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GRAS Notice (GRN) No. 357

<http://www.fda.gov/Food/FoodIngredientsPackaging/GenerallyRecognizedasSafeGRAS/GRASListings/default.htm>

ORIGINAL SUBMISSION

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October 20, 2010


Dr. Paulette Gaynor
Office of Food Additive Safety, GRAS Notification Program
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740-3835

Re: GRAS Notice-Exemption Claim for *Lactobacillus acidophilus* NCFM®

Dear Dr Gaynor:

On behalf of my client, Danisco USA, Inc., please accept the attached documentation, in compliance with the GRAS notification procedure set out in the April 17, 1997 Federal Register (62 FR 18937), as submission of notice of a GRAS exemption claim for the above referenced substance, i.e. use in food of *Lactobacillus acidophilus* NCFM®. As specified in the aforementioned proposed rule, this GRAS notice is submitted in triplicate with each containing: a signed GRAS notice exemption claim; detailed information on the notified substance; and an appendix containing further referenced and substantiating information on the substance.

Please promptly contact me should you have any question regarding the submitted notice. I look forward to receiving acknowledgment of receipt of this notice and to a timely response regarding the noticed substance. Thank you.

Sincerely, 

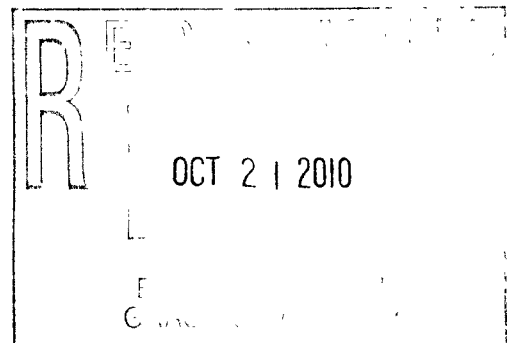
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Robert H. Sindt

Enc.

Cc : Sarah Kraak-Ripple, Danisco USA, Inc.

RHS/bs

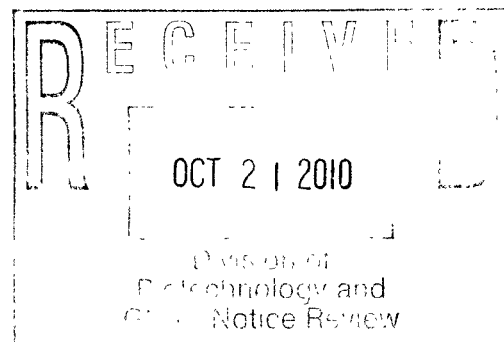


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**GENERALLY RECOGNIZED
AS SAFE NOTICE**

***Lactobacillus acidophilus* NCFM®**

October 2010



Robert H. Sindt

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Washington, D.C. 20036

Phone 202-466-4500 • Fax 202-466-5777 • E-mail rsindt@bobsindtlaw.com

October 20, 2010

Dr. Robert Martin
GRAS Notification Program
Office of Food Additive Safety
Food and Drug Administration
5100 Paint Branch Parkway
College Park, Maryland 20740

Re: GRAS Notice-Exemption Claim for *Lactobacillus acidophilus* NCFM®

Dear Dr. Martin:

On behalf of my client, Danisco USA, Inc. (Danisco), and in accordance with FDA's proposed rule of April 17, 1997 (62 FR 18938) relating to the filing of generally recognized as safe (GRAS) notices, please accept this claim and the attached information, submitted in triplicate, for that purpose as it relates to the use of *Lactobacillus acidophilus* NCFM® in certain foods. Specifically, Danisco claims that use of *Lactobacillus acidophilus* NCFM® as an ingredient in foods, including certain dairy products, functional beverages, nutritional powders, juices, bars, ready-to-eat breakfast cereals, chewing gum and confections (as specified in the detailed information submitted herewith) is exempt from the premarket approval requirements of the Federal Food, Drug and Cosmetic Act based on its determination that such use is GRAS. In conformity with the requirements outlined in the proposed rule, the following information is included with this exemption claim:

- (i) Name and Address of the Notifier:
Danisco USA, Inc.
3329 Agricultural Drive
Madison, WI 53716
- (ii) Common or Usual Name of Notified Substance: *Lactobacillus acidophilus* NCFM®
- (iii) Applicable Conditions of Use: *Lactobacillus acidophilus* NCFM® is manufactured in compliance with current Good Manufacturing Practice as specified in 21 CFR Part 110. *Lactobacillus acidophilus* NCFM® is manufactured through a specific time and temperature controlled fermentation of suitable food grade ingredients with *Lactobacillus acidophilus* NCFM®. It is

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used as an ingredient in foods, including certain dairy products, functional beverages, nutritional powders, juices, bars, ready-to-eat breakfast cereals, chewing gum and confections, at levels not to exceed current good manufacturing practice in accordance with 21 CFR 184.1(b). The targeted use level of foods will be to typically contain 1×10^9 cfu/serving of *Lactobacillus acidophilus* NCFM®. All population age groups, except infants, are expected to consume these foods.

- (iv) Basis for the GRAS Determination: Scientific procedures and supported by a history of common use in foods.
- (v) Availability to FDA of Data and Information that are Basis of Determination: The data and information forming the basis for Danisco's GRAS determination and the exemption claim asserted herein are available for FDA review and copying during reasonable business hours at the following address, or will be sent to FDA upon request:

Robert H. Sindt, Attorney at Law
Suite 400
1850 M Street, NW
Washington, DC 20036
Phone: (202) 466-4500
rsindt@bobsindtlaw.com

Consequently, on the basis of the above specified information, and the additional requested information as specified in the proposed rule and as attached hereto and submitted with this letter, please accept this as Danisco's GRAS notification and claim of exemption from the statutory premarket approval requirements for the use of *Lactobacillus acidophilus* NCFM® as an ingredient in foods, including certain dairy products, functional beverages, nutritional powders, juices, bars, ready-to-eat breakfast cereals, chewing gum and confections. Should you have any questions regarding the submission of this notice, please contact me at the above number. Thank you for your prompt consideration of, and response to, this notice.

Sincerely,

(b) (6)

Robert H. Sindt

RHS:bs

Attachments

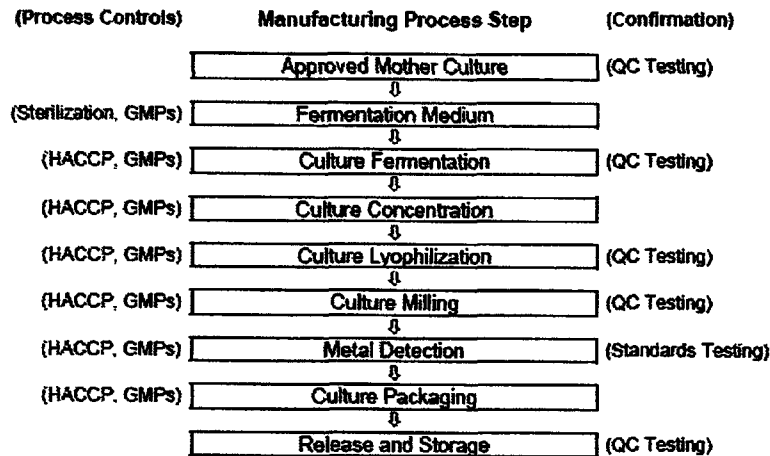
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***L. acidophilus* NCFM®-GRAS NOTICE INFORMATION**

(2) DETAILED INFORMATION ABOUT THE IDENTITY OF THE NOTIFIED SUBSTANCE

- Common and Usual Name of the Food Grade Substance: *Lactobacillus acidophilus* NCFM®
- Chemical Name for *Lactobacillus acidophilus* NCFM®: None
- Chemical Abstract Service (CAS) Registry Number for *Lactobacillus acidophilus* NCFM®: None
- Empirical Formula for *Lactobacillus acidophilus* NCFM®: None
- Structural Formula for *Lactobacillus acidophilus* NCFM®: None
- Quantitative Composition for *Lactobacillus acidophilus* NCFM®: *Lactobacillus acidophilus* NCFM® is a commercially available food ingredient that is produced by culture fermentation utilizing *Lactobacillus acidophilus* NCFM® as the source organism. Use in foods will be targeted to typically contain 1×10^9 cfu/serving of *L. acidophilus* NCFM®.
- Method of Manufacture for *Lactobacillus acidophilus* NCFM®: *Lactobacillus acidophilus* NCFM® is manufactured: in compliance with the U.S. Food and Drug

Administration's current Good Manufacturing Practice guidelines, as specified in 21 CFR, part 110, and in an FDA regulated and inspected facility. All ingredients utilized are food grade or approved for use by the FDA. The manufacturing process is summarized below:



The source organism used is *L. acidophilus* NCFM[®]. The cultures are maintained in the culture bank of Danisco USA Inc. as frozen 1ml. vials at -80°C. Danisco USA Inc. independently verified the identity of each organism. Each seed lot in the culture bank is fully characterized to insure the identity of the seed strains. From the seed vials, Danisco USA Inc. produces concentrated starter for the industrial fermentation.

The product is manufactured through a specific time and temperature controlled fermentation of suitable food grade ingredients with *L. acidophilus* NCFM[®]. Prior to addition of *L. acidophilus* NCFM[®], the mixture is sterilized and cooled to an incubation temperature of 37° C. The mixture is then inoculated with *L. acidophilus* NCFM[®] and allowed to incubate to the fermentation endpoint under constant temperature.

After the required incubation period, the pH is adjusted with ammonium hydroxide, and concentrated via centrifugation. To the concentrated bacterial slurry, food-grade cryoprotectants are added; the material is frozen, and subsequently freeze-dried. The dried cultured product is then packaged and stored in a cool, dry environment.

Release of product for sale according to established specifications is under the responsibility of Danisco Quality Control. Final product testing methods comply with standard Methods for the Examination of Dairy Products of the American Public Health Association.

■ Source Information for *L. acidophilus* NCFM®: The genus *Lactobacillus* is a wide and heterogeneous taxonomic unit, comprising the rod-shaped lactic acid bacteria. This genus encompasses 112 species with a large variety of phenotypic, biochemical and physiological properties. Several species are intentionally introduced in the food chain, being involved in a range of food and feed fermentations and applied as dietary supplements for humans and animals. They are rod-shaped, non-motile and non-spore forming bacteria. Phylogenetic molecular taxonomy and 16S rRNA gene sequence analysis are used for assigning strains to particular species within this genus.

Lactobacillus acidophilus is a homofermentative species that is found in numerous dairy products throughout the world. It is also found as a resident of the intestinal tracts of human and animals. The taxonomy of *L. acidophilus* has undergone significant revisions over the past decade. Strains considered to be of the species “*acidophilus*” were subdivided into 6 species: *acidophilus*, *amylovarus*, *crispatus*, *gallinarum*, *gasseri* and

johnsonii (1; 2). *L. acidophilus* 4356 is the type strain for the species which retained the *acidophilus* name.

Lactobacillus acidophilus NCFM[®] was isolated and characterized in the food microbiology research laboratories at North Carolina State University (NCSU). The strain was isolated from human feces in 1975 (3) and has since been the subject of research at NCSU and other institutions worldwide. NCFM[®] has been shown by several phenotypic (4) and genotypic (5, 6) criteria to be a member of the type A1 *L. acidophilus* species. Hybridization with a species-specific oligonucleotide probe (5' TCTTTCGATGCATCCACA 3'; 7) using slot blots provided further evidence that NCFM[®] belongs to the type A1 *L. acidophilus* group (6). Sequencing of the 16S ribosomal RNA gene of NCFM[®] confirmed its identity as *L. acidophilus* (6; internal Danisco documentation). Fermentation and growth characteristics of NCFM[®] compared to the neotype *L. acidophilus* strain ATCC 4356 are identical, as reported in Sanders et al. 1996 (6). The NCFM[®] DNA has a 34.7% GC ratio. Fermentation results in 34% D- and 66% L-lactic acid for the strain.

