ORIGINAL SUBMISSION

PHARMA FOODS INTERNATIONAL CO.,LTD.
1-49 Goryo-Ohara, Nishikyo-ku,
Kyoto 615-8245 Japan
PHONE : +81-75-394-8605
FAX : +81-75-394-8889

GRN 000595

August 3, 2015

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

Dear Sir or Madam:

Re: GRAS Notice for *gamma*-Aminobutyric Acid (GABA)

In accordance with proposed 21 CFR §170.36 [Notice of a claim for exemption based on a Generally Recognized as Safe (GRAS) determination] published in the *Federal Register* [62 FR18938 (17 April 1997)], I am submitting, as the notifier [Pharma Foods International Co., Ltd., 1-49 Goryo-Ohara, Nishikyo-Ku, Kyoto, 61 5-8245,Japan], a Notice of the determination, on the basis of scientific procedures, that *gamma*-Aminobutyric Acid (GABA) derived from L-glutamate *via Lactobacillus hilgardii* K-3 fermentation, produced by Pharma Foods International (PFI), as defined in the enclosed documents and CD-ROM, is GRAS under specific conditions of use as a food ingredient, and therefore, is exempt from the premarket approval requirements of the *Federal, Food, Drug and Cosmetic Act*. Information setting forth the basis for the GRAS determination, which includes detailed information on the notified substance, a summary of the basis for the GRAS determination, as well as a consensus opinion of an independent panel of experts in support of the safety of GABA under the intended conditions of use, also are enclosed for review by the agency.

I hereby certify that the enclosed electronic files for the GRAS Notice for *gamma*-Aminobutyric Acid (GABA) entitled, "GRAS Exemption Claim for *gamma*-Aminobutyric acid (GABA) for Use as a Food Ingredient in the United States (U.S.)" were scanned for viruses prior to submission and is thus certified as being virus-free using McAfee VirusScan 8.8.

Should you have any questions or concerns regarding this GRAS Notice, please do not hesitate to contact me at any point during the review process so that we may provide a response in a timely manner.

Sincerely,

(b) (6)

Noriko Horie
Director of Sales & Marketing
Pharma Foods International Co., Ltd.
1-49 Goryo-Ohara, Nishikyo-Ku,
Kyoto, 615-8245
Japan
Email: n-tani@pharmafoods.co.jp
Office phone: +81 75 394 8600
Office fax: +81 75 394 0009

PHARMA FOODS INTERNATIONAL CO.,LTD.
1-49 Goryo-Ohara, Nishikyo-ku,
Kyoto 615-8245 Japan
PHONE : +81-75-394-8605
FAX : +81-75-394-8889

August 3, 2015

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

Dear Sir or Madam:

Re: GRAS Notice for *gamma*-Aminobutyric Acid (GABA)

In accordance with proposed 21 CFR §170.36 [Notice of a claim for exemption based on a Generally Recognized as Safe (GRAS) determination] published in the *Federal Register* [62 FR18938 (17 April 1997)], I am submitting, as the notifier [Pharma Foods International Co., Ltd., 1-49 Goryo-Ohara, Nishikyo-Ku, Kyoto, 61 5-8245,Japan], a Notice of the determination, on the basis of scientific procedures, that *gamma*-Aminobutyric Acid (GABA) derived from L-glutamate *via Lactobacillus hilgardii* K-3 fermentation, produced by Pharma Foods International (PFI), as defined in the enclosed documents and CD-ROM, is GRAS under specific conditions of use as a food ingredient, and therefore, is exempt from the premarket approval requirements of the *Federal, Food, Drug and Cosmetic Act.* Information setting forth the basis for the GRAS determination, which includes detailed information on the notified substance, a summary of the basis for the GRAS determination, as well as a consensus opinion of an independent panel of experts in support of the safety of GABA under the intended conditions of use, also are enclosed for review by the agency.

I hereby certify that the enclosed electronic files for the GRAS Notice for *gamma*-Aminobutyric Acid (GABA) entitled, "GRAS Exemption Claim for *gamma*-Aminobutyric acid (GABA) for Use as a Food Ingredient in the United States (U.S.)" were scanned for viruses prior to submission and is thus certified as being virus-free using McAfee VirusScan 8.8.

Should you have any questions or concerns regarding this GRAS Notice, please do not hesitate to contact me at any point during the review process so that we may provide a response in a timely manner.

Sincerely,
(b) (6)

Noriko Horie
Director of Sales & Marketing
Pharma Foods International Co., Ltd.
1-49 Goryo-Ohara, Nishikyo-Ku,
Kyoto, 615-8245
Japan
Email: n-tani@pharmafoods.co.jp
Office phone: +81 75 394 8600
Office fax: +81 75 394 0009

GRAS Exemption Claim for *gamma*-Aminobutyric acid (GABA) for Use as a Food Ingredient in the United States (U.S.)

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

Submitted by:

Pharma Foods International Co., Ltd.
1-49 Goryo-Ohara, Nishikyo-ku
Kyoto, Japan
615-8245

August 3, 2015

GRAS Exemption Claim for a *gamma*-Aminobutyric acid (GABA) for Use as a Food Ingredient in the United States (U.S.)

Table of Contents

List of Appendices

List of Figures and Tables

I GRAS Exemption Claim

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)

As defined herein, *gamma*-aminobutyric acid (GABA) derived from L-glutamate *via* a fermentation process, has been determined by Pharma Foods International Co., Ltd. (PFI) to be Generally Recognized as Safe (GRAS), consistent with Section 201(s) of the *Federal Food, Drug, and Cosmetic Act*. This determination is based on scientific procedures as described in the following sections, under the conditions of intended use as an ingredient in food and beverages. Therefore, the use of PFI's GABA as described below is exempt from the requirement of premarket approval.

Signed,

(b) (6)

August 3, 2015

Noriko Horie
Director of Sales & Marketing
Pharma Foods International Co., Ltd.

Date

B. Name and Address of Notifier

Noriko Horie
Director of Sales & Marketing
Pharma Foods International Co., Ltd.
1-49 Goryo-Ohara, Nishikyo-Ku,
Kyoto, 615-8245
Japan
Email: n-tani@pharmafoods.co.jp
Office phone: +81 75 394 8600
Office fax: +81 75 394 0009

C. Common Name of the Notified Substance

gamma-Aminobutyric acid (GABA)

D. Conditions of Intended Use in Food

PFI intends to market GABA (>80% purity), derived from L-glutamate *via* a *Lactobacillus hilgardii* K-3 fermentation process as a food ingredient in the United States (U.S.) at a level of 100 mg/serving (ranging from 0.04 to 0.67% depending on the serving size) for use in various foods such as snack bars, breakfast cereals, processed cheese, chewing gum, yogurts, hard

and soft candies, and chocolate as well as beverages and beverage bases including carbonated, energy, flavored, powdered and sports drinks, flavored milk and milk drinks, and coffee and tea. GABA is not intended for use in meat and poultry or meat and poultry-containing products. GABA is not to be used or marketed in infant and children's food products.

E. Basis for GRAS Determination

Pursuant to 21 CFR §170.30, GABA has been determined by PFI to be GRAS on the basis of scientific procedures (U.S. FDA, 2014). This GRAS determination is based on data generally available in the public domain pertaining to the safety of GABA for use in food, as discussed herein and in the accompanying documents, and on a consensus among a Panel of Experts[1] who are qualified by scientific training and experience to evaluate the safety of GABA as a component of food [see Appendix A, **Expert Panel Report Concerning the Generally Recognized as Safe (GRAS) Status of *gamma*-Aminobutyric Acid (GABA) for Use as a Food Ingredient**].

F. Availability of Information

The data and information that serve as the basis for this GRAS Notice will be sent to the U.S. Food and Drug Administration (FDA) upon request, or will be available for review and copying at reasonable times at the offices of:

Pharma Foods International Co., Ltd.
1-49 Goryo-Ohara, Nishikyo-ku
Kyoto, 615-8245
Japan

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

II. Detailed Information Regarding the Identity of the Substance

A. Identity

The common or usual name of this product is GABA. PFI's GABA ingredient is a spray-dried white to light yellow crystalline powder produced *via* a *L. hilgardii* K-3 catalyzed fermentation process. The ingredient contains not less than 80% GABA, with the remaining material characterized as being primarily glutamic acid with trace amounts of other free amino acids, carbohydrates, and lipids, which are carry-over products of the fermentation culture.

[1] The Panel of Experts consisted of Prof. Joseph F. Borzelleca, Ph.D. (Virginia Commonwealth University, School of Medicine), Prof. Stephen L. Taylor, Ph.D. (University of Nebraska) and Prof. John A. Thomas, Ph.D. (Indiana University School of Medicine).

Common or Usual Name: GABA

Chemical Name: *gamma*-amino butyric acid

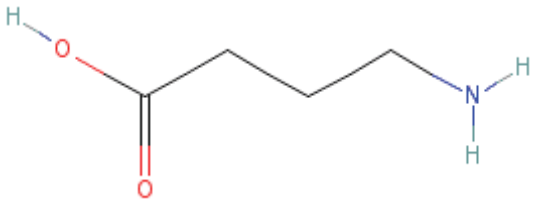
Chemical Abstracts Service (CAS) Number: 56-12-2

Empirical Formula: $C_4H_9NO_2$

Molecular Weight: 103.12

Structural Formula: See Figure II.A-1

Figure II.A-1 Structure of *gamma*-Aminobutyric Acid (GABA)



Physical Properties

The physical properties of GABA are detailed in Table II.A-1.

