
**Completion Date:** 05/22/2019 **Method:** Own method (procedure conform NEN-EN 16995)

\* MOSH/POSH (saturated) C10-16

&lt;1 mg/kg

\* MOSH/POSH (saturated) C17-20

&lt;1 mg/kg

\* MOSH/POSH (saturated) C20-25

&lt;1 mg/kg

\* MOSH/POSH (saturated) C25-35

&lt;1 mg/kg

\* MOSH/POSH (saturated) C35-50

&lt;1 mg/kg

\* MOSH/POSH C20-35 (sum, calculated)

&lt;2 mg/kg

\* MOSH/POSH C10-50 (sum, calculated)

&lt;6 mg/kg

\* MOAH (aromatic) C10-16

&lt;0.15 mg/kg

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Eurofins Sample Code: 468-2019-05150228

Client Sample Code: PO8821059 1103478955

**REPORT OF ANALYSIS**


AR-19-QA-038591-01

**Test Results**

- \* MOAH (aromatic) C16-20
- \* MOAH (aromatic) C20-25
- \* MOAH (aromatic) C25-35
- \* MOAH (aromatic) C35-50
- \* MOAH C16-35 (sum, calculated)
- \* MOAH C10-50 (sum, calculated)

**Result**

<0.15 mg/kg  
<0.15 mg/kg  
<0.15 mg/kg  
<0.15 mg/kg  
<0.5 mg/kg  
<1 mg/kg

**GFF08 - polychlorinated biphenyls (12 WHO PCB + 6 ICES PCB)**
**Completion Date:** 05/20/2019 **Method:** Internal

* PCB 77	<1.08 ng/kg MC12%
* PCB 81	<0.162 ng/kg MC12%
* PCB 105	<2.34 ng/kg MC12%
* PCB 114	<0.318 ng/kg MC12%
* PCB 118	<8.39 ng/kg MC12%
* PCB 123	<0.240 ng/kg MC12%
* PCB 126	<0.150 ng/kg MC12%
* PCB 156	<1.32 ng/kg MC12%
* PCB 157	<0.246 ng/kg MC12%
* PCB 167	<0.659 ng/kg MC12%
* PCB 169	<0.719 ng/kg MC12%
* PCB 189	<0.240 ng/kg MC12%
* WHO(2005)-PCB TEQ (lower-bound)	Not Detected
* WHO(2005)-PCB TEQ (medium-bound)	0.0186 ng/kg MC12%
* WHO(2005)-PCB TEQ (upper-bound)	0.0371 ng/kg MC12%
* PCB 28	<0.0599 µg/kg MC12%
* PCB 52	<0.0599 µg/kg MC12%
* PCB 101	<0.0599 µg/kg MC12%
* PCB 138	<0.0599 µg/kg MC12%
* PCB 153	<0.0599 µg/kg MC12%
* PCB 180	<0.0599 µg/kg MC12%
* Total 6 ndl-PCB (lower-bound)	Not Detected
* Total 6 ndl-PCB (medium-bound)	0.180 µg/kg MC12%
* Total 6 ndl-PCB (upper-bound)	0.360 µg/kg MC12%

**QA0NV - Pesticides - Organotin (LC-MSMS)**
**Completion Date:** 05/23/2019 **Method:** Internal LC-MS/MS

Any opinions/interpretations expressed on the Report of Analysis are outside the scope of this lab's A2LA accreditation.



Eurofins Sample Code: 468-2019-05150228

Client Sample Code: PO8821059 1103478955

**REPORT OF ANALYSIS**



AR-19-QA-038591-01

**Test Results**

- \* Cyhexatin/Azocyclotin (Sum)
- \* Fenbutatin oxide
- \* Fentin compounds calc. as Triphenyltin

**Result**

- <0.01 mg/kg
- <0.01 mg/kg
- <0.01 mg/kg

**QA0PK - Pesticides Quechers (Supplemental)-GC-MS/MS**

Completion Date: 05/21/2019 Method: AOAC 2007.01

- \* Benoxacor <0.01 mg/kg
- \* Chinomethionate <0.01 mg/kg
- \* Chlormephos <0.02 mg/kg
- \* Diclofop-methyl <0.01 mg/kg
- \* Flumiclorac-pentyl <0.010 mg/kg
- \* Fluridone <0.01 mg/kg
- \* Formothion <0.01 mg/kg
- \* Lactofen <0.010 mg/kg
- \* Pyraclofos <0.01 mg/kg
- \* Pyraflufen-ethyl <0.01 mg/kg
- \* Silafluofen <0.01 mg/kg
- \* Thiazopyr <0.01 mg/kg
- \* Vernolate <0.010 mg/kg

