GR

ORIGINAL SUBMISSION

000008

OLSSON FRANK WEEDA
TERMAN BODE MATZ PC
ATTORNEYS AT LAW

SUITE 400
1400 SIXTEENTH STREET, N.W.
WASHINGTON, D. C. 20036 (202) 789-1212
www.ofwlaw.com

PHILIP C. OLSSON
RICHARD L. FRANK
DAVID F. WEEDA (1948-2001)
DENNIS R. JOHNSON
ARTHUR Y. TSIEN
JOHN W. BODE*
STEPHEN D. TERMAN
MARSHALL L. MATZ
MICHAEL J. O'FLAHERTY
DAVID L. DURKIN
NEIL F. O'FLAHERTY
BRETT T. SCHWEMER
STEPHANIE HERSETH SANDLIN*
TISH E. PAHL
ROBERT A. HAHN
EVAN P. PHELPS
GARY H. BAISE
KATHRYN E. BALMFORD
FREDERICK H. BRANDING*

*PRACTICE WITHIN THE DISTRICT OF COLUMBIA IS LIMITED TO MATTERS AND PROCEEDINGS BEFORE FEDERAL COURTS AND AGENCIES

BRUCE A. SILVERGLADE
JOLYDA O. SWAIM
JONATHAN M. WEINRIEB
NANCY W. MATHEWSON

COUNSEL
ROGER R. SZEMRAJ
ANSON M. KELLER
CASPER E. ULDRIKS*

OF COUNSEL
KENNETH D. ACKERMAN
MARK L. ITZKOFF
ELLIOT BELILOS

SENIOR POLICY ADVISOR
JOHN R. BLOCK
CHARLES W. STENHOLM
GEORGE McGOVERN
SALLY S. DONNER
BRENT W. GATTIS
BARBARA J. MASTERS

Mark L. Itzkoff - Of Counsel
Direct (202) 518-6327 / Fax (202) 234-2686
mitzkoff@ofwlaw.com

April 13, 2011

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

RECEIVED
APR 14 2011
BY: (b) (6)

Re: GRAS Notification for Food Ferment Solutions

Dear Sirs:

On behalf of our client, PURAC, we are hereby submitting the enclosed GRAS Notification for Food Ferment Solutions to be used as a food ingredient when used in the applications and under the conditions of use described herein. In compliance with 21 C.F.R. §170.36 (b) (proposed), we are enclosing an original and two copies of this notice.

Should you have any questions regarding this Notice, please do not hesitate to contact me.

Sincerely,

(b) (6)

Mark L. Itzkoff

Attachment
OFW:emp

GRAS Exemption Claim for Food Ferment Solutions

Summary of Data Concerning the Safety and GRAS Determination of Food Ferment Solutions for Use as a Food Ingredient

Submitted to:

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

Prepared by:

Purac
Arkelsedijk 46
P.O. Box 21
Gorinchem, 4200 AA
The Netherlands

April 12, 2011

GRAS Exemption Claim for Food Ferment Solutions

Table of Contents

Page

I. GRAS EXEMPTION CLAIM 4
- I.A Claim of Exemption from the Requirement for Premarket Approval Pursuant to Proposed 21 CFR §170.36(c)(1) [62 FR 18938 (17 April 1997)] (U.S. FDA, 1997) 4
- I.B Name and Address of Notifier 4
- I.C Common Name of the Notified Substance 5
- I.D Conditions of Intended Use in Food and Consumer Exposure 5
  - I.D.1 Current Regulatory Status of Food Ferment Solutions Components and Similar Products 5
  - I.D.2 Intended Use of Food Ferment Solutions and Levels of Use in Foods 8
  - I.D.3 Estimated Dietary Consumption of Food Ferment Solutions Components Based upon Intended Food Uses 12
- I.E Basis for the GRAS Determination 13
- I.F Availability of Information 14

II. DETAILED INFORMATION ABOUT THE IDENTITY OF THE SUBSTANCE 14
- II.A Identity 14
  - II.A.1 Product Identity 14
- II.B Method of Manufacture 15
- II.C Specifications for Food Grade Material 17
  - II.C.1 Product Specifications and Analysis 17
  - II.C.2 Stability 20
  - II.C.3 Additional Characterization 20

III. SELF-LIMITING LEVELS OF USE 21

IV. BASIS FOR GRAS DETERMINATION 21
- IV.A Introduction 21
- IV.B Metabolic Fate of Individual Components 21
- IV.C Safety Aspects of Individual Components 22
  - IV.C.1 Sodium 22
  - IV.C.2 Potassium 23
  - IV.C.3 Calcium 23
  - IV.C.4 Lactate 24
  - IV.C.5 Acetate and Propionate 25
  - IV.C.6 Sugars and Proteins 25
  - IV.C.7 Succinic Acid 26
- IV.D Fermentation Microorganisms 26
  - IV.D.1 *Streptococcus thermophilus* 27
  - IV.D.2 *Bacillus coagulans* 27
  - IV.D.3 *Lactobacillus acidophilus* 27

IV.D.4 *Lactobacillus paracasei* subsp. *paracasei* 27
IV.D.5 *Lactobacillus plantarum* 28
IV.D.6 *Lactobacillus sakei* 28
IV.D.7 *Lactobacillus bulgaricus* 28
IV.D.8 *Propionibacterium freudenreichii* subsp. *shermanii* 28
IV.E Additional Considerations 29
IV.F Summary and Basis for GRAS Conclusion 30

V. REFERENCES 32

Appendix I Expert Panel Consensus Statement Concerning the Generally Recognized as Safe (GRAS) Status of Food Ferment Solutions for the Proposed Food Uses

List of Figures and Tables

Figure II.B-1	Schematic Overview of the Manufacturing Process for Food Ferment Solutions	16
Table I.D.2-1	Summary of the Individual Proposed Food-Uses and Maximum Use-Levels for Individual Components of Food Ferment Solutions in the U.S.	9
Table II.B-1	List of Processing Aids Used in the Manufacture of Food Ferment Solutions	17
Table II.C.1-1	Product Specifications for Food Ferment Solutions	17
Table II.C.1-2	Microbiological Specifications for Food Ferment Solutions	18
Table II.C.1-3	Summary of the Product Analysis for 3 Lots of Food Ferment Solutions (Powder)	19
Table II.C.1-4	Summary of the Product Analysis for 3 Lots of Food Ferment Solutions (Liquid)	19
Table II.C.1-5	Summary of the Microbiological Product Analysis for 3 Lots of Food Ferment Solutions (Liquid) (CFU/g)	20
Table II.C.1-6	Summary of the Microbiological Product Analysis for 3 Lots of Food Ferment Solutions (Powder) (CFU/g)	20

GRAS Exemption Claim for Food Ferment Solutions

I. GRAS EXEMPTION CLAIM

I.A Claim of Exemption from the Requirement for Premarket Approval Pursuant to Proposed 21 CFR §170.36(c)(1) [62 FR 18938 (17 April 1997)] (U.S. FDA, 1997)

Purac has determined that Food Ferment Solutions are Generally Recognized as Safe (GRAS), consistent with Section 201(s) of the *Federal Food, Drug, and Cosmetic Act* (U.S. FDA, 2010a) when used in the applications and under the conditions of use described herein. This determination is based on scientific procedures as described in the following sections, and the views of experts who are qualified by scientific training and experience to evaluate the safety of Food Ferment Solutions as a component of food [see Appendix I, **Expert Panel Consensus Statement Concerning the Generally Recognized as Safe (GRAS) Status of Food Ferment Solutions for the Proposed Food Uses**]. Therefore, the use of Food Ferment Solutions in food as described below is exempt from the requirement of premarket approval.

Signed,

(b) (6)

4/12/11

Name Mr. Ton van Dongen
Title Global Regulatory Manager
Purac

Date: April 12, 2011

I.B Name and Address of Notifier

Name Mr. Ton van Dongen
Title Global Regulatory Manager

Address Purac
Arkelsedijk 46
4206 AC Gorinchem
The Netherlands
Telephone: +31 183 695 730
Facsimile: +31 183 695 607
Email: t.van.dongen@purac.com

REGISTERED UNDER NR. 23036930 CHAMBER OF COMMERCE
purac
pure by nature
Purac Biochem
P.O. Box 21
4200 AA Gorinchem
The Netherlands
GORINCHEM

I.C Common Name of the Notified Substance

The common name of the notified substance is "cultured _____" where the blank is replaced by the name of the natural substrate (dairy sources, sugars, wheat, malt, and fruit- and vegetable-based sources) used in fermentation. The final product is a blend of sodium, potassium and/or calcium salts of lactate, acetate and propionate.

I.D Conditions of Intended Use in Food and Consumer Exposure

I.D.1 Current Regulatory Status of Food Ferment Solutions Components and Similar Products

Food Ferment Solutions consist of blends of organic acids (lactate, acetate and propionate) and their sodium, potassium and calcium salts, as well as sugars, protein, and succinic acid.

I.D.1.1 Lactic Acid and Its Salts

Sodium lactate and potassium lactate are permitted in the U.S. for the following uses:

- As an antimicrobial agent in various meat and meat food products, poultry and poultry food products, except infant formula and infant food, at levels not to exceed 4.8% of the total formulation (9 CFR 424) (U.S. FSIS, 2010);
- As a flavoring agent[1] in various meat and meat food products, poultry and poultry food products, except infant formula and infant food, at levels not to exceed 2% of the total formulation (9 CFR 424) (U.S. FSIS, 2010); and
- As a direct food substance to be used in food as an emulsifier, a flavor enhancer, a flavoring agent or adjuvant, a humectant, and a pH control agent with no limitation other than current Good Manufacturing Practices (cGMP) (21 CFR §184.1768 and 21 CFR §184.1639) (U.S. FDA, 2010b).

In the U.S., calcium lactate is permitted for use as a direct food substance to be used in food as a firming agent, a flavor enhancer, a flavoring agent or adjuvant, a leavening agent, a nutrient supplement, and a stabilizer and thickener with no limitation other than cGMP (21 CFR §184.1207) (U.S. FDA, 2010b).

Lactic acid is affirmed as GRAS as an antimicrobial agent, curing and pickling agent, flavor enhancer, flavoring agent and adjuvant, pH control agent, and as a solvent and vehicle, with no limitation other than cGMP (21 CFR §184.1061) (U.S. FDA, 2010b).

---

[1] As flavoring agents, sodium lactate and potassium lactate are to be used in accordance with the regulations listed in 21 CFR 184.1768 and 21 CFR 184.1639, respectively (U.S. FDA, 2010b).

I.D.1.2 Acetic Acid and Its Salts

Vinegar (primarily containing acetic acid) is considered GRAS in the U.S. when used in accordance with good manufacturing practices (GMP) as it is a common food ingredient in the diet (21 CFR 182.1). Sodium diacetate is approved in the U.S. for use as an antimicrobial in various meat and poultry products at levels not exceeding 0.25% of the total formulation (9 CFR 424) (U.S. FSIS, 2010). Also, sodium acetate is approved in the U.S. for use as a flavoring agent for various meat and poultry products, except infant formulas and infant foods, at levels not exceeding 0.25% in accordance with 21 CFR 184.1721 (9 CFR 424) (U.S. FDA, 2010b; U.S. FSIS, 2010). Acetic acid, produced by fermentation of carbohydrates or by organic synthesis, or produced synthetically *via* oxidation of acetaldehyde or butane, is approved as a direct food substance affirmed as GRAS (21 CFR184.1005) (U.S. FDA, 2010b). It may be used as a curing and pickling agent, a flavoring agent and adjuvant, a pH control agent, a solvent and vehicle additive, and a boiler water additive at levels not to exceed cGMP, resulting in maximal levels of 0.6% of acetic acid in meat products, specifically.

Furthermore, according to the U.S. Department of Agriculture (USDA), potassium acetate is approved as a flavoring agent for various meat and poultry products at levels not exceeding 1.2% of the product formulation (USDA, 2007). Sodium acetate, when used in a mixture with salt, lemon extract, and grapefruit extract, is approved as an antimicrobial agent for ground beef and cooked, cured, or comminuted sausages. The level of this mixture is not to exceed 0.5% of the product formulation (USDA, 2007).

Calcium acetate has been affirmed to be GRAS when used in accordance with GMP, at a maximum level, as served, of 0.2% for baked goods, 0.02% for cheese, 0.2% for gelatins, puddings, and fillings, 0.15% for sweet sauces, toppings, and syrups and 0.0001% for all other food categories (21 CFR 184.1185) (U.S. FDA, 2010b).

I.D.1.3 Propionic Acid and Its Salts

Propionic acid, prepared commercially by chemical synthesis or bacterial fermentation, is affirmed as GRAS (21 CFR 184.1081) (U.S. FDA, 2010b). It may be used in food as an antimicrobial agent or as a flavoring agent with no limitation other than cGMP. Furthermore, calcium propionate is approved in the U.S. as an agent to retard mould growth in pizza crust at levels of 0.32% alone, or in combination based on weight of the flour base used (9 CFR 424) (U.S. FSIS, 2010). Also, calcium propionate as a mould growth inhibitor in fresh pie dough (poultry only) is allowable for use in the U.S. at levels of 0.3% of calcium propionate or sodium propionate alone, or in combination, based on the weight of the flour used. Sodium propionate is deemed to be GRAS for use as an antimicrobial agent in baked goods, nonalcoholic beverages, cheeses, confections and frostings, gelatins, puddings, and fillings, jams and jellies, meat products, and soft candy with no limitations other than current good manufacturing practice (21 CFR 184.1784) (U.S. FDA, 2010b).

I.D.1.4 Sugars

The sugars present in Food Ferment Solutions are sucrose, glucose, fructose, lactose, and galactose). Sucrose is permitted as a direct food substance affirmed as GRAS with no limitations other than cGMP (21 CFR 184.1854) (U.S. FDA, 2010b). Glucose as corn sugar is affirmed to be GRAS with no limitations other than cGMP (21 CFR 184.1857) (U.S. FDA, 2010b). High fructose corn syrup, which is up to 55% fructose, also is affirmed to be GRAS when used as a direct food ingredient with no limitations other than cGMP (21 CFR 184.1866) (U.S. FDA, 2010b). Galactose is one of the dominant monosaccharides present in arabinogalactan, furcelleran, and carrageenan, all of which are food additives permitted for direct addition to food for human consumption with no limitations other than cGMP (21 CFR 172.610, 172.655, 172.620, respectively) (U.S. FDA, 2010b).

I.D.1.5 Proteins

The proteins present in Food Ferment Solutions result from the addition of yeast extract (from bakers yeast) and substrate (*e.g.*, dairy proteins) to the fermentation media. Bakers yeast extract is GRAS for use as a flavoring agent and adjuvant at a level not to exceed 5% in food (21 CFR 184.1983) (U.S. FDA, 2010b).

I.D.1.6 Succinic Acid

Succinic acid is affirmed to be GRAS for use in food in the U.S. with no limitation other than cGMP [cGMP results in a maximum level, as served, of 0.084% in condiments and relishes and 0.0061% in meat products (21 CFR § 184.1091)]. The carboxylate anions of succinic acid (diethyl succinate and dimethyl succinate) are permitted for use as synthetic flavoring substances and adjuvants added to food for direct consumption with no restrictions other than cGMP (21 CFR § 172.515).

I.D.1.7 Corn, Cane, or Beet Sugar Cultured with Bacillus coagulans, Lactobacillus paracasei subsp. paracasei and Propionibacterium freudenreichii subsp. shermanii

The U.S. Food and Drug Administration (FDA) had no questions in response to a GRAS Notice for the production of corn, cane, or beet sugar cultured with *B. coagulans*, *L. paracasei* subsp. *paracasei* and *P. freudenreichii* subsp. *shermanii* (GRN 000240 – U.S. FDA, 2008). This product, also manufactured by Purac, is similar to Food Ferment Solutions in that it is intended for use as a flavoring agent and antimicrobial agent in food products (meat and poultry products), it contains a blend of sodium or potassium organic salts (primarily lactate) and sugars, it is produced *via* fermentation using microorganisms and food-based substrates that also are used in the production of Food Ferment Solutions, and it provides a similarly broad specification range.

I.D.2 Intended Use of Food Ferment Solutions and Levels of Use in Foods

Purac intends to market Food Ferment Solutions in the U.S. in selected food categories, including baked goods and baking mixes, beverages and beverage bases (carbonated and non-carbonated), breakfast cereals, cheeses, coffee and tea, condiments and relishes, fats and oils, fish products (excluding catfish), frozen dairy desserts and mixes, gelatins, puddings, and fillings, grain products and pastas, gravies and sauces, meat products, milk products, plant protein products, poultry products, processed fruits and fruit juices, processed vegetables and vegetable juices, soups and soup mixes, and sweet sauces, toppings, and syrups. Food Ferment Solutions are not intended for use in infant formula or foods. Food Ferment Solutions will be typically added to foods at levels of 0.1 to 4.5%.

The final product composition of the antimicrobial Food Ferment Solutions (*i.e.*, the levels of individual components within the product) will vary depending on the starting materials used (varying levels of fruit, vegetable, *etc.*) and the conditions of the fermentation process. The final product will be tailored, based on the abovementioned factors, to specific customer needs based on the intended food/beverage uses. All products for a specific customer will be internally consistent and all product streams will meet the overall product specifications. As a result of the potential differences in the end product composition, the use-levels will vary and therefore the exposures were calculated on the basis of the individual components rather than the product *per se*. To support the variability in the end product composition, the intakes were determined using the maximum use-levels for each of the individual components in the Food Ferment Solutions. Purac analyzes each lot of Food Ferment Solutions to determine the levels of the product to be included in each food or beverage product. The use levels will not exceed 1,600 ppm or 0.16% for sodium and calcium, 7,500 ppm or 0.75% for potassium, 21,000 ppm or 2.1% for lactate, 6,000 ppm or 0.6% for acetate and propionate, 9,000 ppm or 0.9% for protein, 2,500 ppm or 0.25% for sugar, and 1,000 ppm or 0.1% for succinic acid. The individual proposed food-uses and maximum use-levels for the individual components of Food Ferment Solutions employed in the current intake analysis are summarized in Table I.D.2-1.

Table I.D.2-1 Summary of the Individual Proposed Food-Uses and Maximum Use-Levels for Individual Components of Food Ferment Solutions in the U.S.