Table II.A-1 Physical Properties of *gamma*-Aminobutyric Acid (GABA)

Physical Property	Value	Temperature (°C)	Source
Melting Point	203°C	-	Experimental
pKa Dissociation Constant	4.05	15	Experimental
log P (octanol-water)	-3.17	-	Experimental
Water Solubility	1.30×10^{6} mg/L	25	Experimental
Vapor Pressure	1.98×10^{-8} mmHg	25	Estimated
Henry s Law Constant	9.93×10^{-11} atm-m^3/mole	25	Estimated
Atmospheric OH Rate Constant	3.45×10^{-11} cm^3/molecule-sec	25	Estimated

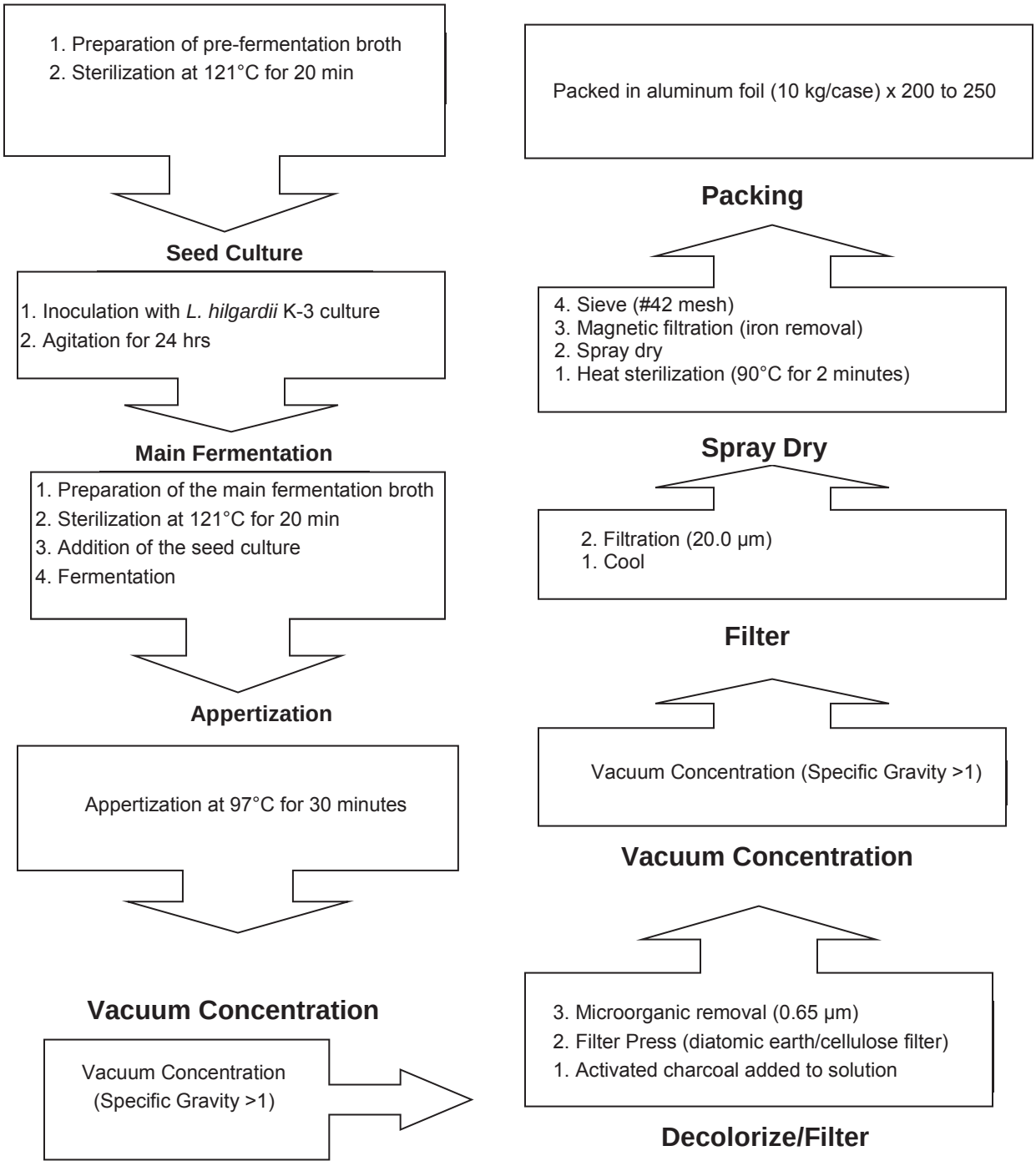
ChemIDplus (2015)

B. Method of Manufacture

A schematic overview of the manufacturing process for GABA is provided in Figure II.B-1 along with a description of the principle production processes. GABA is produced consistent with current Good Manufacturing Practices (cGMP) and an Establishment Inspection Report (EIR) issued by the FDA following inspection of the GABA production facilities concluded that "*there*

were no objectionable conditions observed during the inspection" (U.S. FDA, 2013). The production facility is also certified under ISO9001:2008.

Figure II.B-1 Schematic Overview of the Manufacturing Process for *gamma*-Aminobutyric Acid (GABA)



Initially, a pre-fermentation broth is prepared by dissolving monosodium glutamate, glutamic acid, yeast extract, glucose, and glycerin fatty acid ester in water inside a sealed fermentation vessel. The mixture is then sterilized at a temperature of 121°C for a period of 20 minutes. Following sterilization, the mixture is cooled and the vessel pressure reduced. The fermentation culture (*L. hilgardii* K-3[2]) is then added to the pre-fermentation broth, and is incubated in a sealed fermentation vessel under slight pressure[3] for 24 hours to produce a seed culture for use in the main fermentation. The main fermentation broth is produced using the same raw materials as the pre-fermentation broth, and is sterilized at 121°C for 20 minutes. The seed culture is then added to the main fermentation vessel, and fermentation of glutamate to GABA occurs at room temperature in a sealed vessel, under slight pressure over a few days; during fermentation glutamate is continually added to the fermentation broth. Once fermentation is complete, appertization is conducted by elevating the temperature of the vessel to ~97°C for 30 minutes to inactivate the fermentation organism. After appertization, the vessel is cooled, and the solution then undergoes a vacuum concentration step followed by addition of activated charcoal to the vessel. After brief mixing, the bacteria and large particulates are removed from the solution by filter pressing with a diatomaceous earth/cellulose filter. The heat killed *L. hilgardii* K-3 and other fine particulates are then removed from solution using a 0.65 µm filter[4]. The filtered solution is vacuum concentrated a second time and filtered once again through a 20.0 µm filter to remove any particulates that precipitate during concentration. The solution is stored in polyethylene/nylon bags for transport to the spray-drying tower. The GABA solution is then heat sterilized at 90°C for 2 minutes prior to being spray dried. After spray drying, the product undergoes magnetic filtration at 12,000 G to remove iron particulates[5]. The product then undergoes a partial dry heat sterilization at 90°C for 2 minutes, and after a second round of magnetic filtration (12,000 G) the final product, which is not less than 80% GABA, is produced. The GABA is then sifted through a #42 mesh before storage in aluminum pouches. Alternatively, GABA can be re-formulated to 20% purity as GABA 20 by a 4-fold dilution with the food starch-modified FLO-Max™ 8.

All raw materials and processing aids used in the manufacture of GABA are used in compliance with appropriate U.S. federal regulations (see Table II.B-1).

[2] The fermentation culture undergoes routine quality control monitoring to ensure the absence of contaminating microorganisms and assurance of phenotypic stability.
[3] Sealed vessel under slight pressure represents a critical control point preventing contamination during fermentation.
[4] As indicated by the manufacturer, *L. hilgardii* K-3 is between 2 to 3 µm in size.
[5] Iron removal is a standard practice of the manufacturer. Iron is neither introduced to the product during manufacturing, nor is expected to be present in significant quantities for other reasons.

Table II.B-1 List of Raw Materials and Processing Aids Used in the Manufacture of GABA

Raw Material/Processing Aid	Function	21 CFR Citation or GRAS Number
Lactobacillus hilgardii K-3	Fermentation organism	No regulatory provisions identified for its use in food Safety of source organism for use in the manufacture of PFI's GABA is presented in Section IV.H
L-glutamic acid	Fermentation media	21 CFR §172.320 Amino Acids[a]
Monosodium glutamate	Primary substrate for fermentation	21 CFR §182.1 Substances that are generally recognized as safe
Yeast extract	Nutrient source in fermentation media	21 CFR §172.896 Dried yeasts[b]
Glucose	Nutrient source in fermentation media	21 CFR §184.1857 Corn sugar
Glycerin fatty acid ester	Emulsifier	21 CFR §172.854 Polyglycerol esters of fatty acids
Activated carbon	Purification aid	No federal regulations specific to the intended use were identified Similar uses of activated carbon are considered GRAS for purification and clarification of wine as per 27 CFR §24.246 (US ATTTB, 2014)
Diatomaceous earth/cellulose filter (purification aid) • Cellulose • Natural diatomaceous earths • Perlite • Polypropylene gaskets • <3% polyolefine fibers	Purification aid	Diatomaceous earth and perlite products used to make filtration media are GRAS for use as filter aids in food processing (U.S. FDA, 2002). 21 CFR § 177.1520 Olefin polymers. 21 CFR § 175.300 Resinous and polymeric coatings.
0.65 µm filter (Polysulfone type) (purification aid)	Purification aid	21 CFR § 177.2910 Ultra-filtration membranes. 21 CFR § 177.1655 Polysulfone resins.
20 µm filter (Polyether type) (purification aid)	Purification aid	21 CFR §177.2910 Ultra-filtration membranes. 21 CFR § 177.2260 Filters, resin-bonded.
FLO-Max™ 8 (tapioca starch)	Re-formulation diluent	21 CFR §172.892 Food starch-modified

[a] Permitted for direct addition to food for human consumption at levels not exceeding 12.4% weight of total protein (expressed as free amino acid) of the finished product for nutritive purposes.
[b] A maximum of 0.4 mg/g total folic acid content of yeast permitted.

C. Specifications and Analytical Data

The product specifications established for PFI's GABA are outlined in Table II.C-1. The product is characterized to a purity of not less than 80%, while <5 and 15% is comprised of moisture and ash, respectively. Specifications for heavy metals and microbial contamination are appropriate for use as a food ingredient. Aerobic bacteria and yeast and mold are limited by quality control procedures and are below the established specifications of <1,000 and <300 CFU/g, respectively.

Table II.C-1 Product Specifications for GABA

Specification Parameter	Specification	Method of Analysis
Identity		
Appearance	Light yellow to light brown powder	Visual appearance
gamma-Aminobutyric acid (GABA)	NLT 80%	Amino acid analysis with HPLC (based on Bianchi *et al.*, 1999)
Moisture	<5%	Heated-air drying at normal pressure method (105°C, 5 hours, based on MHLW, 2000, p. 29)
Ash	<15%	Direct ashing method (550 to 600°C, 5 hours, without sulfuric acid, based on MHLW, 2000, p. 7)
Heavy Metals		
Total Heavy Metals	<10 µg/g	Sodium sulfide colorimetry (based on MHLW, 2000, p.24)
Lead	<0.5 µg/g	Atomic Absorption Spectroscopy (based on MHLW, 2000, p. 8)
Microbiological Analysis		
Total Aerobic Counts	<1,000 CFU/g	Methods established by Food Hygiene Guidance (edited by Japan Food Hygiene Association) (MHLW, 2000, p. 32-35)
Yeast and Mold	<300 CFU/g	
Coliform/*Escherichia coli*	Negative	

CFU = colony-forming units; DDTC-Ag = silver diethyldithiocarbamate; HPLC = high-performance liquid chromatography; MHLW, 2000 = Ministry of Health, Labor and Welfare, Japan - Japan's Specifications and Standards for Food Additives (7th ed.); NLT not less than.

Additionally, PFI's GABA will also be used as a re-formulated product, in which GABA (not less than 80% purity) will be diluted approximately 4-fold with FLO-MAX™ 8 Starch to produce a GABA product of not less than 20% purity (GABA 20). The product specifications for GABA 20 are presented in Table II.C-2.