The entire genome of *L. acidophilus* NCFM[®] has been sequenced (8). The complete sequence and corresponding annotation is available from GenBank under accession no CP000033 and, therefore, is available for public reference.

It should be noted that multiple strain designations appear in the literature for NCFM[®] or for single colony isolates of the NCFM[®] parent culture. The designations NCFM[®], N2, NCK56, NCK45, N2 and RL8K are essentially identical strains. The parent NCFM[®] culture carried the NCSU laboratory designation RL8K and was composed of rough and

smooth variants. These variants were designated RL8K-R (bile sensitive) and RL8K-S (bile resistant) upon isolation from the RL8K culture (11). N2 is a smooth, bile-resistant isolate from NCFM[®] selected by scientists at Marschall Products (now Danisco USA Inc.) as a bile-resistant colony, and as this pure culture, is used as the seed for all commercial production runs of NCFM[®]. The mixed parent culture has not been used commercially or for research studies since before 1975. NCK56 is a Klaenhammer laboratory designation for N2, and NCK45 is a Klaenhammer laboratory designation for the parent culture NCFM[®] comprised of rough and smooth colony variants NCFM[®]. These different isolates cannot be differentiated genetically by pulsed field gel electrophoresis (12), 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 (8). In some papers involving NCFM[®], the strain is not identified or NCFM[®] 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[®], 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[®] was abbreviated LBA. Any confusion over use of NCFM[®] in publications included in this document was clarified by personal

communications with company representatives and researchers. In the United States, NCFM® is a registered trademark of the North Carolina Dairy Foundation.

Lastly, consistent with the World Health Organization (WHO) recommendations for cultures, *L. acidophilus* NCFM® has been deposited as safe deposits in two recognized international culture collections (American Type tissue Culture Collection, SD5221 and The German Collection of Microorganisms and Cell Cultures, DSM 22091).

- **Characteristic Properties of *L. acidophilus* NCFM®:** *L. acidophilus* NCFM® is a harmless lactic acid producing bacteria. Commercially, it is produced by fermentation utilizing *L. acidophilus* NCFM®, a safe and suitable bacterium. In powdered form, it has a white to cream color and is typically stored at or below 4°C.
- **Content of Potential Human Toxicants for *L. acidophilus* NCFM®:** None
- **Specifications for Food Grade *L. acidophilus* NCFM®:** *L. acidophilus* NCFM® is a freeze dried powder produced by culture fermentation utilizing *L. acidophilus* NCFM®. *L. acidophilus* NCFM® microbiological specifications /kg are:

Cell count	Greater than 1.5E+11 /g
Non-lactic Count	Less than 5000 /g
Enterococci	Less than 100 /g
Coliforms	Less than 10 /g
E. coli	Negative by test (less than .0.3 /g)
Salmonella	Negative (40 g enrichment)
Staphylococcus (C.P.)	Negative by test (less than 10 /g)
Listeria	Negative (25 g enrichment)

***L. acidophilus* NCFM®-GRAS NOTICE INFORMATION**

(3) INFORMATION ON SELF-LIMITING LEVELS OF USE, IF ANY

- Uses are self-limited to those foods that can sustain living *L. acidophilus* for the shelf life of the food.

***L. acidophilus* NCFM[®]-GRAS NOTICE INFORMATION**

(4) DETAILED SUMMARY OF THE BASIS FOR GRAS DETERMINATION

- (i) Danisco's determination, that the notified uses of *L. acidophilus* NCFM[®] (as an ingredient in foods, including certain dairy products, functional beverages, nutritional powders, juices, bars, ready-to-eat breakfast cereals, chewing gum and confections) are exempt from the premarket approval requirements of the Federal Food, Drug and Cosmetic Act based on its determination that such use is GRAS, is based on scientific procedures as supported by a history of experience based on common use in food. The determination has been confirmed by an independent panel of scientific experts convened by Danisco to conduct such a critical review. Each member of the independent expert panel was qualified by extensive scientific training and experience to evaluate the safety of substances used in food. The independent expert panel's report and determinations, updated to October 2010, is included in its entirety in the Appendix attached hereto.

(A) Safety of *L. acidophilus* NCFM[®]

L. acidophilus NCFM[®] is produced by a fermentation process utilizing seed strains of independently identified *L. acidophilus* NCFM[®] organisms, a safe and suitable lactic acid producing bacterium.

- (1) **Safety and Suitability of Organism.** In conducting its assessment and making its determination, Danisco reviewed the existing regulatory status,

animal studies, human use information, and other published and unpublished studies and information relating to *L. acidophilus* NCFM®.

FDA, EU and scientific consensus on L. acidophilus

In considering the general safety of cultures, and that of *L. acidophilus* NCFM® specifically, several issues were assessed by Danisco, including pathogenicity, toxicity, and the presence of transferable antibiotic resistance genes. Data from animal and human studies were considered, although no animal models for assessment of safety of *Lactobacillus* have been validated (13). Species of the genus *Lactobacillus* are considered to be non-pathogenic, non-toxigenic and have generally been considered safe for use in foods (EFSA, Appendix). Boriello, et al. (14) reviewed data pertinent to safety concerns for these bacteria and concluded that “current evidence suggests that the risk of infection with probiotic lactobacilli or bifidobacteria is similar to that of infection with commensal strains, and that consumption of such products presents a negligible risk to consumers...”. This opinion is echoed in other publications (13; 15). Additionally, the species *L. acidophilus* has been listed in FDA GRAS list, and is proposed for inclusion on the EU QPS list (Appendix). The FDA states:

“Prior sanctions were granted for the use of harmless lactic acid producing bacteria, such as *Lactobacillus acidophilus*, as optional ingredients in specified standardized foods. These bacteria are permitted for use in cultured milk (which includes buttermilk) (§ 131.12), sour cream (§ 131.160), cottage cheese (§ 133.128), and yogurt (§ 131.200), provided that the mandatory cultures of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* are also used in the

yogurt.” (Partial List Of Microorganisms And Microbial-Derived Ingredients That Are Used In Foods; U. S. Food and Drug Administration Center for Food Safety & Applied Nutrition Office of Food Additive Safety, July 2001).”

Animal studies

One study (16) was conducted with two types of gnotobiotic, immunocompromised mice: bg/bg-nu/nu/+ (produce thymus-matured T-cells, euthymic) and bg/bg-nu/nu (athymic). This beige nude mouse model has defects in phagocytic cells and NK cell activity and lacks a functional thymus. In this study, mice, (male and female, adult and neonatal) were inoculated with one of the following strains; *L. acidophilus* NCFM[®], *L. reuteri*, *L. rhamnosus* GG or *Bifidobacterium animalis* by swabbing oral cavity and anal area with culture of 10⁸/ml to create monoassociated mice (germ-free animals, colonized with only one strain). This resulted in colonization of the stomach, small and large intestines. Subsequent generations of mice colonized via exposure to monoassociated mothers and feces. Results of this study revealed:

- no morbidity or mortality in adult and neonatal mice;
- no adverse effects on growth parameters;
- no gross or microscopic pathological changes;
- no abscesses in the stomach or small intestine;
- no deaths in gnotobiotic, immunocompromised mice;
- evidence of induction of immunoglobulins, IgM and IgG;

- translocation of *L. acidophilus* NCFM[®] to other tissues was observed (also LGG and *B. animalis*), but there was no evidence of inflammation or other pathologic findings in tissue sections of translocated mice.

The safety of the *L. acidophilus* NCFM[®] was also evaluated in a colitis mouse model using Trinitrobenzenesulphonic acid (TNBS) to induce colitis. In healthy mice, intra-gastric administration of *L. acidophilus* NCFM[®] did not show any potential adverse effect on mouse activity, weight and colon inflammation. In TNBS-treated mice (mice with very strong colitis), no significant improvement was observed in the group fed *L. acidophilus* NCFM[®]. High doses (10^{10} cfu) of *L. acidophilus* NCFM[®] led to no translocation of the organism or abnormal translocation of the intestinal microflora (17). It may be concluded from the above, that NCFM[®] had no adverse effects in growth, survival, activity and weight of immunocompromised mice. It must also be noted that no translocation was seen at high doses.

Human studies

No human studies have been reported with the express purpose of conducting a safety assessment. However, NCFM[®] has been administered to healthy and compromised patients in the following studies, with no reported adverse incidents related to the treatment protocol.

1. Chronic kidney failure patients with small bowel bacterial overgrowth.

Administered to 22 patients (N=43 total in two arms) at 2×10^{10} cfu/d in the form of enteric coated capsules (18). In some subjects, *L. acidophilus*

NCFM[®] was co-administered with *L. gasseri* BGF204. For both treatments, dimethylamine (DMA) production and the generation of the corresponding carcinogen, nitrosodimethyl-amine (NDMA), was reduced. Changes in patients' weight and % weight gain suggested that *L. acidophilus* can improve nutrition. Hydrogen generation in the small bowel was reduced suggesting that *L. acidophilus* had some effect on altering the resident flora. Twice daily treatment with NCFM[®] and/or BG2FO4 produced no significant side effects in any of the patients over their course of treatment.

2. Mild to moderate symptom irritable bowel syndrome patients. Administered as part of a 2-strain blend along with *Bifidobacterium lactis* Bi-07 in patients with non-constipation IBS, functional diarrhea, or functional bloating. A total of 30 subjects were in the treatment group and consumed 10^{11} cfu of each strain daily. Significant improvement of symptoms of bloating and distention was achieved (19).
3. Feeding of 10 healthy subjects NCFM[®] 10^{10} cfu/d in non-fat dry milk. NCFM[®] was isolated from the feces during feeding (20).
4. Ten healthy subjects were fed 10^{10} cfu/d in milk or water. No difference in fecal isolation of viable NCFM[®] was observed (21).
5. The ability of NCFM[®] to alleviate symptoms of 20 lactose maldigesting children (5-16 yr) was tested in a single blinded study. Symptoms and breath hydrogen excretion were evaluated after consumption of milk with or without 10^{10} cfu total NCFM[®] (11.6g lactose) (22). No difference in breath hydrogen was observed, but NCFM[®] did improve symptoms.

6. Three additional studies were conducted with NCFM[®] to evaluate its impact on improvement of lactose digestion in lactose maldigesters. Kim and Gilliland (23) reported three feeding trials. In the first, subjects were fed milk (5 ml/kg body weight/bid for 6 days) with (2.5×10^8 cfu/ml) or without NCFM[®] (N=6 healthy subjects/group). (Since milk was consumed on a per body weight basis, exact total daily dose could not be calculated from data provided in the paper; however, for a 68 kg person, the dose would have been 1.7×10^{11} cfu/d.). A similar second feeding trial tested lower doses of NCFM[®] (2.5×10^7 cfu/ml or 2.5×10^6 cfu/ml for 6 days). A third trial evaluated 2.5×10^6 cfu/ml for 6 days on 5 new subjects. Improved breath hydrogen excretion was observed for the low and high, but not the middle, doses of NCFM[®] tested. A total of 17 subjects consumed NCFM[®] in the three trials reported in this study. Lin et al. (24) evaluated the impact of NCFM[®] (10^7 or 10^8 cfu/ml) – along with other *L. acidophilus* strains and *S. thermophilus* plus *L. bulgaricus* - on breath hydrogen. Ten healthy lactose maldigesters were each fed 9 test meals on sequential days with 1 day between meals. Therefore, NCFM[®] was only fed for two days to each of 10 subjects. NCFM[®] did not positively impact breath hydrogen at either dose. Lastly, by Savaiano et al. (25) conducted with 9 lactose maldigesters fed 420g milk + NCFM[®] (1.1×10^7 cfu/ml; 420 ml milk consumed) on one day. Consistent with the Lin et al. (24) study, this product did not improve lactose digestion.

