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February 6, 2013

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OFFICE OF
FOOD ADDITIVE SAFETY

Paulette Gaynor (HFS-255)
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition (CFSAN)
U.S. Food and Drug Administration
5100 Paint Branch Parkway
College Park, Maryland 20740-3835

Dear Sir or Madam:

Pursuant to the proposed rule outlined at 62 Fed. Reg. 18939 (April 17, 1997), Avitop GmbH hereby submits notification that the use of pasteurized fermented low-fat milk and skim milk products manufactured with *Bacteroides xylanisolvens* DSM 23964, is exempt from the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act because the notifier has determined that such use is generally recognized as safe (GRAS).

To facilitate your review, this notification is submitted in the format suggested under proposed 21 C.F.R. § 170.36(c) (*see* 62 Fed. Reg. 18961). Three copies of the GRAS Exemption Claim and Additional Information documents are enclosed, pursuant to proposed 21 C.F.R. § 170.36(b) (*see* 62 Fed. Reg. 18961). Also enclosed is an electronic copy (PDF) of the documents.

Sincerely,

(b) (6)

Diane B. McColl
Counsel to Avitop GmbH

DBM/JAR/fjr
0632.002

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GRAS EXEMPTION CLAIM

Avitop GmbH hereby claims that the use of pasteurized fermented low-fat milk and skim milk products manufactured with *Bacteroides xylanisolvens* DSM 23964 is exempt from the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act because the notifier has determined that such use is generally recognized as safe (GRAS).

(1) Name and address of notifier:

Dr. Franzpeter Bracht
Managing Director
Avitop GmbH
Robert-Rössel-Str. 10
13125 Berlin-Buch
Germany

(2) Common or usual names of the substance that is the subject of the GRAS exemption claim:

Pasteurized fermented low-fat milk and skim milk products manufactured with *Bacteroides xylanisolvens* DSM 23964

(3) Product description:

Pasteurized fermented low-fat and skim milk products produced with *B. xylanisolvens* DSM 23964 can be processed and packed for direct consumption as a traditional liquid fermented milk product or spray-dried for use as a fermented milk powder. The fermented milk powder product would be used by food processors in the same manner as yogurt powder.

(4) Applicable conditions of use of the notified substance:

The pasteurized fermented low-fat and skim milk products produced with *B. xylanisolvens* DSM 23964 would be in liquid, semi-liquid or spray-dried powder form. Following pasteurization, optional permissible food ingredients, such as sugars, flavors, dietary fibers, fruit preparations, etc., may be added to the fermented milk products. The spray-dried powder products would be used by manufacturers, much like yogurt powder is used, *e.g.*, in fillings and coatings for foods such as cereals, granola bars, fruits and nuts.

(a) Description of the population expected to consume the substance:

Members of the general population who consume at least one of the food categories described above.

(5) Basis for the GRAS determination:

The basis of this GRAS determination is through scientific procedures.

(6) Review and Copying Statement:

The data and information that are the basis for Avitop GmbH's GRAS determination are available for the Food and Drug Administration's (FDA's) review and copying at reasonable times at the offices of the notifier, or will be sent to FDA upon request.

(b) (6)

Dr. Franzpeter Bracht
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700 Thirteenth Street N.W.
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Phone: 202-737-4291

ADDITIONAL INFORMATION

(1) Identity of the notified substance

(a) Common or usual name

Pasteurized fermented low-fat milk and skim milk products manufactured with *Bacteroides xylanisolvens* DSM 23964

(b) Product description

The pasteurized fermented low-fat and skim milk products produced with *B. xylanisolvens* DSM 23964 would be in liquid, semi-liquid or spray-dried powder form. Low-fat and skim milk products fermented with *B. xylanisolvens* DSM 23964 result in products with softer acidity and texture than conventional yogurt.

(c) Method of manufacture of pasteurized fermented low-fat milk and skim milk products manufactured with *Bacteroides xylanisolvens* DSM 23964

The production of fermented milk products with *B. xylanisolvens* DSM 23964 is conducted under anaerobic conditions following current good manufacturing practice (cGMP). Food-grade glucose may be added during the fermentation process to stimulate the growth of *B. xylanisolvens*. The fermented milk is pasteurized at the end of the fermentation process. Appropriate in-process quality control measures are in place. The quality control of the end product includes standard tests for microbiological purity, as well as a test for viable *B. xylanisolvens*.