Table II.C-2 Product Specifications for GABA 20

Specification Parameter	Specification	Method of Analysis
Identity		
Appearance	White to light yellow powder	Visual appearance
gamma-Aminobutyric acid (GABA)	NLT 20%	Amino acid analysis with HPLC (based on Bianchi *et al.*, 1999)
Moisture	<10%	Heated-air drying at normal pressure method (105°C, 5 hours, based on MHLW, 2000, p. 29)
Ash	<18%	Direct ashing method (550 to 600°C, 5 hours, without sulfuric acid, based on MHLW, 2000, p. 7)
Heavy Metals		
Total Heavy Metals	<10 µg/g	Sodium sulfide colorimetry (based on MHLW, 2000, p.24)
Microbiological Analysis		
Total Aerobic Counts	<1,000 CFU/g	Methods established by Food Hygiene Guidance (edited by Japan Food Hygiene Association) (MHLW, 2000, p. 32-35)
Yeast and Mold	<300 CFU/g	
Coliform/*Escherichia coli*	Negative	

CFU = colony-forming units; DDTC-Ag = silver diethyldithiocarbamate; HPLC = high-performance liquid chromatography; MHLW, 2000 = Ministry of Health, Labor and Welfare, Japan - Japan's Specifications and Standards for Food Additives (7th ed.); NLT not less than.

Representative lots are routinely assayed to ensure compliance with final product chemical, physical, and microbiological specifications. Analysis of 3 non-consecutive lots of GABA and GABA 20 demonstrates that the manufacturing process produces a consistent product that conforms to the established specifications. The results of the batch analyses are presented in Tables II.C-3 and II.C-4 for GABA and GABA 20, respectively, and the Certificates of Analysis are provided in Appendix B (Attachment B-1 and B-2, respectively).

Table II.C-3 Analysis of 3 Manufactured Lots of GABA

Specification Parameter	Specification	Lot Number/Date of Manufacture		
		2J10/ 10/10/2012	4B06/ 2/6/2014	4E21/ 5/21/2014
Identity				
Appearance	Light yellow to light brown powder	Conforms	Conforms	Conforms
GABA (%)	NLT 80	87.2	84.6	89.8
Moisture (%)	<5	2.8	3.0	3.0
Ash (%)	<15	3.0	3.1	3.1
Heavy Metals				
Total Heavy Metals (µg/g)	<10	<10	<10	<10
Lead (µg/g)	<0.5	<0.5	<0.5	<0.5
Arsenic (µg/g)	<2	<2	<2	<2
Microbiological Quality				
Total Aerobic Counts (CFU/g)	<1,000	20	<10	30
Yeast and Mold (CFU/g)	<300	<10	<10	<10
Coliform/*Escherichia coli*	Negative	Negative	Negative	Negative

CFU = colony forming units; GABA = *gamma*-aminobutyric acid; NLT not less than

Table II.C-4 Analysis of 3 Manufactured Lots of GABA 20

Specification Parameter	Specification	Lot Number/Date of Manufacture		
		6A31/ 1/31/2006	6B07/ 2/7/2006	7F15/ 6/15/2007
Identity				
Appearance	White to light yellow powder	Conforms	Conforms	Conforms
GABA (%)	NLT 20	22.9	22.9	21.2
Moisture (%)	<10	4.8	5.6	4.8
Ash (%)	<18	15	14.7	13.1
Heavy Metals				
Total Heavy Metals (µg/g)	<10	<10	<10	<10
Arsenic (µg/g)	<2	<2	<2	<2
Microbiological Quality				
Total Aerobic Counts (CFU/g)	<1,000	<10	10	<10
Yeast and Mold (CFU/g)	<300	<10	<10	<10
Coliform/*Escherichia coli*	Negative	Negative	Negative	Negative

CFU = colony forming units; GABA = *gamma*-aminobutyric acid; NLT not less than

Compositional analysis of one lot of GABA was conducted, as shown in Table II.C-5, indicating that PFI's ingredient comprises not less than 80% GABA, with the remaining material composed of small amounts of free amino acids (7%) as well as moisture (1.9%), ash (3.4%),

carbohydrates (<1%) and lipids (≤1%). Sodium chloride accounts for approximately 90% of the total ash and no minerals were present at levels that would be of toxicological concern.

Table II.C-5 Compositional Analysis of GABA

Analysis Parameter	Results (g/100 g)	Method of Analysis[a]
Compositional Parameters		
gamma-Aminobutyric acid	87.9	Amino acid analysis with HPLC
Total amino acids	7.0	Acid hydrolysis with HPLC
Ash	3.4	Direct incineration method
Moisture	1.9	Heated-air drying at normal pressure
Dietary fiber	0.5	Enzyme-weight method
Total saccharide	0.2	Phenol sulfuric acid method
Lipids	≤0.1	Acid decomposition method
Monoglycerides	ND	Gas chromatography
TOTAL	**101**	
Minerals		
Sodium chloride	3.08	Atomic absorption spectrometer method
Sodium	1.21	Atomic absorption spectrometer method
Potassium	0.287	Atomic absorption spectrometer method
Phosphorus	0.0968	ICP method
Calcium	0.0390	ICP method
Magnesium	0.0137	ICP method
Iron	0.00015	ICP method
Zinc	0.00008	ICP method
Manganese	0.00006	ICP method
Copper	ND	ICP method
TOTAL MINERAL CONTENT	**3.52**	

GABA = *gamma*-aminobutyric acid; HPLC = high-performance liquid chromatography

Analysis of the total acid-hydrolyzed free amino acid content through using high performance liquid chromatography (HPLC) found that the ingredient typically contains a variety of free amino acids and dipeptides that originate from the fermentation medium to which the majority was comprised of unmetabolized glutamate (4.7%) originating from the fermentation process (Table II.C-6). Small amounts of additional amino acids from the fermentation medium were also present each at levels below 0.5%. Further analysis of the total acid-hydrolyzed amino acid content of GABA corresponded on a weight to weight basis to the total free amino acid and dipeptide content of the ingredient indicating that the product is free of contaminating proteins.

Table II.C-6 Detailed Amino Acid Analysis of GABA

Analysis Parameter	Results (g/100 g)[a]		
	Total Amino Acids (Acid Hydrolysis)	Free Amino Acid Content	Polypeptide Content
Arginine	0.02	0.02	ND
Lysine	0.21	0.20	0.01
Histidine	0.04	0.04	ND
Phenylalanine	0.02	0.02	ND
Tyrosine	0.02	0.02	ND
Leucine	0.23	0.23	ND
Isoleucine	0.18	0.16	0.02
Methionine	0.04	0.03	0.01
Valine	0.24	0.24	ND
Alanine	0.42	0.42	ND
Glycine	0.13	0.11	0.02
Proline	0.11	0.11	ND
Glutamic acid	4.70	2.15	2.55
Serine	0.16	0.11	0.05
Threonine	0.14	0.09	0.05
Aspartic acid	0.32	0.17	0.15
Tryptophan	ND	ND	ND
Cysteine	0.02	ND	0.02
TOTAL	**7.00**	**4.12**	**2.88**
Total Amino Acid Content	**7.00**	**7.00**	

GABA = *gamma*-aminobutyric acid; ND = not detected
[a] Amino acid and polypeptide analysis was conducted using high-performance liquid chromatography.

Elimination of the source organism, *L. hilgardii* K-3, from the final product is achieved through the appertization process and subsequent filter sterilization steps. Moreover, lactic acid bacteria such as *L. hilgardii* are highly sensitive to oxidative environments, and thus it is unlikely that the organism would survive the oxidative conditions of spray drying, further ensuring that microbial contamination of *L. hilgardii* K-3 in the final product is prevented. Analysis of the final GABA ingredient confirmed the absence of *L. hilgardii* K-3 (see Appendix C; Attachment C-1). Furthermore, it is not expected that the fermentation conditions would be conducive to contamination of the final product with *L. hilgardii* K-3-derived biogenic amines as many of the biogenic amine precursors (histidine, tyrosine, and phenylalanine) were detected at minimal concentrations of ≤0.04% and analysis of the GABA ingredient confirmed the absence of histamine (see Appendix C; Attachment C-2). Additionally, analysis of *L. hilgardii* K-3 associated microbial by-products including ethylcarbamate and citrulline demonstrated that it was not detected in PFI's final GABA ingredient (see Appendix C; Attachment C-3, C-4, respectively).

Analysis of the bulk stability of GABA in terms of the GABA content and moisture demonstrated that the final product was stable for a period of up to 18 weeks at room temperature (Table II.C-7).

Table II.C-7 GABA Stability Following Storage at Room Temperature (20°C) for 11, 17 and 18 Weeks

Lot Number	Specification (%)	Initial (%)	After stored at 20°C (%)	Difference (%)
GABA Content				
7B09	>80	85.2	87.0 (at 11 weeks)	1.8
6L25		88.1	90.1 (at 17 weeks)	2.0
6K22		87.4	87.8 (at 18 weeks)	0.4
Moisture				
7B09	<5	1.9	2.0 (at 11 weeks)	0.1
6L25	<5	1.6	1.5 (at 17 weeks)	-0.1
6K22	<5	1.7	1.6 (at 18 weeks)	-0.1

GABA = *gamma*-aminobutyric acid

Bulk stability studies also indicate that the GABA 20 formulation is stable for up to 27 months under ambient conditions (Table II.C-8). Based on this the shelf-life was set at 2 years.

Table II.C-8 Bulk Stability of GABA 20 Powder (Lot No. 4H11) Following Storage at Room Temperature for a Period of 27 Months

Months	0	6	12	18	24	27
gamma-Aminobutyric Acid (GABA) (%)	22.1	22.4	22.5	22.1	21.9	22.3

GABA was also determined to be stable under conditions representative of its intended use in foods including a concentration of 5% in aqueous solution under varied pH conditions ranging from 2 to 6 and elevated temperatures of 100 to 120°C (Figures II.C-1 to II.C-3). Thus, GABA is expected to be stable under the proposed conditions of use in foods and beverages.