Food Category	Proposed Food-Uses	Maximum Use level (%)								
		Sodium	Calcium	Potassium	Lactate	Acetate	Propionate	Protein	Sugars	Succinic Acid
Baked Goods and Baking Mixes	Bread and Rolls (all types)	0.16	0.16	0.75	2.1	0.6	0.6	0.9	0.25	0.1
	Cakes	0.16	0.16	0.75	2.1	0.6	0.6	0.9	0.25	0.1
	Cornbread and Tortillas	0.16	0.16	0.75	2.1	0.6	0.6	0.9	0.25	0.1
	Croissants and Pastries	0.16	0.16	0.75	2.1	0.6	0.6	0.9	0.25	0.1
	Crackers (low sodium)	0.16	0.16	0.75	2.1	0.6	0.6	0.9	0.25	0.1
	French Toast, Pancakes, and Waffles	0.16	0.16	0.75	2.1	0.6	0.6	0.9	0.25	0.1
	Pies	0.16	0.16	0.75	2.1	0.6	0.6	0.9	0.25	0.1
	Sweet Breads and Rolls	0.16	0.16	0.75	2.1	0.6	0.6	0.9	0.25	0.1
Beverages and Beverage Bases (Carbonated and non-carbonated)	Carbonated Beverages	0.07	0.07	0.75	0.92	0.6	0.6	0.4	0.25	0.1
	Sports and Isotonic Drinks	0.07	0.07	0.75	0.92	0.6	0.6	0.4	0.25	0.1
	Non Milk-Based Meal Replacement Beverages	0.16	0.16	0.75	2.1	0.6	0.6	0.9	0.25	0.1
Breakfast Cereals	Instant and Regular Hot Breakfast Cereals	0.16	0.16	0.75	2.1	0.6	0.6	0.9	0.25	0.1
Cheeses	Cottage Cheese	0.16	0.16	0.75	2.1	0.6	0.6	0.9	0.25	0.1
	Imitation Cheese	0.16	0.16	0.75	2.1	0.6	0.6	0.9	0.25	0.1
	Processed Cheese and Cheese Spreads	0.16	0.16	0.75	2.1	0.6	0.6	0.9	0.25	0.1
Coffee and Tea	(Iced) Tea	0.07	0.07	0.75	0.92	0.6	0.6	0.4	0.25	0.1
Condiments and Relishes	Olives, Pickles, and other Relishes	0.16	0.16	0.75	2.1	0.6	0.6	0.9	0.25	0.1

Table I.D.2-1 Summary of the Individual Proposed Food-Uses and Maximum Use-Levels for Individual Components of Food Ferment Solutions in the U.S.

Food Category	Proposed Food-Uses	Maximum Use level (%)								
		Sodium	Calcium	Potassium	Lactate	Acetate	Propionate	Protein	Sugars	Succinic Acid
Fats and Oils	Light Margarine (light and salted)	0.09	0.09	0.75	2.1	0.6	0.6	0.9	0.25	0.1
	Salad Dressings (regular and low calorie)	0.16	0.16	0.75	2.1	0.6	0.6	0.9	0.25	0.1
Fish Products	Fish- and Shellfish-Based Meals (excluding catfish)	0.16	0.16	0.75	2.1	0.6	0.6	0.9	0.25	0.1
Frozen Dairy Desserts and Mixes	Frozen Yogurt	0.16	0.16	0.75	2.1	0.6	0.6	0.9	0.25	0.1
Gelatins, Puddings, and Fillings	Gelatin Desserts	0.16	0.16	0.75	2.1	0.6	0.6	0.9	0.25	0.1
	Puddings, Custards, and Mousses	0.16	0.16	0.75	2.1	0.6	0.6	0.9	0.25	0.1
Grain Products and Pastas	Granola, Breakfast, and Meal Replacement Bars	0.16	0.16	0.75	2.1	0.6	0.6	0.9	0.25	0.1
	Grain-Based Meals	0.16	0.16	0.75	2.1	0.6	0.6	0.9	0.25	0.1
	Pastas and Noodles	0.16	0.16	0.75	2.1	0.6	0.6	0.9	0.25	0.1
Gravies and Sauces	Tomato-Based Sauces	0.16	0.16	0.75	2.1	0.6	0.6	0.9	0.25	0.1
	Gravies and Other Sauces	0.16	0.16	0.75	2.1	0.6	0.6	0.9	0.25	0.1
Meat Products	Meat-Based Prepared Foods	0.16	0.16	0.75	2.1	0.6	0.6	0.9	0.25	0.1
Milk Products	Flavored Milk and Milk Drinks	0.16	0.16	0.75	2.1	0.6	0.6	0.9	0.25	0.1
	Sour Cream and Dips	0.16	0.16	0.75	2.1	0.6	0.6	0.9	0.25	0.1
	Yogurt	0.16	0.16	0.75	2.1	0.6	0.6	0.9	0.25	0.1
	Yogurt Drinks[1]	0.16	0.16	0.75	2.1	0.6	0.6	0.9	0.25	0.1
Plant Protein Products	Plant Protein Products	0.16	0.16	0.75	2.1	0.6	0.6	0.9	0.25	0.1

000020

Table I.D.2-1 Summary of the Individual Proposed Food-Uses and Maximum Use-Levels for Individual Components of Food Ferment Solutions in the U.S.

Food Category	Proposed Food-Uses	Maximum Use level (%)								
		Sodium	Calcium	Potassium	Lactate	Acetate	Propionate	Protein	Sugars	Succinic Acid
Poultry Products	Poultry-Based Prepared Foods	0.16	0.16	0.75	2.1	0.6	0.6	0.9	0.25	0.1
Processed Fruits and Fruit Juices	Fruit Drinks and Ades	0.07	0.07	0.75	0.92	0.6	0.6	0.4	0.25	0.1
	Fruit Juices (including citrus) and Nectars	0.16	0.16	0.75	2.1	0.6	0.6	0.9	0.25	0.1
	Processed Fruits and Fruit Mixtures	0.16	0.16	0.75	2.1	0.6	0.6	0.9	0.25	0.1
	Raw Fruits and Berries (Surface-Treated)	0.16	0.16	0.75	2.1	0.6	0.6	0.9	0.25	0.1
Processed Vegetables and Vegetable Juices	Processed Vegetables and Vegetable Mixtures	0.16	0.16	0.75	2.1	0.6	0.6	0.9	0.25	0.1
	Raw Vegetables (Surface-Treated)	0.16	0.16	0.75	2.1	0.6	0.6	0.9	0.25	0.1
	Vegetable Juices	0.16	0.16	0.75	2.1	0.6	0.6	0.9	0.25	0.1
Soups and Soup Mixes	Soups	0.16	0.16	0.75	2.1	0.6	0.6	0.9	0.25	0.1
Sweet Sauces, Toppings, and Syrups	Sweet Sauces, Toppings, and Syrups	0.16	0.16	0.75	2.1	0.6	0.6	0.9	0.25	0.1

[1] No food codes were identified for yogurt drinks; therefore, surrogate codes of fruit smoothie drinks were used to represent the food codes in this category.

000021

I.D.3 Estimated Dietary Consumption of Food Ferment Solutions Components Based upon Intended Food Uses

Estimates for the intake of the individual components of Food Ferment Solutions were based on the proposed food-uses and use-levels in conjunction with food consumption data included in the National Center for Health Statistics' (NCHS) 2003-2004 and 2005-2006 National Health and Nutrition Examination Survey (NHANES) (CDC, 2006, 2009; USDA, 2009).

The proposed food-uses for Food Ferment Solutions were widely consumed by the total U.S. population with 16,246 potential users identified (97.3% of the total U.S. population. All survey respondents (100%) were identified in each of the individual population groups, with the exception of the infant population group (76.6% users). As a result of the high percentage of users identified within all population groups, except for the infant population group, the intake estimates for the all-person (data not shown) and all-user categories were similar; therefore, only the all-user results are discussed.

The consumption of all proposed food-uses by the total U.S. population resulted in an estimated mean all-user intake of sodium and calcium in Food Ferment Solutions of 1.4 g/person/day (24 mg/kg body weight/day). The estimated mean all-user intakes of potassium, lactate, acetate, propionate, protein, sugars, and succinic acid in Food Ferment Solutions were equivalent to 9.0, 18.3, 7.2, 7.2, 7.9, 3.0, and 1.2 g/person/day, respectively (152, 321, 122, 122, 137, 51, and 20 mg/kg body weight/day, respectively).

When heavy consumers (90th percentile) were assessed, the 90th percentile all-user intake estimates of sodium and calcium in Food Ferment Solutions from all proposed food-uses by the total population were 2.3 g/person/day (45 mg/kg body weight/day). The estimated 90th percentile all-user intakes of potassium, lactate, acetate, propionate, protein, sugars, and succinic acid in Food Ferment Solutions at the 90th percentile were 15.8, 30.2, 12.7, 12.7, 12.9, 5.3, and 2.1 g/person/day, respectively (281, 590, 225, 225, 253, 94, and 37 mg/kg body weight/day, respectively).

The intake methodology used is generally considered to be "worst case" as a result of several conservative assumptions made in consumption estimates. For example, it is often assumed that all food products within a food category contain the ingredient at the maximum specified level of use. Furthermore, while the intake estimations were conducted using maximum use-levels for each component of Food Ferment Solutions, in reality, the composition of Food Ferment Solutions varies in that for several intended uses, some components are absent or present at low levels. For example, sodium and potassium are present at low levels in applications intended for dairy products as calcium is the preferred cation for these applications. In contrast, calcium can only be used at low levels in meat products due to the risk of disintegration of the food. Such an approach provides for a highly conservative exposure assessment.

It also is well established that the length of a dietary survey affects the estimated consumption of individual users. Short-term surveys, such as the typical 2- or 3-day dietary surveys, overestimate the consumption of food products that are consumed relatively infrequently.

It should be noted that for several proposed food uses, Food Ferment Solutions will be used as a direct replacement for existing lactate products that are currently in the food supply; therefore, exposure to lactate from the consumption of these foods is not expected to increase.

Based on the above-described limitations of the intake methodology, it is expected that the actual intakes of the individual components of Food Ferment Solutions from the proposed uses will be considerably lower than indicated in the exposure estimates.

I.E Basis for the GRAS Determination

Pursuant to 21 CFR §170.35, Food Ferment Solutions have been determined by Purac to be GRAS on the basis of scientific procedures (U.S. FDA, 2010b). This determination is based on the views of experts who are qualified by scientific training and experience to evaluate the safety of Food Ferment Solutions as a component of food [see Appendix I, **Expert Panel Consensus Statement Concerning the Generally Recognized as Safe (GRAS) Status of Food Ferment Solutions for the Proposed Food Uses**].

At the request of Purac, an Expert Panel (the "Panel") of independent scientists, qualified by their relevant national and international experience and scientific training to evaluate the safety of food ingredients, was specially convened on January 19, 2011 to conduct a critical and comprehensive evaluation of the available pertinent data and information, and determine whether the intended uses of Food Ferment Solutions as a food ingredient are safe and suitable and would be GRAS based on scientific procedures.

The Panel consisted of the following qualified scientific experts: Professor Eric A. Johnson (University of Wisconsin-Madison), Professor John Doull (University of Kansas) and Professor John Thomas (Indiana University School of Medicine).

The Expert Panel convened on behalf of Purac, independently and collectively, critically evaluated the data and information summarized herein and concluded that the intended uses in traditional foods described herein Food Ferment Solutions, meeting appropriate food-grade specifications and manufactured according to cGMP, are safe and suitable and GRAS based on scientific procedures. It is also the Expert Panel's opinion that other qualified and competent scientists reviewing the same publicly available toxicological and safety information would reach the same conclusion.

Food Ferment Solutions are GRAS based on scientific procedures for their intended use as a food ingredient; therefore, they are excluded from the definition of a food additive and thus may

be marketed and sold for their intended purpose in the United States without the promulgation of a food additive regulation under 21 CFR.

I.F Availability of Information

The detailed data and information that serve as a basis for this GRAS determination will be provided to the FDA upon request, or are available for the FDA's review and copying during reasonable business hours at the offices of:

Name	Mr. Ton van Dongen
Title	Global Regulatory Manager
Address	Purac Arkelsedijk 46 4206 AC Gorinchem The Netherlands
Telephone:	+31 183 695 730
Facsimile:	+31 183 695 607
Email:	t.van.dongen@purac.com

Should the FDA have any questions or additional information requests regarding this notification, Purac will supply these data and information.

II. DETAILED INFORMATION ABOUT THE IDENTITY OF THE SUBSTANCE

II.A Identity

II.A.1 Product Identity

A product composed of lactic acid (2-hydroxypropanoic acid), monosodium salt and 2-hydroxypropanoic acid, monopotassium salt, with 2-hydroxypropanoic acid, monocalcium salt, the sodium, potassium and calcium salt of acetic acid, and propionic acid, as well as proteins, sugars, and succinic acid.

Common or Usual Name:
"Cultured _____" where the blank is replaced by the name of the natural substrate (dairy sources, sugars, wheat, malt, and fruit- and vegetable-based sources) used in fermentation. The resulting product is a blend of sodium, potassium and/or calcium salts of lactate, acetate and propionate.

Chemical Abstracts Service (CAS) Numbers for Components:

Sodium lactate:	72-17-3
Potassium lactate:	996-31-6
Calcium acetate:	62 -54-4
Calcium lactate:	814-80-2
Lactic acid:	50-21-5
Sodium acetate:	127-09-3
Potassium acetate:	127-08-2
Sodium propionate:	137-40-6
Potassium propionate:	327-62-8
Calcium propionate:	4075-81-4
Glucose:	50-99-7
Fructose:	57-48-7
Sucrose:	57-50-1
Lactose:	63-42-3
Galactose:	26566-61-0
Succinic acid:	110-15-6

Chemical and Physical Characteristics

A liquid or powder with a savory, sweet odor and a color depending on the type of raw material used.

II.B Method of Manufacture

Food Ferment Solutions are produced by the fermentation of natural food sources (such as caramel, dairy sources (lactose, whey and whey permeate, milk, milk solids, yogurt), fruit- and vegetable-based sources (including juices, pastes, and peels), honey[2], maple syrup, molasses, starch (from barley, corn, malt, potato, rice, tapioca, and wheat), sugars (from corn, beet, palm or sugar cane), and wheat). The substrate is fermented to organic acids, by individual microorganisms including *Streptococcus thermophilus, Bacillus coagulans*, *Lactobacillus acidophilus*, *Lactobacillus paracasei* subsp. *paracasei*, *Lactobacillus plantarum*, *Lactobacillus sakei*, *Lactobacillus bulgaricus* and *Propionibacterium freudenreichii* subsp. *shermanii*, or mixtures of these strains.

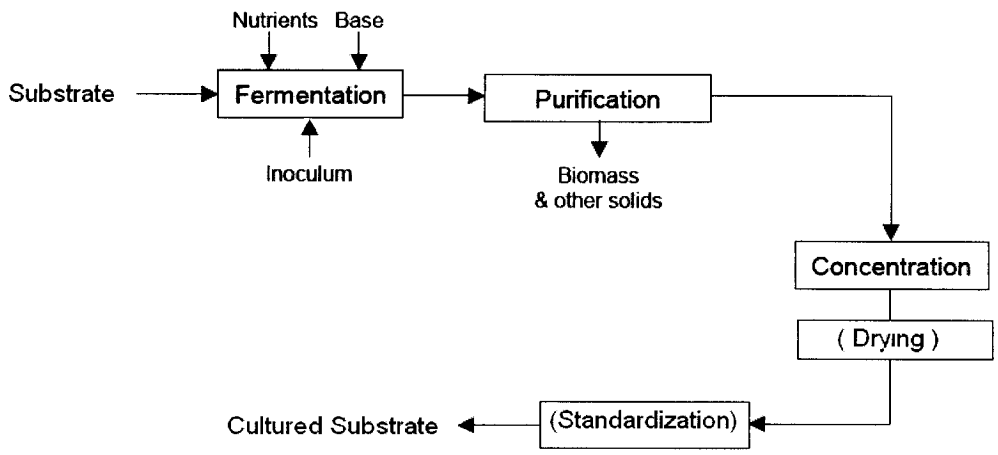
Following fermentation, a suitable base (permitted for use in the U.S. under 21 CFR 184) is added to neutralize the liquid. The fermentation end product is purified to remove biomass, ions, and other impurities; this is followed by concentration of the product. To produce Food Ferment Solutions in powder form, the product undergoes spray drying and spray

[2] A botulinum spore sterilization procedure will be undertaken when honey is used as a substrate.

agglomeration. Carriers that are permitted for use in the U.S. under 21 CFR 184 are utilized during this process. For Food Ferment Solutions in solution form, the product can be mixed with water or other Food Ferment Solutions, and the pH can be adjusted (using acids or bases permitted for use in the U.S. under 21 CFR 182 and 21 CFR 184) to obtain the desired product specification. Pasteurization, sterilization, and purification are applied as needed to ensure that Food Ferment Solutions meet the product specifications.

The general process route for the production of Food Ferment Solutions is outlined in Figure II.B-1.

Figure II.B-1 Schematic Overview of the Manufacturing Process for Food Ferment Solutions



All processing aids used in the manufacture of Food Ferment Solutions are used in compliance with appropriate federal regulations, as indicated in Table II.B-1.

Table II.B-1 List of Processing Aids Used in the Manufacture of Food Ferment Solutions	
Processing Aids Used in the Manufacture of Food Ferment Solutions	**Reference to Appropriate Use in Food**
Lime	21 CFR 184.1205
Coagulants	21 CFR 182.1073, 182.1810, 182.6760
Flocculating Agents	21 CFR 173.5
Sulfuric Acid	21 CFR 184.1095
Ion Exchange Resins	21 CFR 173.25
Ultrafiltration Membranes	21 CFR 177.2910
Activated Carbon	No specific regulations pertaining to activated carbon; however, it is an accepted processing aid in food oil manufacture (GRAS Notice No. GRN 000138 – U.S. FDA, 2004)

II.C Specifications for Food Grade Material

II.C.1 Product Specifications and Analysis

The product specifications for Food Ferment Solutions are presented in Tables II.C.1-1 and II.C.1-2, and include physical, chemical, and microbiological specifications.