7. Healthy volunteers (N=20 in the treatment group) taking antibiotics were fed blend of 5 strains for 20 days with no adverse incidents reported (26).
NCFM[®] was fed at levels of 5×10^9 cfu/d.
8. Healthy male volunteers (N=21, 22 or 23; publication states “4 or 5 per group”) were fed milk containing different levels of NCFM[®] and levels of lactobacilli were evaluated in fecal samples obtained before, during and after feeding in three separate feeding trials (27). The dose fed ranged from 2.4×10^9 cfu/d to 3.8×10^{11} cfu/d. No adverse incidents were reported from feeding. Increased levels of facultative lactobacilli were observed during feeding, especially in subjects with low baseline levels. [Gilliland et al. (3) evaluated methods to detect NCFM[®] in human feces, but this study did not report a separate feeding trial.]
9. Healthy volunteers (Goldin and Gorbach (28) found that daily consumption of milk containing NCFM[®] (10^9 cfu/d; N=21 healthy volunteers) for 4 weeks resulted in a 2- to 4-fold reduction in the activity β -glucuronidase, nitroreductase, and azoreductase in the feces. Similar results on fecal activity of β -glucuronidase and nitroreductase were observed when NCFM was added to the omnivorous diet of human subjects (29). Goldin and Gorbach (30) fed 7 healthy subjects on a standard “Western diet” $\sim 10^{10}$ cfu/d NCFM[®] for 1 month. A one-month run in and wash out period were conducted as well. Reductions in fecal activities of β -glucuronidase and nitroreductase were observed.
10. Newcomer et al. (31) tested the ability of NCFM[®] to alleviate symptoms of irritable bowel syndrome and lactose maldigestion. Although no benefit was

observed, this crossover study provides additional evidence for safe consumption of NCFM[®] by 89 test subjects (61 for IBS trial, 18 for lactose malabsorption trial and 10 healthy controls). IBS subjects consumed 2.9×10^9 cfu NCFM[®] in 720 ml milk/d for 10 weeks. Lactose maldigesters consumed on average 1.5 glasses of milk (4×10^6 cfu/ml) per day for 2 one-week periods.

11. The ability of *L. acidophilus* NCFM[®] and *L. acidophilus* La-14 to stimulate specific immunity has been evaluated in a human study measuring primary immune reaction following vaccination (32). One week prior to oral vaccination with cholera vaccine, healthy volunteers received either a placebo (maltodextrin, n=20) or *L. acidophilus* NCFM[®] (n=9) or *L. acidophilus* La-14 (n=9). Supplementation with *L. acidophilus* NCFM[®] or La-14 or the placebo started on day 0 and continued for 21 days. The subjects consumed two capsules a day with 10^{10} cfu *L. acidophilus* NCFM[®] or two capsules a day with 10^{10} cfu *L. acidophilus* La-14 or two capsules a day with maltodextrin (control). Supplementation with *L. acidophilus* NCFM[®] tended to increase the specific serum IgA for the period D21-D28 (P=0.09) compared to the placebo group and supplementation with La-14 significantly increased the specific serum IgG for the period D21-D28 (P=0.01).
12. The efficacy of *L. acidophilus* NCFM[®] was also tested either alone, or as part of a two-strain blend along with *B. animalis* subsp. *lactis* Bi-07, in a study of 3-5 year old children measuring the impact on cold/flu symptom reduction. Children receiving only NCFM[®] (n=110) or NCFM[®]/Bi-07 (n=112) had

significant reductions in the incidence and duration of cold/flu symptoms with no adverse events reported (33).

13. In a study reported by Fisberg et al (34), *L. acidophilus* NCFM® was part of a synbiotic group (also combined with *B. lactis* Bi-07 and fructooligosaccharides at 0.5 g/L after reconstitution) that evaluated the incidence, duration of illness, and anthropometrics in children who received a nutritional supplement with or without synbiotics. In this double-blind, randomized study of 616 children aged 1-6 years old, both study feedings were well tolerated and the overall incidence of adverse events was very low, with none of the adverse events considered as study-related. The dose in this study was not well communicated in the manuscript but is estimated to be greater the one billion probiotics per day.

14. *Lactobacillus acidophilus* NCFM® has also been tested in another synbiotic study where the combination of *B. lactis* Bi-07, *Lactobacillus acidophilus* NCFM®, and fructooligosaccharides was used as the symbiotic arm (35). In this study, 129 children aged 1-6 who were acutely ill and receiving antibiotic therapy were randomized to receive a nutritional supplement with or without synbiotics or a fruit-flavored drink. The dose was the same as in the aforementioned study. In terms of tolerance, the authors determined that the supplements were well tolerated with no significant change from baseline in terms of symptomology or adverse events.

It may be concluded from the above that ingestion of *Lactobacillus acidophilus* NCFM[®] at daily doses up to 3.8×10^{11} cfu/day for six days (high dosage for short duration), as well as 1×10^{10} cfu/day over six months (lower dosage for longer duration), failed to induce any adverse signs or symptoms.

Transferable antibiotic resistance

Although there is negligible concern for translocation, toxigenicity, or any adverse nutritional activity from consumption of *L. acidophilus* NCFM[®], the presence of transferable antibiotic resistance genes was also 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 (8) in NCFM[®].

Lactobacillus acidophilus is not listed higher than 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/>).

The highest level of intake of NCFM[®] that has been demonstrated to be safe in humans (No observable Adverse Effect Level) and published in the peer-reviewed literature is 3×10^{11} cfu/day (27).

(2) Supporting Recent Safe History of Use in Food. *Lactobacillus* species have a long history of safe use when consumed as part of dairy food and supplement products with thirty-six (36) species listed in IDF Bulletin No. 377: *Inventory of Microorganisms with a Documented History of Use in Food* (9). And, *Lactobacillus acidophilus* has been added to human food since at least 1950 and is very common in dairy products worldwide including the US where the organism is the most common *Lactobacillus* in yogurt products (9).

More specifically, *Lactobacillus acidophilus* NCFM[®] is a strain available in conventional foods (e.g., milk, yogurt, and toddler formula) and dietary supplements. It has been commercially available in the United States since the mid-1970s with no adverse incidents reported. The strain has been characterized extensively and its qualities and safety have been evaluated *in vitro* and *in vivo* (animal and human studies). Also, *L. acidophilus* NCFM[®] has been safely added globally to dairy products and dietary supplements for at least 25 years without a report of adverse effect on consumers (27, 33).

(3) Probable Consumption/Effect of *L. acidophilus* NCFM[®] in Diet. Uses will be limited to foods that can sustain living *L. acidophilus* NCFM during shelf life, and are currently envisioned to include 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 10⁹ cfu/250g serving throughout the

shelf life of the product. The initial addition level may be as high as 5×10^{10} cfu/250g serving (i.e. 2×10^8 cfu/g) in order to insure at least 10^9 cfu/250g serving remains viable over the product shelf life.

Realistically, Danisco believes that there will be limited types of foods that will be available containing the strain and thus, the safety margin developed above is highly conservative. Also, consumers are very likely only to consume the food such that they achieve the daily benefit of products containing *L. acidophilus* NCFM[®]. For instance, in the beverage category Danisco does not envision *L. acidophilus* containing products competing with the myriad of functional food beverages on the market today because of either product incompatibility or cost. Because of anticipated marketing, few products in a given category will likely contain the strain. Based on these assumptions, consumers will most probably consume a single 250 g serving to achieve the benefit thus ingesting approximately 5×10^{10} cfu per day.

Danisco calculated that at a maximum level of 5×10^{10} cfu/250g serving, a consumer could consume six servings of products containing *L. acidophilus* NCFM[®] and would be below the level shown to be tolerated by humans in safety studies.

(B) Information That May Appear Inconsistent With GRAS Determination:

Danisco is not aware of information that appears to be inconsistent with the determination of safety or general recognition of safety for the present or

proposed uses of *L. acidophilus* NCFM®. It should be noted, however, that *Lactobacillus* species are a rare cause of endocarditis in adults in the absence of such supplementation, but that *Lactobacillus* sp. are widely considered safe for use in otherwise healthy persons (10).

(C) Expert Consensus for GRAS Determination for *L. acidophilus* NCFM®:

To further its internal safety and GRAS determinations of the subject food uses of *Lactobacillus acidophilus* NCFM®, Danisco convened a panel of independent scientists (“Expert Panel”), qualified by their relevant national and international experience and scientific training, to evaluate the safety of food and food ingredients, to conduct a critical and comprehensive evaluation of the available pertinent published literature and other information on *Lactobacillus acidophilus*. Danisco asked the Panel to determine, based on its review, the safety and the GRAS status of the intended uses of *Lactobacillus acidophilus* NCFM® in various foods. The Expert Panel consisted of Professor Emeritus of Pharmacology and Toxicology, Joseph F. Borzelleca, Ph.D. (Virginia Commonwealth University, School of Medicine); Professor Emeritus, Food Science, Michael W. Pariza, Ph.D. (University of Wisconsin- Madison); and Walter Glinsmann, M.D. (Glinsmann Inc., and formerly of the USFDA). The panel of scientific experts confirmed Danisco’s determination of the safety and general recognition of safety of the present and proposed uses of *Lactobacillus acidophilus* NCFM®. (See Appendix for Expert Panel Report).

Specifically, in making its determination, the Expert Panel stated that it had “independently and collectively, critically examined the GRAS Dossier, a comprehensive package of data and information compiled from the literature and other published sources through May 2009 and recently updated through August 2010 and other technical information, prepared by Sarah Kraak-Ripple of Danisco”. Such information “included a description of *L. acidophilus* NCFM®, the Danisco manufacturing process, history of safe use in food, intended use-levels, intake estimates and safety information, including animal feeding studies and human studies.”

In addition and based on their view that it would be relevant to their consideration, the panel asked Dr. Pariza to conduct an analysis utilizing the recognized decision tree that he developed with Dr. Eric Johnson for evaluating the safety of organisms utilized in enzyme preparations. Regarding this analysis, the panel stated “One of the Expert Panelists, Dr. Michael W. Pariza, analyzed the available information on *L. acidophilus* NCFM® (using the Pariza-Johnson Decision Tree (Pariza, M.W. and Johnson, A.E. Evaluating the Safety of Microbial Enzyme Preparations Used in Food Processing: Update for a New Century, Reg. Tox. and Pharm. 33:173-186, 2001) (Attachment 1) and concluded “A NOAEL cannot be determined for a living microorganism that grows in the gastrointestinal tract of the host after ingestion. However, in 25 years of experience with this strain as a starter culture and probiotic, there have been no reported adverse effects in humans, a result that is also supported by short-term clinical trials. Therefore we conclude that *Lactobacillus acidophilus* NCFM® is

ACCEPTED.” The other Expert Panelists concurred with this conclusion.” (See Appendix).

In summarizing its extensive review of safety the Expert Panel stated that “Clinical trials and safety studies in animals involving different doses and forms of *Lactobacillus acidophilus* demonstrate that *Lactobacillus acidophilus* in dairy foods and dietary supplements is safe for human consumption. In the worst-case scenario, a consumer could consume up to six servings of foods containing 5×10^{10} cfu/250g serving *L. acidophilus* at the beginning of shelf life, and still be highly unlikely to consume at the level shown to be tolerated by humans in safety studies where subjects ingested 3×10^{11} cfu/day for a period of six days without adverse effect.”

Further, the Expert Panel found “*Lactobacillus acidophilus* strain NCFM® is non-pathogenic and non-toxigenic; it lacks infectivity in immunocompromised, neonatal animal models; it lacks transferable antibiotic resistance genes as determined by total genomic sequencing; and it failed to cause any adverse effects in healthy and diseased human volunteers. *L. acidophilus*, including strain NCFM®, has a safe history of use (common use) in food, including dietary supplements, for over 30 years of reported commercial use. The safety of *L. acidophilus* is supported by its presence on both proposed QPS and FDA GRAS lists, and a general consensus in the published literature that *Lactobacillus* species are safe for use in foods for the generally healthy population.”