Figure II.C-1 Bulk Stability of GABA Powder (Lot No. 4H11) Under Heated Conditions Over a Period of 60 Minutes

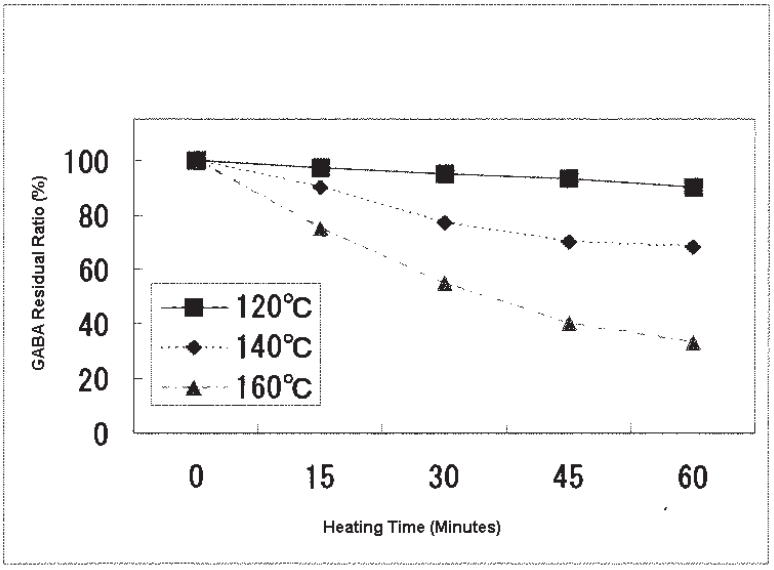


Figure II.C-2 Stability of a 5% GABA Solution (Lot No. 4H11) Under Heated Conditions for a Period of 120 Minutes

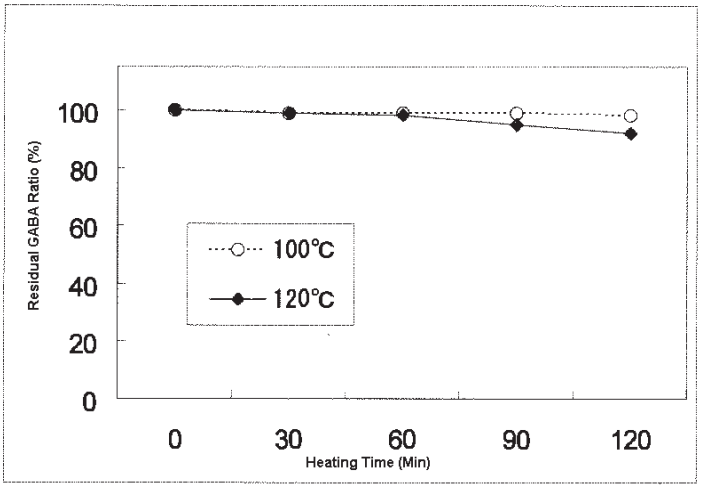
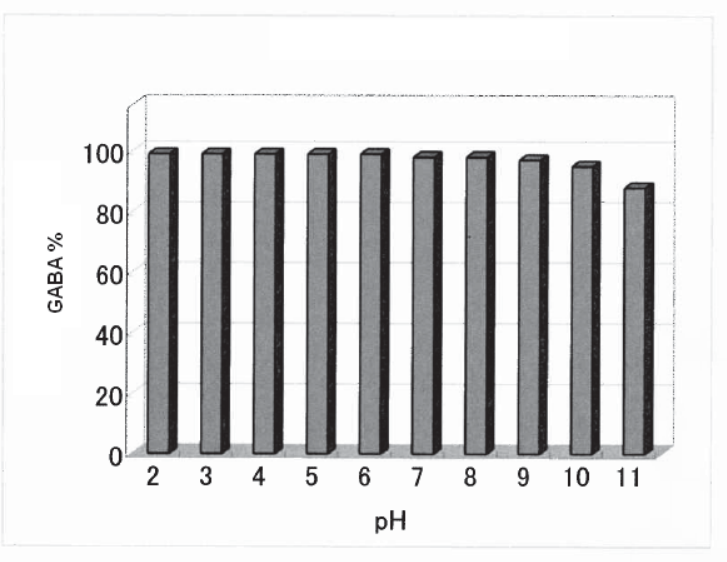


Figure II.C-3 Stability of a 5% GABA Solution (Lot No. 4H11) Under Varied pH Conditions



III. Self-Limiting Levels of Use

The levels of use of GABA are limited due to food formulation issues, as the ingredient is expected to impart an undesirable flavor to the food when incorporated at high concentrations. Specific thresholds for these effects have not been determined.

IV. Basis for GRAS Determination

Determination of the GRAS status of GABA is on the basis of scientific procedures. Information supporting the general recognition of the safe use of GABA includes:

- Data pertaining to the identity, intended use, and estimated intake of GABA;
- Background occurrence of GABA as a natural or added component of various foods consumed in the U.S. and Japan, including estimates of background dietary exposures;
- Information characterizing the kinetics and metabolic fate of GABA, including data on the capacity of GABA to cross the blood-brain-barrier from the systemic circulation;
- The entirety of animal and human studies assessing the safety of GABA consumption; and
- Data pertaining to the safety of *L. hilgardii* K-3 for use in the production of a food ingredient.

Moreover, these data were reviewed by a Panel of Experts, qualified by scientific training and experience to evaluate the safety of GABA as a component of food, who concluded that the

proposed uses of GABA are safe and suitable and would be GRAS based on scientific procedures [see Appendix A **Expert Panel Report Concerning the Generally Recognized as Safe (GRAS) Status of *gamma*-Aminobutyric Acid (GABA) for Use as a Food Ingredient**]. A summary of the data is presented herein.

A. Estimated Intake of GABA

Background Dietary Consumption of GABA

As shown in Table IV.A-1, GABA exists naturally in many different foods at low levels, and in higher levels in fermented food products. Examples of GABA-rich foods include tomatoes, potatoes, melons, and the traditional Korean and Japanese food, kimchi and miso. These foods have been reported to contain from 27 to 305 mg GABA/100 g food product (Steward *et al.,* 1949; Fox, 1995; Loiudice *et al.,* 1995; Hayakawa *et al.,* 1997; Matumoto *et al.,* 1997; Nomura *et al.,* 1998; Akastu, 2000; Murcia *et al*., 2001; Nakamura *et al*., 2006). In addition, since the conversion of glutamate to GABA is reported for many lactic acid bacteria, other foods are likely to contain appreciable amounts of GABA as a result of the processing methods used to produce them (*e.g.*, lactic acid-fermented foods, such as cured meats and cheeses). For example, certain cheeses such as Kompansiti cheese have been reported to contain levels of GABA as high as 602 mg/100 g.

Table IV.A-1 Reported GABA Content of Various Foods[a]

Food Item	GABA Content (mg/100 g)	Food Item	GABA Content (mg/100 g)
Green Tea Leaf (dried)	100 to 200	Cucumber	7.2
Melon	74.5	Tofu	6.4
Tomato	62.6	Soy bean (green)	6.4
San Marzano Tomato	51 - 305	Carrot	6.2
Kimchi (fermented)	59.4	Radish	5.5
Vegetable Juice	56.0	Persimmon	5.5
Tomato Juice	51.0	Green pepper	4.4
Miso (soy paste)	48.0	Green bean	4.3
Potato	24 - 61	Kiwi	3.3
Kimchi (lightly pickled)	27.5	Unpolished rice	3.0
Grapes	23.2	Lettuce	1.9
Egg Plant	20.0	Peach	1.7
Mandarin Orange	17.5	Onion	1.4
Rice Vinegar	15.0	Spinach	1.3
Chocolate	14.5	Chinese cabbage	1.0
Amanatsu orange	12.1	Rice (polished)	1.0
Navel orange	11.5	Milk	0.05
Germinated rice	10.0	Canned coffee	0.02

Table IV.A-1 Reported GABA Content of Various Foods[a]

Food Item	GABA Content (mg/100 g)	Food Item	GABA Content (mg/100 g)
Pumpkin	9.7	Cheddar	4.8
Cabbage	8.2	Gouda	17.7
Shitake mushroom	8.2	Parmigiano cheese	170
Loquat	8.0	Kompanisti cheese	602
Broccoli	3.1	Potato Tuber	54

[a] Adapted from Steward *et al.* (1949), Fox (1995); Hayakawa *et al.* (1997), Nakamura *et al*. (2006); Matumoto *et al.* (1997), Akastu (2000); Nomura *et al.* (1998); Loiudice *et al.* (1995); Murcia *et al*. (2001); independent analysis conducted by Pharma Foods International Co., Ltd.

Using annual *per capita* consumption data for the available foods above (Stofberg and Grundschober, 1987) and published information on the GABA content of various foods, the average U.S. intake of GABA from its natural occurrence was estimated to be 136 mg/person/day (equivalent to 2.1 mg/kg body weight/day for a 65 kg individual). While the consumption data is old, this value is likely to be an overestimation of GABA intakes as more recent estimates of the background intake of GABA from natural dietary sources using Japanese survey data (Japan National Health and Nutrition Examination Survey 2005) (MHLW, 2007a,b) were determined to be lower (80.20 mg/person/day), despite Japanese diets containing more fermented foods relative to North American diets.

Food Uses of GABA World-Wide

Many GABA-enriched foods have been launched onto the Japanese market over the past 20 years with positive consumer reception. Products that currently are enriched with GABA in Japan include tea, germinated rice products, pickles, fermented milk, and chocolate (as detailed in Table IV.A-2. The GABA content in these products ranges from approximately 10 mg/100 g in germinated rice to 280 mg/50 g in chocolate.

Table IV.A-2 Examples of GABA-Enriched Foods and Beverages Currently Sold in Japan

Food	Product Name	Manufacturer	GABA Content	Year Launched
Tea	GABRON-TEA	OSK, IKT	200 mg/100 g	1986
Germinated Rice	Sprouted Brown Rice	Domer Inc.	10 mg/100 g	1998
Germinated Rice	Brown Rice "HATSUGA-MAI"	FANCL Corp	10 mg/100 g	1999
Germinated Rice Extract	ORYZA-GABA21	ORYZA Oil and Chemical Co.	250 mg/100 g	1998
Pickles	"TSUKEMONO-HYAKUSEN"	Fujicco Co., Ltd	50 mg/100 g	2002
Drinks	Score-Aid	Okura Chemi-Tech Corp.	100 mg/350 mL	2003
Fermented Milk	PRETIO	Yakult Honsha Co., Ltd	10 mg/100 mL	2004
Chocolate	Mental Balance Chocolate GABA	EZAKI GLICO Co., Ltd.	280 mg/50 g	2005

GABA = *gamma*-aminobutyric acid

Use of GABA as a Dietary Supplement

GABA is a dietary ingredient in a number of dietary supplement products currently available on the U.S. market as a principle ingredient or as a component of multiple ingredients. A preliminary review of dietary supplement products indicates that GABA is present in supplemental products at levels ranging from 100 to 750 mg/capsule or tablet, and recommended intakes are typically 750 mg GABA/person/day (Table IV.A-3). The consumption of up to 1,500 to 5,000 mg of GABA/day has been indicated for some dietary supplement products, without recommended durations for use.

PFI's GABA has been marketed in dietary supplements available in the U.S. for approximately 8 years. U.S. companies that manufacture dietary supplements containing PFI's GABA include Jarrow Formulas® and Biosynergy Health Alternatives with recommended daily intakes from these supplemental products up to 600 mg GABA/day. In the U.S., the annual sales of PFI's GABA as an ingredient for use in dietary supplements ranges from 1 to 2 metric tons.