Table II.C.1-1 Product Specifications for Food Ferment Solutions		
Combination Overview	**Food Ferment Solutions Product as is**	**Analysis Method**
Composition		
Sodium (% w/w)	0 to 22%	M12.3.45E – Metals in Purac-PLN-G products by ICP
Potassium (% w/w)	0 to 19%	AM12.3.45E – Metals in Purac-PLN-G products by ICP
Calcium (% w/w)	0 to 20%	AM12.3.45E – Metals in Purac-PLN-G products by ICP
Lactate (% w/w)	0 to 50%	VD001- Determination of the content in lactate, acetate, and propionate in propionate fermentate
Acetate (% w/w)	0 to 20%	
Propionate (% w/w)	0 to 45%	
Protein (% w/w)	0 to 8%	AMCAL008 - Determination of the protein content
Sugars (% w/w)[a]	0 to 5%	RDT-A-0023 – Determination of total residual sugars using spectrophotometry
Succinic acid	0 to 4%	VD001- Determination of the content in lactate, acetate, and propionate in propionate fermentate
Moisture (%)[b]	0 to 15%	RDT-A-0035 – Karl Fischer water determinations Mettler DL38

Table II.C.1-1 Product Specifications for Food Ferment Solutions		
Combination Overview	**Food Ferment Solutions Product as is**	**Analysis Method**
Purity		
pH (10%)	3 to 9	VD009 – Determination of pH and pH10%
Lead (ppm)	≤ 2	AM12.3.45E – Metals in Purac-PLN-G products by ICP

[a] Sugar components (fructose, sucrose, lactose, glucose, galactose) are from natural food sources used as starting materials.
[b] Applicable to powder products only.
Constituents not listed in the table occur at levels below 0.5%. Maximum level of all unlisted constituents combined is ≤5%.

Table II.C.1-2 Microbiological Specifications for Food Ferment Solutions		
Specification Parameter	**Specification**	**Analysis Method**
Mesophilic bacteria	<1,000 CFU/g	AMCAL004 – Yeasts, moulds, and aerobic mesophilic bacteria in Purac
Moulds	<100 CFU/g	
Yeasts	<100 CFU/g	
Escherichia coli	absent in 1 g	ISO 16649-2
Salmonella	absent in 25 g	2000.07 AOAC
Staphylococcus aureus	absent in 1 g	ISO 6888-2

CFU = colony forming units

Food Ferment Solutions encompass a range of solutions that meet the diverse need of different market segments. For each food and beverage product application, Purac manufactures a product that contains organic acids (*i.e.*, lactate, acetate, and/or propionate) and cations (*i.e.*, sodium, potassium, and/or calcium) at levels required for the intended use. pH levels also are tailored to each application. Purac ensures that all batches of Food Ferment Solutions intended for a specific application are consistent with respect to composition. Moreover, all batches are in compliance with the specifications listed in Tables II.C.1-1 and II.C.1-2. The advised maximum use-levels for Food Ferment Solutions comply with and do not exceed the maximum use-levels for the individual components summarized in Table I.D.2-1.

Analytical results from testing non-consecutive batches for Food Ferment Solutions (3 batches in powder form and 3 batches in solution form) demonstrate that for each general food application, the manufacturing process generates a product that is qualitatively similar but with a quantitative change in the individual components that can be produced consistently and that meets the chemical, physical and microbiological specifications (Tables II.C.1-3 and II.C.1-4).

Table II.C.1-3 Summary of the Product Analysis for 3 Lots of Food Ferment Solutions (Powder)				
Specification Parameter	**Product Specification**	**Manufacturing Lot**		
		Food Ferment Solutions 1003001143	**Food Ferment Solutions 1008002121**	**Food Ferment Solutions 1008002123**
Sodium	0 to 22%	1.3	2.3	1.5
Potassium	0 to 19%	0.78	0.86	0.53
Calcium	0 to 20%	13.0	12.2	12.2
Lactate	0 to 50%	44.1	43.6	44.0
Acetate	0 to 20%	4.5	4.6	4.6
Propionate	0 to 45%	10.2	10.6	10.3
Protein (% w/w)	0 to 8%	4.3	5.3	3.4
Sugars[a]	0 to 5%	<5	<5	<5
Succinic acid	0 to 4%	0.8	0.7	0.8
Lead	≤2 ppm	<1	<1	<1
Moisture (%w/w)	0 to 15	<15	<15	<15

[a] Sugar components (fructose, sucrose, lactose, glucose, galactose) are from natural food sources used as starting materials.
Constituents not listed in the table occur at levels below 0.5%.

Table II.C.1-4 Summary of the Product Analysis for 3 Lots of Food Ferment Solutions (Liquid)				
Specification Parameter	**Product Specification***	**Manufacturing Lot**		
		Food Ferment Solutions 0910002224	**Food Ferment Solutions 091091277**	**Food Ferment Solutions 0904000597**
Sodium	0 to 22%	8.1	7.2	7.5
Potassium	0 to 19%	0.6	0.4	0.5
Calcium	0 to 20%	0.0	0.0	0.0
Lactate	0 to 50%	23.9	26.6	26.3
Acetate	0 to 20%	5.2	5.6	5.7
Propionate	0 to 45%	12.9	13.4	14.0
Protein (% w/w)	0 to 8%	5.6	3.6	5.0
Sugars[a]	0 to 5%	<5	<5	<5
Succinic acid	0 to 4%	0.4	0.6	0.6
pH (10%)	3 to 9	4.9	4.6	4.6
Lead	≤2 ppm	<1	<1	<1

* Balance of product to 100% is water
[a] Sugar components (fructose, sucrose, lactose, glucose, galactose) are from natural food sources used as starting materials.
Constituents not listed in the table occur at levels below 0.5%.

Microbiological analyses representing 3 non-consecutive batches of Food Ferment Solutions in liquid and powder forms from dairy sources, sugars, wheat, malt, and fruit- and vegetable-based sources are presented in Tables II.C.1-5 and II.C.1-6.

Table II.C.1-5 Summary of the Microbiological Product Analysis for 3 Lots of Food Ferment Solutions (Liquid) (CFU/g)

Parameter	Product Specification	Manufacturing Lot		
		Lot# 0910002224	Lot# 0901091277	Lot# 0904000597
Mesophilic bacteria	<1,000 CFU/g	<1,000	<1,000	<1,000
Yeasts and moulds	<100 CFU/g	<100	<100	<100
Escherichia coli	Absent in 1 g	Absent in 1 g	Absent in 1 g	Absent in 1 g
Salmonella	Absent in 25 g	Absent in 25 g	Absent in 25 g	Absent in 25 g
Staphylococcus aureus	Absent in 1 g	Absent in 1 g	Absent in 1 g	Absent in 1 g

Table II.C.1-6 Summary of the Microbiological Product Analysis for 3 Lots of Food Ferment Solutions (Powder) (CFU/g)

Parameter	Product Specification	Manufacturing Lot		
		Lot# 1003001143	Lot# 1008002121	Lot# 1008002123
Mesophilic bacteria	<1,000 CFU/g	<1,000	<1,000	<1,000
Yeasts and moulds	<100 CFU/g	<100	<100	<100
Escherichia coli	Absent in 1 g	Absent in 1 g	Absent in 1 g	Absent in 1 g
Salmonella	Absent in 25 g	Absent in 25 g	Absent in 25 g	Absent in 25 g
Staphylococcus aureus	Absent in 1 g	Absent in 1 g	Absent in 1 g	Absent in 1 g

II.C.2 Stability

The stability of Food Ferment Solutions was tested under ambient conditions (25°C with a relative humidity of 60%) and in an accelerated shelf-life study (40°C with a relative humidity of 75%). In each study, samples of Food Ferment Solutions were analyzed at 0, 1, 3, 6, and 12 months for several parameters related to physical and chemical properties, as well as composition. Results indicate that Food Ferment Solutions are stable for a period of at least 1 year under typical and accelerated storage conditions.

II.C.3 Additional Characterization

For the manufacture of Food Ferment Solutions, Purac only sources fruits and vegetables that comply with Regulation (EC) No. 396/2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin (EC, 2005) [amended by Regulation (EC) No. 299/2008] (EC, 2008).

III. SELF-LIMITING LEVELS OF USE

The use of Food Ferment Solutions is limited by taste contribution in the food.

IV. BASIS FOR GRAS DETERMINATION

IV.A Introduction

Food Ferment Solutions contain sodium, potassium, and calcium salts of lactate, acetate, and propionate. Based on their ionic nature, these salts dissociate into their constituent cations and anions in the gastrointestinal tract; therefore, the safety of the ions may be considered separately. Thus, this report presents the safety data pertaining to sodium, potassium, calcium, lactate, acetate, and propionate. When available, data specific to the major components of Food Ferment Solutions, namely sodium lactate, potassium lactate, and calcium lactate, are provided. The safety of the exposure to other components of Food Ferment Solutions (*i.e.*, protein and sugars) also is discussed.

All of the components of Food Ferment Solutions naturally occur or are added to a number of foods in the U.S. As such, these components are currently consumed on a regular basis by the U.S. population.

IV.B Metabolic Fate of Individual Components

Absorbed sodium and potassium largely are excreted in the urine and the balance of their levels is dependent on various hormones and systems. For example, the renin-angiotensin-aldosterone axis is stimulated by reduced salt intake, blood volume, or blood pressure, and acts to promote sodium retention and reabsorption in the kidney. Similarly, the sympathetic nervous system is also activated under conditions of sodium depletion and suppressed when excess sodium is present. In contrast, atrial natriuretic peptide is released in response to elevated blood volume and serves to counteract the renin-angiotensin-aldosterone system. Sodium homeostasis is also maintained by the kallikrein-kinin system, several intrarenal mechanisms, and other factors regulating renal and medullary blood flow.

Excess potassium is removed by increasing excretion into the urine; whereas, a potassium deficiency would result in decreased urinary excretion (Sheng, 2000). Rate of excretion *via* the kidneys is dependent on the secretion of potassium ions by renal tubular cells of the cortical collecting tubules (Rodríguez-Soriano, 1995) and is regulated by the hormone aldosterone.

Calcium absorption and utilization are influenced by age, physical activity, nutritional status, food source, nutrient-nutrient interactions, menopausal status, and caffeine intake (IOM, 1997; PDRNS, 2008). Further, bones and teeth, which contain 99% of the body stores of calcium, are

continuously undergoing remodeling as calcium becomes deposited into the bones and reabsorbed by the body (IOM, 1997). Dietary intake and the above-mentioned factors play a role in levels of free circulating calcium and excretion in the urine.

Lactic acid is produced as an intermediate of anaerobic metabolism through the metabolism of pyruvate. Orally administered sodium lactate has been reported to achieve maximal absorption after 4 hours. Once absorbed, L-lactate may be converted to glycogen. Less than 1% of L-lactate is excreted in the urine in comparison to D-lactate, where up to 30% may be excreted in the urine.

Propionate is metabolized and utilized similar to fatty acids (JECFA, 1967). Acetic acid is absorbed through the gastrointestinal tract and completely oxidized by tissues in the metabolic pathways that involve the production of ketone bodies (JECFA, 1967). These organic acids are components of various metabolic pathways of the body.

The sugars and proteins contained in Food Ferment Solutions are metabolized through well-established pathways.

Succinic acid, as succinate, is naturally occurring throughout the human body and plays a key role in energy production *via* the citric acid cycle (Gibala *et al.*, 1998).

IV.C Safety Aspects of Individual Components

IV.C.1 Sodium

The tolerable upper limit (UL) for sodium, established by the Food and Nutrition Board of the Institute of Medicine (IOM), ranges from 1.5 to 2.2 g/day for children, and is 2.3 g/day for adolescents and adults, based on the effects of sodium on blood pressure (IOM, 2004a). The estimated mean and 90th percentile total population intakes of sodium from the proposed uses of Food Ferment Solutions are 1.4 and 2.3 g/person/day, respectively, in the total population. These levels are similar to or below the UL for sodium.

While the use of Food Ferment Solutions may increase the daily consumption of sodium, it is not expected that the total dietary consumption of sodium will exceed the UL to an appreciable extent due to the conservative nature of the daily intake estimates. Additionally, Purac has indicated that the total sodium content of foods and beverages are likely to remain constant despite the addition of Food Ferment Solutions, as food and beverage manufacturers intend to maintain the flavor profile of the food or beverage. This will be accomplished by reducing the sodium content added from other sources. Based on this information, it is anticipated that the consumption of sodium from the proposed uses of Food Ferment Solutions will not be substantially higher than current sodium intakes and thus will not pose a safety concern.

IV.C.2 Potassium

The IOM did not establish a UL in its evaluation of potassium (IOM, 2004b). The intake of potassium from foods poses no risk to healthy individuals with normal kidney function as excess potassium is readily excreted in the urine.

The majority of the safety data on potassium is related to its beneficial effects on hypertension. In its review of potassium (NRC, 1989), the Committee on Diet and Health reviewed numerous epidemiological and animal studies investigating the effect of dietary potassium levels on hypertension and concluded that "an intake of ≥3.5 g/day of elemental potassium is associated with a beneficial effect, and no threshold for this effect is known".

As increased potassium intakes are considered to have a positive effect on hypertension, and there are no reported adverse effects associated with the estimated potassium intakes from the proposed uses of Food Ferment Solutions (18.9 g/day in male teenager heavy-consumers), the consumption of potassium contained in Food Ferment Solutions does not pose a safety concern.

IV.C.3 Calcium

The recent UL values for calcium, established by the IOM, range from 1 to 3 g/day in infants, children, and adolescents, and from 2 to 2.5 g/day in adults (IOM, 2011). The indicators selected for the UL values were calcium excretion for younger age groups, and kidney stone formation for older age groups (IOM, 2011).

While some evidence indicates calcium consumption contributes to the risk of kidney stones, it has been suggested that dietary calcium actually has a protective effect against kidney stones because it binds dietary oxalate, thus reducing oxalate excretion (FAO/WHO, 2002). The Food and Agriculture Organization/World Health Organization (FAO/WHO) (2002) stated that the risk of kidney stones from dietary hypercalciuria should be negligible as urinary calcium levels increase very slowly with intake. The IOM also indicated that various dietary and non-dietary factors are associated with kidney stone formation; as such, the available data are difficult to interpret (IOM, 2011). Based on the proposed uses of Food Ferment Solutions, the mean and 90th percentile all-user intakes of calcium were estimated to be 1.4 and 2.3 g/person/day, respectively, in the total population. These levels are similar to the UL for calcium.

While the addition of Food Ferment Solutions to the proposed food uses will increase the daily consumption of calcium, it is not expected that the total dietary consumption of calcium will exceed the UL to an appreciable extent because the estimated intakes of calcium from the proposed uses of Food Ferment Solutions are based on several conservative assumptions and thus are considered to be 'worst case' estimates. Additionally, the broad specification for calcium (0 to 20%) indicates that calcium will not be present in some of the Food Ferment

Solutions products; therefore, the actual intakes of calcium from the proposed uses are expected to be lower than indicated in the exposure estimates (for example, see Table II.C.1-4). Moreover, given the conservative nature of the UL for calcium, the consumption of calcium from the proposed uses of Food Ferment Solutions is not anticipated to pose a risk to human health.

IV.C.4 Lactate

In reviewing the safety of lactic acid and its sodium, potassium, and calcium salts, the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 1974a,b[3]) concluded that it was "unnecessary to set ADI limits" for these additives since lactic acid is a normal constituent of food and a normal intermediary metabolite in humans. In another review of the safety of lactic acid and calcium lactate conducted by the Federation of American Societies for Experimental Biology (FASEB) (1978) for the FDA, similar conclusions were drawn by the Select Committee indicating that lactic acid and calcium lactate were safe for use by "individuals beyond infancy when they are used at levels that are now current or that might reasonably be expected in the future".

Lactic acid is a normal intermediary metabolite that is widely distributed in the body (FASEB, 1978). It has been shown that the major tissues that generate lactate (*i.e.*, brain, erythrocytes, and muscle) produce 46 mg lactic acid/kg body weight/hour or 77 g/day in adults (assuming a body weight of 70 kg) (Kreisberg *et al.*, 1970). The total turnover rate of lactate in humans (used to assess the amount of lactate produced in the body) is estimated to be 82 mg/kg body weight/hour or 140 g/day in adults (assuming a body weight of 70 kg) (Kreisberg *et al.*, 1970). Also, the capacity of the liver to absorb lactate from the bloodstream can range from 100 to 400 g/day, depending on the fasting state of the individual (Berry, 1967). These findings indicate that the human body has the capacity to handle large amounts of lactic acid.

Very young infants may have difficulty handling lactate in the diet, as they have limited capacity to produce bicarbonate to neutralize excess lactic acid (JECFA, 1967). FASEB (1978) reviewed a number of studies in which lactic acid had been used to acidify infant feeding formulas given to full and pre-term infants. These studies involved formulas made with either L(+) or DL-lactate, with dose levels ranging from 600 to 1,275 mg/kg body weight/day for periods of up to 36 days. Most of the studies lacked comparative control groups. In the studies involving DL-lactate, metabolic acidosis and reduced growth were reported, mostly in pre-term infants but also in some full-term infants. Reduced growth rate, however, did not occur in the absence of metabolic acidosis. The studies involving L(+)-lactate formulas did not report either metabolic acidosis or reduced growth rates. Purac has indicated that one of the fermentation organisms used in the production of Food Ferment Solutions (*B. coagulans*) produces L-lactic acid instead

[3] http://apps.who.int/ipsc/database/evaluations/search.aspx

of D-lactic acid. Moreover, Food Ferments Solutions are not intended for use in infant formula or foods.

Based on the existing regulatory status of lactic acid and sodium, potassium, and calcium lactate, the evaluations of lactic acid and its salts by JECFA and FASEB, and the endogenous production of lactic acid in the human body, exposure to lactic acid and its sodium, potassium, and calcium salts from the uses of Food Ferment Solutions is not anticipated to be associated with a safety concern.

IV.C.5 Acetate and Propionate

These organic acids occur naturally in the diet and are permitted for use in food in the U.S., with no limitation other than cGMP (21 CFR §184.1005 and 21 CFR §184.1081) (U.S. FDA, 2010b), such that their consumption under the intended conditions of use is not expected to be of safety concern.

It also should be noted that JECFA evaluated the following salts of acetate and propionate, and concluded that no Acceptable Daily Intake (ADI) levels were required to be set for these compounds for the specified uses (JECFA, 1973, 1974a,b, 1997):

- sodium acetate (acidity regulator);
- sodium propionate (preservative);
- potassium acetate (acidity regulator or preservative);
- potassium propionate (preservative);
- calcium acetate (acidity regulator, preservative, or stabilizer);
- calcium propionate (preservative);
- acetic acid (flavoring agent, acidifier, antimicrobial preservative); and
- propionic acid (antimold and antirope agent; preservative; flavoring).

IV.C.6 Sugars and Proteins

The sugars present in Food Ferment Solutions (fructose, sucrose, lactose, glucose, galactose) are from the natural food sources used as a starting material. It is not anticipated that there will be a safety concern associated with these sugars as they are already consumed as regular components of the diet.