Finally, the Expert Panel reached its conclusion of safety and general recognition of safety for the proposed food uses of *Lactobacillus acidophilus* NCFM® by

stating “We, the Expert Panel, have individually and collectively critically evaluated the available information on *Lactobacillus acidophilus* strains including strain NCFM[®] prepared by Danisco and other information deemed appropriate and conclude that the intended uses in food of strain NCFM[®], manufactured consistent with current Good Manufacturing Practice (cGMP) and meeting the specifications presented in the dossier, are safe and suitable.

We further conclude that the intended uses of strain NCFM[®], manufactured consistent with cGMP and meeting the specifications presented in the dossier, are Generally Recognized As Safe (GRAS) based on scientific procedures and supported by a history of common use in foods.

It is our opinion that other qualified experts would concur with these conclusions.”

Based on the information contained in the exemption claim, the above additional and supplementary information, and the information contained in the Appendix attached hereto, a clear and ample basis exists to support Danisco’s determination, confirmed by the Expert Panel, of general recognition of safety for the food uses, present and proposed herein, of *Lactobacillus acidophilus* NCFM[®].

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L. acidophilus NCFM® GRAS NOTICE
APPENDIX

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REPORT OF THE EXPERT PANEL ON THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF THE INTENDED FOOD USES OF LACTOBACILLUS ACIDOPHILUS NCFM®

INTRODUCTION

Danisco convened a panel of independent scientists ("Expert Panel"), qualified by their relevant national and international experience and scientific training to evaluate the safety of food and food ingredients, to conduct a critical and comprehensive evaluation of the available pertinent published literature and other information on *Lactobacillus acidophilus*. Danisco asked the Panel to determine, based on its review, the safety and the GRAS status of the intended uses of *Lactobacillus acidophilus* NCFM® in various foods. The Expert Panel consisted of Professor Joseph F. Borzelleca, Ph.D. (Virginia Commonwealth University, School of Medicine), Wisconsin Distinguished Professor of Food Microbiology and Toxicology Michael W. Pariza, Ph.D. (University of Wisconsin- Madison), and Walter Glinsmann, M.D. (Glinsmann Associates and formerly of the USFDA). *Curricula vitae* of the Panel are provided in Attachment 1.

The Expert Panel, independently and collectively, critically examined the GRAS Dossier, a comprehensive package of data and information compiled from the literature and other published sources through May 2009 and recently updated through August 2010 and other technical information, prepared by Sarah Kraak-Ripple of Danisco. This latter information included a description of *L. acidophilus* NCFM®, the Danisco manufacturing process, history of safe use in food, intended use-levels, intake estimates and safety information, including animal feeding studies and human studies.

Following independent, critical evaluation of such data and information, and other pertinent information, the Expert Panel unanimously concluded that the intended uses in food of *Lactobacillus acidophilus* NCFM®, produced consistent with current Good Manufacturing Practice (cGMP) and meeting the specifications presented in the GRAS Dossier, are safe and suitable. The Expert Panel further unanimously concluded that these intended uses are GRAS based on scientific procedures and supplemented by a demonstrated history of safe use in food. A summary of the basis for the Expert Panel's conclusion is provided below.

Product Identification and Characterization/Description of *Lactobacillus acidophilus* NCFM

Lactobacillus acidophilus NCFM® was isolated and characterized in the food microbiology research laboratories at North Carolina State University (NCSU). It is defined by its genome sequence, as determined by Altermann et al (Proc. Natl. Acad. Sci. USA 102:3906-12, 2005). The strain was isolated from human feces in 1975 and has since been the subject of research at NCSU and other institutions worldwide. NCFM® has been shown by several phenotypic and genotypic 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® belongs to the type A1 *L. acidophilus* group. Sequencing of the 16S ribosomal RNA gene of NCFM® confirmed its identity as *L. acidophilus*. Fermentation and growth characteristics of NCFM® and the neotype strain ATCC 4356 are identical. The NCFM® DNA has a 34.7% GC ratio. Fermentation results in 34% D- and 66% L-lactic acid.

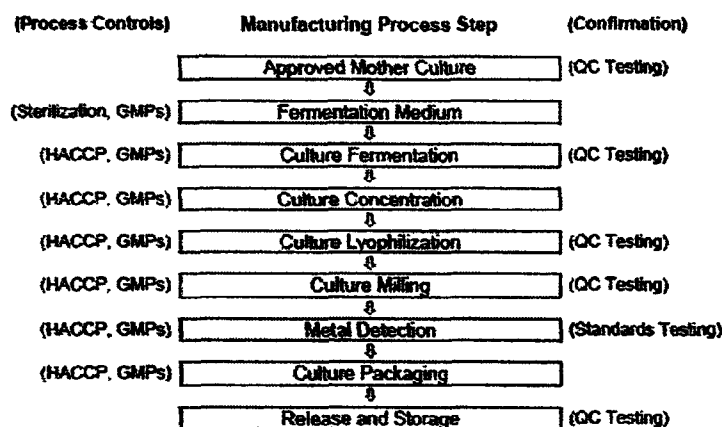


The entire genome of *L. acidophilus* NCFM® has been sequenced (8). The complete sequence and corresponding annotation is available from GenBank under accession no CP000033 and therefore is available for public reference.

Consistent with the World Health Organization (WHO) recommendations for cultures, *L. acidophilus* NCFM® has been deposited as safe deposits in two recognized international culture collections (American Type tissue Culture Collection, SD5221 and The German Collection of Microorganisms and Cell Cultures, DSM 22091).

Manufacturing and Specifications (including stability)

NCFM® is manufactured in accordance with the U.S. Food & Drug Administration's current Good Manufacturing Practice (cGMP) guidelines in an FDA regulated and inspected facility. The manufacturing process is summarized below.



Only approved food grade or equivalent materials are used in the manufacture of these products. The source organism used is *L. acidophilus* NCFM®. The culture is maintained in the culture bank of Danisco USA Inc. as frozen 1ml. vials at -80°C. Danisco USA Inc. independently verifies the identity of each organism. Each seed lot in the culture bank is fully characterized to insure the identity of the seed strains. From the seed vials, Danisco USA Inc. produces concentrated starter for the industrial fermentation.

The product is manufactured through a specific time and temperature controlled fermentation of suitable food grade ingredients with *L. acidophilus* NCFM®.

Prior to the addition of *L. acidophilus* NCFM®, the mixture is sterilized and cooled to an incubation temperature of 37° C. The mixture is then inoculated with *L. acidophilus* NCFM® and allowed to incubate to the fermentation endpoint under constant temperature.

After the required incubation period, the pH is adjusted with ammonium hydroxide, and bacteria are concentrated via centrifugation. To the concentrated bacterial slurry, food-grade cryoprotectants are added; the material is frozen; and subsequently freeze-dried. The dried cultured product is then packaged and stored in a cool, dry environment.

Release of product for sale according to established specifications is under the responsibility of Danisco Quality Control. Final product testing methods comply with standard Methods for the Examination of Dairy Products of the American Public Health Association.



Stability testing of cultures is done on a regular basis by Danisco Innovation. The results for *L. acidophilus* NCFM® shelf live testing is as follows: at room temperature freeze-dried NCFM had 77% recovery after 2 years storage. This would be considered excellent stability for a probiotic powder. At 30°C storage, the same powder had 33% recovery. This is considered very good. This type of stability allows the deliverability of a target amount of live culture throughout the shelf life of the final food product.

One of the Expert Panelists, Dr. Michael W. Pariza, analyzed the available information on *L. acidophilus* NCFM® using the Pariza-Johnson Decision Tree (Pariza, M.W. and Johnson, A.E. Evaluating the Safety of Microbial Enzyme Preparations Used in Food Processing: Update for a New Century, Reg. Tox. and Pharm. 33:173-186, 2001) (Attachment 1) and concluded "A NOAEL cannot be determined for a living microorganism that grows in the gastrointestinal tract of the host after ingestion. However, in 25 years of experience with this strain as a starter culture and probiotic, there have been no reported adverse effects in humans, a result that is also supported by short-term clinical trials. Therefore we conclude that *Lactobacillus acidophilus* NCFM® is ACCEPTED." The other Expert Panelists concurred with this conclusion.

Intended Uses, Use Levels, and Exposure Estimates

Uses are self-limited to those foods that can sustain living *L. acidophilus* for the shelf life of the food. These include dairy products, functional beverages, nutritional powders, juices, bars, RTE breakfast cereals, chewing gum, and confections. Meat and meat products, poultry and poultry products and infant formulas are not being considered. Based on its market analysis, Danisco estimates that relatively few foods and beverages within each category will be developed with *L. acidophilus*. These cultures, in general have a relatively high cost which will limit the breadth of their applications. Also, based on marketing estimates, Danisco believes it is reasonable to assume that consumption will be for the express purpose of ingesting the proper amount of the organisms to achieve the claimed benefit – generally in a single serving per day.

Foods will be targeted to typically contain 1×10^9 cfu/serving of *L. acidophilus*. Since the cell count may decrease over the shelf life of some foods, such as fruit juices with a low pH, a use level of up to about 5×10^{10} cfu/serving may be utilized for products likely to experience such phenomenon.

At a maximum use level where all foods contained 5×10^{10} cfu/250g serving (i.e. 2×10^8 cfu/g) at the beginning of shelf life, a consumer could consume six servings of products containing *L. acidophilus* NCFM® and would be below the level shown to be tolerated by humans in safety studies. Realistically, there will be limited types of foods that will be available containing the strain and consumers are very likely only to consume the foods that enable them to achieve the daily benefits of *L. acidophilus*. For instance, in the beverage category it is not envisioned that *L. acidophilus* containing products will compete with the myriad of functional food beverages on the market today because of either product incompatibility or cost. Danisco indicates that because of anticipated marketing, few products in a given category will likely contain the strain. Based on these assumptions, consumers will most probably consume a single serving to achieve the benefit, thus ingesting approximately 10^{10} to 1×10^{11} cfu per day.



Safety Assessment

History of Use in Food

Lactobacillus species have a long history of safe use when consumed as part of dairy food and supplement products. There are thirty-six (36) species listed in IDF Bulletin No. 377: *Inventory of Microorganisms with a Documented History of Use in Food*.

More specifically, *Lactobacillus acidophilus* has been added to human food since at least 1950 and is very common in dairy products worldwide including the US where the organism is the most common *Lactobacillus* in yogurt products. *Lactobacillus* sp. are non-pathogenic and non-toxicogenic and are considered safe. *L. acidophilus* NCFM® has been safely added to foods globally in dairy products and dietary supplements for at least 25 years and not a single case of adverse effects on consumers has been reported. It is noted, however, that *Lactobacillus* species may be a rare cause of endocarditis in adults in the absence of such supplementation.

Multiple strain designations appear in the literature for NCFM® or for single colony isolates of the NCFM® parent culture. The designations NCFM®, N2, NCK56, NCK45, N2 and RL8K are essentially identical strains. The parent NCFM® culture carried the NCSU laboratory designation RL8K and was composed of rough and smooth variants. These variants were designated RL8K-R (bile sensitive) and RL8K-S (bile resistant) upon isolation from the RL8K culture. N2 is a smooth, bile-resistant isolate from NCFM® selected by scientists at Marschall Products (now Danisco USA Inc.) as a bile-resistant colony, and pure culture is used as the seed for all commercial production runs of NCFM®. The mixed parent culture has not been used commercially or for research studies since before 1975. NCK56 is a Klaenhammer laboratory designation for N2, and NCK45 is a Klaenhammer laboratory designation for the parent culture NCFM® comprised of rough and smooth colony variants NCFM®. These different isolates cannot be differentiated genetically by pulsed field gel electrophoresis, a technique that 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. In some papers involving NCFM®, the strain is not identified, or NCFM® 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®, 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® was abbreviated LBA. Any confusion over use of NCFM® in publications included in this document was clarified by personal communications with company representatives and researchers. In the United States NCFM® is a registered trademark of the North Carolina Dairy Foundation.