Table IV.A-3 Examples of Dietary Supplement Products in the U.S. Identified as Containing GABA as a Dietary Ingredient

Product	Ingredient(s) [Form]	Directions for Use	Amount of GABA (mg)/ serving	Daily Intake of GABA (mg)
AST Sports Science GABA	GABA [powder]	3 to 5 scoops daily	3,000 to 5,000	3,000 to 5,000
Biosynergy Health Alternatives Natural GABA	GABA, magnesium stearate, silicon dioxide, maltodextrin [capsule]	1 to 3 capsules daily	200	600
Jarrow Formulas GABA Soothe	GABA, theanine, Ashwagandha extract, cellulose, magnesium stearate, silicon dioxide [capsule]	1 capsule daily	100	100
Jarrow Formulas Sleep Optimizer	GABA, L-tryptophan, lemon balm, hops flower, melatonin, magnesium stearate, silicon dioxide [capsule]	2 capsules daily before bedtime	50	100
KAL GABA	GABA, various fillers [tablet]	1 tablet daily	750	750
Swanson Health Products GABA	GABA, gelatin, magnesium stearate [capsule]	1 capsule daily	500	500
Source Naturals GABA	GABA [capsule or tablet]	1 tablet daily	750	750
TwinLabs GABA Plus	GABA, niacin, inositol, silica, magnesium and fillers [capsule]	2 to 5 capsules daily	100	500
Ultimate Nutrition GABA	GABA, Cellulose, dicalcium phosphate, gelatin and magnesium stearate [capsule]	2 tablets daily	750	1,500
Vitamin Shoppe GABA	GABA, various fillers [capsule]	1 tablet daily	750	750

Intended Conditions of Use of PFI's GABA in Food and Proposed Levels of Use

PFI intends to market GABA as a food ingredient in the U.S. in a variety of food and beverages at a use level of 100 mg GABA per serving (or 0.04 to 0.67% GABA depending on the serving size). The individual proposed food-uses and use-levels for GABA employed in the current intake analysis are summarized in Table IV.A-4. Food codes representative of each proposed food-use were chosen from the 2011-2012 National Health and Nutrition Examination Survey (NHANES) (CDC, 2014; USDA, 2014). Food codes were grouped in food-use categories according to Title 21, Section §170.3 of the Code of Federal Regulations (U.S. FDA, 2014). It should be noted that GABA is not intended for use in meat and poultry or meat and poultry-containing products. GABA is also not intended for use in infant and children's foods.

Table IV.A-4 Summary of the Individual Proposed Food-Uses and Use-Levels for *gamma*-Aminobutyric Acid (GABA) in the U.S. (NHANES 2011-2012 Data)

Food Category	Food-Uses	Serving Size[a]	Proposed Use Level	
			(per serving)	(%)
Baked Goods and Baking Mixes	Snack Bars	40 g	100 mg	0.25
Beverages and Beverage Bases	Carbonated Drinks	240 mL	100 mg	0.0416
	Energy Drinks (including shot drinks)[b]	240 mL (58 mL[c])	100 mg	0.0416 (0.172)
	Flavored Drinks	240 mL	100 mg	0.0416
	Powder Drinks (including protein powder)	240 mL	100 mg	0.0416
	Sports Drinks, Isotonic Drinks	240 mL	100 mg	0.0416
Breakfast Cereals	Breakfast Cereals	15 g (puffed) 30 g (extruded) 55 g (biscuit)	100 mg	0.667 0.333 0.182
Cheeses	Processed Cheese[d]	30 g	100 mg	0.3
Chewing Gum	Chewing Gum	40 g	100 mg	0.25
Coffee and Tea	Coffee, Specialty Coffee, and Instant Coffee	240 mL	100 mg	0.0416
	Tea and Instant Tea	240 mL	100 mg	0.0416
Hard Candy and Cough Drops	Candies (Hard)	40 g	100 mg	0.25
Milk Products	Flavored Milk and Milk Drinks	240 mL	100 mg	0.0416
	Yoghurts[d]	225 g	100 mg	0.044
Processed Vegetables and Vegetable Juices	Vegetable Juices	240 mL	100 mg	0.0416
Soft Candy	Candies (Soft)	40 g	100 mg	0.25
	Chocolates[d]	40 g	100 mg	0.25

[a] RACC refers to Reference Amounts Customarily Consumed per eating occasion – 21 CFR §101.12 (U.S. FDA, 2014). When a range of values is reported for a particular food-use, particular foods within that food-use may differ with respect to their RACC
[b] There were no food codes identified for energy shot drinks within the NHANES dataset
[c] Values taken from product websites
[d] This food-use represents non-standardized food products; however, in order to obtain a conservative intake estimate, surrogate codes for the standardized food products were chosen.

Estimated Intake from the Proposed Conditions of Use

Estimates for the anticipated dietary exposure to GABA as an ingredient in foods and beverages under the proposed conditions was generated by applying the maximum proposed use level indicated for each proposed food use as presented in Table IV.A-4 using the 2011-2012 National Health and Nutrition Examination Survey (NHANES) data (CDC, 2014; USDA, 2014). During the intake calculations it was assumed that GABA was 100% pure. Although the assumption would result in a slight overestimation of intakes, it was performed for ease of comparison to available safety data.

Within the NHANES survey data, approximately 97.4% of the total U.S. population was identified as consumers of GABA from the proposed food uses and all individual age groups evaluated in the current intake assessment. Infants had the lowest proportion of users (70.1%), and greater than 96.2% of all other population groups consisted of users of those food products in which GABA is currently proposed for use (see Table IV.A-5). Consumption of GABA-containing foods by the total U.S. population would result in estimated mean and 90th percentile all-user intakes of GABA of 429 and 811 mg/person/day, respectively (see Table IV.A-5). Of the individual population groups, male adults were determined to have the greatest mean and 90th percentile all-user intakes of GABA on an absolute basis, at 535 and 978 mg/person/day, respectively, while infants had the lowest mean and 90th percentile all-user intakes of 140 and 298 mg/person/day, respectively.

Table IV.A-5 Summary of the Estimated Daily Intake of *gamma*-Aminobutyric Acid (GABA) from Proposed Food-Uses in the United States by Population Group (NHANES 2011-2012 Data)

Population Group	Age Group (Years)	All-Person Consumption (mg/person/day)		All-Users Consumption (mg/person/day)			
		Mean	90th Percentile	% Users	n	Mean	90th Percentile
Infants	0 to 2	98	245	70.1	447	140	298
Children	3 to 11	259	482	98.1	1,501	264	485
Female Teenagers	12 to 19	327	634	96.2	515	339	641
Male Teenagers	12 to 19	388	694	98.9	506	392	694
Female Adults	20 and up	416	766	98.5	2,176	422	766
Male Adults	20 and up	529	975	98.8	2,054	535	978
Total Population	All Ages	418	807	97.4	7,199	429	811

On a body weight basis, infants were identified as having the highest mean and 90th percentile all-user intakes of any population group, of 11.1 and 23.1 mg/kg body weight/day, respectively, while children's intakes of GABA on a body weight basis are 9.9 and 18.5 mg/kg body weight/day at the mean and 90th percentile, respectively (see Table IV.A-6). However, it should be noted that PFI's GABA is not intended to be marketed or used in infant and children foods; thus the actual infant and children consumption of GABA is expected to be highly limited. While an estimate of the consumption of GABA on a body weight basis in infants and children from all proposed food uses has been included for completeness, it is considered to be a gross over-estimate of the actual expected intake of GABA by infants and children from addition to food. This is supported in that the lowest intakes on an absolute basis were determined to be in infants and children. Female teenagers had the lowest mean all-user intake at 5.6 mg/kg body weight/day, while female adults had the lowest 90th percentile all-user intake at 10.3 mg/kg body weight/day.

Table IV.A-6 Summary of the Estimated Daily Per Kilogram Body Weight Intake of *gamma*-Aminobutyric Acid (GABA) from Proposed Food-Uses in the United States by Population Group (NHANES 2011-2012 Data)

Population Group	Age Group (Years)	All-Person Consumption (mg/kg bw/day)		All-Users Consumption (mg/kg bw/day)			
		Mean	90th Percentile	% Users	n	Mean	90th Percentile
Infants	0 to 2	7.8	20.7	70.2	446	11.1	23.1
Children	3 to 11	9.7	18.4	98.1	1,501	9.9	18.5
Female Teenagers	12 to 19	5.4	10.7	96.2	504	5.6	10.8
Male Teenagers	12 to 19	5.9	11.3	98.9	503	6.0	11.3
Female Adults	20 and up	5.7	10.2	98.5	2,153	5.8	10.3
Male Adults	20 and up	6.1	11.1	98.8	2,036	6.2	11.2
Total Population	All Ages	6.4	12.3	97.4	7,143	6.6	12.4

bw = body weight

B. Pharmacokinetics and Metabolic Fate

Data pertaining to the expected metabolic fate and kinetics of GABA were reviewed in order to assess the absorption, distribution, metabolism and excretion under the intended conditions of use in foods and beverages.

Pharmacokinetics

There is limited information detailing the absorption and kinetic properties of orally administered GABA. Evidence of specific GABA transporters in the rat jejunum has been reported (Nácher *et al.*, 1994); however, the maximum plasma levels of GABA following oral administration of GABA in rats were 20-fold less than those observed following intraperitoneal administration of the same dose, suggesting that absorption and/or bioavailability in rodents is low (van Gelder and Elliott, 1958). The kinetics of GABA administered *via* the intraperitoneal or intravenous route were demonstrated to be similar among rats, rabbits, and cats, with rapid clearance rates and half-lives of approximately 20 minutes (van Gelder and Elliott, 1958). The tissue distribution of GABA following intraperitoneal administration was similar in the rat and mouse, where GABA was distributed primarily to the liver, kidneys, and muscle. In mice, significant GABA levels were also detected in the urinary bladder, gastrointestinal wall, pituitary gland, and cartilage of the spine, ribs, and trachea; however, the GABA levels rapidly diminished post-injection (van Gelder and Elliott, 1958; Hespe *et al.*, 1969). It should be noted, that the methodology used to measure GABA in these studies (*i.e.,* inhibition of a spontaneous active crayfish stretch receptor and radioactivity) was non-qualitative, and information distinguishing GABA *versus* metabolites of GABA, or potential bacterial degradation products could not be determined. No evidence of GABA bioaccumulation or organ-specific retention was observed in any of the reviewed

studies[6]. No information detailing the absorption and kinetics of GABA consumption in humans was identified in the published literature.

Metabolic Fate

GABA is an endogenous compound, and given its importance as a neurotransmitter, metabolism of the molecule has been well characterized in humans and animals. The liver is considered to be the primary metabolic site of extra-cerebral GABA, and rats display a large capacity for GABA uptake by this organ. For example, *ex vivo* liver perfusion concentrations had to be increased 60-fold above baseline plasma levels (84 ng/mL) before saturation of GABA uptake occurred in rats (Schafer *et al.*, 1981; Ferenci *et al.*, 1988). The catabolism of GABA occurs exclusively *via* GABA transaminase where the compound is used as a carbon source in the tricarboxylic acid cycle (TCA), resulting in carbon dioxide (CO_2) as the primary waste product (Patel *et al.*, 2005). The catabolism of GABA is indirect and requires the presence of α-ketoglutarate, the only known acceptor of the GABA nitrogen. The transamination of GABA with α-ketoglutarate by GABA transaminase results in the formation of glutamate, succinic semialdehyde, and CO_2. The liberated glutamate can then be used for protein metabolism (in the brain, glutamate is converted to glutamine and recycled back into GABA) or converted to glutamine and used as an energy source entering the TCA cycle. Succinic semialdehyde is further oxidized *via* succinic semialdehyde decarboxylase to produce succinic acid, which is also used as a carbon source for energy in the TCA cycle (Roberts and Baxter, 1958; Waagepetersen *et al.*, 1999; Patel *et al.*, 2005). Thus, GABA is essentially utilized as an energy source by the body, and is metabolized to innocuous compounds.