The pasteurized fermented milk product may then be processed and packed for direct consumption as a traditional liquid fermented milk product, or spray-dried for use as a fermented milk powder. The fermented milk powder product would be used by food processors in the same manner as a yogurt powder.

(i) *B. xylanisolvens* DSM 23964 microorganism

B. xylanisolvens is a natural component of the normal human fecal microflora (Qin et al., 2010; Marschal et al., 2011; Ponnusamy et al., 2011; Kulagina et al., 2012). *B. xylanisolvens* accounts for a significant proportion of all *Bacteroides* occurring in human feces (Qin et al., 2010). *Bacteroides* in turn is estimated to account for about a quarter of all anaerobic microorganisms residing in the human colon (Salyers, 1984). While certain strains of *Bacteroides fragilis* may be pathogenic and toxigenic (Sears, 2001; Brook, 1989; Sánchez et al., 2012), *Bacteroides* in general act largely as nonpathogenic

commensals[1] (van Belkum et al., 1999). Some *Bacteroides* species may even favorably affect the immune system (reviewed in Wexler, 2007) and the intestinal epithelial homeostasis (Hamady et al., 2010; Hudcovic et al., 2009).

B. xylanisolvens DSM 23964 has originally been isolated from the feces of a healthy, adult subject. *B. xylanisolvens* DSM 23964 is manufactured as a starter culture for use as a starter culture for the production of pasteurized fermented low-fat milk and skim-milk products.

(d) Characteristic properties

Low-fat and skim milk products fermented with *B. xylanisolvens* DSM 23964 result in products with softer acidity and texture than conventional yogurt. The pasteurized fermented low-fat and skim milk products produced with *B. xylanisolvens* DSM 23964 would be in liquid, semi-liquid, or spray-dried powder form.

When used in liquid-form, pasteurized fermented low-fat milk and skim milk products manufactured with *B. xylanisolvens* DSM 23964 are alternatives to regular low-fat or non-fat yogurt, acidophilus milk, or cultured buttermilk.

When used in spray-dried form, pasteurized fermented low-fat milk and skim milk products manufactured with *B. xylanisolvens* DSM 23964 are used like yogurt powder as an ingredient of certain processed foods (*e.g.* in fillings and coatings).

(e) Any content of potential human toxicants

None.

(f) Specifications for *Bacteroides xylanisolvens* DSM 23964 products

Microbiological specifications of the fermented milk product

Specification Parameter	Specification	Analysis Method
Aerobic mesophile bacteria (cfu/g)	< 330	ASU L 00.00-88/PCA/30°C/72h
Yeast and molds (cfu/g)	< 10	ASU L 01.00-37/YGC/25°C/72-120h
E. coli (cfu/g)	< 10	ISO 16649-2/TBX/44°C/24h
Coagulase (+) Staphylococcus spp. (cfu/g)	< 10	ASU L 00.00-55/mod. BP/37°C/48h
Presumptive Bacillus cereus (cfu/g)	< 10	ASU L 00.00-

[1] *Bacteroides gingivalis* and *B. asaccharolyticus* for which a cytotoxic activity has been reported, are now re-classified as *Porphorymonas gingivalis* and *asaccharolyticus*, respectively (Pissiotis et al., 2007; van Steenbergen et al., 1982).

		25/PEMBA/37°C/48h
Clostridium perfringens (cfu/g)	< 10	ASU L 00.00-57/TSC/37°C/24h
Salmonella spp. (25 g)	Absent	ASU L 00.00-20
Listeria monocytogenes (cfu/g)	< 100	ASU L 00.00-22
cfu = colony-forming units		

(2) Information regarding any self-limiting levels of use

None.

(3) Probable consumption of pasteurized fermented low-fat and skim milk products manufactured with *Bacteroides xylanisolvens* DSM 23964

Estimates of the potential intake of non-fat milk solids, which would result from the consumption of fermented milk products produced with *B. xylanisolvens* DSM 23964 as starter culture, were calculated using US food consumption data. Applying a "worst case" approach, it was assumed that low-fat or non-fat milk cultured with *B. xylanisolvens* DSM 23964 would substitute for all yogurt, buttermilk and acidophilus milk that is currently consumed, be it as such, *i.e.* in semi-liquid or frozen form, or as a spray-dried powder, *i.e.* as an ingredient of certain processed foods.