As mentioned previously, the proteins present in Food Ferment Solutions result from the addition of yeast extract (from bakers' yeast) to the fermentation media as a nutrient or from food-based substrates (*i.e.*, dairy products). These proteins are already consumed in the diet (from breads containing yeast or from dairy products) and thus are not expected to pose a risk when consumed. The estimated all-user 90th percentile total population intake of protein

(12.9 g/person/day) is well below the total median dietary intake of protein in the adult U.S. population (55 to 101 g/person/day) (IOM, 2005).

IV.C.7 Succinic Acid

Succinic acid is permitted for use in food in the U.S. with no limitation other than cGMP. cGMP results in a maximum level, as served, of 0.084% in condiments and relishes and 0.0061% in meat products (21 CFR § 184.1091). The carboxylate anions of succinic acid (diethyl succinate and dimethyl succinate) are permitted for use as synthetic flavoring substances and adjuvants added to food for direct consumption with no restrictions other than cGMP (21 CFR § 172.515).

It also should be noted that JECFA evaluated the following carboxylate anions of succinic acid and concluded that no ADI levels were required to be set for these compounds for the specified uses (JECFA, 1998, 1999).

- Diethyl succinate (flavoring agent), FEMA No. 2377
- Dimethyl succinate (flavoring agent), FEMA No. 2396
- Monomethyl succinate (flavoring agent), FEMA No. 3810.

Based on the evaluations by JECFA and the permitted use in the U.S. of diethyl succinate and dimethyl succinate for use as a flavoring substance and adjuvant at levels consistent with cGMP, the consumption of succinic acid from the proposed uses of Food Ferment Solutions is not expected to pose a safety concern.

IV.D Fermentation Microorganisms

As mentioned, in the production of Food Ferment Solutions, food grade substrates are fermented to the organic acid blend by a selection of one or more of the microorganisms *Streptococcus thermophilus, Bacillus coagulans*, *Lactobacillus acidophilus*, *Lactobacillus paracasei* , *Lactobacillus plantarum*, *Lactobacillus sakei*, *Lactobacillus bulgaricus*, and *Propionibacterium freudenreichii*. These are standard microorganisms used in the food industry for the production of enzymes, fermented dairy, meat and fish products and are also used as probiotics. The FDA had no questions in response to a GRAS Notice for the production of corn, cane, or beet sugar cultured with *B. coagulans*, *L. paracasei* subsp. *paracasei* and *P. freudenreichii* subsp. *shermanii* (GRN 000240 – U.S. FDA, 2008).

No endogenous antimicrobials (*e.g.*, bacteriocins) are produced during the fermentation process.

IV.D.1 *Streptococcus thermophilus*

S. thermophilus is a common starter bacteria used in cheese and yogurt production and has been safely consumed by humans (including infants) as a probiotic (Saavedra *et al.*, 2004; Delorme, 2008; Liu *et al.*, 2010). Genomic sequencing confirms that virulence-related and obvious antibiotic resistance genes are absent or inactivated in this strain, in comparison to other *Streptococcus* strains (Bolotin *et al.*, 2004). These bacteria are listed in the FDA yogurt standard (21 CFR 131.200, 131.203, 131.206; U.S. FDA, 2010b), and have been concluded to be GRAS for use in infant formulas (GRN 000049, U.S. FDA, 2002).

IV.D.2 *Bacillus coagulans*

B. coagulans is listed in 21 CFR 184.1372 as a non-pathogenic and non-toxigenic microorganism that is approved for use in the U.S. for the production of insoluble glucose isomerase enzyme preparations, which are used in the production of high fructose corn syrup (U.S. FDA, 2010b). Additionally, *B. coagulans* is designated by the American Type Culture Collection (ATCC) as a Biosafety Level 1 microorganism, indicating that it is not known to cause disease in adult humans (ATCC, 2010). Furthermore, *B. coagulans* also has been reported to be used to ferment the African oil bean (*Pentoclethra macrophylla Bentham*) seeds to ugba, a traditional Nigerian food (Isu and Njoku, 1997).

IV.D.3 *Lactobacillus acidophilus*

Lactobacillus strains have a history of use in food production, with large levels of viable bacteria present in many foods, including yogurt, fermented milk, and cheeses (Holzapfel *et al.,* 2001; Bulut *et al.,* 2005; Sánchez *et al.,* 2005, 2006; Rojo-Bezares *et al.,* 2006).

L. acidophilus is a lactic acid bacterium that is isolated from and used as a probiotic in a range of foods, such as milk and yogurt, used in the fermentation of meat products and has a long history of safe use, with reported beneficial health effects in animals and humans (Arihara *et al.*, 1998; Sanders and Klaenhammer, 2001; Oyetayo *et al.*, 2003; Bernardeau *et al.*, 2008).

IV.D.4 *Lactobacillus paracasei* subsp. *paracasei*

L. paracasei subsp. paracasei is a lactic acid bacterium that is present in the human gastrointestinal tract and shown to be well tolerated in feeding studies with healthy human subjects (*i.e.,* infants, adults, elderly) and may be a potentially beneficial probiotic (Crittenden *et al.*, 2002; Ljungh and Wadström, 2006).

In a randomized, double-blind, placebo controlled, parallel dose-response study, adults provided high doses (up to 10^{11} CFU/day) of *L. paracasei* subsp. *paracasei* and *Bificobacterium animalis* subsp. *lactis*, given in combination, were reported to have no adverse effects associated with

the probiotic supplementation (Larsen *et al.,* 2006). Furthermore, *L. paracasei* was not recovered in any of the fecal samples of the subjects, suggesting that *L. paracasei* did not survive during passage through the intestinal tract.

IV.D.5 *Lactobacillus plantarum*

L. plantarum is present in the human mucosa and gastrointestinal tract and has been isolated from and utilized for the production of fermented foods and cheeses, with potential beneficial characteristics to human health as a probiotic (Ennahar *et al.*, 1996; Molin, 2001; Kleerebezem *et al.*, 2003; Essid *et al.*, 2009). *L. plantarum* also has been reported to have anti-microbial properties against pathogenic microorganisms (Ennahar *et al.*, 1996).

IV.D.6 *Lactobacillus sakei*

L. sakei is a lactic acid bacterium that occurs naturally in meat and fish and is commonly used in the manufacture of fermented meat products (Chaillou *et al.*, 2005; Hüfner *et al.*, 2007; McLeod *et al.*, 2010).

IV.D.7 *Lactobacillus bulgaricus*

L. bulgaricus (also known as *Lactobacillus delbrueckii* ssp. *bulgaricus*) is a very similar yet distinct lactic acid bacteria from the *L. acidophilus* species (Wheater, 1955; van de Guchte *et al.*, 2006). *L. bulgaricus* is an important bacterial strain in yogurt production and other fermented foods and is reported to have beneficial health effects as a probiotic, including cooperative properties with *S. thermophilus* (van de Guchte *et al.*, 2006; Hickson *et al.*, 2007; Zeng *et al.*, 2008; Liu *et al.*, 2010).

These bacteria are listed in the FDA yogurt standard as being required to be present in yogurts (21 CFR 131.200, 131.203, 131.206; U.S. FDA, 2010b).

IV.D.8 *Propionibacterium freudenreichii* subsp. *shermanii*

P. freudenreichii subsp. *shermanii* is used to produce the characteristic qualities in Swiss and Emmentaler cheese and is described generically as propionic acid-producing bacteria (propionibacteria) in the standards of identity for these cheeses (21 CFR 133.195) (U.S. FDA, 2010b). *P. freudenreichii* and other propionibacteria are used in food as probiotics. In response to a previous GRAS notice for skim milk or dextrose cultured with *P. freudenreichii* subsp. *shermanii* for use as an antimicrobial agent in various foods (GRN 000128), the FDA had no questions (U.S. FDA, 2003).

Professor Eric Johnson (Food Research Institute, University of Wisconsin-Madison) reviewed the scientific and medical literature and considered that *B. coagulans*, *L. paracasei* subsp.

paracasei and *P. freudenreichii* subsp. *shermanii* used in the production of Purac's product VERDAD are not toxigenic or pathogenic (GRN 000240 – U.S. FDA, 2008).

IV.E Additional Considerations

Lactates have been reported to extend shelf life and inhibit the outgrowth of pathogens such as *Salmonella enteritidis*, *Listeria monocytogenes* and *Clostridium botulinum* (Maas *et al.,* 1989; Shelef, 1991; Unda *et al.,* 1991; Meng and Genigeorgis, 1993, 1994; Shirazinejad and Ismail, 2010). A study was conducted to demonstrate the efficacy of Food Ferment Solutions in controlling *S. enteritidis* in a "ready to eat" food model containing a homogenous mixture of spinach, potatoes and chicken, incubated at 12°C over 21 days. As an inoculation study, the food models were injected with 0, 0.5, 1.0, or 1.5% Food Ferment Solutions and evaluated for *S. enteritidis* growth. No appreciable growth was reported in any of the Food Ferment Solutions samples over the course of the 21-day testing period, while the control sample had significant growth of *S. enteritidis* within 7 days. These data confirm that Food Ferment Solutions are efficacious in controlling microbial safety of food products at the proposed use levels.

Another study was conducted to demonstrate the efficacy of Food Ferment Solutions in controlling *L. monocytogenes* in a "ready to eat" food model containing a homogenous mixture of spinach, potatoes and chicken, incubated at 4°C over 60 days. As an inoculation study, the food models were injected with 0, 1.0, or 1.5% Food Ferment Solutions and evaluated for *L. monocytogenes* growth. A significant increase in *L. monocytogenes* was observed in the control after approximately 10 days, while 1.0% Food Ferments Solutions resulted in significant growth only after 45 days. No significant growth was observed after 60 days in the sample injected with 1.5% Food Ferment Solutions. These data further confirm that Food Ferment Solutions are efficacious in controlling microbial safety of food products at the proposed use levels.

A final study was conducted by Purac to evaluate the antimicrobial properties of Food Ferment Solutions against *L. monocytogenes* in cottage cheese. Cottage cheese (90% curd, 10% cream) was produced and blended with 0%, 0.3%, or 0.5% (w/w) Food Ferment Solutions. All samples were adjusted to pH 5.2 to minimize the pH effect. The samples were inoculated with *L. monocytogenes* (from cheese and food isolates) and stored at 45°F (7°C) for 3 weeks.

During this study, 2 log growth of *L. monocytogenes* was observed in the control sample within 7 days. The samples containing 0.3% or 0.5% did not support growth of *L. monocytogenes*, as no significant growth was observed over the course of the test period. These data confirm that Food Ferment Solutions are efficacious in controlling microbial safety of cottage cheese at the proposed use levels.

IV.F Summary and Basis for GRAS Conclusion

The GRAS determination for Food Ferment Solutions is based on scientific procedures. Food Ferment Solutions are produced from the fermentation of dairy sources (milk, milk solids, whey powder, lactose), sugars (from corn, beet, or sugar cane), wheat, malt, and fruit- and vegetable-based sources (including juices, pastes, and peels) using bacteria that are either approved for use in food in the U.S. or have a long history of use in foods. Furthermore, these microorganisms are considered non-pathogenic and non-toxigenic.

Food Ferment Solutions undergo various processing techniques that result in a final product that is standardized to contain primarily organic acids, *i.e.* sodium lactate and potassium lactate, with lower levels of calcium lactate and sodium, potassium, and calcium salts of acetate and propionate in solution.

Small amounts of proteins, sugars, and succinic acid also are present. These compounds are approved for use in food in the U.S., are major components of foods with regulatory status in the U.S., are commonly consumed in the diet, or are endogenous to the body.

The sodium, potassium, and calcium salts of lactate, acetate, and propionate are expected to be hydrolyzed *in vivo* into their respective components. These components are absorbed in the small intestine and either excreted in the urine, or are utilized within the body for energy, cellular signaling and/or bone structure.

The final product composition of the antimicrobial Food Ferment Solutions will vary depending on the starting materials used and conditions of the fermentation process. Due to the potential differences in the end product composition, the use-levels will vary and therefore the exposures were calculated on the basis of the individual components rather than the product *per se.* To support the variability in the end product composition, the intakes were determined using the maximum use-levels for each of the individual components in the Food Ferment Solutions. As a result, the estimated intakes are much higher than the maximum intake expected from the use of Food Ferment Solutions.

Purac analyzes each lot of Food Ferment Solutions to determine the levels of the product to be included in each food or beverage product. Based on the maximum use-levels of each component of Food Ferment Solutions, the estimated mean all-user intakes of sodium, calcium, potassium, lactate, acetate, propionate, protein, sugars, and succinic acid in Food Ferment Solutions were 1.4, 1.4, 9.0, 18.3, 7.2, 7.2, 7.9, 3.0, and 1.2 g/person/day, respectively (24, 24, 152, 321, 122, 122, 137, 51, and 20 mg/kg body weight/day, respectively). The estimated 90th percentile all-user intakes of sodium, calcium, potassium, lactate, acetate, propionate, protein, sugars, and succinic acid in Food Ferment Solutions at the 90th percentile were 2.3, 2.3, 15.8, 30.2, 12.7, 12.7, 12.9, 5.3, and 2.1 g/person/day, respectively (45, 45, 281, 590, 225, 225, 253, 94, and 37 mg/kg body weight/day, respectively). These values are an over-estimate of the

actual levels that would be consumed from the use of Food Ferment Solutions because of several conservative assumptions made in the intake estimates. These include assumptions that all of the food products in which Food Ferment Solutions are proposed for use will contain the ingredients, and that Food Ferment Solutions will be used at the maximum level in all food products.

The safety of the individual components of Food Ferment Solutions under the intended conditions of use is supported by the regulatory or GRAS status of its components in food in the U.S., as well as their natural occurrence in the diet. Evaluations by authoritative bodies and results of available toxicological and human studies further corroborate the safety of the components.

The estimated intakes of sodium and calcium based on the proposed uses of Food Ferment Solutions are similar to or below their respective ULs established by the IOM. Given the conservative nature of the intake methodology used, the actual intakes of sodium and calcium are expected to be lower than indicated in the exposure estimates.

Based on the evaluations by authoritative bodies, indicating the beneficial effect of potassium on hypertension, the consumption of potassium from the proposed uses of Food Ferment Solutions is not anticipated to pose a safety concern. The safety of lactic acid and its sodium, potassium, and calcium salts from the intended uses of Food Ferment Solutions also is supported by evaluations of lactic acid and its salts by authoritative bodies, the permitted uses of lactic acid and sodium, potassium, and calcium lactate in food in the U.S., and the endogenous production of lactic acid in the human body.

With respect to the remaining minor components of Food Ferment Solutions, acetate and propionate occur naturally in the diet and are permitted for use in food in the U.S. Additionally, the sugars, proteins, and succinic acid present in Food Ferment Solutions are either permitted for use in food in the U.S. or are major components of foods that are already consumed in the diet. Therefore, their consumption under the intended conditions of use is not expected to be of safety concern.

Based on the entirety of the available scientific data, it is concluded that Food Ferment Solutions are safe for their intended use in food. The data and information summarized in this dossier demonstrate that Food Ferment Solutions, meeting appropriate food-grade specifications, would be GRAS based on scientific procedures under the conditions of intended use in foods, as described herein.

V. REFERENCES

Arihara K, Ota H, Itoh M, Kondo Y, Sameshima T, Yamanaka H, et al. (1998). *Lactobacillus acidophilus* group lactic acid bacteria applied to meat fermentation. J Food Sci 63(3):544-547.

ATCC (2010). [Search for Bacillus coagulans, ATCC nos.10545; 11369; 12245; 15949; 23498; 31284; 35670; 8038; BAA-738; 53595; 11014; 7050]. In: *The Global Bioresource Center*™. Manassas (VA): ATCC. Available from: http://www.atcc.org/ATCCAdvancedCatalogSearch/AllCollectionSearch/tabid/454/Default.aspx [Last accessed: December 20, 2010].

Bernardeau M, Vernoux JP, Henri-Dubernet S, Guéguen M (2008). Safety assessment of dairy microorganisms: the *Lactobacillus* genus. Int J Food Microbiol 126(3):278-285.

Berry MN (1967). The liver and lactic acidosis. Proc R Soc Med 60(12):1260-1262.

Bolotin A, Quinquis B, Renault P et al., (2004). Complete sequence and comparative genome analysis of the dairy bacterium *Streptococcus thermophilus*. Nature Biotech 22(12):1554:1558.

Bulut C, Gunes H, Okuklu B, Harsa S, Kilic S, Coban HS et al. (2005). Homofermentative lactic acid bacteria of a traditional cheese, Comlek peyniri from Cappadocia region. J Dairy Res 72(1):19-24.

CDC (2006). *Analytical and Reporting Guidelines: The National Health and Nutrition Examination Survey (NHANES)*. Hyattsville (MD): Centers for Disease Control and Prevention (CDC), National Center for Health Statistics (NCHS). Available at: http://www.cdc.gov/nchs/data/nhanes/nhanes_03_04/nhanes_analytic_guidelines_dec_2005.pdf.

CDC (2009). *National Health and Nutrition Examination Survey (NHANES): 2005-2006*. Hyattsville (MD): Centers for Disease Control and Prevention (CDC), National Center for Health Statistics (NCHS). Available at: http://www.cdc.gov/nchs/nhanes/nhanes2005-2006/nhanes05_06.htm.

Chaillou S, Champomier-Vergès MC, Cornet M, Crutz-Le Coq AM, Dudez AM, Martin V et al. (2005). The complete genome sequence of the meat-borne lactic acid bacterium Lactobacillus sakei 23K. Nat Biotechnol 23(12):1527-1533.

Crittenden R, Saarela M, Matto J, Ouwehand AC, Salminen S, Pelto L et al. (2002). *Lactobacillus paracasei* subsp. *paracasei* F19: Survival, ecology and safety in the human intestinal tract-A survey of feeding studies within the PROBDEMO project. Microb Ecol Health Dis 14(Suppl. 3):22-26.

Delorme C (2008). Safety assessment of dairy microorganisms: *Streptococcus thermophilus*. Int J Food Microbiol. 126(3): 274-277.

EC (2005). European Commission. Plant Protection - Pesticide Residues - Regulation (EC) No 396/2005. Brussels, Belgium: European Commission. Available at: http://ec.europa.eu/food/plant/protection/pesticides/regulation_ec_396_2005_en.htm.

EC (2008). Regulation (EC) No 299/2008 of the European Parliament and of the Council of 11 March 2008 amending Regulation (EC) No 396/2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin, as regards the implementing powers conferred on the Commission (1) OJ C 161, 13.7.2007, p. 45. 2008. Regulation (EC) No 178/2006 (OJ L 29, 2.2.2006, p. 3). Off J Eur Union 53(L97):67-71. Available at: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2008:097:0067:0071:en:PDF.