GABA and the Blood-Brain Barrier (BBB)

Considering the importance of GABA as an inhibitory neurotransmitter in the brain, a crucial issue pertaining to the safety of systemic exposure to GABA is the ability of the compound to cross the BBB. Based on an extensive body of evidence, the ability of the BBB to prevent GABA permeation from the plasma to the brain, although not absolute, is substantial and not significantly affected by exogenously administered GABA (van Gelder and Elliott, 1958; Hespe *et al.*, 1969; Kuriyama and Sze, 1971; Oldendorf, 1971; Frey and Löscher, 1980; Krantis, 1984; Al-Sarraf, 2002; Al-Awadi *et al.*, 2006). For example, in the rat, a 1,250-fold increase in the dose of GABA administered intravenously (from 0.004 to 5 mg/kg body weight) resulted in an increase in the levels of GABA in the cerebrospinal fluid (CSF) of only 30-fold (Al-Awadi *et al.*, 2006). The poor dose-response relationship between the administered dose and GABA permeation across the BBB was also reported in radiokinetic studies, where increases in plasma concentrations of GABA displayed little effect on the overall permeation rates of GABA across the BBB of rats (Krantis, 1984). Even under experimental conditions where GABA permeation rates were increased between 3- to 16-fold [*e.g.*, in neonatal rats or in

[6] Retention of radioactivity in the Harder's glands was noted during autoradiography studies by Hespe *et al.* (1969); however, this organ is not relevant to humans.

Spontaneously Hypertensive Rats (SHR)], the increased permeation rate was not reflected in increased GABA in the central nervous system (Al-Sarraf, 2002; Al-Awadi *et al.*, 2006). These observations may be explained by studies conducted by Kakee *et al.* (2001) that showed that the efflux rate of GABA though the BBB of rats exceeded influx by approximately 16-fold. Furthermore, in a study conducted by Kuriyama and Sze (1971), only 8.8% of the administered dose of GABA was observed in the brains of male albino rabbits 1 hour following sterotaxically injection of radiolabeled GABA (5 mg/kg body weight) into the left ventricle of the brain. Moreover, in animals pre-treated with amino-oxcyacetic acid hemihydrochloride (AOAA), a GABA transaminase inhibitor, 40% of the injected GABA radioactivity was detected in the whole brain, indicating the importance of GABA transaminase in removing excess GABA from the brain. Interestingly, in AOAA-treated animals, most of the radioactivity was identified unabsorbed within the ventricles (site of injection) suggesting the ability of GABA to migrate from the CSF of the ventricles to other areas of the brain is limited.

Strong evidence supporting the inability of GABA to cross the blood brain barrier is also presented by Roberts *et al*. (1958). The authors administered [^{14}C] GABA *via* interperitoneal injection to adult female mice after complete GABA transaminase blockade. Using autoradiography, the majority of GABA was shown to accumulate in the liver, and levels exceeding background were observed in the kidney, urinary bladder, gastrointestinal wall, pituitary gland and cartilage of the vertebrae, ribs and tracheal rings. No evidence of GABA in the brain was observed with this autoradiographic technique. Slight species differences in BBB permeability of GABA were suggested by van Gelder and Elliott (1958), where GABA entry into the brain may be even more restricted in higher order mammals. In contrast to rodents, where small amounts of GABA have been shown to enter the brain, van Gelder and Elliott (1958) reported that the levels of GABA in the CSF were undetectable 30 minutes following the intravenous administration of high doses of GABA (200 mg/kg body weight) to monkeys.

Given the poor permeation rates of the BBB to GABA, the fact that GABA efflux rates are 16-fold greater than GABA influx rates, that GABA transaminase has an efficient capacity to rapidly metabolize large doses of intracerebrally-administered GABA, and observations that large doses of intracerebrally-administered GABA in the presence of GABA transaminase inhibitors do not result in GABA diffusing to other regions of the brain, it is unlikely that GABA would accumulate in the brain to any significant degree following oral consumption of GABA. Thus, on the basis of the pharmacokinetic and metabolic information reviewed from the available literature, the exposure to GABA under the intended conditions of use is unlikely to result in adverse effects in humans mediated by accumulation of GABA in the CNS or any other organ system, since the data from the available studies suggest that GABA is poorly absorbed from the gastrointestinal tract, is unable to cross the BBB to any physiologically-meaningful degree and is rapidly metabolized and cleared from the body.

C. Studies in Animals

Acute Toxicity Studies

A number of studies have demonstrated that GABA is of low acute oral toxicity. In mice, the oral median lethal dose (LD_{50}) for GABA has been reported to be high, ranging from >1 to 12 g/kg body weight (Oshima *et al.,* 1965; Frey and Löscher, 1980).

A single-dose, oral toxicity study was conducted by Japan Food Research Laboratories (JFRL) (JFRL, 2002 [unpublished]) in 4-week-old male and female Wistar rats (10/sex/group) using a study design based on the Organization for Economic Co-operation and Development (OECD) guidelines for the testing of chemicals (OECD Guideline 401) (OECD, 1987). The test article used in the study was GABA 20. Animals received a single gavage of GABA 20 dissolved in water to provide a dose of 5,000 mg/kg body weight of GABA 20 (corresponding to an acute GABA exposure of 1,000 mg/kg body weight). A control group received equal volumes of water. Standard clinical monitoring and observations of mortality were recorded over a 14-day period. No differences in body weights were observed on Days 7 and 14 for both male and female test animals relative to controls. No evidence of morbidity or incidence of mortality was reported, and hence the LD_{50} of GABA 20 in rats was >5,000 mg/kg body weight (or approximately >1,000 mg GABA/kg body weight).

28-Day Studies

Sauchi *et al*. (2009) evaluated the toxicity of PFI's GABA in a 28-day repeat dose toxicity study using male and female Wistar rats. This study was not conducted in accordance to Good Laboratory Practices (GLP) or any other accepted international standard. The test article used in the study was GABA 20, which as previously described, is PFI's GABA ingredient diluted to 20% purity, and identical to the GABA source used in the acute toxicity study of JFRL (2002 [unpublished]). Rats (32 days of age) were randomized to 1 of 2 groups (20/sex/group) receiving standard rat chow (control) or feed supplemented with 1% GABA-20 [providing approximately 1,000 mg GABA-20/kg body weight/day (U.S. FDA, 1993), which would correspond to approximately 200 mg GABA/kg body weight/day]. Standard clinical monitoring, body weight gain, and food consumption were recorded throughout the course of the experiment. On Day 28, hematological and biochemical tests were performed and standard gross pathology and measurement of organ weights also were conducted. Histopathological testing was performed for brain, heart, liver, kidney, testes, and ovaries. Over the course of the study, no significant differences in weight gain were reported between the treated and control groups, and no differences in average food consumption were observed. No signs of morbidity were observed and no mortality was reported. There were no changes in hematology or biochemical parameters for either male or female treated animals relative to controls; however, it should be noted that a number of biochemical parameters were below the detection limit of the analytical technique in both control and treatment groups, including alanine aminotransferase

(ALT)[7], lactate dehydrogenase (LDH), bilirubin, and cholesterol, indicating potential limitations to the analytical sensitivity and methodology. There were no abnormal findings noted during necropsy. The only significant difference in organ weights was absolute and relative testis weights, which were increased from control by 7 and 6% (p<0.05), respectively; however, this increase was reported by the authors to be within the range of historical control data. Histopathological investigations were also unremarkable. On the basis of these findings, the no-observed-adverse-effect level (NOAEL) for PFI's GABA was determined to be 200 mg/kg body weight/day, the only dose tested.

Kato *et al.* (2005) conducted a 28-day study in male and female Crj:CD(SD) rats. Following a 1-week acclimatization period, male rats weighing 179.9 to 195.4 g and female rats weighing 145.5 to 163.1 g were randomized to 1 of 3 groups (4 animals/sex/group) to receive gavage doses of unfermented milk (skim milk), fermented milk containing 10 mg GABA/100 mL (DGB10), or fermented milk containing 25 mg GABA/100 mL (DGB25). The GABA-containing milk was prepared by fermenting skim milk with *Lactobacillus casei* and *Lactococcus lactis*, and the final product was then formulated with added maltitol, pectin, and soy polysaccharide. Gavage volumes were 20 mL fluid/kg body weight for each treatment group such that the rats allocated to the DGB10 and DBG25 treatment groups received 2 and 5 mg GABA/kg body weight, respectively. The animals were housed under standard animal care conditions, and were permitted *ad libitum* access to feed and water. Rats were observed for signs of toxicity on a daily basis and feed intake, water consumption, and body weights were evaluated once a week. Routine clinical chemistry, hematology, and urinalysis were performed. At the end of the study period, the animals were necropsied, selected organs were weighed, and tissues were preserved for histopathological examination.

No significant differences in body weight gain were observed between groups. Other than a slight decrease (<10%; p<0.05) in food intake in DGB25 females on Day 11 and decreased (<10%; p<0.05) water intake for DGB10 females on Day 4, no significant differences in food or water intakes were noted. With the exception of a slight (2.4%; p<0.01) increase in urine specific gravity in DGB25 males, quantitative and qualitative urinalysis was unremarkable among groups. The change observed in urine specific gravity was modest, did not correlate with changes in urine protein or glucose, and was not observed in females, and therefore was considered a spurious finding by the authors. A few sporadic significant changes in hematological parameters (*i.e.*, prothrombin time, mean corpuscular hemoglobin concentration, hematocrit, basophil number, and percent basophil) were noted; however, the changes were modest (<10% for all parameters except basophil number, which decreased from 0.01 to 0.00), in many cases were not dose responsive, and in all cases did not occur in both sexes. Therefore, the various changes were not considered toxicologically relevant. There were no toxicologically relevant changes in serum biochemistry. ALT levels were increased by ~15% in the DGB25 females; however, the effect was not observed in males, and did not correlate with

[7] Also commonly referred to as glutamate pyruvate transaminase (GPT).

other changes in liver biochemistry parameters. Brain and pituitary weights were increased in the DGB25 males by 4% (p<0.01) and 15% (p<0.05), respectively, with a 12% (p<0.05) increase in pituitary weight relative to body weight. Additionally, a 12% increase (p<0.05) in total adrenal weight was observed in DBG25 males. As similar dose-related trends were not observed in females and the changes were modest, the findings of increased brain, pituitary, and adrenal weights were determined by the authors not to be biologically relevant. Other changes in organ weights [decreased heart weight in DBG10 males (6%, p<0.05), increased heart weight in DBG25 females (9%, p<0.05), and decreased relative testes weight in DBG10 males (11%, p<0.05)], were also determined to be not compound-related, in consideration of the lack of dose-response and modest magnitude of the changes. Microscopic pathology did not reveal any increased incidence of pathological findings in treated animals relative to unfermented milk controls. Thus, a NOAEL of 5 mg GABA/kg body weight/day, the highest dose tested, was established under these study conditions.