The results of these intake calculations reveal a current mean and 90th percentile intake of non-fat, milk-derived solids of 5.4 and 15.3 g/d, respectively, for users of all fermented milk products combined. On a per kg bodyweight basis and on average of all age groups, the mean and 90th percentile user of such products consumes 0.104 and 0.288 g/kg bw/d, respectively, of non-fat milk derived solids. The highest intake occurs in 2 – 5 year old children with a milk solids intake from all fermented milk products combined of 0.383 and 0.964 g/kg bw/d for the mean and 90th percentile user, respectively.

A standard plain low-fat or non-fat milk yogurt contains about 14 g nonfat milk solids per 100 g of the food as consumed. Cultured low-fat milk produced with *B. xylanisolvens* DSM 23964 contains about 0.5 x 10^{12} heat-inactivated cells per 100 ml. In terms of bacterial cell weight (lyophilized), this cell number corresponds to about 150-300 mg.[2] In other words, 14 g non-fat milk solids contain about 150-300 mg *B. xylanisolvens* DSM 23964. It follows from these figures that the mean and 90th percentile consumer of yogurt (*i.e.*, users only, age ≥2 years) who consumes the *B. xylanisolvens* DSM 23964 fermented milk product, ingests about 11.4 and 22.5 g/d milk solids and about 180 – 360 mg heat inactivated *B. xylanisolvens* DSM 23964.

[2] Values of 1.7 x 10^{12} cells/g and 3.5 x 10^{12} cells/g lyophilized biomass have been reported for *B. xylanisolvens* DSM 23964 (Leuscher, 2010c at p. 16 – 17). For comparison, a value of 4.3 ± 1.2 x 10^{12} cells/g dry weight has been reported for *E. coli* (Holme, 1957).

In comparison to yogurt, the daily intake of non-fat milk solids with buttermilk and acidophilus milk is more than 15 times lower for both the mean and 90th percentile consumer and each age group. The yogurt consumer is, therefore, the consumer with the potentially highest intake of *B. xylanisolvens* DSM 23964.

The estimated intake of *B. xylanisolvens* DSM 23964 cells from its intended use in low-fat and non-fat yogurt is shown for the mean consumer, per age group, is shown below. While the intake of *B. xylanisolvens* DSM 23964 is about the same in the different age groups (4×10^{11} cells/d), its intake per kg bodyweight is highest in 2-5 year old children (2×10^{10} cells/kg bw/d).[3]

Estimated intake of non-fat milk solids and heat-inactivated *B. xylanisolvens* DSM 23964 in the mean user of yogurt, per age group

	Non-fat milk solids		*B. xylanisolvens* DSM 23964	
Age group, gender	g/d	mg/kg bw/d	cells/d[1)]	cells/kg bw/d
2 - 5 y	9.5	570	3.4×10^{11}	2.0×10^{10}
6 - 10 y	9.6	357	3.4×10^{11}	1.3×10^{10}
11 - 19 y, m	12.7	225	4.5×10^{11}	0.80×10^{10}
11 - 19 y, f	11.0	190	3.9×10^{11}	0.68×10^{10}
> 20 y, m	12.1	147	4.3×10^{11}	0.52×10^{10}
> 20 y, f	11.7	171	4.2×10^{11}	0.61×10^{10}
all	11.4	225	4.1×10^{11}	0.80×10^{10}

[1)] Calculated on basis of the assumption that 100 ml pasteurized fermented low-fat or non-fat milk product contains 14 g non-fat milk solids and 0.5×10^{12} *B. xylanisolvens* DSM 23964 cells

(4) Detailed summary of the basis for the notifier's determination that a particular use of the notified substance is exempt from the premarket approval requirements of the Federal Food, Drug and Cosmetic Act because such use is GRAS.

[3] It may be noted, for comparison, that the density of *Bacteroides* in human stool is on average 5×10^{11} CFU/g dry weight (Finegold et al., 1983).

A substantial body of evidence supports the safety of pasteurized milk products that have been obtained by fermentation with *B. xylanisolvens* DSM 23964 as follows:

B. xylanisolvens accounts for a significant proportion of all Bacteroides occurring in human feces (Qin et al., 2010). *B. xylanisolvens* DSM 23964 has been isolated from the feces of a healthy human. Despite the wide occurrence of *B. xylanisolvens* in feces, there are no reports associating this species with human (or animal) disease.