Ennahar S, Aoude-Werner D, Sorokine O, Van Dorsselaer A, Bringel F, Hubert JC et al. (1996). Production of pediocin AcH by Lactobacillus plantarum WHE 92 isolated from cheese. Appl Environ Microbiol 62(12):4381-4387.

Essid I, Medini M, Hassouna M (2009) Technological and safety properties of *Lactobacillus plantarum* strains isolated from a Tunisian traditional salted meat. Meat Sci 81(1): 203-208.

FAO/WHO (2002). *Human Vitamin and Mineral Requirements*. Report of a Joint FAO/WHO Expert Consultation, Bangkok, Thailand. Rome, Italy: Food and Agricultural Organization of the United Nations (FAO) / World Health Organization (WHO). Available at: http://www.fao.org/docrep/004/y2809e/y2809e00.htm.

FASEB (1978). *Evaluation of the Health Aspects of Lactic Acid and Calcium Lactate as Food Ingredients*. (FDA/BF-78/108; PB238-713; SCOGS-11). Prepared by Bethesda (MD):Federation of American Societies for Experimental Biology (FASEB), Life Sciences Research Office (LSRO) for Washington (DC):U.S. Food and Drug Administration (U.S. FDA), Bureau of Food.

Gibala MJ, MacLean DA, Graham TE, Saltin B (1998). Tricarboxylic acid cycle intermediate pool size and estimated cycle flux in human muscle during exercise. Am J Physiol 275(2 Part 1):E235-E242.

Hickson M, D'Souza AL, Rajkumar C, Bulpitt CJ, Muthu N, Rogers TR et al. (2007). Use of probiotic *Lactobacillus* preparation to prevent diarrhoea associated with antibiotics: randomised double blind placebo controlled trial. Br Med J 335(7610):80-83.

Holzapfel WH, Haberer P, Geisen R, Bjorkroth J, Schillinger U (2001). Taxonomy and important features of probiotic microorganisms in food and nutrition. Am J Clin Nutr 73(2, Suppl.):365S-373S.

Hüfner E, Markieton T, Chaillou S, Crutz-Le Coq AM, Zagorec M, Hertel C (2007). Identification of Lactobacillus sakei genes induced during meat fermentation and their role in survival and growth. Appl Environ Microbiol 73(8):2522-2531.

IOM (1997). *Dietary Reference Intakes for Calcium, Phosphorus, Magnesium, Vitamin D, and Fluoride*. National Academy of Sciences, Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, Food and Nutrition Board, Institute of Medicine (IOM). Washington (DC):National Academy Press (NAP). Available at: http://www.nap.edu/books/0309063507/html/index.html.

IOM (2004a). Sodium and chloride. In: *Dietary Reference Intake for Water, Potassium, Sodium, Chloride, and Sulfate*. Washington (DC): National Academy of Sciences, Food and Nutrition Board, Institute of Medicine (IOM), National Academy Press (NAP), pp. 269-423. Available at: http://books.nap.edu/catalog.php?record_id=10925.

IOM (2004b). Potassium. In: *Dietary Reference Intake for Water, Potassium, Sodium, Chloride, and Sulfate*. Washington (DC): National Academy of Sciences, Food and Nutrition Board, Institute of Medicine (IOM), National Academy Press (NAP), pp. 186-268. Available at: http://books.nap.edu/catalog.php?record_id=10925.

IOM (2005). Protein and amino acids. In: *Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids (Macronutrients)*. Washington (DC): Institute of Medicine (IOM) of the National Academies / The National Academy Press (NAP), pp. 589-768. Available at: http://fnic.nal.usda.gov/nal_display/index.php?info_center=4&tax_level=4&tax_subject=256&topic_id=1342&level3_id=5141&level4_id=10588.

IOM (2011). *Dietary Reference Intakes for Calcium and Vitamin D*. Washington (DC): National Academy of Science (NAS), Institute of Medicine (IOM), Food and Nutrition Board.

Isu NR, Njoku HO (1997). An evaluation of the microflora associated with fermented African oil bean (*Pentaclethra macrophylla* Bentham) seeds during ugba production. Plant Foods Hum Nutr 51(2):145-157.

JECFA (1967). Acetic acid, glacial. In: *Toxicological Evaluation of Some Antimicrobials, Antioxidants, Emulsifiers, Stabilizers, Flour-Treatment Agents, Acids and Bases*. Joint FAO/WHO Expert Committee on Food Additives (JECFA) 9th (Dec. 13-20, 1965, Rome) & 10th Meetings (Oct. 11-18, 1966, Geneva). (FAO Nutrition Meetings Report Series, No. 40A,B&C). Geneva, Switz.: Food and Agriculture Organization of the United Nations (FAO) / World Health Organization (WHO). Available at: http://www.inchem.org/documents/jecfa/jecmono/40abcj37.htm.

JECFA (1973). Sodium acetate, Sodium propionate, Potassium acetate, Potassium propionate, Calcium acetate, Calcium propionate. In: *Evaluations of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) [Database]*. Geneva, Switz.: Joint FAO/WHO Expert Committee on Food Additives (JECFA) / Food and Agriculture Organization of the United Nations (FAO) / World Health Organization (WHO), International Programme on Chemical Safety (IPCS). Available at: http://apps.who.int/ipsc/database/evaluations/search.aspx [Search for chemical].

JECFA (1974a). *Toxicological Evaluation of Some Food Additives Including Anticaking Agents, Antimicrobials, Antioxidants, Emulsifiers and Thickening Agents*. 17th Meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), June 25-July 4, 1973, Geneva, Switz. (WHO Food Additives Series no 5). Rome, Italy: Food and Agriculture Organization of the United Nations (FAO). Available at: http://www.inchem.org/documents/jecfa/jecmono/v05je01.htm.

JECFA (1974b). Potassium lactate (solution), Sodium lactate (solution), Calcium lactate. In: *Evaluations of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) [Database]*. Geneva, Switz.: Joint FAO/WHO Expert Committee on Food Additives (JECFA) / Food and Agriculture Organization of the United Nations (FAO) / World Health Organization (WHO), International Programme on Chemical Safety (IPCS). Available at: http://apps.who.int/ipsc/database/evaluations/search.aspx [Search for chemical].

JECFA (1997). Propionic Acid. In: *Evaluations of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) [Database]*. Geneva, Switz.: Joint FAO/WHO Expert Committee on Food Additives (JECFA). Available at: http://www.inchem.org/documents/jecfa/jeceval/jec_2015.htm.

JECFA (1998). Mono-methyl succinate. In: *Evaluations of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) [Database]*. Geneva, Switz.: Joint FAO/WHO Expert Committee on Food Additives (JECFA) / Food and Agriculture Organization of the United Nations (FAO) / World Health Organization (WHO), International Programme on Chemical Safety (IPCS). Available at: http://apps.who.int/ipsc/database/evaluations/search.aspx [Search for chemical].

JECFA (1999). Diethyl succinate, Dimethyl succinate. In: *Evaluations of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) [Database]*. Geneva, Switz.: Joint FAO/WHO Expert Committee on Food Additives (JECFA) / Food and Agriculture Organization of the United Nations (FAO) / World Health Organization (WHO), International Programme on Chemical Safety (IPCS). Available at: http://apps.who.int/ipsc/database/evaluations/search.aspx [Search for chemical].

Kleerebezem M, Boekhorst J, van Kranenburg R, Molenaar D, Kuipers OP, Leer R et al. (2003). Complete genome sequence of *Lactobacillus plantarum* WCFS1. Proc Natl Acad Sci U S A 100(4):1990-1995.

Kreisberg RA, Pennington LF, Boshell BR (1970). Lactate turnover and gluconeogenesis in normal and obese humans. effect of starvation. Diabetes 19(1):53-63.

Larsen CN, Nielsen S, Kaestel P, Brockmann E, Bennedsen M, Christensen HR et al. (2006). Dose-response study of probiotic bacteria *Bifidobacterium animalis* subsp *lactis* BB-12 and *Lactobacillus paracasei* subsp *paracasei* CRL-341 in healthy young adults. Eur J Clin Nutr 60(11):1284-1293.

Liu JE, Zhang Y, Zhang J, Dong PL, Chen M, Duan ZP (2010). Probiotic yogurt effects on intestinal flora of patients with chronic liver disease. Nurs Res 59(6):426-432.

Ljungh A, Wadström T (2006). Lactic acid bacteria as probiotics. Curr Issues Intest Microbiol 7(2):73-89.

Maas MR, Glass KA, Doyle MP (1989). Sodium lactate delays toxin production by Clostridium botulinum in cook-in-bag turkey products. Appl Environ Microbiol 55(9):2226-2229.

McLeod A, Zagorec M, Champomier-Vergès MC, Naterstad K, Axelsson L (2010). Primary metabolism in *Lactobacillus sakei* food isolates by proteomic analysis. BMC Microbiol 10:120. doi: 10.1186/1471-2180-10-120.

Meng J, Genigeorgis CA (1993). Modeling lag phase of nonproteolytic Clostridium botulinum toxigenesis in cooked turkey and chicken breast as affected by temperature, sodium lactate, sodium chloride and spore inoculum. Int J Food Microbiol 19(2):109-122.

Meng J, Genigeorgis CA (1994). Delaying toxigenesis of Clostridium botulinum by sodium lactate in 'sous-vide' products. Lett Appl Microbiol 19(1):20-23.

Molin G (2001). Probiotics in foods not containing milk or milk constituents, with special reference to *Lactobacillus plantarum* 299v. Am J Clin Nutr 73(2, Suppl.):380S-385S.

NRC (1989). Water and electrolytes - Potassium. In: *Recommended Dietary Allowances, 10th edition*. Washington (DC): National Academy Press (NAP) / Food and Nutrition Board; Commission on Life Sciences, National Research Council (NRC), pp. 255-257. Available at: http://books.nap.edu/openbook.php?record_id=1349&page=247.

Oyetayo VO, Adetuyi FC, Akinyosoye FA (2003). Safety and protective effect of *Lactobacillus acidophilus* and *Lactobacillus casei* used as probiotic agent *in vivo*. Afr J Biotechnol 2(11):448-452.

PDRNS (2008). Potassium. In: *PDR® for Nutritional Supplements, 2nd edition*. Montvale (NJ): Physicians' Desk Reference (PDR), pp. 509-512.

Rodríguez-Soriano J (1995). Potassium homeostasis and its disturbances in children. Pediatr Nephrol 9(3):364-374.

Rojo-Bezares B, Sáenz Y, Poeta P, Zarazaga M, Ruiz-Larrea F, Torres C (2006). Assessment of antibiotic susceptibility within lactic acid bacteria strains isolated from wine. Int J Food Microbiol 111(3):234-240.

Saavedra JM, Abi-Hanna A, Moore N, Yolken RH (2004). Long-term consumption of infant formulas containing live probiotic bacteria: tolerance and safety. Am J Clin Nutr 79(2):261-267.

Sánchez I, Seseña S, Poveda JM, Cabezas L, Palop L (2005). Phenotypic and genotypic characterization of lactobacilli isolated from Spanish goat cheeses. Int J Food Microbiol 102(3):355-362.

Sánchez I, Seseña S, Poveda JM, Cabezas L, Palop L (2006). Genetic diversity, dynamics, and activity of *Lactobacillus* community involved in traditional processing of artisanal Manchego cheese. Int J Food Microbiol 107(3):265-273.

Sanders ME, Klaenhammer TR (2001). Invited review: the scientific basis of *Lactobacillus acidophilus* NCFM functionality as a probiotic. J Dairy Sci 84(2):319-331.

Shelef LA (1991). Antimicrobial effects of lactates: a review. J Food Protect 57(5):445-450.

Sheng HP (2000). Sodium chloride, and potassium (Chapter 30). In: Stipanik, MH, editor. *Biochemical and Physiological Aspects of Human Nutrition*. Philadelphia (PA):W.B. Saunders Company, pp. 686-710.

Shirazinejad A, Ismail N (2010). Effect of lactate treatments on survival of food-borne pathogens in frozen shrimp (*Penaeus merguiensis*). Am J Agric Biol Sci 5(2):242-246.

U.S. FDA (1997). Substances generally recognized as safe; Proposed rule (21 CFR Parts 170, 184, 186, and 570) [Docket No. 97N-0103]. Fed Regist (US) 62(74):18937-18964.

U.S. FDA (2002). *Agency Response Letter GRAS Notice No. GRN 000049 [Bifidobacterium lactis strain Bb12 and Streptococcus thermophilus strain Th4].* Submitted by Glendale (CA): Nestle USA to College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety and Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: http://www.fda.gov/Food/FoodIngredientsPackaging/GenerallyRecognizedasSafeGRAS/GRASListings/ucm153759.htm.

U.S. FDA (2003). *Agency Response Letter GRAS Notice No. GRN 000128 [*Skim milk or dextrose cultured with *Propionibacterium freudenreichii* subsp. *shermanii].* Submitted by Cranbury (NJ): Rhodia Inc. to College Park (MD): U.S. Food and Drug Administration (FDA), Center for Food Safety and Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: http://www.fda.gov/Food/FoodIngredientsPackaging/GenerallyRecognizedasSafeGRAS/GRASListings/ucm153945.htm.

U.S. FDA (2004). *Agency Response Letter GRAS Notice No. GRN 000138 [Fish Oil].* Submitted by Bedford (NS): Ocean Nutrition Canada Ltd. to College Park (MD): U.S. Food and Drug Administration (FDA), Center for Food Safety and Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: http://www.fda.gov/Food/FoodIngredientsPackaging/GenerallyRecognizedasSafeGRAS/GRASListings/ucm153965.htm.

U.S. FDA (2008). *Agency Response Letter GRAS Notice No. GRN 000240 [Corn, cane, or beet sugar cultured with Lactobacillus paracasei subsp. paracasei, Bacillus coagulans LA-1, or Propionibacterium freudenreichii subsp. shermanii, or mixtures of these microorganisms].* Submitted by Gorinchem, The Netherlands: Purac to College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety and Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: http://www.fda.gov/Food/FoodIngredientsPackaging/GenerallyRecognizedasSafeGRAS/GRASListings/ucm153929.htm.

U.S. FDA (2010a). Federal Food, Drug, and Cosmetic Act (FD&C Act): Subchapter II: Definitions. Sec. 201. [21 USC §321]: [Including amendments to Feb. 1, 2010]. In: *U.S. Code—Title 21—Food and Drug, Chapter 9.* Rockville (MD): U.S. Food and Drug Administration (U.S. FDA). Available at: http://www.fda.gov/RegulatoryInformation/Legislation/FederalFoodDrugandCosmeticActFDCAct/FDCActChapterslandIIShortTitleandDefinitions/ucm086297.htm.

U.S. FDA (2010b). U.S. Code of Federal Regulations (CFR). Title 21—Food and Drugs (Food and Drug Administration). Washington (DC): U.S. Government Printing Office (GPO). Available at: http://www.access.gpo.gov/nara/cfr/cfr-table-search.html#page1 [see table below for CFR sections].

Table of CFR Sections Referenced (Title 21—Food and Drugs)		
Part	**Section §**	**Section Title**
131—Milk and cream	131.200	Yogurt
	131.203	Lowfat yogurt
	131.206	Nonfat yogurt
133—Cheeses and related cheese products	133.195	Swiss and emmentaler cheese
170—Food additives	170.35	Affirmation of generally recognized as safe (GRAS) status
172—Food additives permitted for direct addition to food for human consumption	172.515	Synthetic flavoring substances and adjuvants
	172.610	Arabinogalactan
	172.620	Carrageenan
	172.655	Furcelleran
173—Secondary direct food additives permitted in food for human consumption	173.5	Acrylate-acrylamide resins
	173.25	Ion-exchange resins
177—Indirect food additives: Polymers	177.2910	Ultra-filtration membranes
182—Substances generally recognized as safe	182.1	Substances that are generally recognized as safe
	182.1073	Phosphoric acid
	182.1810	Sodium tripolyphosphate
	182.6760	Sodium hexametaphosphate
184—Direct food substances affirmed as generally recognized as safe	184.1005	Acetic acid
	184.1061	Lactic acid
	184.1081	Propionic acid
	184.1091	Succinic acid
	184.1095	Sulfuric acid
	184.1185	Calcium acetate
	184.1205	Calcium hydroxide
	184.1207	Calcium lactate
	184.1372	Insoluble glucose isomerase enzyme preparations
	184.1639	Potassium lactate
	184.1721	Sodium acetate
	184.1768	Sodium lactate
	184.1784	Sodium propionate
	184.1854	Sucrose
	184.1857	Corn sugar
	184.1866	High fructose corn syrup
	184.1983	Bakers yeast extract

U.S. FSIS (2010). Part 424—Preparation and Processing Operations. In: *U.S. Code of Federal Regulations (CFR). Title 9—Animals and Animal Products*. Washington (DC): U.S. Food and Drug Administration (U.S. FDA), U.S. Government Printing Office (GPO), Food Safety and Inspection Service (FSIS). Available at: http://ecfr.gpoaccess.gov/cgi/t/text/text-idx?c=ecfr&sid=ecaa2e39b14b12f6665ef866d17f35d3&tpl=/ecfrbrowse/Title09/9cfr424_main_02.tpl.

Unda JR, Molins RA, Walker HW (1991). Clostridium sporogenes and Listeria monocytogenes: survival and inhibition in microwave-ready beef roasts containing selected antimicrobials. J Food Sci 56(1):198-205.

USDA (2007). Safe and Suitable Ingredients Used in the Production of Meat and Poultry Products. (FSIS Directive 7120.1, revision 3). Washington (DC): United States Department of Agriculture (USDA), Food Safety and Inspection Service. Available at: http://www.fsis.usda.gov/OPPDE/rdad/FSISDirectives/7120.1.pdf.

USDA (2009). *What We Eat In America: National Health and Nutrition Examination Survey (NHANES): 2003-2004, 2005-2006*. Riverdale (MD): U.S. Department of Agriculture (USDA). Available at: http://www.ars.usda.gov/Services/docs.htm?docid=13793.

van de Guchte M, Penaud S, Grimaldi C, Barbe V, Bryson K, Nicolas P et al. (2006). The complete genome sequence of Lactobacillus bulgaricus reveals extensive and ongoing reductive evolution. PNAS 103(24):9274-9279.

Wheater DM (1955). The Characteristics of *Lactobacillus acidophilus* and *Lactobacillus bulgaricus*. J Gen Microbiol 12:123-132.