**QA0PJ - Pesticides Quechers (Supplemental)-LC-MS/MS**

Completion Date: 05/23/2019 Method: AOAC 2007.01

- \* Azamethiphos <0.010 mg/kg
- \* Bensulfuron methyl <0.01 mg/kg
- \* Benzovindiflupyr <0.010 mg/kg
- \* Chlorfluazuron <0.01 mg/kg
- \* Chlorimuron-Ethyl <0.010 mg/kg
- \* Dinotefuran <0.01 mg/kg
- \* Enamectin <0.01 mg/kg
- \* Ethametsulfuron-methyl <0.01 mg/kg
- \* Ethiprole <0.01 mg/kg
- \* Fluazinam <0.01 mg/kg
- \* Indaziflam <0.01 mg/kg
- \* Isopyrazam <0.01 mg/kg
- \* Mesosulfuron-methyl <0.010 mg/kg
- \* Metconazole <0.01 mg/kg

Any opinions/interpretations expressed on the Report of Analysis are outside the scope of this lab's A2LA accreditation.

Eurofins Sample Code: 468-2019-05150228

Client Sample Code: PO8821059 1103478955

**REPORT OF ANALYSIS**


AR-19-QA-038591-01

**Test Results**

- \* Metrafenone
- \* Nicosulfuron
- \* Phoxim
- \* Pyroxasulfone
- \* Saflufenacil
- \* Sedaxane
- \* Triasulfuron
- \* Tribenuron-methyl
- \* Zoxamide

**Result**

<0.01 mg/kg  
<0.010 mg/kg  
<0.01 mg/kg  
<0.01 mg/kg  
<0.01 mg/kg  
<0.01 mg/kg  
<0.010 mg/kg  
<0.010 mg/kg  
<0.010 mg/kg

**QA0LN - Phthalate Compounds (GC-MSMS)**
**Completion Date:** 05/17/2019 **Method:** Internal method

- \* Benzyl butyl phthalate (BBP) <0.05 mg/kg
- \* Dibutyl phthalate (DBP) <0.05 mg/kg
- \* Dibutyladipate <0.05 mg/kg
- \* Diethyl hexyl phthalate (DEHP) <0.05 mg/kg
- \* Diethyladipate <0.05 mg/kg
- \* Diethylhexyl adipate (DEHA) <0.05 mg/kg
- \* Diethyl phthalate (DEP) <0.05 mg/kg
- \* Di-isobutyl phthalate (DiBP) <0.05 mg/kg
- \* Di-isobutyladipate <0.05 mg/kg
- \* Diisodecylphthalate (DIDP) <0.05 mg/kg
- \* DiisoHeptylphthalate (DiHP) <0.05 mg/kg
- \* Diisononylphthalate (DINP) <0.05 mg/kg
- \* Dimethyl phthalate (DMP) <0.05 mg/kg
- \* Di-n-octylphthalate (DNOP) <0.05 mg/kg
- \* Dinonyl phthalate (DNP) <0.05 mg/kg
- \* Dipentylphthalate (DPP) <0.05 mg/kg
- \* Di-n-hexyl phthalate <0.05 mg/kg

**GFTE1 - TEQ-Totals WHO-PCDD/F and PCB**
**Completion Date:** 05/20/2019 **Method:** Internal

- \* WHO(2005)-PCDD/F+PCB TEQ (lower-bound) Not Detected
- \* WHO(2005)-PCDD/F+PCB TEQ (medium-bound) 0.0495 ng/kg MC12%
- \* WHO(2005)-PCDD/F+PCB TEQ (upper-bound) 0.0989 ng/kg MC12%


Any opinions/interpretations expressed on the Report of Analysis are outside the scope of this lab's A2LA accreditation.

Eurofins Sample Code: 468-2019-05150228

Client Sample Code: PO8821059 1103478955

**REPORT OF ANALYSIS**

AR-19-QA-038591-01

**Test Results***\*This is not covered by our current A2LA accreditation.***Result**Respectfully Submitted,  
Eurofins Central Analytical LaboratoriesResults shown in this report relate solely to the item  
submitted for analysis.  
Uncertainty can be obtained upon request.  
Victoria Siegel, Analytical Service ManagerTESTING CERT  
#2993-01

Any opinions/interpretations expressed on the Report of Analysis are outside the scope of this lab's A2LA accreditation.

## Certificate of Analysis

Date: 09 Jan 2020

Our ref. no.: 0

Your ref.