Regulatory Status of *L. acidophilus*.

FDA, EU and scientific consensus on *L. acidophilus*

Species of the genus *Lactobacillus* are considered to be non-pathogenic, non-toxicogenic and have generally been considered safe for use in foods (e.g., EFSA, FDA). Boriello, et al. (2003) reviewed data pertinent to safety concerns for these bacteria and concluded that "current evidence suggests that the risk of infection with probiotic lactobacilli or bifidobacteria is similar



to that of infection with commensal strains, and that consumption of such products presents a negligible risk to consumers..." This opinion is echoed in other publications. Additionally, the species *L. acidophilus* has been listed in FDA's GRAS list, and is proposed for inclusion on the EU QPS list. The FDA states:

"Prior sanctions were granted for the use of harmless lactic acid producing bacteria, such as *Lactobacillus acidophilus*, as optional ingredients in specified standardized foods. These bacteria are permitted for use in cultured milk (which includes buttermilk) (§ 131.12), sour cream (§ 131.160), cottage cheese (§ 133.128), and yogurt (§ 131.200), provided that the mandatory cultures of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* are also used in the yogurt." (Partial List Of Microorganisms And Microbial-Derived Ingredients That Are Used In Foods; U. S. Food and Drug Administration Center for Food Safety & Applied Nutrition Office of Food Additive Safety, July 2001)."

Animal studies

The results of a 12 week study with two types of gnotobiotic, immunocompromised, male and female mice, bg/bg-nu/nu/+ (produce thymus-matured T-cells, euthymic) and bg/bg-nu/nu (athymic), where male and female mice were inoculated with NCFM[®] by one swabbing of the oral cavity and anal area with culture of 10⁸ ml, revealed the following:

- no morbidity or mortality in adult and neonatal mice;
- no adverse effects on growth parameters;
- no gross or microscopic pathological changes;
- no abscesses in the stomach or small intestine;
- no deaths in gnotobiotic, immunocompromised mice;
- evidence of induction of immunoglobulins, IgM and IgG.
- Translocation of *L. acidophilus* NCFM[®] to other tissues was observed (also LGG and *B. animalis*), but there was no evidence of inflammation or other pathologic findings in tissue sections of translocated mice.

The safety of the *L. acidophilus* NCFM[®] was also evaluated in a colitis mouse model using trinitrobenzenesulphonic acid (TNBS) to induce colitis. In healthy BALB/c male and female mice, intra-gastric administration of *L. acidophilus* NCFM[®] at 10¹⁰ cfu/day for five days did not show any potential adverse effect on mouse activity, weight and colon inflammation. In TNBS-treated mice (mice with severe colitis), no significant improvement was observed in the group fed *L. acidophilus* NCFM[®]. High doses (10¹⁰ cfu/kg bw/day for four consecutive days) of *L. acidophilus* NCFM[®] did not lead to translocation of the organism or abnormal translocation of the intestinal microflora.

It may be concluded from the above, that NCFM[®] had no adverse effects in growth, survival, activity and weight of immunocompromised mice. It must also be noted that no translocation was seen at high doses, which contributes to the evidence of safety for this strain.

Human studies

Human safety studies of NCFM[®] have not been reported. However, NCFM[®] has been administered to healthy male and female children and adults and to children and adults with irritable bowel syndrome, lactose maldigestion, or cold/flu symptoms in at least 35 clinical efficacy studies at doses up to 3.8 x 10¹¹ cfu /day for six days and no adverse incidents related to treatment were reported in healthy or diseased subjects. The highest intake of NCFM[®] that has



been demonstrated to be safe in healthy human volunteers, the No Observable Adverse Effect Level, and published in the peer-reviewed literature, was 3.8×10^{11} cfu /day for six days. If the consumer has gut barrier dysfunction or is immunocompromised, consultation with a physician before use is advised.

Transferable antibiotic resistance

Although there is negligible concern for translocation, toxigenicity, or any adverse nutritional activity from consumption of *L. acidophilus* NCFM®, 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 that 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®.

***Lactobacillus acidophilus* is not listed higher than Biosafety Level 1 (CDC/NHI Guidelines "Biosafety in Microbiological and Biomedical Laboratories", 4th edition, 1999, section III. The American Biological Safety Association as defines Biosafety Level 1 (BSL-1): "Well characterized agent not consistently known to cause disease in healthy adult humans or minimal potential hazard to laboratory personnel and the environment."**Summary

Clinical trials and safety studies in animals involving different strains and doses of *Lactobacillus acidophilus* demonstrate that *Lactobacillus acidophilus* in dairy foods and dietary supplements is safe for human consumption. In the worst-case scenario, a consumer could consume up to six servings of foods containing 5×10^{10} cfu/250g serving *L. acidophilus* at the beginning of shelf life, and still be highly unlikely to consume at the level shown to be tolerated by humans in safety studies where subjects ingested 3×10^{11} cfu/day for a period of six days without adverse effect.

Lactobacillus acidophilus strain NCFM® is non-pathogenic and non-toxigenic; it lacks infectivity in immunocompromised, neonatal animal models; it lacks transferable antibiotic resistance genes as determined by total genomic sequencing; and it failed to cause any adverse effects in healthy and diseased human volunteers. *L. acidophilus*, including strain NCFM®, has a history of safe use (common use) in food, including dietary supplements, for at least 30 years of reported commercial use. The safety of *L. acidophilus* is supported by its presence on both proposed QPS and FDA GRAS lists, and a general consensus in the published literature that *Lactobacillus* species are safe for use in foods for the generally healthy population.

**Conclusion**

We, the Expert Panel, have individually and collectively critically evaluated the available information on *Lactobacillus acidophilus* strains including strain NCFM® prepared by Danisco and other information deemed appropriate and conclude that the intended uses in food of strain NCFM®, manufactured consistent with current Good Manufacturing Practice (cGMP) and meeting the specifications presented in the dossier, are safe and suitable.

We further conclude that the intended uses of *L. acidophilus* NCFM® manufactured consistent with cGMP and meeting the specifications presented in the dossier, are Generally Recognized As Safe (GRAS) based on scientific procedures and supported by a history of common use in foods.

It is our opinion that other qualified experts would concur with these conclusions.

(b) (6)

Signature:

Date: 09 October 2010

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

(b) (6)

Signature:

Date: 12 Oct. 2010

Walter H. Glinsmann, M.D.
President
Glinsmann Inc.
Arlington, Virginia

(b) (6)

Signature:

Date: 13 Oct 2010

Michael W Pariza, Ph.D.
~~Distinguished~~ Professor Emeritus
University of Wisconsin/Madison
Madison, Wisconsin

000041



This analysis is based on the Decision Tree of MW Pariza and EA Johnson (2001): *Evaluating the Safety of Microbial Enzyme Preparations Used in Food Processing: Update for a New Century*, Regulatory Toxicology and Pharmacology, 33:173-186. Decision points that do not pertain are deleted. In this analysis, *Lactobacillus acidophilus* NCFM® is both the "production strain" and the "test article."

1. Is the production strain genetically modified?

If no, go to 6. **NO**

6. Is the production strain derived from a safe lineage, as previously demonstrated by repeated assessment via this evaluation procedure?

If no, go to 7. **NO**

7. Is the organism nonpathogenic?

If yes, go to 8. **YES**

8. Is the test article free of antibiotics?

If yes, go to 9. **YES**

9. Is the test article free of oral toxins known to be produced by other members of the same species?

If yes, go to 11. **YES (This species does not produce toxins that act via the oral route.)**

11. Is the NOAEL for the test article in appropriate oral studies sufficiently high to ensure safety?

If yes, the test article is **ACCEPTED**. A NOAEL cannot be determined for a living microorganism that grows in the host after feeding. However, in 25 years of experience with this strain as a starter culture and probiotic, there have been no reported adverse effects in humans, a result that is also supported by short-term clinical trials. Therefore we conclude that *Lactobacillus acidophilus* NCFM® is **ACCEPTED**.

000042

Michael W. Pariza Consulting LLC
7102 Valhalla Trail
Madison, WI 53719
(608) 271-5169
mwpariza@gmail.com

March 1, 2010

Sarah F. Kraak-Ripple
Manager, Corporate Regulatory Affairs
Danisco USA, Inc.
3329 Agriculture Drive
Madison, WI 53716

Dear Dr. Kraak-Ripple:

I am writing in regard to your request for an evaluation of the safety of Danisco's *Lactobacillus acidophilus* NCFM[®] for direct addition to various foods including, but not limited to, dairy products, functional beverages, nutritional powders, juices, bars, RTE breakfast cereals, chewing gum, and confections.

In conducting this evaluation I considered the biology of *L. acidophilus*, relevant information available in the peer-reviewed scientific literature, and information that you provided regarding the lineage and cloning history for *L. acidophilus* NCFM[®].

Lactobacillus acidophilus is a gram positive non-spore forming bacterium that occurs naturally in the human gastrointestinal tract. It does not produce toxins and is only rarely observed as an opportunistic pathogen for otherwise healthy subjects. To the contrary, *L. acidophilus* is associated with antibacterial activity against acknowledged bacterial pathogens, for example *Staphylococcus aureus*. *Lactobacillus acidophilus* has been added to human food since at least 1950, including the US where this organism is the most common *Lactobacillus* in yogurt.

In the 1970s, *L. acidophilus* NCFM[®] was isolated from human feces in the food microbiology research laboratories at North Carolina State University (NCSU). It has since been defined by its genome sequence, as determined by Altermann et al (Proc. Natl. Acad. Sci. USA 102:3906-12, 2005). This strain has been safely added to dairy products and dietary supplements throughout the world for at least 25 years, with not a single reported case of adverse effect on consumers.

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An analysis of *L. acidophilus* NCFM[®], based on the decision tree of MW Pariza and EA Johnson (2001): *Evaluating the Safety of Microbial Enzyme Preparations Used in Food Processing: Update for a New Century*, Regulatory Toxicology and Pharmacology, 33:173-186, is attached. In this analysis, *L. acidophilus* NCFM[®] is both the "production strain" and the "test article."

From these considerations, I conclude that Danisco's *L. acidophilus* NCFM[®] is safe for direct addition to various foods including, but not limited to, dairy products, functional beverages, nutritional powders, juices, bars, RTE breakfast cereals, chewing gum, and confections. It is my professional opinion that other qualified experts would also concur in this conclusion.

Please note that this is a professional opinion directed at safety considerations only and not an endorsement, warranty, or recommendation regarding the possible use of the subject product by you or others.

Sincerely,

(b) (6)



Michael W. Pariza
Member, Michael W. Pariza Consulting, LLC
Professor Emeritus, Food Science
University of Wisconsin-Madison

000044

This analysis is based on the Decision Tree of MW Pariza and EA Johnson (2001): *Evaluating the Safety of Microbial Enzyme Preparations Used in Food Processing: Update for a New Century*, Regulatory Toxicology and Pharmacology, 33:173-186. Decision points that do not pertain are deleted. In this analysis, *Lactobacillus acidophilus* NCFM® is both the "production strain" and the "test article."

1. Is the production strain genetically modified?

If no, go to 6. **NO**

6. Is the production strain derived from a safe lineage, as previously demonstrated by repeated assessment via this evaluation procedure?

If no, go to 7. **NO**

7. Is the organism nonpathogenic?

If yes, go to 8. **YES**

8. Is the test article free of antibiotics?

If yes, go to 9. **YES**

9. Is the test article free of oral toxins known to be produced by other members of the same species?

If yes, go to 11. **YES** (This species does not produce toxins that act via the oral route.)