ZengJ, Li YQ, Zuo XL, Zhen YB, Yang J, Liu CH (2008). Clinical trial: effect of active lactic acid bacteria on mucosal barrier function in patients with diarrhoea-predominant irritable bowel syndrome. Aliment Pharmacol Ther 28(8):994-1002.

Appendix I

Expert Panel Consensus Statement Concerning the Generally Recognized as Safe (GRAS) Status of Food Ferment Solutions for the Proposed Food Uses

Expert Panel Consensus Statement Concerning the Generally Recognized as Safe (GRAS) Status of Food Ferment Solutions for the Proposed Food Uses

February 14, 2011

INTRODUCTION

At the request of Purac, an Expert Panel (the "Panel") of independent scientists, qualified by their relevant national and international experience and scientific training to evaluate the safety of food ingredients, was specially convened to conduct a critical and comprehensive evaluation of the available pertinent data and information, and determine whether, under the conditions of intended use as a food ingredient, Food Ferment Solutions would be Generally Recognized as Safe (GRAS), based on scientific procedures. The Panel consisted of the below-signed qualified scientific experts: Professor Eric A. Johnson (University of Wisconsin-Madison), Professor John Doull (University of Kansas) and Professor John Thomas (Indiana University School of Medicine).

The Panel, independently and collectively, critically examined a comprehensive package of scientific information and data compiled from the literature and other published sources. This information was presented in a dossier [Documentation Supporting the Evaluation of PuraQ Food Ferment Solutions as Generally Recognized as Safe (GRAS) for Use as a Food Ingredient] that was submitted by Purac to the Panel. In addition, the Panel evaluated other information deemed appropriate or necessary, including data and information provided by Purac. The data evaluated by the Panel included information pertaining to the method of manufacture and product specifications, analytical data, intended use levels in specified food products, consumption estimates for all intended uses, and a comprehensive assessment of the available scientific literature pertaining to the safety of Food Ferment Solutions and their component ingredients.

Following independent and collaborative critical evaluation of such data and information, the Panel unanimously concluded that under the conditions of intended use in traditional foods described herein, Food Ferment Solutions, meeting appropriate food-grade specifications, and manufactured in accordance with current Good Manufacturing Practice (cGMP), are GRAS based on scientific procedures. A summary of the basis for the Panel's conclusion, excluding confidential data and information, is provided below.

SUMMARY AND BASIS FOR GRAS

Purac intends to market Food Ferment Solutions, containing a blend of sodium, potassium, and calcium salts of lactate, acetate and propionate, as well as sugars, proteins, and succinic acid, as a flavoring and antimicrobial agent in the United States (U.S.). Food Ferment Solutions have a broad application range that meets the needs of different market segments. As each food and beverage application has specific requirements with respect to preservation, pH, taste, *etc.*, Food Ferments provide a range of solutions that meet individual needs.

Food Ferment Solutions are intended for use at levels of 0.1 to 4.5% in baked goods and baking mixes, beverages and beverage bases (carbonated and non-carbonated), breakfast cereals, cheeses, coffee and tea, condiments and relishes, fats and oils, fish products (excluding catfish), frozen dairy desserts and mixes, gelatins, puddings, and fillings, grain products and pastas, gravies and sauces, meat products, milk products, plant protein products, poultry products, processed fruits and fruit juices, processed vegetables and vegetable juices, soups and soup mixes, and sweet sauces, toppings, and syrups. Food Ferment Solutions are not intended for use in infant formula or foods.

Food Ferment Solutions are produced from the fermentation of food-based materials, including caramel, dairy sources (lactose, whey and whey permeate, milk, milk solids, yogurt), fruit- and vegetable-based sources (including juices, pastes, and peels), honey, maple syrup, molasses, starch (from barley, corn, malt, potato, rice, tapioca, and wheat), sugars (from corn, beet, palm or sugar cane), and wheat using bacteria that are either permitted for use in food in the U.S. or have a long history of use in foods. The bacteria used in the fermentation process will include one or a combination of the following organisms: *Streptococcus thermophilus, Bacillus coagulans*, *Lactobacillus acidophilus*, *Lactobacillus paracasei* subsp. *paracasei*, *Lactobacillus plantarum*, *Lactobacillus sakei*, *Lactobacillus bulgaricus,* and *Propionibacterium freudenreichii* subsp. *shermani*. These microorganisms are considered non-pathogenic and non-toxigenic.

Following fermentation, various processing and purification techniques are applied as needed to produce final products that meet the need of the food and beverage application for which they are intended. The final products contain primarily sodium lactate and potassium lactate, with lower levels of calcium lactate and sodium, potassium, and calcium salts of acetate and propionate in solution or powder form. Small amounts of proteins, sugars, and succinic acid also are present. All reagents and processing aids used in the manufacture of Food Ferment Solutions are food-grade materials. The specifications for Food Ferment Solutions are provided in Appendix A.

For each food and beverage product application, Purac manufactures a blend that contains organic acids (*i.e.*, lactate, acetate, and/or propionate) and cations (*i.e.*, sodium, potassium, and/or calcium) at levels required for the intended use. pH levels also are tailored to each application. Purac ensures that all batches of Food Ferment Solutions intended for a specific

application are consistent with respect to composition. Moreover, all batches of Food Ferment Solutions meet the specifications listed in Appendix A.

In the U.S., lactic acid, acetic acid, propionic acid, and their salts are permitted for various uses in food. Proteins, sugars, and succinic acid are similarly permitted for use in food in the U.S. These are major components of foods and are commonly consumed in the diet, or are endogenous to the body.

The U.S. Food and Drug Administration had no questions in response to a GRAS Notice for the production of corn, cane, or beet sugar cultured with *B. coagulans*, *L. paracasei* subsp *paracasei* and *P. freudenreichii* subsp *shermanii* (GRN 000240 – U.S. FDA, 2008). This product, also manufactured by Purac, is similar to Food Ferment Solutions in that it is intended for use as a flavoring agent and antimicrobial agent in food products (meat and poultry products), it contains a blend of sodium or potassium organic salts (primarily lactate) and sugars, it is produced *via* fermentation using microorganisms and food-based substrates that also are used in the production of Food Ferment Solutions, and it provides a similarly broad specification range.

The consumption of the individual components of Food Ferment Solutions from all proposed food uses was estimated using the National Center for Health Statistics' (NCHS) 2003-2004 and 2005-2006 National Health and Nutrition Examination Surveys (NHANES) (CDC, 2006, 2009; USDA, 2006, 2008). Consumption data and information pertaining to the individual proposed food-uses of Food Ferment Solutions were used to estimate the all-person and all-user intakes of the individual components of Food Ferment Solutions for specific demographic groups and for the total U.S. population.

The final composition of the antimicrobial Food Ferment Solutions (*i.e.*, the levels of individual components within the product) will vary depending on the starting materials used (varying levels of fruit, vegetable, *etc.*) and the conditions of the fermentation process. As a result of the potential differences in the composition of Food Ferment Solutions, the use-levels will vary and therefore the exposures were calculated on the basis of the individual components rather than each solution *per se*. To support the variability in the composition of Food Ferment Solutions, the intakes were determined using the maximum use-levels for each of the individual components in the Food Ferment Solutions. Purac analyzes each lot of Food Ferment Solutions to determine the levels of the product to be included in each food or beverage product. The use levels will not exceed 1,600 ppm or 0.16% for sodium and calcium, 7,500 ppm or 0.75% for potassium, 21,000 ppm or 2.1% for lactate, 6,000 ppm or 0.6% for acetate and propionate, 9,000 ppm or 0.9% for protein, 2,500 ppm or 0.25% for sugar, and 1,000 ppm or 0.1% for succinic acid.

The consumption of all proposed food-uses by the total U.S. population resulted in an estimated mean all-user intake of sodium and calcium in Food Ferment Solutions of 1.4 g/person/day

(2.3 g/day in the 90th percentile users). The estimated mean all-user intake of potassium, lactate, acetate, propionate, protein, sugars and succinic acid in Food Ferment Solutions were equivalent to 9.0, 18.3, 7.2, 7.2, 7.9, 3.0, and 1.2 g/person/day, respectively (with the estimated 90th percentile consumer intakes of 15.8, 30.2, 12.7, 12.7, 12.9, 5.3, and 2.1 g/person/day, respectively). These values are an over-estimate of the actual levels that would be consumed from the use of Food Ferment Solutions because of several conservative assumptions made in the intake estimates. These include assumptions that all of the food products in which Food Ferment Solutions is proposed for use will contain the ingredients (at their maximum use level), and that Food Ferment Solutions will be used at the maximum level in all food products.

As mentioned, Food Ferment Solutions contain sodium, potassium, and calcium salts of lactate, acetate, and propionate. These salts are ionic and will dissociate *in vivo* into their respective components, sodium, calcium, potassium, lactate, acetate and propionate. These components are absorbed in the small intestine and either excreted in the urine, or are utilized within the body for energy, cellular signaling and/or bone structure.

The safety of Food Ferment Solutions under the intended conditions of use is supported by the permitted uses of its components in food in the U.S., as well as their natural occurrence and regular consumption in the diet. Evaluations by authoritative bodies and results of available studies further corroborate the safety of the components.

The estimated intakes of sodium based on the proposed uses of Food Ferment Solutions are similar to or lower than the tolerable upper intake level (UL) established by the Institute of Medicine (IOM) of 2.3 g/day (IOM, 2004). Given the conservative nature of the intake methodology used, the actual intakes of sodium are expected to be lower than indicated in the exposure estimates. Additionally, Purac has indicated that the total sodium content of foods and beverages are likely to remain constant despite the addition of Food Ferment Solutions, as food and beverage manufacturers intend to maintain the flavor profile of the food or beverage. This will be accomplished by reducing the sodium added from other sources.

In regards to calcium, the mean and 90th percentile all-user intakes of calcium were estimated to be 1.4 and 2.3 g/person/day, respectively, in the total population. These values are below the UL for calcium for children, adolescents, and adults [19 to 50 years of age] (2.5 to 3.0 g/day) (IOM, 2010). The estimated heavy-user intake is slightly higher than the UL for adults over the age of 50 (2.0 g/day); however, it is not expected that the total dietary consumption of calcium will exceed the UL to an appreciable extent because the estimated intakes of calcium from the proposed uses of Food Ferment Solutions are based on several conservative assumptions and thus are considered to be 'worst case' estimates. Additionally, the broad specification for calcium (0 to 20%) indicates that calcium will not be present in some of the Food Ferment Solutions products; therefore, the actual intakes of calcium from the proposed uses are expected to be lower than indicated in the exposure estimates. Moreover, given the

conservative nature of the UL for calcium, the consumption of calcium from the proposed uses of Food Ferment Solutions is not anticipated to pose a risk to human health.

Based on the evaluations by authoritative bodies, indicating the beneficial effect of potassium on hypertension, the consumption of potassium from the proposed uses of Food Ferment Solutions is not anticipated to pose a safety concern. The safety of lactic acid and its sodium, potassium, and calcium salts from the intended uses of Food Ferment Solutions also is supported by evaluations of lactic acid and its salts by authoritative bodies, the permitted uses of lactic acid and sodium, potassium, and calcium lactate in food in the U.S., and the endogenous production of lactic acid in the human body.

With respect to the remaining minor components of Food Ferment Solutions, acetate and propionate occur naturally in the diet and are permitted for use in food in the U.S. Additionally, the sugars, proteins, and succinic acid present in Food Ferment Solutions are either permitted for use in food in the U.S. or are major components of foods that are already consumed in the diet. Therefore, their consumption under the intended conditions of use is not expected to be of safety concern.

Based on the entirety of the available scientific data, it is concluded that Food Ferment Solutions are safe for its intended use in food. The data and information summarized in this document demonstrate that Food Ferment Solutions, meeting appropriate food-grade specifications, would be GRAS based on scientific procedures under the conditions of intended use in foods, as described herein.

CONCLUSION

We, the Expert Panel, have independently and collectively critically evaluated the data and information summarized above and conclude that Food Ferment Solutions, meeting appropriate food-grade specifications presented in the supporting dossier [Documentation Supporting the Evaluation of Food Ferment Solutions as Generally Recognized as Safe (GRAS) for Use as a Food Ingredient], and manufactured consistent with current Good Manufacturing Practices (cGMP), are safe and suitable.

We further conclude that the intended uses of Food Ferment Solutions meeting appropriate food-grade specifications presented in the supporting dossier and manufactured consistent with cGMP are GRAS based on scientific procedures.

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

(b) (6)

Prof. John Doull, M.D., Ph.D.
University of Kansas Medical Center

2/28/2011
Date

(b) (6)

Prof. Eric A. Johnson, Sc.D.
University of Wisconsin-Madison

3/4/11
Date

(b) (6)

Prof. John Thomas, Ph.D.
Indiana University School of Medicine

3/3/11
Date

REFERENCES

CDC (2006). *Analytical and Reporting Guidelines: The National Health and Nutrition Examination Survey (NHANES).* Hyattsville (MD): Centers for Disease Control and Prevention (CDC), National Center for Health Statistics (NCHS). Available at: http://www.cdc.gov/nchs/data/nhanes/nhanes_03_04/nhanes_analytic_guidelines_dec_2005.pdf.

CDC (2009). *National Health and Nutrition Examination Survey (NHANES): 2005-2006.* Hyattsville (MD): Centers for Disease Control and Prevention (CDC), National Center for Health Statistics (NCHS). Available at: http://www.cdc.gov/nchs/nhanes/nhanes2005-2006/nhanes05_06.htm.

IOM (2004). Sodium and chloride. In: *Dietary Reference Intake for Water, Potassium, Sodium, Chloride, and Sulfate.* Washington (DC): National Academy of Sciences, Food and Nutrition Board, Institute of Medicine (IOM), National Academy Press (NAP), pp. 269-423. Available at: http://books.nap.edu/catalog.php?record_id=10925.

IOM (2010). *Dietary Reference Intakes for Calcium and Vitamin D.* Washington (DC): National Academy of Science (NAS), Institute of Medicine (IOM), Food and Nutrition Board.

U.S. FDA (2008). *Agency Response Letter GRAS Notice No. GRN 000240 [Corn, cane, or beet sugar cultured with Lactobacillus paracasei subsp. paracasei, Bacillus coagulans LA-1, or Propionibacterium freudenreichii subsp. shermanii, or mixtures of these microorganisms].* Submitted by Gorinchem, The Netherlands: Purac to College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety and Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: http://www.fda.gov/Food/FoodIngredientsPackaging/GenerallyRecognizedasSafeGRAS/GRASListings/ucm153929.htm.

USDA (2006). *What We Eat In America: National Health and Nutrition Examination Survey (NHANES): 2003-2004.* Riverdale (MD): U.S. Department of Agriculture (USDA). Available at: http://www.ars.usda.gov/Services/docs.htm?docid=13793.

USDA (2008). *What We Eat In America: National Health and Nutrition Examination Survey (NHANES): 2005-2006.* Riverdale (MD): U.S. Department of Agriculture (USDA). Available at: http://www.ars.usda.gov/Services/docs.htm?docid=13793.

Appendix A

Product Specifications for Food Ferment Solutions

Table A-1 Product Specifications for Food Ferment Solutions		
Combination Overview	**Food Ferment Solutions Product as is**	**Analysis Method**
Composition		
Sodium (% w/w)	0 to 22%	M12.3.45E – Metals in Purac-PLN-G products by ICP
Potassium (% w/w)	0 to 19%	AM12.3.45E – Metals in Purac-PLN-G products by ICP
Calcium (% w/w)	0 to 20%	AM12.3.45E – Metals in Purac-PLN-G products by ICP
Lactate (% w/w)	0 to 50%	VD001- Determination of the content in lactate, acetate, and propionate in propionate fermentate
Acetate (% w/w)	0 to 20%	
Propionate (% w/w)	0 to 45%	
Protein (% w/w)	0 to 8%	AMCAL008 - Determination of the protein content
Sugars (% w/w)[a]	0 to 5%	RDT-A-0023 – Determination of total residual sugars using spectrophotometry
Succinic acid	0 to 4%	VD001- Determination of the content in lactate, acetate, and propionate in propionate fermentate
Moisture (%)[b]	0 to 15%	RDT-A-0035 – Karl Fischer water determinations Mettler DL38
Purity		
pH (10%)	3 to 9	VD009 – Determination of pH and pH10%
Lead (ppm)	≤ 2	AM12.3.45E – Metals in Purac-PLN-G products by ICP
Advised addition level (%w/w)	0.1 to 4.5	

[a] Sugar components (fructose, sucrose, lactose, glucose, galactose) are from natural food sources used as starting materials.
[b] Applicable to powder products only.
Constituents not listed in the table occur at levels below 0.5%. Maximum level of all unlisted constituents combined is ≤5%. Exposure to these constituents would be below the threshold of concern.

Table A-2 Microbiological Specifications for Food Ferment Solutions		
Specification Parameter	**Specification**	**Analysis Method**
Mesophilic bacteria	<1,000 CFU/g	AMCAL004 – Yeasts, moulds, and aerobic mesophilic bacteria in Purac
Moulds	<100 CFU/g	
Yeasts	<100 CFU/g	
Escherichia coli	absent in 1 g	ISO 16649-2
Salmonella	absent in 25 g	2000.07 AOAC
Staphylococcus aureus	absent in 1 g	ISO 6888-2

CFU = colony forming units

SUBMISSION END

PHILIP C. OLSSON
RICHARD L. FRANK
DAVID F. WEEDA (1948-2001)
DENNIS R. JOHNSON
ARTHUR Y. TSIEN
STEPHEN D. TERMAN
MARSHALL L. MATZ
MICHAEL J. O'FLAHERTY
DAVID L. DURKIN
NEIL F. O'FLAHERTY
BRETT T. SCHWEMER
STEPHANIE HERSETH SANDLIN*
TISH E. PAHL
ROBERT A. HAHN
EVAN P. PHELPS
GARY H. BAISE
KATHRYN E. BALMFORD
FREDERICK H. BRANDING*

*PRACTICE WITHIN THE DISTRICT OF COLUMBIA IS LIMITED TO MATTERS AND PROCEDURES BEFORE FEDERAL COURTS AND AGENCIES

OFW LAW
OLSSON FRANK WEEDA TERMAN MATZ PC

THE WATERGATE
600 NEW HAMPSHIRE AVENUE, N.W.
SUITE 500
WASHINGTON, D.C. 20037

(202) 789-1212 • FAX (202) 234-3550
WWW.OFWLAW.COM

BRUCE A. SILVERGLADE
JOLYDA O. SWAIM
JONATHAN M. WEINRIEB

NANCY W. MATHEWSON
COUNSEL
ROGER R. SZEMRAJ
ANSON M. KELLER
CASPER E. ULDRIKS
OF COUNSEL
KENNETH D. ACKERMAN
MARK L. ITZKOFF
ELLIOT BELILOS
SENIOR POLICY ADVISOR
JOHN R. BLOCK
CHARLES W. STENHOLM
GEORGE McGOVERN
SALLY S. DONNER
BRENT W. GATTIS
BARBARA J. MASTERS, D.V.M.