90-Day Studies

Takeshima *et al.* (2014) investigated the safety of PFI's GABA (PharmaGABA™ produced by fermentation using *Lactobacillus hilgardii* K-3; 91.6% purity) in 6-week-old SPF Sprague-Dawley (Crl:CD (SD)) rats. This study was conducted in accordance to GLP (OECD, 1998a) and OECD guideline 408 (OECD, 1998b). Animals (10/sex/group) were administered 0, 500 (low-dose), 1,250 (mid-dose), or 2,500 (high-dose) mg GABA/kg body weight/day by gavage (vehicle not reported) for 13 weeks. Animals were monitored twice daily for mortality, general condition, and clinical signs, while body weight and feed intake were measured weekly. Twenty-four hour urine analyses and ophthalmology examinations were conducted during Week 13 on 5 rats/sex/group. Hematology and clinical chemistry parameters, organ weight, and macroscopic and histological tissue evaluations (the latter in control and high-dose groups only) were conducted upon necropsy.

Through the duration of the study GABA was well-tolerated. In the high-dose group, diarrhea and salivation occurred sporadically (in 5 males and 1 female, and 8 males and 7 females, respectively) which were not considered to be compound-related. Significantly reduced body weight was observed in high-dose males at Weeks 2 and 4 in comparison to the untreated group; however, given that the reduction in body weight corresponded to a significant reduction in feed intake in high-dose males during study Weeks 1, 4, and 9, and the changes were subtle (approximately 6 to 17%), they were not considered to be toxicologically relevant. Moreover, no significant differences in body weight or feed intake were observed in any of the female rats.

Despite some statistically significant changes in several hematology parameters in GABA-treated rats compared to the untreated control group (*i.e.,* increased hemoglobin, mean corpuscular hemoglobin, and platelet count in high-dose males; increased red and white blood cell count in mid- and high-dose females; and decreased mean corpuscular volume and mean corpuscular hemoglobin in mid- and high-dose females), these changes were modest and

remained within historical control ranges, and were therefore considered not toxicologically relevant. The prothrombin times in high-dose males and females were elevated (statistically significant) and were slightly above the historical control ranges; however, the magnitude of the differences from controls was minimal (~1 sec.), and no additional evidence of impaired coagulation was reported (*e.g.,* no changes in activated partial thromboplastin time or signs of hemorrhage were observed). Hence, this finding was ascribed no toxicological significance. Likewise, several statistically significant effects on clinical chemistry parameters observed in the GABA-treated rats were only modestly changed in comparison to the untreated group and/or were confined to a single sex (*i.e.,* increased potassium in low- and high-dose males; decreased sodium and chloride in high-dose males; decreased sodium in high-dose females; and decreased total protein, albumin, and albumin:globulin ratio in high-dose females). Alkaline phosphatase values were significantly, but modestly, elevated (about 1.5-fold) in the mid- and high-dose females in comparison to the controls, however, this observation was not considered a compound-related effect as it was not evident in males, lacked a clear-dose response relationship, and was not accompanied by other changes in liver enzymes or by histological changes in the liver, bone, or intestinal tract, which are potential sources of serum alkaline phosphatase activity. No compound-related changes were observed in terms of urinalysis parameters, nor were differences seen upon ophthalmologic evaluation.

Significant increases in absolute and relative liver weights (in mid-dose males) and increased relative kidney weights (in high-dose males) were reported. However, as these changes lacked a dose-response relationship and were not observed in females, they were not considered to be compound-related. Macroscopic and histological evaluation revealed sporadic changes that were mild to moderate in severity and were not considered an effect of the GABA treatment as they were unremarkable from the control group. On the basis of these findings, the NOAEL for GABA in rats was determined to be 2,500 mg/kg body weight/day, the highest dose tested.

A 90-day sub-chronic toxicity study was conducted in rats using the same GABA-containing fermented milk as in the previous 28-day repeated-dose study reported by Kato *et al.* (2005). The experiment was conducted in the same strain of rat (Crj:CD(SD)), and at 5 weeks of age, the animals were randomized to 1 of 4 groups (4/sex/group). Each animal received 1 of 4 gavage treatment regimens: distilled water (control), unfermented milk (negative control), fermented milk containing GABA at a concentration of 10 mg/100 mL (DGB10), or fermented milk containing GABA at a concentration of 25 mg/100 mL (DGB25). Gavage volumes were 20 mL fluid/kg body weight for each group such that rats allocated to treatment groups DGB10 and DBG25 received 2 and 5 mg GABA/kg body weight, respectively. Animal housing, GABA treatment, and toxicological endpoints were identical to those detailed for the 28-day study.

No significant differences in body weight or food and water intake were reported. Quantitative measurements of biochemical and histological urine parameters revealed no compound-related adverse effects. Urine volume was significantly ($p<0.05$) increased by approximately 30 and

45% in the male rats receiving DGB10 and DGB25, respectively. The increase in urine volume was associated with a slight (approximately 1%; $p<0.05$) decrease in urine specific gravity in the DGB25 group. The changes in urine volume and specific gravity did not occur in females. The authors stated that hypotension has been reported to occur with GABA administration, an effect that is often associated with diuretic effects (Shimizu *et al.*, 1959). Since the observed changes in urine volume were not associated with other adverse effects of urine function, and were not observed in females, the observation was reported to be toxicologically insignificant. Likewise, changes in urinary creatinine in DGB10 males (+10%, $p<0.05$) and sodium in DGB10 females (-18%, $p<0.05$) were modest, did not occur in both sexes, and did not show a dose-responsive effect and were therefore determined to be not compound-related. No abnormalities following ophthalmologic analysis were observed. Hemoglobin concentration was significantly lower in the DGB-treated females relative to the unfermented milk controls; however, the observation was slight (<5%; $p<0.01$) and was not observed in males. In the absence of changes for other indices indicative of anemia, the decrease in hemoglobin concentration observed in females was determined to be toxicologically insignificant. Sporadic decreases ($p<0.05$) in differential leukocyte counts (lymphocyte, neutrophil, and eosinophil) were reported in the DGB25 female animals relative to the unfermented milk group; however, the effects were not dose responsive, were not observed in males, and were not significantly decreased relative to distilled water controls, and therefore, the changes were considered to be a spurious finding and biologically irrelevant. Biochemical indices were unremarkable.

Sporadic increases in organ weights were noted. Specifically, elevations were observed in total lung weight in the DGB10 males (absolute weight; +11%, $p<0.01$) and adrenal weights in the DBG-females (absolute weight, +19%, $p<0.01$; relative weight, +21%, $p<0.05$) relative to the unfermented milk controls; however, these changes were not observed in both sexes, were not dose-responsive, and in the case of the adrenals were not significantly changed relative to distilled water controls and hence were considered to be toxicologically insignificant. Macroscopic pathology was unremarkable for all groups. On the basis of the study results reported, the NOAEL was 5 mg/kg body weight, the highest dose tested.

Chronic Studies

Tower (1960) reviewed chronic toxicity studies of GABA conducted by Merck Sharp and Dohme Research laboratories. These studies consisted of the oral administration of GABA at dose levels up to 10 mM/kg (1 g GABA/kg) body weight/day for chronic administration to rats and dogs, and the intravenous administration to unanesthetized dogs at similar doses. At termination of the study, complete autopsies revealed no signs of toxicity or untoward effects in any of the treated animals. While no other details were presented, Tower concluded that the data reviewed demonstrated the lack of toxicity of GABA during chronic administration.

No chronic toxicity studies of GABA were identified in the literature. However, in chronic toxicity studies of GABA transaminase inhibitors administered orally to rats (doses up to 300 mg/kg

body weight/day for 1 year), dogs (doses up to 200 mg/kg body weight/day for 1 year) and primates (doses up to 300 mg/kg body weight/day for 16 months or 100 mg/kg body weight/day for 6 years), to elevate plasma and tissue GABA levels, no evidence of peripheral neurotoxicity were reported upon assessment of standard toxicological parameters (Gibson *et al*., 1990). GABA transaminases are responsible for conversion of GABA to glutamate, and the administration of pharmacological inhibitors of the enzyme, *e.g*., γ-vinyl-GABA (GVG), impairs GABA turnover leading to significant increases in endogenously produced GABA (Gibson *et al.*, 1990; Qume and Fowler, 1996). For example, the administration of GVG to rats has been shown to increase brain GABA levels by 200 to 300%[8], and in liver and plasma GABA levels by 300 to 1,500% and 200 to 300% respectively (Qume and Fowler, 1996). In the study conducted by Gibson *et al*. (1990), CSF levels of GABA in rats increased 400 and 800% relative to controls for animals treated with GABA at doses of 50 and 300 mg/kg body weight/day, respectively, over 4 weeks. Likewise, in dogs, CSF GABA levels increased from approximately 400 to >2,000% above controls in animals receiving the compound for 16 weeks at doses of 50 and 200 mg/kg body weight/day, respectively. While it was unclear why decreased weight gain and food consumption was observed in rats following chronic GVG administration, along with a dose-dependent increase of tonic convulsions (usually a result of handling or external stimuli) in animals receiving ≥200 mg/kg body weight/day throughout the course of the experiment, this effect was not observed in the dog or monkey. In the dog, no remarkable changes were observed in hematology, clinical chemistry, or opthalmoscopic measures. Although a dose-dependent neural vacuolation was observed, this did not translate to peripheral neural toxicity. In monkeys administered GVG for up to 16 months, the only compound-related adverse effect reported was transitory loose stools in the high-dose group (300 mg/kg body weight/day); clinical chemistry, hematology and urine analyses were all within control values, and histopathological changes were unremarkable (Gibson *et al.*, 1990). No evidence of neuropathology was reported in monkeys orally administered 100 mg GVG/kg body weight/day for 6 years (Gibson *et al*., 1990). However, it should be noted that GABA levels in the CSF were not significantly increased relative to the levels in the control group; this was speculated by the authors to be due to a reduction in the absorption of GVG in the monkey and a decrease in the capacity to inhibit GABA transaminase activity. While these studies show some effects upon elevation of GABA in the brain, they are of limited relevance as it is important to recognize that administration of a GABA transaminase inhibitor is not the same as administering GABA itself and orally administered GABA is poorly absorbed and does not cross the BBB.

D. Mutagenicity and Genotoxicity

Given that GABA is an endogenous compound present at substantial levels in the brain and is readily detectable in the systemic circulation, it is not expected to be either mutagenic or genotoxic.

[8] Note that GVG crosses the blood brain barrier, and therefore increases brain GABA levels.

Only one mutagenicity study was identified in the literature, a Rec assay using *Bacillus subtilis* strains H17 (Rec+) and M45 (Rec-), that assessed the potential mutagenicity of GABA, using GABA-containing fermented milk products, DGB10 and DGB25, which contained GABA at 10 and 25 mg/100 mL, respectively (Osawa *et al.*, 2005). DGB25 was added to paper disks at levels of 46.9 to 750 mg, and DGB10 at levels of 18.75 to 300 mg. Both studies were conducted in the presence and absence of metabolic activation. There was no evidence of mutagenicity at either concentration of GABA milk. Based on the Rec assay, GABA is not mutagenic.