11. Is the NOAEL for the test article in appropriate oral studies sufficiently high to ensure safety?

If yes, the test article is **ACCEPTED**. A NOAEL cannot be determined for a living microorganism that grows in the host after feeding. However, in 25 years of experience with this strain as a starter culture and probiotic, there have been no reported adverse effects in humans, a result that is also supported by short-term clinical trials. Therefore we conclude that *Lactobacillus acidophilus* NCFM® is **ACCEPTED**.



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Food

Partial List Of Microorganisms And Microbial-Derived Ingredients That Are Used In Foods

July 2001

Food ingredients may be "food additives" that are approved by FDA for specific uses or GRAS (generally recognized as safe) substances. A substance may be GRAS only if its general recognition of safety is based on the views of experts qualified to evaluate the safety of the substance. GRAS status may be based either on a history of safe use in food prior to 1958 or on scientific procedures, which require the same quantity and quality of evidence as would be required to obtain a food additive regulation. Because GRAS status may be either affirmed by FDA or determined independently by qualified experts, FDA's regulations do not include all GRAS Ingredients and the specific uses described in the GRAS regulations may not be comprehensive for the listed ingredients.

The list below includes some ingredients that are not listed in 21 CFR but have been the subject of opinion letters from FDA to individuals who asked whether FDA would object to the use of the ingredient in food on the basis of an independent GRAS determination. Because the list is not updated on a regular basis, questions about the regulatory status of microorganisms or microbial-derived ingredients that are not on this list may be directed to us via electronic mail at Premarkt@fda.hhs.gov

The following list, which derives partially from FDA's regulations in Title 21 of the Code of Federal Regulations (21 CFR), includes approved food additives, substances whose GRAS status has been affirmed by FDA and substances that FDA listed as GRAS based on a history of safe use in food. In addition, microorganisms and microbial-derived ingredients may be the subject of a GRAS notice. For further information, consult the summary listing of GRAS ingredients.

The following is a compilation of food additives listed in Title 21 of the Code of Federal Regulations (21 CFR) Part 172 and 173, which are derived from microorganisms. This list also includes seaweed sources. Conditions for their use are prescribed in the referent regulations and are predicated on the use of good manufacturing practices.

To access the specific regulations listed below, type in the title number, use the links below to access the Government Printing Office web site.

Table 1. Food Additives Derived from Microorganisms listed in 21 CFR 172 and 173

Regulation in 21 CFR	Ingredient
§172.155	Natamycin derived from <i>Streptomyces natalensis</i> and <i>Streptomyces chattanoogensis</i>
§172.325	Bakers yeast protein derived from <i>Saccharomyces cerevisiae</i>
§172.590	Yeast-malt sprout extract, derived from <i>Saccharomyces cerevisiae</i> , <i>Saccharomyces fragilis</i> , <i>Candida utilis</i>
§172.620	Carrageenan, a hydrocolloid extracted from the following members of the families <i>Gigartinaeae</i> and <i>Soliericeae</i> of the class <i>Rodophyceae</i> (red seaweed): <i>Chondrus crispus</i> , <i>Chondrus ocellatus</i> , <i>Eucheuma cottonii</i> , <i>Eucheuma spinosum</i> , <i>Gigartina acicularis</i> , <i>Gigartina pistillata</i> , <i>Gigartina radula</i> , <i>Gigartina stellata</i>
§172.655	Furcelleran, the refined hydrocolloid extracted from <i>Furcellaria fastigiata</i> of the class <i>Rodophyceae</i> (red seaweed)
§172.695	Xanthan Gum derived from <i>Xanthomonas campestris</i>
§172.725	Gibberellic acid derived by fermentation from <i>Fusarium moniliforme</i>
§172.896	Dried yeasts, <i>Saccharomyces cerevisiae</i> , <i>Saccharomyces fragilis</i> , and dried torula yeast, <i>Candida utilis</i>
§172.898	Bakers yeast glycan from <i>Saccharomyces cerevisiae</i>
§173.110	Amyloglucosidase derived from <i>Rhizopus niveus</i> for use in degrading gelatinized starch into constituent sugars
§173.120	Carbohydrase and cellulase derived from <i>Aspergillus niger</i> for use in clam and shrimp processing
§173.130	Carbohydrase derived from <i>Rhizopus oryzae</i> for use in the production of dextrose from starch
§173.135	Catalase derived from <i>Micrococcus lysodeikticus</i> for use in the manufacture of cheese
§173.140	Esterase-lipase derived from <i>Mucor miehei</i> var. Cooney et Emerson as a flavor enhancer in cheeses, fats and oils, and milk products
§173.145	Alpha-galactosidase derived from <i>Mortierella vinaceae</i> var. <i>raffinoseutilizer</i> for use in the production of sucrose from sugar beets
§173.150	Milk-clotting enzymes, microbial for use in the production of cheese (Milk-clotting enzymes are derived from <i>Endothia parasitica</i> , <i>Bacillus cereus</i> , <i>Mucor pusillus</i> Lindt and <i>Mucor miehei</i> and <i>Aspergillus oryzae</i> modified to contain the gene for aspartic proteinase from <i>Rhizomucor miehei</i> var Cooney et Emerson
§173.160	<i>Candida guilliermondii</i> as the organism for fermentation production of citric acid
§173.165	<i>Candida lipolytica</i> for fermentation production of citric acid.
§173.280	A solvent extraction process for recovery of citric acid from <i>Aspergillus niger</i> fermentation liquor

The following is a compilation of GRAS affirmed substances listed in 21 CFR part 184 which are derived from microorganisms. This list also includes seaweed sources. Conditions for their use are prescribed in the referent regulations and are predicated on the use of nonpathogenic and nontoxicogenic strains of the respective organisms and on the use of current good manufacturing practice (184.1(b)). Please be aware that not all GRAS substances have been recorded as such and so this does not represent a complete list of all microbial derived GRAS food ingredients.

Table 2. Substances Derived from Microorganisms Affirmed by FDA as Generally Recognized as Safe in 21 CFR184

Section in 21 CFR	Ingredient or Substance
§184.1005	Acetic acid may be produced by fermentation
§184.1011	Alginate acid made from certain brown algae
§184.1012	Alpha-amylase enzyme preparation from <i>Bacillus stearothermophilus</i> used to hydrolyze edible starch to produce maltodextrin and nutritive carbohydrate sweeteners.
§184.1027	Mixed carbohydrase and protease enzyme product derived from <i>Bacillus licheniformis</i> for use in hydrolyzing proteins and carbohydrate in the preparation of alcoholic beverages, candy, nutritive sweeteners and protein hydrolysates
§184.1061	Lactic acid may be produced by fermentation
§184.1081	Propionic acid from bacterial fermentation
§184.1115	Agar-agar, extracted from a number of related species of red algae class <i>Rhodophyceae</i>
§184.1120	Brown algae, to be used dried as a flavor enhancer, are seaweeds of the species: <i>Anelopus japonicus</i> , <i>Eisenia bicyclis</i> , <i>Hizikia fusiforme</i> , <i>Kjellmaniella gyrate</i> , <i>Laminaria angustata</i> , <i>Laminaria longiruris</i> , <i>Laminaria longissima</i> , <i>Laminaria ochotensis</i> , <i>Laminaria clausonila</i> , <i>Laminaria saccharina</i> , <i>Laminaria digitata</i> , <i>Laminaria japonica</i> , <i>Macrocystis pyrifera</i> , <i>Petalonia fascia</i> , <i>Scytosiphon lome</i>
§184.1121	Red algae, to be used dried as a flavor enhancer, are seaweeds of the species: <i>Gloiopeltis furcata</i> , <i>Porphyra crispata</i> , <i>Porphyra deudata</i> , <i>Porphyra perforata</i> , <i>Porphyra suborbiculata</i> , <i>Porphyra tenera</i> , <i>Rhodymenis palmata</i>

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- §184.1133 Ammonium alginate from certain brown algae
- §184.1187 Calcium alginate from certain brown algae
- §184.1318 Glucono delta-lactone, by oxidation of D-glucose by microorganisms that are nonpathogenic and nontoxicogenic to man or other animals. These include but are not restricted to *Aspergillus niger* and *Acetobacter suboxydans*
- §184.1372 Insoluble glucose isomerase enzyme preparations are derived from recognized species of precisely classified, nonpathogenic, and nontoxicogenic microorganisms, including *Streptomyces rubiginosus*, *Actinoplanes missouriensis*, *Streptomyces olivaceus*, *Streptomyces olivochromogenes*, and *Bacillus coagulans* grown in a pure culture fermentation that produces no antibiotic
- §184.1387 Lactase enzyme preparation from *Candida pseudotropicalis* for use in hydrolyzing lactose to glucose and galactose
- §184.1388 Lactase enzyme preparation from *Kluyveromyces lactis* (previously called *Saccharomyces lactis*) for use in hydrolyzing lactose in milk
- §184.1420 Lipase enzyme preparation from *Rhizopus niveus* used in the interesterification of fats and oils.
- §184.1538 Nisin preparation from *Lactococcus lactis* Lancefield Group N for use as an antimicrobial agent to inhibit the outgrowth of *Clostridium botulinum* spores and toxin formation in pasteurized cheese spreads.
- §184.1610 Potassium alginate, the potassium salt of alginic acid, derived from certain brown algae
- §184.1685 Rennet (animal derived) and chymosin preparation from *Escherichia coli* K-12, *Kluyveromyces marxianus* var. *lactis* or *Aspergillus niger* var. *awamori* to coagulate milk in cheeses and other dairy products
- §184.1695 Riboflavin biosynthesized by *Eremothecium ashbyii*
- §184.1724 Sodium alginate, the sodium salt of alginic acid, derived from certain brown algae
- §184.1848 Butter starter distillate from milk cultures of *Streptococcus lactis*, *Streptococcus cremoris*, *Streptococcus lactis* subspecies *diacetylactis*, *Leuconostoc citovorum*, *Leuconostoc dextranum*
- §184.1924 Urease enzyme preparation from *Lactobacillus fermentum* for use in the production of wine
- §184.1945 Vitamin B12 from *Streptomyces griseus*
- §184.1950 Vitamin D, produced by ultraviolet irradiation of ergosterol isolated from yeast and related fungi
- §184.1983 Bakers Yeast extract from *Saccharomyces cerevisiae*
- §184.1985 Aminopeptidase enzyme preparation from *Lactococcus lactis* used as an optional ingredient for flavor development in the manufacture of cheddar cheese.

The following GRAS affirmed substances are listed in 21 CFR Part 186 and are affirmed for use as substances added indirectly to food. Conditions for their use are prescribed in the referent regulations and are predicated on the use of nonpathogenic and nontoxicogenic strains of the respective organisms and on the use of current good manufacturing practice (186.1(b)).

Table 3. Substances Derived from Microorganisms Affirmed by FDA as Generally Recognized as Safe for Indirect Uses in 21 CFR186

Section in 21 CFR	Substance
§186.1275	Dextrans, made by fermentation of sucrose by <i>Leuconostoc mesenteroides</i> strain NRRL B-512(F)
§186.1839	Sorbose, made by oxidation of sorbitol by <i>Acetobacter xylinum</i> or by <i>Acetobacter suboxydans</i>

The following is a compilation of microbial derived enzymes which the FDA recognized as GRAS in opinion letters issued in the early 1960's. The opinions are predicated on the use of nonpathogenic and nontoxicogenic strains of the respective organisms and on the use of current good manufacturing practice.