September 19, 2011

BY ELECTRONIC MAIL

Dr. Shayla West-Barnette,
Consumer Safety Officer
Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740-3835

Re: GRAS Notification for Food Ferment Solutions (GRN 378)

Dear Dr. West-Barnette:

As per our telephone conversation and recommendations regarding intakes and future follow-up, enclosed is "Amendment to GRAS Exemption Claim for Food Ferment Solutions". This document will replace Sections I.D.2 (Intended Use of Food Ferment Solutions and Levels of Use in Foods) and I.D.3 (Estimated Dietary Consumption of Food Ferment Solutions Components Based upon Intended Food Uses) of the Generally Recognized as Safe (GRAS) Notice for Food Ferment Solutions that was submitted on April 12, 2011 (GRN 378).

In the original Notification, exposures were calculated assuming that each of the individual components would be present at the maximum permissible level in every application. This is not how Food Ferment Solution products will be formulated. The levels of each component of Food Ferment Solutions will be tailored to the needs of the intended application. Thus, the estimated exposures to the individual components presented in the original Notification were gross overestimates of the actual intakes.

In the enclosed document, we have provided intake estimates that more accurately reflect the actual consumption of the major components of Food Ferment Solutions from the intended uses. These estimates were achieved by calculating intakes of four (4) examples of Food Ferment Solution products that are intended for use in specific food applications. These examples are representative of several proposed food uses. Each of these example products is tailored, with respect to composition, to the intended food uses. For example, PuraQ RS20 P, which is intended for use in cheese, contains calcium at levels of 11.0 to 14.0%, as calcium is the preferred cation for dairy products, but contains

no sodium or potassium. Similarly, sodium is the only cation present in PuraQ RV75, which will be used in breads.

These examples demonstrate that some components in Food Ferment Solutions formulations are absent from the products or present at levels well below the maximum specified levels as outlined above. As a result, the actual exposure to the major components of Food Ferment Solutions in each example product is far lower than originally estimated using the maximum levels of each component. Future Food Ferment Solutions formulations would be expected to result in dietary intakes that are similar to those estimated in the attached submission.

It should be noted that for several of the proposed uses, Food Ferment Solutions will be used as a direct replacement for existing lactate products that are currently in the food supply. Since the Food Ferment Solutions products will be replacing the current lactate products, there will be no net increase in lactate intake resulting from these uses. Since the attached dietary exposure calculation assumes that all uses of the Food Ferment Solution products will be new uses of lactates, the actual increase in exposure to lactate will be lower than indicated in the exposure estimates.

In addition, Purac has developed Food Ferment Solution products with lower sodium content by substituting either potassium or calcium for sodium. As demonstrated by the example products, sodium is either absent from some Food Ferment Solution applications or is present at levels lower than indicated in the general product specifications. Using these products in place of current sodium lactates will result in lower sodium intakes than originally estimated.

I trust that the revised intake methodology using various food product applications shows that the dietary intakes of these components from the proposed uses are not expected to be of any safety concern. Please do not hesitate to contact me should you have further questions.

Sincerely,

(b) (6)

Mark L. Itzkoff
Counsel for Purac

OFW:emp

Amendment to GRAS Exemption Claim for Food Ferment Solutions

I.D.2 Intended Use of Food Ferment Solutions and Levels of Use in Foods

Purac intends to market Food Ferment Solutions in the United States (U.S.) in selected food categories, including baked goods and baking mixes, beverages and beverage bases (carbonated and non-carbonated), breakfast cereals, cheeses, coffee and tea, condiments and relishes, fats and oils, fish products (excluding catfish), frozen dairy desserts and mixes, gelatins, puddings, and fillings, grain products and pastas, gravies and sauces, meat products, milk products, plant protein products, poultry products, processed fruits and fruit juices, processed vegetables and vegetable juices, soups and soup mixes, and sweet sauces, toppings, and syrups. Food Ferment Solutions are not intended for use in infant formula or foods. Food Ferment Solutions will be typically added to foods at levels of 0.1 to 4.5%.

Purac reviewed the intended applications and specific lots of Food Ferment Solutions intended for use in each application to determine the potential dietary intake for each constituent of Food Ferment Solutions that might result from each food or beverage product. While the concentration of each component in a specific Food Ferment Formulation will vary, the level of use of the Food Ferment Solution in each final product will also vary. Taking both factors into consideration, Purac has determined that the constituent concentrations in the final products will not exceed 1,600 ppm or 0.16% for sodium and calcium, 7,500 ppm or 0.75% for potassium, 21,000 ppm or 2.1% for lactate, 6,000 ppm or 0.6% for acetate and propionate, 9,000 ppm or 0.9% for protein, 2,500 ppm or 0.25% for sugar, and 1,000 ppm or 0.1% for succinic acid. The individual proposed food-uses and maximum use-levels for the individual components of Food Ferment Solutions are summarized in Table I.D.2-1.

Table I.D.2-1 Summary of the Individual Proposed Food-Uses and Maximum Use-Levels for Individual Components of Food Ferment Solutions in the U.S.

Food Category	Proposed Food-Uses	Maximum Use level (%)								
		Sodium	Calcium	Potassium	Lactate	Acetate	Propionate	Protein	Sugars	Succinic Acid
Baked Goods and Baking Mixes	Bread and Rolls (all types)	0.16	0.16	0.75	2.1	0.6	0.6	0.9	0.25	0.1
	Cakes	0.16	0.16	0.75	2.1	0.6	0.6	0.9	0.25	0.1
	Cornbread and Tortillas	0.16	0.16	0.75	2.1	0.6	0.6	0.9	0.25	0.1
	Croissants and Pastries	0.16	0.16	0.75	2.1	0.6	0.6	0.9	0.25	0.1
	Crackers (low sodium)	0.16	0.16	0.75	2.1	0.6	0.6	0.9	0.25	0.1
	French Toast, Pancakes, and Waffles	0.16	0.16	0.75	2.1	0.6	0.6	0.9	0.25	0.1
	Pies	0.16	0.16	0.75	2.1	0.6	0.6	0.9	0.25	0.1
	Sweet Breads and Rolls	0.16	0.16	0.75	2.1	0.6	0.6	0.9	0.25	0.1
Beverages and Beverage Bases (Carbonated and non-carbonated)	Carbonated Beverages	0.07	0.07	0.75	0.92	0.6	0.6	0.4	0.25	0.1
	Sports and Isotonic Drinks	0.07	0.07	0.75	0.92	0.6	0.6	0.4	0.25	0.1
	Non Milk-Based Meal Replacement Beverages	0.16	0.16	0.75	2.1	0.6	0.6	0.9	0.25	0.1
Breakfast Cereals	Instant and Regular Hot Breakfast Cereals	0.16	0.16	0.75	2.1	0.6	0.6	0.9	0.25	0.1
Cheeses	Cottage Cheese	0.16	0.16	0.75	2.1	0.6	0.6	0.9	0.25	0.1
	Imitation Cheese	0.16	0.16	0.75	2.1	0.6	0.6	0.9	0.25	0.1
	Processed Cheese and Cheese Spreads	0.16	0.16	0.75	2.1	0.6	0.6	0.9	0.25	0.1
Coffee and Tea	(Iced) Tea	0.07	0.07	0.75	0.92	0.6	0.6	0.4	0.25	0.1
Condiments and Relishes	Olives, Pickles, and other Relishes	0.16	0.16	0.75	2.1	0.6	0.6	0.9	0.25	0.1

Table I.D.2-1 Summary of the Individual Proposed Food-Uses and Maximum Use-Levels for Individual Components of Food Ferment Solutions in the U.S.

Food Category	Proposed Food-Uses	Maximum Use level (%)								
		Sodium	Calcium	Potassium	Lactate	Acetate	Propionate	Protein	Sugars	Succinic Acid
Fats and Oils	Light Margarine (light and salted)	0.09	0.09	0.75	2.1	0.6	0.6	0.9	0.25	0.1
	Salad Dressings (regular and low calorie)	0.16	0.16	0.75	2.1	0.6	0.6	0.9	0.25	0.1
Fish Products	Fish- and Shellfish-Based Meals (excluding catfish)	0.16	0.16	0.75	2.1	0.6	0.6	0.9	0.25	0.1
Frozen Dairy Desserts and Mixes	Frozen Yogurt	0.16	0.16	0.75	2.1	0.6	0.6	0.9	0.25	0.1
Gelatins, Puddings, and Fillings	Gelatin Desserts	0.16	0.16	0.75	2.1	0.6	0.6	0.9	0.25	0.1
	Puddings, Custards, and Mousses	0.16	0.16	0.75	2.1	0.6	0.6	0.9	0.25	0.1
Grain Products and Pastas	Granola, Breakfast, and Meal Replacement Bars	0.16	0.16	0.75	2.1	0.6	0.6	0.9	0.25	0.1
	Grain-Based Meals	0.16	0.16	0.75	2.1	0.6	0.6	0.9	0.25	0.1
	Pastas and Noodles	0.16	0.16	0.75	2.1	0.6	0.6	0.9	0.25	0.1
Gravies and Sauces	Tomato-Based Sauces	0.16	0.16	0.75	2.1	0.6	0.6	0.9	0.25	0.1
	Gravies and Other Sauces	0.16	0.16	0.75	2.1	0.6	0.6	0.9	0.25	0.1
Meat Products	Meat-Based Prepared Foods	0.16	0.16	0.75	2.1	0.6	0.6	0.9	0.25	0.1
Milk Products	Flavored Milk and Milk Drinks	0.16	0.16	0.75	2.1	0.6	0.6	0.9	0.25	0.1
	Sour Cream and Dips	0.16	0.16	0.75	2.1	0.6	0.6	0.9	0.25	0.1
	Yogurt	0.16	0.16	0.75	2.1	0.6	0.6	0.9	0.25	0.1
	Yogurt Drinks[1]	0.16	0.16	0.75	2.1	0.6	0.6	0.9	0.25	0.1
Plant Protein Products	Plant Protein Products	0.16	0.16	0.75	2.1	0.6	0.6	0.9	0.25	0.1

Table I.D.2-1 Summary of the Individual Proposed Food-Uses and Maximum Use-Levels for Individual Components of Food Ferment Solutions in the U.S.

Food Category	Proposed Food-Uses	Maximum Use level (%)								
		Sodium	Calcium	Potassium	Lactate	Acetate	Propionate	Protein	Sugars	Succinic Acid
Poultry Products	Poultry-Based Prepared Foods	0.16	0.16	0.75	2.1	0.6	0.6	0.9	0.25	0.1
Processed Fruits and Fruit Juices	Fruit Drinks and Ades	0.07	0.07	0.75	0.92	0.6	0.6	0.4	0.25	0.1
	Fruit Juices (including citrus) and Nectars	0.16	0.16	0.75	2.1	0.6	0.6	0.9	0.25	0.1
	Processed Fruits and Fruit Mixtures	0.16	0.16	0.75	2.1	0.6	0.6	0.9	0.25	0.1
	Raw Fruits and Berries (Surface-Treated)	0.16	0.16	0.75	2.1	0.6	0.6	0.9	0.25	0.1
Processed Vegetables and Vegetable Juices	Processed Vegetables and Vegetable Mixtures	0.16	0.16	0.75	2.1	0.6	0.6	0.9	0.25	0.1
	Raw Vegetables (Surface-Treated)	0.16	0.16	0.75	2.1	0.6	0.6	0.9	0.25	0.1
	Vegetable Juices	0.16	0.16	0.75	2.1	0.6	0.6	0.9	0.25	0.1
Soups and Soup Mixes	Soups	0.16	0.16	0.75	2.1	0.6	0.6	0.9	0.25	0.1
Sweet Sauces, Toppings, and Syrups	Sweet Sauces, Toppings, and Syrups	0.16	0.16	0.75	2.1	0.6	0.6	0.9	0.25	0.1

[1] No food codes were identified for yogurt drinks; therefore, surrogate codes of fruit smoothie drinks were used to represent the food codes in this category.

While the maximum use-levels for each of the individual components in the Food Ferment Solutions are presented in the table above, the final product composition of the antimicrobial Food Ferment Solutions (*i.e.*, the levels of individual components within the product) will vary depending on the starting materials used (varying levels of fruit, vegetable, *etc.*) and the conditions of the fermentation process. The final product will be tailored, based on the abovementioned factors, to specific customer needs and will be based upon the targeted food product matrix. All products for a specific customer will be internally consistent and all product streams will meet the overall product specifications.

The composition of Food Ferment Solutions will change to meet the requirements of the intended uses; in some formulations specific components will be absent or only present at low levels. For example, calcium is the preferred cation for dairy products, thus sodium and potassium will present at low levels in formulations intended for use in dairy applications. Similarly, calcium can only be used at low levels in meat products due to the risk of disintegration of the food. No formulation will contain all of the components of Food Ferment Solutions at the maximum use-levels.

The following tables (Tables I.D.2-2 to I.D.2-5) provide four examples of Food Ferment Solutions products that are intended for use in specific food applications. These applications are representative of several proposed food uses. Levels of the major components (*i.e.*, sodium, potassium, calcium, lactate, acetate, and propionate) are presented for each example. These examples demonstrate that the levels of each component of Food Ferment Solutions vary depending on the intended application, and that in several applications, some components are absent from or only present at levels well below the maximum concentration. The compositions of other Food Ferment Solution applications developed by Purac also support the wide ranges indicated in the product specifications (data not shown).

PuraQ RV75 is intended for use in baked goods and baking mixes, specifically bread (all types). The maximum use level of PuraQ RV75 in these applications is 1.5%.

Table I.D.2-2 Composition of PuraQ RV75

Specification Parameter	Specification (% w/w)
Sodium	7 to 9
Potassium	0
Calcium	0
Lactate	23 to 28
Acetate	5.1 to 6.4
Propionate	12.7 to 14.7

PuraQ RS20P is intended for use in cheeses, specifically soft cheese, sour cream, and cottage cheese. The maximum use level of PuraQ RS20P in these applications is 1%.

Table I.D.2-3 Composition of PuraQ RS20P	
Specification Parameter	**Specification (% w/w)**
Sodium	0
Potassium	0
Calcium	11.0 to 14.0
Lactate	40 to 46
Acetate	3 to 6
Propionate	10 to 12

PuraQ NV 15 is intended for use in fats and oils, specifically salad dressings. The maximum use level of PuraQ NV 15 is 3%.

Table I.D.2-4 Composition of PuraQ NV 15	
Specification Parameter	**Specification (% w/w)**
Sodium	0
Potassium	15 to 17
Calcium	0
Lactate	29 to 33
Acetate	4.3 to 4.9
Propionate	0

PuraQ RV70 is intended for use in fish products (fish- and shellfish-based prepared meals and salads), grain products and pastas (grain-based prepared meals and salads), meat products (meat-based prepared meals and salads), and poultry products (poultry-based prepared meals and salads). The maximum use level of PuraQ RV70 in these applications is 2%.

Table I.D.2-5 Composition of PuraQ RV70	
Specification Parameter	**Specification (% w/w)**
Sodium	≤ 8
Potassium	≤1
Calcium	0
Lactate	38 to 42
Acetate	0
Propionate	4.5 to 5.2

The estimated intakes were determined using the maximum use-levels for the four examples of Food Ferment Solutions products. Intakes of the individual components were calculated based on the compositional information provided in the tables above.

I.D.3 Estimated Dietary Consumption of Food Ferment Solutions Components Based Upon Intended Food Uses

Estimates for the intake of the individual components of Food Ferment Solutions were based on the proposed food-uses and use-levels in conjunction with food consumption data included in the National Center for Health Statistics' (NCHS) 2003-2004 and 2005-2006 National Health and Nutrition Examination Survey (NHANES) (CDC, 2006, 2009; USDA, 2009).

Estimates for the intake of the individual components of Food Ferment Solutions were based on the proposed food-uses and use-levels in conjunction with food consumption data included in the National Center for Health Statistics' (NCHS) 2003-2004 and 2005-2006 National Health and NHANES (CDC, 2006, 2009; USDA, 2006, 2008). NHANES are conducted as a continuous, annual survey, and are released in 2 year cycles. Each year about 7,000 people from 15 different locations across the U.S. are interviewed, and approximately 5,000 complete the health examination component of the survey. Any combination of consecutive years of data collection is a representative sample of the U.S. population. It is well established that the length of a dietary survey affects the estimated consumption of individual users and that short-term surveys, such as the typical 1-day dietary survey, overestimate consumption over longer time periods (Anderson, 1988). Because two 24 hour dietary recalls administered on 2 non-consecutive days (Day 1 and Day 2) are available from the NHANES 2003-2004, 2005-2006 survey, these data were used to generate estimates for the current intake analysis.

Consumption data from individual dietary records, detailing food items ingested by each survey participant, were collated by computer and used to generate estimates for the intake of the components of Food Ferment Solutions by the U.S. population. Estimates for the daily intake of the components of Food Ferment Solutions represent projected 2-day averages for each individual from Day 1 and Day 2 of NHANES 2003-2004 data; these average amounts comprised the distribution from which mean and percentile intake estimates were produced. Mean and percentile estimates were generated incorporating survey weights in order to provide representative intakes for the entire U.S. population. "All-person intake" refers to the estimated intake of the components of Food Ferment Solutions averaged over all individuals surveyed, regardless of whether they consumed food products containing Food Ferment Solutions, and therefore includes "zero" consumers (those who reported no intake of food products containing Food Ferment Solutions during the 2 survey days). "All-user intake" refers to the estimated intake of the components of Food Ferment Solutions by those individuals consuming food products containing Food Ferment Solutions, hence the "all-user" designation. Individuals were considered users if they consumed 1 or more food products containing Food Ferment Solutions on either Day 1 or Day 2 of the survey.

Within all population groups, the all-user intake estimates were greater than the all-person intake estimates; therefore, based on a worst-case scenario, only the all-user results are presented and discussed.

I.D.3.1 Estimated Dietary Consumption of PuraQ RV75 Components

Approximately 74.7% of the total U.S. population was identified as potential consumers of the individual components of PuraQ RV75 from proposed food-uses (12,479 actual users identified). The estimated all-user intakes of the individual major components of PuraQ RV75 are presented in Tables I.D.3.1-1 and I.D.3.1-2.