Examinations of *B. xylanisolvens* DSM 23964 for virulence plasmids and potential virulence determinants known to occur in certain *Bacteroides* species, such as the *bft* enterotoxin and the polysaccharide PS A, showed absence in this strain (Ulsemer, 2012a). The absence of pathogenicity was confirmed in an abscess formation test in mice (Ulsemer, 2012b).

Heat-inactivated and not heat-inactivated *B. xylanisolvens* DSM 23964 did not exert a mutagenic effect in standard Ames tests and were not clastogenic in human peripheral lymphocytes with and without metabolic activation (Ulsemer, 2012b). No signs of cytotoxicity were observed in the clastogenicity test of heat-inactivated and not heat-inactivated *B. xylanisolvens* DSM 23964 with peripheral human lymphocytes (Ulsemer, 2012b).

The results of a 90-day oral toxicity test in mice also demonstrates that *B. xylanisolvens* DSM 23964 is not toxigenic. Daily intakes of 2.8×10^{12} and 3.6×10^{12} not heat-inactivated and heat-inactivated bacteria per kg body weight, i.e. the highest dose levels tested, were found to be NOAELs in male and female mice, respectively (Ulsemer, 2012b).

The human tolerance of pasteurized fermented low-fat milk has been demonstrated in a 3-week placebo-controlled human study. In the high dose group, which consumed 1×10^{12} cells/day with the fermented milk product, no adverse effects on the analyzed clinical chemical parameters were observed and there were no subjective side-effects that could be attributed to the treatment (Ulsemer, 2012c).

Recently, a specially-convened panel of independent experts (the "Expert Panel"), qualified by scientific training and experience to evaluate the safety of food ingredients, independently and collectively critically evaluated the published and unpublished scientific information and data deemed appropriate or pertinent to the safety of pasteurized milk products fermented with *B. xylanisolvens* DSM 23964 under the conditions of intended use in food. The Expert Panel also evaluated data and information concerning the method of manufacturing of the *B. xylanisolvens* starter culture, the conditions of fermented and the pasteurization of the final fermented milk product, the

product specifications and analytical data, and the specific conditions of intended use of the pasteurized fermented milk product in foods.

The Expert Panel unanimously concluded that under the conditions of intended use in foods, the pasteurized milk product fermented with *B. xylanisolvens* DSM 23964 meeting appropriate food grade specifications and manufactured in accordance with current good manufacturing practice, is "generally recognized as safe" (GRAS) based on scientific procedures. A copy of the Expert Panel's GRAS opinion is provided in Appendix 1.

(5) References

Brook I. (1989). Pathogenicity of the *Bacteroides fragilis* group. Ann. Clin. Lab. Sci. 19(5): 360-376.

Finegold S.M., Sutter V.L. and Mathisen G.E. (1983). Normal indigenous intestinal flora. In: Human Intestinal Microflora in Health and Disease, ed. Hentges D.J., Academic Press, New York, pp. 3-32.

Hamady Z.Z.R., Scott N., Farrar M.D., Holland K.T., Whitehead T. and Carding S.R. (2010). Xylan-regulated delivery of human keratinocyte growth factor-2 to the inflamed colon by the human anaerobic commensal bacterium *Bacteroides ovatus*. Gut 59: 461-469.

Holme T. (1957). Continuous culture studies on glycogen synthesis in *Escherichia coli B*. Acta Chem. Scand. 11: 1225-1228.

Hudcovic T., Kozáková H., Kolinská J., Stepánková R., Hrncir T and Tlaskalová-Hogenová H. (2009). Monocolonization with *Bacteroides ovatus* protects immunodeficient SCID mice from mortality in chronic intestinal inflammation caused by long-lasting dextran sodium sulfate treatment. Physiol. Res. 58: 101-110.

Kulagina E.V., Efimov B.A., Maximox P.Y., Kafarskaia L.I., Chaplin A.V. and Shkoporov A.N. (2012). Species composition of *Bacteroidales* order bacteria in the feces of healthy people of various ages. Biosci. Biotechnol. Biochem. 76(1): 169-171.

Marschal M., Schumacher U. and Autenrieth I. (2011). Improvement of identification of anaerobes by MALDI-TOF mass spectrometry analysis. 21st European Congress of Clinical Microbiology and Infectious Diseases (ECCMID) 27th International Congress of Chemotherapy (ICC). 09 May 2011.