Table 4. Substances Derived from Microorganisms Recognized by FDA as Generally Recognized as Safe in Opinion Letters

Enzyme
Carbohydrase, cellulase, glucose oxidase-catalase, pectinase, and lipase from <i>Aspergillus niger</i>
Carbohydrase and protease from <i>Aspergillus oryzae</i>
Carbohydrase and protease from <i>Bacillus subtilis</i>
Invertase from edible baker's yeast or brewer's yeast (<i>Saccharomyces cerevisiae</i>)

The following is a compilation of foods for human consumption listed in 21 CFR Parts 131, 133, 136 and 137 that may contain or be derived from microorganisms.

Table 5. Foods for human consumption that may contain or be derived from microorganisms listed in 21 CFR Parts 131, 133, 136, and 137

Section in 21 CFR	Standardized Food
§131.111	Acidified milk, with or without the addition of characterizing microbial organisms, and aroma - and flavor - producing microbial culture. Conditions for their use are prescribed in the referent regulations
§131.200	Yogurt made by the lactic acid-producing bacteria <i>Lactobacillus bulgaricus</i> and <i>Streptococcus thermophilus</i>
§131.106	Blue cheese, characterized by the presence of the mold <i>Penicillium roquefortii</i>
§133.113	Cheddar cheese, subjected to the action of a lactic acid producing bacterial culture and clotting enzymes of animal, plant or microbial origin used in curing or flavor development
§136.110	Bread, rolls, and buns may contain as optional ingredients lactic-acid producing bacteria
§137.105	Flour may contain alpha-amylase obtained from the fungus <i>Aspergillus oryzae</i>

Prior sanctions were granted for the use of harmless lactic acid producing bacteria, such as *Lactobacillus acidophilus*, as optional ingredients in specified standardized foods. These bacteria are permitted for use in cultured milk (which includes buttermilk) (§ 131.112), sour cream (§ 131.160), cottage cheese (§ 133.128), and yogurt (§ 131.200), provided that the mandatory cultures of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* are also used in the yogurt.

Links on this page:

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Appendix A: *L. acidophilus* species is on EFSA's QPS list

From QPS document, EFSA, Appendix A - Assessment of gram-positive non-sporulating bacteria *The EFSA Journal* (2007) 587, Qualified Presumption of Safety

http://www.efsa.europa.eu/cs/BlobServer/Scientific_Opinion/sc_appendixa_qps_en.pdf?ssbinary=true

Lactobacillus

The genus *Lactobacillus* is a wide and heterogeneous taxonomic unit, comprising the rod-shaped lactic acid bacteria. This genus encompasses more than 100 different species with a large variety of phenotypic, biochemical and physiological properties. Many of the species are significant constituents of the normal gut flora of humans and livestock although their occurrence and numbers are host dependent. Several species of the genus are intentionally introduced in the food chains, being involved in a range of food and feed fermentations and applied as probiotics for humans and animals.

Taxonomic unit defined

As for other lactic acid bacteria, lactobacilli belong to the phylum *Firmicutes*. They are rod shaped, non-motile and non-sporeformers. Classically, the *Lactobacillus* genus is divided into three groups: group 1, obligate homofermentative, group 2, facultative heterofermentative and group 3 obligate heterofermentative (for a review, see Axelsson 2004). The application of phylogenetic molecular taxonomy and 16S rRNA gene sequence analysis resulted in several changes within the taxonomy of this genus, with an increase in the number of species. At present 112 species belong to the genus *Lactobacillus*. Several molecular methods are available for the identification of lactobacilli to species level.

Is the body of knowledge sufficient?

The characteristics and habitat of most of *Lactobacillus* species are well known. Some of the species of this genus have a long history of apparent safe use in industrial and agricultural applications. Lactobacilli are used as starter cultures in a variety of food fermentation, such as dairy products, fermented and cured meats, fermented vegetables, sourdough and silage. Moreover, they are among the dominant populations in microbial communities of traditional fermented foods, being part of the natural starter cultures. Increased information on this genus is being derived from the sequence analysis of several genomes of *Lactobacillus* species.

Are there safety concerns?

Members of the *Lactobacillus* genus are daily consumed in large quantities in a variety of fermented foods by people of all ages, ethnic groups and health status with apparently no ill effects. Apart from their possible involvement in the development of dental caries, lactobacilli have generally been considered to be non-pathogenic. However, there have been an increasing number of reports that these organisms might occasionally be involved in human disease (Sharpe, Hill *et al.* 1973; Gasser 1994; Salminen, Rautelin *et al.* 2006). A variety of different *Lactobacillus* species has been recovered from human clinical specimens. These include *L. rhamnosus*, *L. fermentum*, *L. plantarum*, *L. casei*, *L. jensenii*, *L. salivarius*, *L. gasseri*, *L. salivarius*, and *L. acidophilus*. Clinical conditions from which these species were derived were chiefly subacute endocarditis and bacteremia or systemic septicemia, but also included abscesses, chorioamnionitis, and urosepsis (Lorenz, Appelbaum *et al.* 1982; Dickgiesser, Weiss *et al.* 1984; Salminen, Tynkkynen *et al.* 2002; Salminen, Rautelin *et al.* 2004; Salminen, Rautelin *et al.* 2006). Even the strain *L. rhamnosus* ATCC 53103,

used as human probiotic, has occasionally been encountered in clinical specimens such as blood or pus samples (Rautio, Jousimies-Somer *et al.* 1999; Salminen, Tynkkynen *et al.* 2002; Salminen, Rautelin *et al.* 2004; De Groote, Frank *et al.* 2005; Salminen, Rautelin *et al.* 2006). However, Salminen and co-workers (Salminen, Rautelin *et al.* 2006) demonstrated that increased probiotic use of *L. rhamnosus* ATCC 53103 had not led to an increase in *Lactobacillus* bacteraemia. Furthermore, it has been demonstrated that strains isolated from clinical samples, show phenotypic differences from probiotic *L. rhamnosus* strains (Klein, Hack *et al.* 1995; Ouwehand, Saxelin *et al.* 2004). Many of the patients with apparent *Lactobacillus* infection were immunocompromised or had other severe underlying illnesses. As far as endocarditis due to lactobacilli is concerned, this infection usually develops on the basis of preceding anatomical alterations of the heart valves. There are indications, however, that good adhesion properties of lactobacilli and, thus, of probiotic strains, might be a potential risk for bacteremia (Apostolou, Kirjavainen *et al.* 2001). In conclusion, most of the *Lactobacillus* species described to date can rightly be considered to be non-pathogenic to humans (Bernardeau, Guguen *et al.* 2006). Only certain strains of *L. rhamnosus* may be considered to be potential human opportunistic pathogens because they not only affect severely immunocompromised, but also immunologically healthy individuals with a history of rheumatic endocarditis or heart valve replacement.

Several examples of antibiotic resistant lactobacilli isolated from food or from the gut of animals exist. Acquired genes for antibiotic resistance have been detected in *Lactobacillus* species: *tet*(M) has been found in *L. plantarum*, *L. brevis*, *L. sakei* and *L. curvatus* (Danielsen 2002; Gevers, Danielsen *et al.* 2003) and *tet*(S) in *L. plantarum* (Huys, D'Haene *et al.* 2006). Erythromycin resistance determinants *erm*(B) has been found in *L. plantarum*, *L. salivarius*, *L. animalis*, *L. fermentum*, *L. reuteri* (Axelsson, Ahrne *et al.* 1988; Fons, Hege *et al.* 1997; Gevers, Danielsen *et al.* 2003; Martel, Meulenaere *et al.* 2003). Moreover, the gene coding for the bifunctional aminoglycoside-modifying enzyme AAC(6')-APH(2'') was detected in *L. salivarius* and *L. acidophilus* (Tenorio, Zarazaga *et al.* 2001) and chloramphenicol resistance gene *cat* was identified in *L. reuteri* (Lin, Fung *et al.* 1996). Obligate and facultative heterofermentative lactobacilli, and *L. salivarius*, are intrinsically resistant to vancomycin and other glycopeptide antibiotics.

Several genetic determinants for antibiotic resistance in *Lactobacillus* are harboured by extrachromosomal elements (Lin, Fung *et al.* 1996; Danielsen 2002; Gevers, Danielsen *et al.* 2003; Gfeller, Roth *et al.* 2003; Huys, D'Haene *et al.* 2006). However, transferable elements encoding resistances of clinical relevance, such as to the glycopeptides have been excluded for some probiotic *L. reuteri* and *L. rhamnosus* strains (Klein, Hallmann *et al.* 2000).

Livestock. No report can be found on safety concerns related to lactobacilli in animals

Can the safety concerns be excluded?

There are apparently no specific safety concerns regarding a number of *Lactobacillus* species which have a long history of apparent safe use in the food chain. Susceptibility to antibiotics should be assessed as defined by the EFSA opinion for each strain (EFSA 2005).

4.5 Units proposed for QPS status

Due to the long history of safe use the following species are proposed for QPS status:

L. acidophilus, *L. amylolyticus*, *L. amylovorus*, *L. alimentarius*, *L. aviaries*, *L. brevis*, *L. buchneri*, *L. casei*, *L. crispatus*, *L. curvatus*, *L. delbrueckii*, *L. farciminis*, *L. fermentum*, *L. gallinarum*, *L. gasseri*, *L. helveticus*, *L. hilgardii*, *L. johnsonii*, *L. kefiranoferiens*, *L. kefir*, *L. mucosae*, *L. panis*, *L.*

paracasei, *L. paraplantarum*, *L. pentosus*, *L. plantarum*, *L. pontis*, *L. reuteri*, *L. rhamnosus*, *L. sakei*, *L. salivarius*, *L. sanfranciscensis* and *L. zeae*.

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SUBMISSION END

000052

From: rsindt@bobsindtlaw.com
To: [Harry, Molly *](#)
Cc: [Carlson, Susan](#); [Flamm, Edwin G](#); ["Kraak-Ripple"](#); [Joseph F. Borzelleca Ph. D. \(josephfborzelleca@comcast.net\)](#)
Subject: RE: GRAS notice on *Lactobacillus acidophilus* strain ATCC SD5221 (GRN 000357)
Date: Thursday, January 20, 2011 11:03:29 AM
Attachments: [Responses to FDA Questions -1-11.pdf](#)

Ms. Harry,
Attached please find a document, submitted on behalf of my client, Danisco USA, Inc., that contains Danisco's replies to FDA's questions you sent me by way of your email of December 14, 2010. We would ask that the responses be appended to the original notice as filed and be utilized as clarifying/amending the notice, where and as appropriate, in FDA's continuing review of the notice. Please promptly notify me should FDA have any other questions regarding these responses or any other portions of the notice.

Further, a comment is in order regarding your indication that FDA intends to use the name *Lactobacillus acidophilus* strain ATCC SD 5221 vs. *Lactobacillus acidophilus* NCFM. It should be noted that the strain was identified and named NCFM by North Carolina State University and that the NCFM is a registered trademark of the North Carolina Dairy Foundation of NCSU. Danisco is a licensee for use of the NCFM strain. Initial identity and commercial use of the strain has been associated with the NCFM name and Danisco believes that the common and usual name of the strain is NCFM. Consequently, we would ask that you consider this information in your further review of the notice.

Again, we apologize for the amount of time, which was necessitated in providing this information. I would appreciate your acknowledging receipt of this information. Thank you.

Bob Sindt
Robert H. Sindt
Attorney at Law
1850 M St., NW, Suite 400
Washington, DC 20036
Phone: (202) 466-4500
Fax: (202) 466-5777
rsindt@bobsindtlaw.com

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From: Harry, Molly * [Molly.Harry@fda.hhs.gov]
Sent: Tuesday, January 18, 2011 11:48 AM
To: rsindt@bobsindtlaw.com; 'Kraak-Ripple'
Cc: Carlson, Susan; Flamm, Edwin G
Subject: RE: GRAS notice on *Lactobacillus acidophilus* strain ATCC SD5221 (GRN 000357)

Hi Mr. Sindt,

This is to remind you that we are still waiting for your response to our December 14, 2010 e-mail (below) seeking clarifications regarding the GRAS notice on *Lactobacillus acidophilus* strain ATCC SD5221 (GRN 000357), that you submitted on behalf of Danisco USA, Inc. To enable the review team complete the review of the notice, please provide the requested information/clarification soon.