Material:	1286076	HOWARU Dophilus PN 200B - 1 KG
Batch No.:	1102066715	Best before date: 30 Apr 2015
		Production date: 30 Apr 2013

Test	Result	Specification	Unit	Reference
<b>Physical/Chemical Specifications</b>				
Appearance	Conforms	Conforms		VISUAL EXAMINATION
Water Activity	0.076	< 0.180		ROTRONIC METHOD
Foreign matter	Absent	Absent		.
<b>Microbiological Specifications</b>				
Viable Cell count	4.10E+11	> 2.00E+11	/g	ISO 7889/IDF 117
Non Lactic count	< 1000	< 1000	/g	ISO 13559
Enterococcus count	< 100	< 100	/g	SMEDP
Sulfite reducing anaerobes	< 100	< 100	/g	ISO 15213
Bacillus cereus count	< 100	< 100	/g	AOAC
Yeast and Mold count	< 50	< 50	/g	USP29, NF24, 2006
Enterobacteria, neg. in 10 g	Negative	Negative		ISO 21528
Enterobact. sakazakii, neg. in 10 g	Negative	Negative		ISO 22964
Staph. aureus, neg. by test (<10/g)	Negative	Negative		AOAC
Salmonella, neg. in 40 g	Negative	Negative		AOAC
Listeria, neg. in 25 g	Negative	Negative		AOAC



**Certificate of Analysis**

Date: 09 Jan 2020

Our ref. no.: 0

Your ref.

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Material:	1286076	HOWARU Dophilus PN 200B - 1 KG
Batch No.:	1102066715	Best before date: 30 Apr 2015
		Production date: 30 Apr 2013

---

Date of release: 28 May 2013

**Comments**

Exceeds 200 billion CFU/gm of freeze-dried Lb. acidophilus NCFM.

Each probiotic intermediate is confirmed to the genus/species level (or sub-species level where applicable) based on DNA fingerprinting analysis prior to milling and blending.

Refer to the Product Description (PD) for other non-batch related information. This product batch has been manufactured and released in compliance with FSSC 22000.

The material property data reported in this COA are representative values obtained in laboratory tests conducted on product sample taken from the batch produced. DuPont certifies that the values reported are the results of the tests conducted. DuPont makes no warranties regarding those values or the product in this COA, including as to fitness for a particular purpose or merchantability of goods produced from this supplied product or the supplied product itself, except for warranties expressly stated in the DuPont Product Description (PD) or otherwise agreed in writing by DuPont. All other warranties are specifically excluded. DuPont's standard terms and conditions apply to the sale of the supplied product unless otherwise agreed in writing by DuPont.

  
**Phil Ihrke**

Quality Control Department

**Certificate of Analysis**

Date: 09 Jan 2020

Our ref. no.: 0

Your ref.

---

Material:	1286155	HOWARU Dophilus PN 30B - 1 KG
Batch No.:	1102684485	Best before date: 04 Jan 2018
		Production date: 05 Jan 2016

---

Test	Result	Specification	Unit	Reference
<b>Physical/Chemical Specifications</b>				
Appearance	Conforms	Conforms		VISUAL EXAMINATION
Water Activity	0.031	< 0.180		ROTRONIC METHOD
Foreign matter	Absent	Absent		.
<b>Microbiological Specifications</b>				
Viable Cell count	6.50E+10	> 3.00E+10	/g	ISO 7889/IDF 117
Non Lactic count	< 1000	< 1000	/g	ISO 13559
Enterococcus count	< 100	< 100	/g	SMEDP
Sulfite reducing anaerobes	< 100	< 100	/g	ISO 15213
Bacillus cereus count	< 100	< 100	/g	AOAC
Yeast and Mold count	< 50	< 50	/g	USP
Enterobacteria, neg. in 10 g	Negative	Negative		ISO 21528
Enterobact. sakazakii, neg. in 10 g	Negative	Negative		ISO 22964
Staph. aureus, neg. by test (<10/g)	Negative	Negative		AOAC
Salmonella, neg. in 40 g	Negative	Negative		AOAC
Listeria, neg. in 25 g	Negative	Negative		AOAC



**Certificate of Analysis**

Date: 09 Jan 2020

Our ref. no.: 0

Your ref.

---

Material:	1286155	HOWARU Dophilus PN 30B - 1 KG
Batch No.:	1102684485	Best before date: 04 Jan 2018
		Production date: 05 Jan 2016

---

Date of release: 12 Jan 2016

**Comments**

Exceeds 30 billion CFU/gm of freeze-dried Lb. acidophilus NCFM in a base of rice maltodextrin.

Each probiotic intermediate is confirmed to the genus/species level (or sub-species level where applicable) based on DNA fingerprinting analysis prior to milling and blending.

Refer to the Product Description (PD) for other non-batch related information. This product batch has been manufactured and released in compliance with FSSC 22000.

The material property data reported in this COA are representative values obtained in laboratory tests conducted on product sample taken from the batch produced. DuPont certifies that the values reported are the results of the tests conducted. DuPont makes no warranties regarding those values or the product in this COA, including as to fitness for a particular purpose or merchantability of goods produced from this supplied product or the supplied product itself, except for warranties expressly stated in the DuPont Product Description (PD) or otherwise agreed in writing by DuPont. All other warranties are specifically excluded. DuPont's standard terms and conditions apply to the sale of the supplied product unless otherwise agreed in writing by DuPont.