Pissiotis E. and Spngberg L.S.W. (1991). Toxicity of sonicated extracts of *Bacteroides gingivalis* on human pulpal cells and L929 cells in vitro. J. Endodon. 17(11): 553-560.

Ponnusamy K., Choi J.N., Kim J., Lee S.Y. and Lee C.H. (2011). Microbial community and metabolomic comparison of irritable bowel syndrome faeces. J. Med. Microbiol. 60: 817-827.

Qin J., Li R. et al., (2010). A human gut microbial gene catalogue established by metagenomic sequencing. Nature 464: 59-65.

Salyers A.A. (1984). Bacteroides of the human lower intestinal tract. Ann. Rev. Microbiol. 38: 293-313.

Sánchez E., Laparra J.M. and Sanz Y. (2012). Discerning the role of *Bacteroides fragilis* in celiac disease pathogenesis. Am. Soc. Microbiol. Published online ahead of print on 6 July 2012 doi:10.1128/AEM.00563-12.

Sears C. L. (2001). The toxins of *Bacteroides fragilis*. Toxicon 39: 1737-1746.

Ulsemer P. Toutounian K., Schmidt J., Karsten U. and Goletz S. (2012a). Preliminary safety evaluation of a new *Bacteroides xylanisolvens* isolate. Appl. Environ. Microbiol. 78(2): 528-535.

Ulsemer P., Toutounian K., Schmidt J., Leuschner J., Karsten U. and Goletz S. (2012b). Safety assessment of the commensal strain *Bacteroides xylanisolvens* DSM 23964. Regul. Toxicol. Pharmacol. 62: 336-346.

Ulsemer P., Toutounian K., Kressel G., Schmidt J., Karsten U., Hahn A. and Goletz S. (2012c). Safety and tolerance of *Bacteroides xylanisolvens* DSM 23964 in healthy adults. Beneficial Microbes 3(2): 99-111.

Van Belkum A., Leszczynski P., Vogel M., Verbrugh H. and Meisel-Mikolajczyk F. (1999). An archival case of clinically significant infection in a surgical patient by enterotoxigenic *Bacteroides fragilis*: PCR amplification of the metalloprotease enterotoxin gene from non-viable lyophilizates. Clin. Microbiol. Infect. 5: 117-118.

Van Steenbergen T.J.M., den Ouden M.D., Touw J.J.A. and de Graaff J. (1982). Cytotoxic activity of *Bacteroides gingivalis* and *Bacteroides asaccharolyticus*. J. Med. Microbiol. 5: 253-258.

Wexler H.M. (2007). *Bacteroides*: the good, the bad, and the nitty-gritty. Clin. Microbiol. Rev. 20(4): 593-621.

Expert Panel Consensus Statement concerning the "Generally Recognized as Safe" (GRAS) status of pasteurized fermented low-fat milk and skim milk products manufactured with *Bacteroides xylanisolvens* DSM 23964

Prepared for:

Avitop GmbH
Robert Rössle Str. 10
D-13125 Berlin
Germany

Date: October 22, 2012

Introduction

As members of a panel of independent experts qualified by national and international training and experience to evaluate the safety of food and food ingredients (the "Expert Panel"), we were asked by Avitop GmbH ("Avitop") to determine whether the proposed uses of pasteurized fermented low-fat milk and skim milk products produced with *Bacteroides xylanisolvens* DSM 23964 as a starter culture are "generally recognized as safe" ("GRAS"), based on scientific procedures. For purposes of our evaluation, "safe" or "safety" means that there is a reasonable certainty that the product is not harmful under the intended conditions of use, as stated in 21 C.F.R. 170.3(i). *Curricula vitae* evidencing our expert qualifications for evaluating the safety of food ingredients are available upon request.

We independently and collectively critically evaluated published data on the identity and characteristic properties of *B. xylanisolvens*, specifically *B. xylanisolvens* DSM 23964, as well as the safety of fermented products produced with the use of *B. xylanisolvens* DSM 23964 as a starter culture at levels of exposure corresponding to those of traditional fermented low-fat and skim milk products. In addition, we evaluated other information that the Expert Panel deemed appropriate, necessary or pertinent to safety, including information about the production and use of *B. xylanisolvens* DSM 23964 as starter culture as well as data on the nutritional equivalence of spray-dried skim milk cultured with *B. xylanisolvens* DSM 23964 and regular spray-dried fermented milk products.