Table I.D.3.1-1 Summary of the Estimated All-User Daily Intake of Individual Components of PuraQ RV75 from Proposed Food-Uses in the U.S. (2003-2004, 2005-2006 NHANES Data)

Population Group	Age (years)	Percent Users	Actual # of Users	All-User Mean Consumption (mg)					
				Sodium	Potassium	Calcium	Lactate	Acetate	Propionate
Infants	0 to 2	42.0	804	33	-	-	103	24	54
Children	3 to 11	80.9	2,211	59	-	-	183	42	96
Female Teenagers	12 to 19	72.4	1,438	69	-	-	216	49	114
Male Teenagers	12 to 19	76.2	1,479	82	-	-	256	58	135
Female Adults	20 and up	79.0	3,383	61	-	-	190	43	100
Male Adults	20 and up	82.4	3,164	87	-	-	270	62	142
Total Population	All Ages	74.7	12,479	71	-	-	220	50	116
				All-User 90th Percentile Consumption (mg)					
Infants	0 to 2	42.0	804	69	-	-	214	49	113
Children	3 to 11	80.9	2,211	105	-	-	328	75	172
Female Teenagers	12 to 19	72.4	1,438	134	-	-	418	96	220
Male Teenagers	12 to 19	76.2	1,479	154	-	-	479	109	252
Female Adults	20 and up	79.0	3,383	117	-	-	363	83	191
Male Adults	20 and up	82.4	3,164	160	-	-	498	114	262
Total Population	All Ages	74.7	12,479	135	-	-	420	96	221

Table I.D.3.1-2	Summary of the Estimated All-User Daily Per Kilogram Body Weight Intake of Individual Components of PuraQ RV75 from Proposed Food-Uses in the U.S. (2003-2004, 2005-2006 NHANES Data)								
Population Group	**Age (years)**	**Percent Users**	**Actual # of Users**	**All-User Mean Consumption (mg/kg bw)**					
				Sodium	**Potassium**	**Calcium**	**Lactate**	**Acetate**	**Propionate**
Infants	0 to 2	42.0	804	2.7	-	-	8.4	1.9	4.4
Children	3 to 11	80.9	2,211	2.2	-	-	6.8	1.6	3.6
Female Teenagers	12 to 19	72.4	1,438	1.2	-	-	3.7	0.8	2.0
Male Teenagers	12 to 19	76.2	1,479	1.3	-	-	4.0	0.9	2.1
Female Adults	20 and up	79.0	3,383	0.9	-	-	2.7	0.6	1.4
Male Adults	20 and up	82.4	3,164	1.0	-	-	3.2	0.7	1.7
Total Population	All Ages	74.7	12,479	1.2	-	-	3.7	0.8	1.9
				All-User 90th Percentile Consumption (mg/kg bw)					
Infants	0 to 2	42.0	804	5.3	-	-	16.5	3.8	8.7
Children	3 to 11	80.9	2,211	4.1	-	-	12.8	2.9	6.8
Female Teenagers	12 to 19	72.4	1,438	2.3	-	-	7.2	1.7	3.8
Male Teenagers	12 to 19	76.2	1,479	2.4	-	-	7.4	1.7	3.9
Female Adults	20 and up	79.0	3,383	1.7	-	-	5.2	1.2	2.8
Male Adults	20 and up	82.4	3,164	1.9	-	-	6.0	1.4	3.1
Total Population	All Ages	74.7	12,479	2.4	-	-	7.4	1.7	3.9

I.D.3.2 Estimated Dietary Consumption of PuraQ RS20P Components

Approximately 13.6% of the total U.S. population was identified as potential consumers of the individual components of PuraQ RS20P from proposed food-uses (2,272 actual users identified). The estimated all-user intakes of the individual major components of PuraQ RS20P are presented in Tables I.D.3.2-1 and I.D.3.2-2.

Table I.D.3.2-1 Summary of the Estimated All-User Daily Intake of Individual Components of PuraQ RS20P from Proposed Food-Uses in the U.S. (2003-2004, 2005-2006 NHANES Data)

Population Group	Age (years)	Percent Users	Actual # of Users	All-User Mean Consumption (mg)					
				Sodium	Potassium	Calcium	Lactate	Acetate	Propionate
Infants	0 to 2	5.4	104	-	-	25	82	11	21
Children	3 to 11	12.0	328	-	-	34	113	15	30
Female Teenagers	12 to 19	15.0	299	-	-	34	111	14	29
Male Teenagers	12 to 19	8.9	172	-	-	51	167	22	44
Female Adults	20 and up	19.2	823	-	-	46	151	20	39
Male Adults	20 and up	14.2	546	-	-	47	154	20	40
Total Population	All Ages	13.6	2,272	-	-	44	145	19	38
				All-User 90th Percentile Consumption (mg)					
Infants	0 to 2	5.4	104	-	-	85	281	37	73
Children	3 to 11	12.0	328	-	-	79	260	34	68
Female Teenagers	12 to 19	15.0	299	-	-	76	249	33	65
Male Teenagers	12 to 19	8.9	172	-	-	128	421	55	110
Female Adults	20 and up	19.2	823	-	-	129	423	55	110
Male Adults	20 and up	14.2	546	-	-	109	359	47	94
Total Population	All Ages	13.6	2,272	-	-	109	359	47	94

Table I.D.3.2-2	Summary of the Estimated All-User Daily Per Kilogram Body Weight Intake of Individual Components of PuraQ RS20P from Proposed Food-Uses in the U.S. (2003-2004, 2005-2006 NHANES Data)								
Population Group	**Age (years)**	**Percent Users**	**Actual # of Users**	**All-User Mean Consumption (mg/kg bw)**					
				Sodium	**Potassium**	**Calcium**	**Lactate**	**Acetate**	**Propionate**
Infants	0 to 2	5.4	104	-	-	2.1	6.8	0.9	1.8
Children	3 to 11	12.0	328	-	-	1.4	4.6	0.6	1.2
Female Teenagers	12 to 19	15.0	299	-	-	0.6	1.9	0.2	0.5
Male Teenagers	12 to 19	8.9	172	-	-	0.7	2.4	0.3	0.6
Female Adults	20 and up	19.2	823	-	-	0.6	2.1	0.3	0.5
Male Adults	20 and up	14.2	546	-	-	0.5	1.8	0.2	0.5
Total Population	All Ages	13.6	2,272	-	-	0.7	2.3	0.3	0.6
				All-User 90th Percentile Consumption (mg/kg bw)					
Infants	0 to 2	5.4	104	-	-	6.3	20.6	2.7	5.4
Children	3 to 11	12.0	328	-	-	2.8	9.1	1.2	2.4
Female Teenagers	12 to 19	15.0	299	-	-	1.1	3.8	0.5	1.0
Male Teenagers	12 to 19	8.9	172	-	-	2.1	6.9	0.9	1.8
Female Adults	20 and up	19.2	823	-	-	1.7	5.5	0.7	1.4
Male Adults	20 and up	14.2	546	-	-	1.2	4.0	0.5	1.0
Total Population	All Ages	13.6	2,272	-	-	1.7	5.5	0.7	1.4

I.D.3.3 Estimated Dietary Consumption of PuraQ NV 15 Components

Approximately 27.9% of the total U.S. population was identified as potential consumers of the individual components of PuraQ NV 15 from proposed food-uses (4,666 actual users identified). The estimated all-user intakes of the individual major components of PuraQ NV 15 are presented in Tables I.D.3.3-1 and I.D.3.3-2.

Table I.D.3.3-1 Summary of the Estimated All-User Daily Intake of Individual Components of PuraQ NV15 from Proposed Food-Uses in the U.S. (2003-2004, 2005-2006 NHANES Data)

Population Group	Age (years)	Percent Users	Actual # of Users	All-User Mean Consumption (mg)					
				Sodium	Potassium	Calcium	Lactate	Acetate	Propionate
Infants	0 to 2	7.3	140	-	33	-	65	10	-
Children	3 to 11	22.5	614	-	70	-	136	20	-
Female Teenagers	12 to 19	28.9	575	-	125	-	242	36	-
Male Teenagers	12 to 19	21.8	422	-	131	-	253	38	-
Female Adults	20 and up	38.8	1,663	-	109	-	212	32	-
Male Adults	20 and up	32.6	1,252	-	122	-	237	35	-
Total Population	All Ages	27.9	4,666	-	111	-	216	32	-
				All-User 90th Percentile Consumption (mg)					
Infants	0 to 2	7.3	140	-	75	-	145	22	-
Children	3 to 11	22.5	614	-	163	-	317	47	-
Female Teenagers	12 to 19	28.9	575	-	300	-	582	86	-
Male Teenagers	12 to 19	21.8	422	-	300	-	582	86	-
Female Adults	20 and up	38.8	1,663	-	226	-	439	65	-
Male Adults	20 and up	32.6	1,252	-	287	-	558	83	-
Total Population	All Ages	27.9	4,666	-	240	-	465	69	-

Table I.D.3.3-2	Summary of the Estimated All-User Daily Per Kilogram Body Weight Intake of Individual Components of PuraQ NV15 from Proposed Food-Uses in the U.S. (2003-2004, 2005-2006 NHANES Data)								
Population Group	**Age (years)**	**Percent Users**	**Actual # of Users**	**All-User Mean Consumption (mg/kg bw)**					
				Sodium	**Potassium**	**Calcium**	**Lactate**	**Acetate**	**Propionate**
Infants	0 to 2	7.3	140	-	2.5	-	4.9	0.7	-
Children	3 to 11	22.5	614	-	2.6	-	5.1	0.8	-
Female Teenagers	12 to 19	28.9	575	-	2.1	-	4.0	0.6	-
Male Teenagers	12 to 19	21.8	422	-	1.9	-	3.6	0.5	-
Female Adults	20 and up	38.8	1,663	-	1.5	-	3.0	0.4	-
Male Adults	20 and up	32.6	1,252	-	1.4	-	2.7	0.4	-
Total Population	All Ages	27.9	4,666	-	1.6	-	3.2	0.5	-
				All-User 90th Percentile Consumption (mg/kg bw)					
Infants	0 to 2	7.3	140	-	5.6	-	10.9	1.6	-
Children	3 to 11	22.5	614	-	6.2	-	12.0	1.8	-
Female Teenagers	12 to 19	28.9	575	-	4.4	-	8.4	1.3	-
Male Teenagers	12 to 19	21.8	422	-	4.7	-	9.1	1.4	-
Female Adults	20 and up	38.8	1,663	-	3.3	-	6.4	1.0	-
Male Adults	20 and up	32.6	1,252	-	3.0	-	5.8	0.9	-
Total Population	All Ages	27.9	4,666	-	3.5	-	6.7	1.0	-

I.D.3.4 Estimated Dietary Consumption of PuraQ RV70 Components

Approximately 70.4% of the total U.S. population was identified as potential consumers of the individual components of PuraQ RV70 from proposed food-uses (11,760 actual users identified). The estimated all-user intakes of the individual major components of PuraQ RV70 are presented in Tables I.D.3.4-1 and I.D.3.4-2.

Table I.D.3.4-1 Summary of the Estimated All-User Daily Intake of Individual Components of PuraQ RV70 from Proposed Food-Uses in the U.S. (2003-2004, 2005-2006 NHANES Data)

Population Group	Age (years)	Percent Users	Actual # of Users	All-User Mean Consumption (mg)					
				Sodium	Potassium	Calcium	Lactate	Acetate	Propionate
Infants	0 to 2	41.0	784	114	14	-	600	-	74
Children	3 to 11	75.3	2,057	226	28	-	1,188	-	147
Female Teenagers	12 to 19	77.1	1,532	299	37	-	1,567	-	194
Male Teenagers	12 to 19	81.5	1,582	423	53	-	2,223	-	275
Female Adults	20 and up	71.2	3,048	276	34	-	1,447	-	179
Male Adults	20 and up	71.8	2,757	406	51	-	2,129	-	264
Total Population	All Ages	70.4	11,760	322	40	-	1,692	-	209
				All-User 90th Percentile Consumption (mg)					
Infants	0 to 2	41.0	784	223	29	-	1,224	-	152
Children	3 to 11	75.3	2,057	460	58	-	2,417	-	299
Female Teenagers	12 to 19	77.1	1,532	584	73	-	3,068	-	380
Male Teenagers	12 to 19	81.5	1,582	880	110	-	4,622	-	572
Female Adults	20 and up	71.2	3,048	538	67	-	2,822	-	349
Male Adults	20 and up	71.8	2,757	798	100	-	4,192	-	519
Total Population	All Ages	70.4	11,760	652	81	-	3,421	-	423

Table I.D.3.4-2	Summary of the Estimated All-User Daily Per Kilogram Body Weight Intake of Individual Components of PuraQ RV70 from Proposed Food-Uses in the U.S. (2003-2004, 2005-2006 NHANES Data)								
Population Group	**Age (years)**	**Percent Users**	**Actual # of Users**	**All-User Mean Consumption (mg/kg bw)**					
				Sodium	**Potassium**	**Calcium**	**Lactate**	**Acetate**	**Propionate**
Infants	0 to 2	41.0	784	9.2	1.1	-	48.2	-	6.0
Children	3 to 11	75.3	2,057	8.3	1.0	-	43.5	-	5.4
Female Teenagers	12 to 19	77.1	1,532	5.1	0.6	-	26.5	-	3.3
Male Teenagers	12 to 19	81.5	1,582	6.5	0.8	-	34.1	-	4.2
Female Adults	20 and up	71.2	3,048	3.8	0.5	-	20.1	-	2.5
Male Adults	20 and up	71.8	2,757	4.8	0.6	-	25.2	-	3.1
Total Population	All Ages	70.4	11,760	5.1	0.6	-	26.8	-	3.3
				All-User 90th Percentile Consumption (mg/kg bw)					
Infants	0 to 2	41.0	784	18.3	2.3	-	95.9	-	11.9
Children	3 to 11	75.3	2,057	16.9	2.1	-	88.9	-	11.0
Female Teenagers	12 to 19	77.1	1,532	10.1	1.3	-	53.0	-	6.6
Male Teenagers	12 to 19	81.5	1,582	13.1	1.6	-	68.9	-	8.5
Female Adults	20 and up	71.2	3,048	7.9	1.0	-	41.3	-	5.1
Male Adults	20 and up	71.8	2,757	9.4	1.2	-	49.5	-	6.1
Total Population	All Ages	70.4	11,760	10.5	1.3	-	55.2	-	6.8

Table I.D.3.4-3 summarizes the estimated all-user total population daily intake of the individual major components of Food Ferment Solutions from the 4 example products.

Table I.D.3.4-3 Estimated All-User Total Population Daily Intake of Individual Major Components of Food Ferment Solutions

	Estimated intake from PuraQ RV75 (mg)		Estimated intake from PuraQ RS20P (mg)		Estimated intake from PuraQ NV15 (mg)		Estimated intake from PuraQ RV70 (mg)	
Component	**Mean**	**90th Percentile**	**Mean**	**90th Percentile**	**Mean**	**90th Percentile**	**Mean**	**90th Percentile**
Sodium	71	135	-	-	-	-	322	652
Potassium	-	-	-	-	111	240	40	81
Calcium	-	-	44	109	-	-	-	-
Lactate	220	420	145	359	216	465	1,692	3,421
Acetate	50	96	19	47	32	69	-	-
Propionate	116	221	38	94	-	-	209	423

The above-described examples represent 4 of the Food Ferment Solution applications that will be utilized in the proposed food categories. The intake estimations reflect the variation in composition between example products and the fact that, in several applications, the components are absent from the product or only present at levels well below the maximum specified levels. Food Ferment Solutions products developed in the future would be expected to result in intakes that are similar to example applications. It is highly unlikely that all of the foods for which Food Ferment Solutions are proposed for use will be consumed on a daily basis.

Even using the model Food Ferment Solutions products to calculate the dietary intake, the intakes presentedabove will still over-estimate consumer exposure. In general, it is well understood that several conservative assumptions are made in estimating anticipated human consumption. For example, it is often assumed that all food products within a food category contain the ingredient at the maximum specified level of use. It also is well established that the length of a dietary survey affects the estimated consumption of individual users. Short-term surveys, such as the typical 2- or 3-day dietary surveys used in the NHANES survey, overestimate the consumption of food products that are consumed relatively infrequently.

It also should be noted that for several proposed food uses, Food Ferment Solutions will be used as a direct replacement for existing lactate products that are currently in the food supply; therefore, exposure to lactate from the consumption of these foods will be the same. Sodium lactate and potassium lactate are permitted in the U.S. in various meat and meat food products, poultry and poultry food products, except infant formula and infant food as an antimicrobial agent (maximum level of 4.8% of the total formulation) and as a flavoring agent (maximum level of 2% of the total formulation) (9 CFR 424) (U.S. FSIS, 2010). Sodium lactate and potassium

lactate also are permitted for use as an emulsifier, a flavor enhancer, a flavoring agent or adjuvant, a humectant, and a pH control agent with no limitation other than current Good Manufacturing Practices (cGMP) (21 CFR §184.1768 and 21 CFR §184.1639) (U.S. FDA, 2010b). Additionally, calcium lactate is permitted for use as a direct food substance to be used in food as a firming agent, a flavor enhancer, a flavoring agent or adjuvant, a leavening agent, a nutrient supplement, and a stabilizer and thickener with no limitation other than cGMP (21 CFR §184.1207) (U.S. FDA, 2010b).

With an increasing demand to reduce the sodium content in food products, Purac has developed ferment products with a lower sodium content. This is achieved by substituting sodium with either potassium or calcium. Furthermore, replacement by either potassium or calcium is dependent on the solubility characterization. As demonstrated in Tables I.D.2-2 to I.D.2-5, sodium is absent from some of the Food Ferment Solutions applications or is present at levels lower than indicated in the general product specifications.

In addition, acetate, propionate, and lactate have varying antimicrobial activity with a different working spectrum, with acetate and propionate being overall more effective. Acetate acts to control bacteria, yeast, and some molds, propionate can control bacteria, molds, and some yeast, while lactate only functions in controlling bacterial growth. Given the greater efficacy and broader spectrum of activity associated with acetate and propionate compared to lactate, it is expected that Food Ferment Solutions will contain acetate and propionate in preference to lactate, thereby reducing lactate consumption.

Based on the above-described limitations of the intake methodology, as well as knowing that Food Ferment Solutions will replace existing lactate products in the U.S. food supply, it is expected that the actual intakes of the individual components of Food Ferment Solutions from the proposed uses will be lower than indicated in the exposure estimates.