**Phil Ihrke**

Quality Control Department

**Certificate of Analysis**

Date: 09 Jan 2020

Our ref. no.: 0

Your ref.

---

Material:	1286155	HOWARU Dophilus PN 30B - 1 KG
Batch No.:	1103390466	Best before date: 27 Dec 2020
		Production date: 28 Dec 2018

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Test	Result	Specification	Unit	Reference
<b>Physical/Chemical Specifications</b>				
Appearance	Conforms	Conforms		VISUAL EXAMINATION
Water Activity	0.030	< 0.180		ROTRONIC METHOD
Foreign matter	Absent	Absent		.
<b>Microbiological Specifications</b>				
Viable Cell count	6.10E+10	> 3.00E+10	/g	ISO 7889/IDF 117
Non Lactic count	< 1000	< 1000	/g	ISO 13559
Enterococcus count	< 100	< 100	/g	SMEDP
Sulfite reducing anaerobes	< 100	< 100	/g	ISO 15213
Bacillus cereus count	< 100	< 100	/g	AOAC
Yeast and Mold count	< 50	< 50	/g	USP
Enterobacteria, neg. in 10 g	Negative	Negative		ISO 21528
Enterobact. sakazakii, neg. in 10 g	Negative	Negative		ISO 22964
Staph. aureus, neg. by test (<10/g)	Negative	Negative		AOAC
Salmonella, neg. in 40 g	Negative	Negative		AOAC
Listeria, neg. in 25 g	Negative	Negative		AOAC

---

**Certificate of Analysis**

Date: 09 Jan 2020

Our ref. no.: 0

Your ref.

---

Material:	1286155	HOWARU Dophilus PN 30B - 1 KG
Batch No.:	1103390466	Best before date: 27 Dec 2020
		Production date: 28 Dec 2018

---

Date of release: 08 Jan 2019

**Comments**

Exceeds 30 billion CFU/gm of freeze-dried Lb. acidophilus NCFM in a base of rice maltodextrin.

Each probiotic intermediate is confirmed to the genus/species level (or sub-species level where applicable) based on DNA fingerprinting analysis prior to milling and blending.

Refer to the Product Description (PD) for other non-batch related information. This product batch has been manufactured and released in compliance with FSSC 22000.

The material property data reported in this COA are representative values obtained in laboratory tests conducted on product sample taken from the batch produced. DuPont certifies that the values reported are the results of the tests conducted. DuPont makes no warranties regarding those values or the product in this COA, including as to fitness for a particular purpose or merchantability of goods produced from this supplied product or the supplied product itself, except for warranties expressly stated in the DuPont Product Description (PD) or otherwise agreed in writing by DuPont. All other warranties are specifically excluded. DuPont's standard terms and conditions apply to the sale of the supplied product unless otherwise agreed in writing by DuPont.

**Phil Ihrke**

Quality Control Department

February 14, 2020

Stephanie Hice, PhD  
Center for Food Safety and Applied Nutrition  
Office of Food Additive Safety  
U.S. Food and Drug Administration

Re: Response to questions regarding GRAS Notice No. 000865

Dear Dr. Hice:

Below please find DuPont Nutrition and Biosciences' (hereafter referred to as DuPont) response to the question received on February 4, 2020 regarding GRAS Notice No. 000865 for *Lactobacillus acidophilus* ATCC SD5221 (also referred to as NCFM).

*Question 1: The notifier provides a specification for Salmonella serovars, listed as negative by test in 40 grams. The method referenced is AOAC 2004.03. We note, that this method requires pre-enrichment to initiate the growth of salmonellae. The method states, "... procedure must be performed as described in [AOAC] 967.26 (see 17.9.02) or as in Bacteriological Analytical Manual"; both AOAC 967.26 and the Bacteriological Analytical Manual Chapter 5: Salmonella are based on the analysis of a 25-gram test portion. Please clarify the use of 40-gram sample size in the specification Salmonella serovars.*

Response: We have modified the method AOAC 2004.03 to include a larger sample size (from 25 grams to 40 gram). The additional sample size provides better assurance of detection. USP sampling allows for 10 gram increments. Note that the enrichment dilution follows the same percentage as described in both AOAC 967.26 (see 17.9.02) and in Chapter 5 of the Bacteriological Analytical Manual.

Please let me know if there are any additional questions about DuPont's GRAS notification GRN 865.

Kind regards,



Elizabeth McCartney  
Regulatory Affairs Specialist  
DuPont Nutrition and Biosciences