Our summary and conclusions resulting from this critical review are presented below.

Summary

- *B. xylanisolvens* DSM 23964 is a gram-negative, anaerobic and non-spore forming commensal of the human intestinal microbiota. Like other *Bacteroides*, it is unable to degrade starch but it degrades xylane to acetate, propionate and succinate. The *B. xylanisolvens* strain DSM 23964 that is the subject of this GRAS evaluation was isolated from the feces of a healthy human subject. This strain has been found to be suitable for the use as starter culture in the production of fermented milk products.

- The classification of the microorganism *B. xylanisolvens* is based on the examination of its phenotypical and biochemical properties by sequencing its 16S rRNA genes and by DNA-DNA hybridization with *B. xylanisolvens* DSM 18836 and closely

related *Bacteroides* species. The classification has been confirmed by the German Resource Center for Biological Materials (DSMZ) where the strain is deposited under number DSM 23964.

- The production of fermented milk products with *B. xylanisolvens* DSM 23964 is conducted under anaerobic conditions following current good manufacturing practice (cGMP). Food-grade glucose may be added during the fermentation process to stimulate the growth of *B. xylanisolvens*. The fermented milk is pasteurized at the end of the fermentation process. Appropriate in-process quality control measures are in place. The quality control of the end product includes standard tests for microbiological purity as well as a test for viable *B. xylanisolvens*.

  The pasteurized fermented milk product may then be processed and packed for direct consumption as a traditional liquid fermented milk product or spray-dried for use as a fermented milk powder. The fermented milk powder product would be used by food processors in the same manner as a yogurt powder.

- The potential intake of non-fat milk solids that would result from the consumption of fermented milk products produced with *B. xylanisolvens* DSM 23964 was estimated using United States food consumption data. It was assumed that low-fat or non-fat milk cultured with *B. xylanisolvens* DSM 23964 would substitute for all yogurt, buttermilk and acidophilus milk products that are currently consumed in the United States in semi-liquid or frozen form, or as spray-dried powder in certain processed foods (see attached Food Codes List).

  The mean and 90th percentile intakes of non-fat, milk-derived solids for users of all fermented milk products combined is 5.4 g/d and 15.3 g/d, corresponding to 0.104 g/kg bw/day and 0.228 g/kg bw/d, respectively. The highest intake occurs in 2 - 5 year old children with a milk solids intake from all fermented milk products combined of 0.383 g/kg bw/d and 0.964 g/kg bw/d for the mean and 90th percentile user, respectively.

- The estimated daily intake of heat-inactivated *B. xylanisolvens* DSM 23964 cells from its intended use in low-fat and non-fat yogurt-like fermented milk products is similar in the different age groups (4×10^{11} cells/d) but, if expressed per kg bodyweight, it is highest in 2 - 5 year old children (2×10^{10} cells/kg bw/d).

- The macro- and micro-nutrient content of spray-dried pasteurized skim milk fermented with *B. xylanisolvens* DSM 23964 is substantially equivalent to that of regular spray-dried fermented milk products.

- There are no reports which would associate this species with human (or animal) disease.

  Virulence plasmids and potential virulence determinants known to occur in certain *Bacteroides* species, such as the *bft* enterotoxin and the polysaccharide PS A, are absent in *B. xylanisolvens* DSM 23964. In an abscess formation test in mice, *B. xylanisolvens* DSM 23964 was found to be non-pathogenic.

  B. xylanisolvens DSM 23964 and heat-inactivated *B. xylanisolvens* DSM 23964 did not exert a mutagenic effect in standard Ames tests and were not clastogenic in human peripheral lymphocytes with and without metabolic activation.

  No signs of cytotoxycity were observed in a clastogenicity test of *B. xylanisolvens* DSM 23964 and heat-inactivated *B. xylanisolvens* DSM 23964 with peripheral human lymphocytes.

  In a 90-day oral toxicity test in mice, *B. xylanisolvens* DSM 23964 was not toxigenic. Daily intakes of 2.8×10^{12} *B. xylanisolvens* DSM 23964 bacteria and 3.6×10^{12} heat-inactivated *B. xylanisolvens* DSM 23964 bacteria per kg body weight, i.e. the highest doses tested, were found to represent the "no observed adverse effect levels" ("NOAELs").