Please let me know if you have any questions.

Sincerely,
Molly Harry, M.S.
Division of Biotechnology

From: rsindt@bobsindtlaw.com [mailto:rsindt@bobsindtlaw.com]
Sent: Thursday, December 23, 2010 4:10 PM
To: Harry, Molly *
Cc: Gaynor, Paulette M; Flamm, Edwin G; Srinivasan, Jannavi; Martin, Robert L; Sarah F. Kraak-Ripple (sarah.kraak-ripple@danisco.com)
Subject: RE: GRAS notice on *Lactobacillus acidophilus* strain ATCC SD5221 (GRN 000357)

Ms. Harry,
Unfortunately, my ability to respond within the 10 days requested does not appear doable due to the intervening holidays and inability to contact all the parties necessary to properly reply to your comments and to supply the clarifying information you have requested. It is my hope and expectation, however, to be able to reply by or before your indicated return to your office in early January. It is my present intention to reply by email with a request that the clarifying information be appended to and appropriately amend/clarify the original filed document.

Thank you and we look forward to responding.
Bob Sindt

Robert H. Sindt
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Washington, DC 20036
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From: Harry, Molly * [Molly.Harry@fda.hhs.gov]
Sent: Tuesday, December 14, 2010 4:01 PM
To: rsindt@bobsindtlaw.com
Cc: Gaynor, Paulette M; Flamm, Edwin G; Srinivasan, Jannavi; Martin, Robert L
Subject: GRAS notice on *Lactobacillus acidophilus* strain ATCC SD5221 (GRN 000357)

Dear Mr. Sindt,

Our review of the GRAS notice on *Lactobacillus acidophilus* strain ATCC SD5221 (GRN 000357), that you submitted on behalf of Danisco USA, Inc. (Danisco) is ongoing. We want you to be aware that, as indicated in the acknowledgement letter, FDA will be using the name *Lactobacillus acidophilus* strain ATCC SD5221. During the pre-submission meeting, FDA recommended that Danisco not include their intellectual property information (i.e., □) by the strain name as FDA's review of the notice would not be an approval or a license, also because the notice would be made available to the general public on FDA's internet site.

Currently, we have identified some areas in the submission that we would like to bring to your attention for clarification, those items are listed below.

1. On pages 0007 and 0031 of the notice, the notifier states that *L. acidophilus* strain NCFM was isolated from human feces in 1975. The article by Gilliland et al., (Appl Microbiol 30:541-545, 1975) reports that the organism was isolated from human feces but does not give a year. The article by Altermann et al., (Proc Natl Acad Sci 102:3906-3912, 2005) which reports on the genomics of the organism states that *L. acidophilus* strain NCFM has been produced commercially since 1972. **Please clarify this inconsistency.**
2. The title of the article by Leyer et al., (Pediatrics 124:e172-e179) is reported in the Bibliography

section (page 0029) as: ***A randomized controlled trial of probiotics on cold and flu-like symptom incidence and duration in children***, and no year is reported. The title should read: ***Probiotic Effects on Cold and Influenza-Like Symptom Incidence and Duration in Children***, the year of publication is 2009. **Please update the bibliography.**

3. Page 0009 of the notice reports that *L. acidophilus* strain NCFM is deposited in the American Type Culture Collection (ATCC) as SD5221. On pages 0007 and 0008 of the notice, the notifier states that the parent NCFM culture carried the North Carolina State University laboratory designation RL8K and was composed of rough and smooth variants. The purified RL8K-S (bile resistant) culture, designated ATCC700396, is the organism that was subjected to chromosomal DNA sequencing (Altermann et al., Proc Natl Acad Sci vol 102: 3906-3912, 2005). Additionally, the study that gave children (3-5 years of age) *L. acidophilus* for 6 months (Leyer et al., Pediatrics vol 124:e172-e179, 2009) also used the organism deposited as ATCC 700396. In both cases the articles report that *L. acidophilus* strain NCFM was used. If there is no detectable genetic difference between ATCC SD5221 and 700396, could it be reported in the notice that *L. acidophilus* strain NCFM is deposited in the ATCC under both SD5221 and 700396?
4. On page 0002, the food categories listed include "certain dairy products" and "functional beverages". Please specify the dairy products, and the beverages respectively that the substance *Lactobacillus acidophilus* strain ATCC SD5221 is intended for use in. Also, FDA recommends that Danisco drop the food category "nutritional powders" from the intended uses as this is not a program area considered by the Office of Food Additive Safety. This program area (i.e., dietary supplements) is under the purview of the Office of Nutrition, Labeling, and Dietary Supplements. **Please provide an updated list of food categories.**
5. On page 0003 of the notice, the notifier states that: "All population age groups, except infants, are expected to consume these foods." **Please clarify whether this statement means that infant formula is excluded from the intended uses in the notice.**

Please provide the requested information/clarification within ten (10) days. Please address your response to all as I will be on leave starting from December 16, 2010 and will return on January 10, 2011.

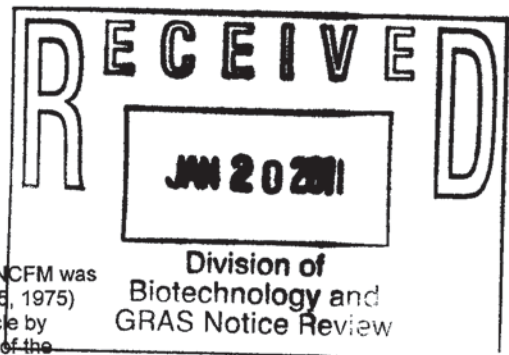
Please let me know if you have any questions.

Sincerely,

Molly Harry, M.S.

Division of Biotechnology

and GRAS Notice Review



1. On pages 0007 and 0031 of the notice, the notifier states that *L. acidophilus* strain NCFM was isolated from human feces in 1975. The article by Gilliland et al., (Appl Microbiol 30:541-545, 1975) reports that the organism was isolated from human feces but does not give a year. The article by Altermann et al., (Proc Natl Acad Sci 102:3906-3912, 2005) which reports on the genomics of the organism states that *L. acidophilus* strain NCFM has been produced commercially since 1972. Please clarify this inconsistency.

Response: Consultation with the North Carolina Dairy Foundation, Inc. at North Carolina State University indicates that the *L. acidophilus* NCFM strain was first isolated in the "early 1970's", and that it was first utilized commercially in a lowfat milk in 1975. Therefore, pages 007 and 0031 of the notice should have stated that the NCFM strain was isolated in the "early 1970's". We are not able to confirm that the date for commercial production cited in the Altermann et al article is accurate, as we have only been able to obtain from the North Carolina Dairy Foundation the historical information mentioned.

2. The title of the article by Leyer et al., (Pediatrics 124:e172-e179) is reported in the Bibliography section (page 0029) as: ***A randomized controlled trial of probiotics on cold and flu-like symptom incidence and duration in children***, and no year is reported. The title should read: ***Probiotic Effects on Cold and Influenza-Like Symptom Incidence and Duration in Children***, the year of publication is 2009. Please update the bibliography.

Response: The title to Reference #33 of the notice's bibliography should read:
Leyer, G., Li, S., Reifer, C., Mubasher, M., and A.O. Ouwehand. 2009. Probiotic Effects on Cold and Influenza-Like Symptom Incidence and Duration in Children. Pediatrics 124:e172-e179.

3. Page 0009 of the notice reports that *L. acidophilus* strain NCFM is deposited in the American Type Culture Collection (ATCC) as SD5221. On pages 0007 and 0008 of the notice, the notifier states that the parent NCFM culture carried the North Carolina State University laboratory designation RL8K and was composed of rough and smooth variants. The purified RL8K-S (bile resistant) culture, designated ATCC700396, is the organism that was subjected to chromosomal DNA sequencing (Altermann et al., Proc Natl Acad Sci vol 102: 3906-3912, 2005). Additionally, the study that gave children (3-5 years of age) *L. acidophilus* for 6 months (Leyer et al., Pediatrics vol 124:e172-e179, 2009) also used the organism deposited as ATCC 700396. In both cases the articles report that *L. acidophilus* strain NCFM was used. If there is no detectable genetic difference between ATCC SD5221 and 700396, could it be reported in the notice that *L. acidophilus* strain NCFM is deposited in the ATCC under both SD5221 and 700396?

Response: ATCC SD5221 and ATCC700396 are indeed the same strains, being produced from the same parent seed collection and utilizing the same manufacturing process at our facility. When the NCFM strain was deposited into the Safe Deposit collection of ATCC, it was given the SD identifying number SD5221. The ATCC

reference number 700396 is referring to a patent deposit of the same strain made during the genome sequencing project. Hence, it would be accurate to state that the NCFM strain has been deposited at the ATCC under both the SD5221 and 700396 numbers.

4. On page 0002, the food categories listed include "certain dairy products" and "functional beverages". Please specify the dairy products, and the beverages respectively that the substance *Lactobacillus acidophilus* strain ATCC SD5221 is intended for use in. Also, FDA recommends that Danisco drop the food category "nutritional powders" from the intended uses as this is not a program area considered by the Office of Food Additive Safety. This program area (i.e., dietary supplements) is under the purview of the Office of Nutrition, Labeling, and Dietary Supplements. **Please provide an updated list of food categories.**

Response: The food category "certain dairy products" is intended to include cheeses, milk drinks, and milk products. The food category "functional beverages" is intended to include water and teas. For further clarification, the term "juices" is intended to include fruit juices, nectars, aides and drinks. Finally, Danisco concurs with FDA's recommendation and requests that the food category "nutritional powders" contained in the notice be deleted. Consequently, we ask that FDA consider the applicable food use categories as clarified and revised by this response in its further consideration of the notice:

5. On page 0003 of the notice, the notifier states that: "All population age groups, except infants, are expected to consume these foods." **Please clarify whether this statement means that infant formula is excluded from the intended uses in the notice.**

Response: Infant formula is not proposed as an intended use for the NCFM strain under the notice.



From: rsindt@bobsindtlaw.com [mailto:rsindt@bobsindtlaw.com]

Sent: Thursday, March 31, 2011 12:08 PM

To: Harry, Molly *; Carlson, Susan

Cc: Sarah F. Kraak-Ripple (sarah.kraak-ripple@danisco.com); Joseph F. Borzelleca Ph. D. (josephfborzelleca@comcast.net)

Subject: GRN 000357

Dr. Carlson and Ms. Harry,

This will respond to your recent call and our discussion wherein you requested that I provide further clarity on three points regarding the pending GRAS Notice (000357) for certain food uses of *Lactobacillus acidophilus* NCFM, filed on behalf of my client, Danisco USA, Inc. The clarification is as follows:

- 1) The food category "water" contained in the revised intended food use categories clarified in my email reply of January 21, 2011 is meant to refer to use in bottled water;
- 2) The food category "fruit juices, nectars, aides and drinks" contained in the revised intended food use categories clarified in my email reply of January 21, 2011 is meant for all those foods to contain a percentage of fruit juice; and
- 3) It appears that an inadvertent error occurred in transcription of the Expert Panel's final report that resulted in a apparent oversight in the concluding paragraph on page 3 (page 33 of GRN 000357) of the Expert Panel report referring to an estimated daily use level. The final clause on page 3 of the report should have stated "thus ingesting approximately 5×10^{10} cfu per day." This correction has been confirmed by the Chair of the Expert Panel.

I would ask that these clarifying responses be appended to the original notice as filed and be utilized as clarifying/amending the notice, where and as appropriate, in FDA's continuing review of the notice. Please promptly notify me should FDA have any other questions regarding these responses or any other portions of the notice. Thank you.
Bob Sindt

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