  In a 3-week placebo-controlled human tolerance study the high dose group consumed pasteurized fermented low-fat milk with 1×10^{12} *B. xylanisolvens* DSM 23964 cells/day without adverse effects on the analyzed clinical chemical parameters. There were no subjective treatment-related side-effects reported.

Conclusion

We, the undersigned expert panel members, are qualified by relevant national and international scientific education and experience to evaluate the safety of foods and food ingredients, including foods obtained by fermentation. We individually and collectively critically evaluated a comprehensive package of publicly available scientific materials compiled from the literature and other public sources by Albert Bär, Ph.D. and summarized above. We also considered any additional data and information that we deemed pertinent to safety of the intended food uses of the pasteurized fermented low-fat milk and skim milk products produced with *Bacteroides xylanisolvens* DSM 23964. Following independent, critical evaluation of the data and information, we convened as an Expert Panel on August 1, 2012 *via* teleconference.

We recognized that *B. xylanisolvens* is a regular commensal microorganism of the human fecal microbiota and that there are no reports which would associate this microorganism with human or animal disease.

We concluded that the *B. xylanisolvens* strain DSM 23964 has been adequately identified and characterized. The available phenotypic and genotypic data do not raise concerns regarding the safety of ingestion of this bacterium. Direct evidence of the safety of pasteurized fermented milk products with *B. xylanisolvens* DSM 23964 is provided by genotoxicity tests of *B. xylanisolvens* DSM 23964 cells and heat-heat-inactivated *B. xylanisolvens* DSM 23964 cells, a standard 90-day toxicity test with suspensions of *B. xylanisolvens* DSM 23964 cells and heat-inactivated *B. xylanisolvens* DSM 23964 cells in mice, as well as a human tolerance study with ingestion of pasteurized, spray-dried low-fat milk cultured with *B. xylanisolvens* DSM 23964 at doses of up to 1×10^{12} cells/day for a period of six weeks.

Basis for GRAS Determination

Therefore, we unanimously conclude that the food uses of pasteurized low-fat and skim milk products fermented with *B. xylanisolvens* DSM 23964 are safe. We further unanimously concluded that the pasteurized low-fat milk and skim milk products fermented with *B. xylanisolvens* DSM 23964 are Generally Recognized As Safe (GRAS) based on scientific procedures.

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

(b) (6)

Signature [redacted] Date: 12 November 2012

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

Signature: _____ Date: _____

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

Signature: _____ Date: _____

Michael W. Pariza, Ph.D.
Director Emeritus, Food Research Institute
Emeritus Professor, Food Science
University of Wisconsin-Madison
Madison, Wisconsin

Signature: _____ Date: _____

Gary M. Williams, M.D.
Professor of Pathology
Environmental Pathology & Toxicology
New York Medical College

Basis for GRAS Determination

Therefore, we unanimously conclude that the food uses of pasteurized low-fat and skim milk products fermented with *B. xylanisolvens* DSM 23964 are safe. We further unanimously concluded that the pasteurized low-fat milk and skim milk products fermented with *B. xylanisolvens* DSM 23964 are Generally Recognized As Safe (GRAS) based on scientific procedures.

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

Signature: _____ Date: _____

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

(b) (6)

Signature [redacted] Date: Nov. 12, 2012

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

Signature: _____ Date: _____

Michael W. Pariza, Ph.D.
Director Emeritus, Food Research Institute
Emeritus Professor, Food Science
University of Wisconsin-Madison
Madison, Wisconsin

Signature: _____ Date: _____

Gary M. Williams, M.D.
Professor of Pathology
Environmental Pathology & Toxicology
New York Medical College

Basis for GRAS Determination

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Signature: _____ Date: _____

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

Signature: _____ Date: _____

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

(b) (6)

Signature: _____ Date: 14 November 2012

Michael W. Pariza, Ph.D.
Director Emeritus, Food Research Institute
Emeritus Professor, Food Science
University of Wisconsin-Madison
Madison, Wisconsin

Signature: _____ Date: _____

Gary M. Williams, M.D.
Professor of Pathology
Environmental Pathology & Toxicology
New York Medical College

Basis for GRAS Determination

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

Signature: _____ Date: _____

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

Signature: _____ Date: _____

Walter H. Glinsmann, M.D.
President
Glinsmann Inc.
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Signature: _____ Date: _____

Michael W. Pariza, Ph.D.
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(b) (6)

Signature: [redacted] Date: 20 November 2012

Gary M. Williams, M.D.
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SUBMISSION END