E. Human Studies

A number of studies reported on the effect of GABA supplementation in humans primarily in the context of its effects on blood pressure and stress reduction. Most reports have also included extensive safety-related endpoints (*i.e.*, full tabulated summaries of all biochemical, hematological, and urinalysis parameters) that demonstrate that GABA was well-tolerated at doses up to 120 mg/day for up to 12 weeks or up to 250 mg/day for up to 30 days. In addition GABA was reported to be well tolerated in 3 studies at high doses of 5 to 18 g/day for up to 4 days with only minor side effects reported. A summary of the studies of GABA consumption in supplements and in various GABA enriched foods is presented in Table IV.E-1.

Table IV.E-1 Human Studies Evaluating the Supplemental Use of GABA or GABA-Enriched Foods						
Study Design/ Health Status/ Age	**Number of Subjects/ Group**	**GABA Dose (mg/day) [Delivery Matrix]**	**Duration of GABA Intake**	**Major Findings**	**Reported Side Effect (Incidence GABA/ Control)**	
GABA Supplements						
PCT Healthy subjects 21 to 53 years	Control: 14 (sex NR) GABA = 1M, 16F	0 or 5,000 [delivery method NR]	Single dose	• No safety parameters measured. • Increased plasma GH ($p<0.0001$) relative to control. Effect was transient, returning to control levels by 180 min.	• Transient burning sensation of breathl lasting a few min ingestion. • Slight tiredness and muscular weakne legs following rep	
PCT Healthy subjects 18 to 65 years	Control = 1M, 17F GABA = 2M, 17F	0 or 5,000 [dissolved in water]	Single dose	• No clinically significant changes in pulse rate or blood pressure. • GABA did not affect glucose concentrations during an insulin tolerance test. • Increased growth hormone level, but no change in plasma prolactin level *vs*. control.		
	GABA = 8F	18,000 [dissolved in water]	4 days			
RPCT crossover design Healthy subjects 18 to 52 years	3M, 9F	0, 5,000 or 10,000 [dissolved in water]	Single dose	• Dose-responsive increases in immunoreactive insulin and glucagon (maximum of 60 and 40%, respectively, compared to baseline; $p<0.01$). Effect was transient and returned to baseline within 180 minutes. • No changes in blood glucose.		
Uncontrolled intervention Healthy subjects 20 to 49 years	N 21 (sex NR)	250 [hard capsules]	30 days	• No differences were observed in standard hematological and biochemical parameters *vs*. baseline levels.	• No AE reported.	

Table IV.E-1 Human Studies Evaluating the Supplemental Use of GABA or GABA-Enriched Foods

Study Design/ Health Status/ Age	Number of Subjects/ Group	GABA Dose (mg/day) [Delivery Matrix]	Duration of GABA Intake	Major Findings	Reported Side Effect (Incidence GABA/ Control)	
RPCT, DB Mild hypertension 20 to 70 years	**Study 1:** Control: 6M, 7F 20 mg: 3M, 9F 40 mg: 6M, 7F 80 mg:6M, 7F	0, 20, 40, or 80 [tablet]	4 weeks	• Decreased SBP in groups given 40 and 80 mg/day (-3% and -5%, respectively, *vs*. baseline; p<0.01) • Reduced DBP (-6%, p<0.01) with 80 mg GABA intake *vs*. baseline. • No significant changes in standard hematology and clinical chemistry tests and urinalysis.	• No AE reported.	
	Study 2: Control: 11M, 12F GABA: 10 M, 13F	0 or 80 [tablet]	8 weeks	• No changes in plasma GABA levels relative to controls. • Decrease SBP (~6 to 10 mmHg; p<0.05) at Weeks 4, 6 and 8 relative to controls. • No significant changes in standard hematology and clinical chemistry tests and urinalysis.	• No AE reported.	
RPCT High-normal BP or mild hypertension Control: 54.7 ± 8.6 years (mean) GABA: 53.8 ± 8.5 years (mean)	Control: 16 M, 27 F GABA: 15M, 30F	0 or 80 [tablets]	12 weeks	• SBP and DBP transiently decreased (~5%, *p*<0.01) in GABA group compared to placebo (at Week 10 and 12, respectively). • GABA-related effects on BP were abolished 4 weeks post-administration. • No significant changes in plasma GABA level, pulse, body weight, hematology, clinical chemistry or urinalysis.	• Symptoms includ on soles (1/0), co diarrhea (0/2), loos passage (2/0), ras rash on face (0/1), (1/1), back itching (1 headache (1/0), he stomach (0/1); • All effects were ju to correlate with te compound.	
Uncontrolled study Subjects with various neurologic impairments Age NR	11 subjects (sex NR)	~56,000 (or 0.8 g/kg bw/day, no other details reported)	3 months to 2 years	• No evidence of chronic or cumulative toxicity. • Peak serum levels of ranged from approximately 60 to 80 µM/100 mL between 1 to 2 hours post GABA consumption. Cerebral spinal fluid levels of GABA ranged from 0 to 21 µM/100 mL. GABA was undetectable in the urine.	• Initial flushing par of the extremities, generalized malai nausea lasting betw to 90 min after G consumption. O occasion, emesi diarrhea (2 subje • Tolerance to thes	

Table IV.E-1 Human Studies Evaluating the Supplemental Use of GABA or GABA-Enriched Foods

Study Design/ Health Status/ Age	Number of Subjects/ Group	GABA Dose (mg/day) [Delivery Matrix]	Duration of GABA Intake	Major Findings	Reported Side Effect (Incidence GABA/ Control)	
					occurred within a f weeks.	
GABA-Enriched Chlorella						
Open-label, uncontrolled study Mild hypertension 48.8 years (mean)	10 (sex NR)	30 [GABA-enriched Chlorella tablets]	8 weeks	• Significant reduction in SBP at Weeks 4 (~4.9; $p<0.05$) and 8 (~6.1%; $p<0.01$). • No changes in bw, heart rate, and DBP. • General hematological and biochemical tests of kidney and liver function were unremarkable.	• Not reported.	
RPCT, DB intervention High-normal BP or borderline hypertension Placebo: 47.9±9.5 years (mean) GABA: 50.1±9.2 years (mean)	Control: 26M, 14F GABA: 18M, 22F	0 or 20 [GABA-rich *Chlorella* tablets]	12 weeks	• Decrease in SBP in the GABA group in comparison to baseline measures (~12%, $p<0.01$) and placebo group (~7%, $p<0.05$) at Week 12. • Significant reduction in DBP in the GABA group in comparison to baseline values (~10%, $p<0.01$) • Reduction in SBP and DBP occurred as early as 4 weeks of treatment. • No differences in heart rate or BMI were observed. • No changes in hematology, serum biochemistry, and urinalysis evaluations.	• Symptoms observ and transient diarr and loose stools • Determined to be no related to the test-compound.	
GABA-Containing Fermented Drinking Water						
RPCT, DB intervention normal BP or mild/moderate hypertension	Normotensive Control: 3M, 4F GABA: 7M, 1F Mild to moderate hypertensive	0 or 70 [fermented water beverage containing vinegar and peptides from	12 weeks	• At Week 12, in the mild/moderate hypertensive subjects, decrease in SBP (~5.6%, p<0.05) and DBP (~11.5%, p<0.01) in the GABA group *vs*. baseline. Reductions in SBP (-4.2%, p<0.05) and DBP (-8.5%, p<0.01) were also	• No AE reported.	

Table IV.E-1 Human Studies Evaluating the Supplemental Use of GABA or GABA-Enriched Foods

Study Design/ Health Status/ Age	Number of Subjects/ Group	GABA Dose (mg/day) [Delivery Matrix]	Duration of GABA Intake	Major Findings	Reported Side Effect (Incidence GABA/ Control)	
Normotensive control: 49.3±10.5 years (mean) GABA:45.1 ± 3.3 years (mean) Hypertensive Control: 46.9±3.2 years (mean) GABA:45.7 ± 9.8 years (mean)	Control: 10M, 1F GABA: 5M, 5F	dried bonito]		observed in the mild/moderate hypertensive subjects consuming fermented water only *vs*. baseline. • Reductions in BP ($p<0.05$)observed as early as 4 and 6 weeks for the fermented water only and GABA enriched fermented drinking water group, respectively. • No changes in BP were observed in the normotensive subjects. • No differences in heart rate. • Changes in hematology, serum biochemistry, and urinalysis parameters were within normal ranges.		
GABA-Enriched Fermented Milk						
Study 1: Uncontrolled study Healthy subjects 36.5 ± 9.7 years (mean)	8M	**Study 1:** 11.5 to 12.8 [as 100 mL of GABA-containing fermented milk[a]]	8 weeks	• No significant changes in BP for healthy adults receiving GABA in Study 1or 3. • HR increased in Study 3 GABA group (9%, $p<0.001$) after 2 weeks treatment *vs*. baseline, but no difference from control. • No significant effect on defecation frequency with single excessive intake. • Increased tendency of mild abdominal symptoms. • No significant changes in hematological and biochemical tests.	**Study 1:** • Increased urinat fullness (1/0).	
Study 2: PCT, crossover design Healthy subjects 32 ± 6 years (mean)	6M, 6F	**Study 2:** 0 or 34.5 to 38.4 [as 300 of GABA-containing fermented milk[a]; placebo is regular milk]	Single dose		**Study 2:** • Temporal abdomi symptoms includ gurgling sounds flatus discharge in	
Study 3: PCT	8 men, 8 women (number/ group	**Study 3:** 0 or 34.5 to 38.4	2 weeks		**Study 3:** • No AE reported.	

Table IV.E-1 Human Studies Evaluating the Supplemental Use of GABA or GABA-Enriched Foods

Study Design/ Health Status/ Age	Number of Subjects/ Group	GABA Dose (mg/day) [Delivery Matrix]	Duration of GABA Intake	Major Findings	Reported Side Effect (Incidence GABA/ Control)	
Healthy subjects Control: 31 ± 4 years (mean) GABA: 30 ± 5 years (mean)	NR)	[as 300 of GABA-containing fermented milk[a]; placebo is regular milk]				
RPCT Mild hypertension 28 to 81 years	Control[10]: 10M, 7F GABA[b]: 10M, 8F	0 or 10 to 12 [as 100 mL GABA-containing fermented milk[c]]	12 weeks	• SBP decreased in time-dependent manner in GABA group *vs*. control (~12 mmHg at Week 12; $p<0.05$). • No significant change in DBP relative to control. However, a time-dependent reduction in DBP (mean of -7.2 mmHg; $p<0.05$) was observed in the GABA group *vs*. baseline from Week 2 onwards. • No significant changes in heart rate, body weight, hematological and blood chemistry, and urinalysis.	• No side effects or noteworthy change or after intake pe	
PCT, DB Mild hypertension Control: 52±12 years (mean) GABA: 52 ± 11 years (mean)	Control: 23M, 20F; GABA: 22M, 21 F	0 or ≥10 [provided as 100 mL GABA-enriched fermented milk[d]]	12 weeks	• Decreased SBP (~6%; $p<0.01$) and DBP (~5%; $p<0.05$) in GABA group *vs*. control after 6 weeks of intake and remained stable until the end of treatment. • No significant changes in body weight, hematology or urinalysis.	• Symptoms includ diarrhea for more days (2/3), cold (lumbago (1/0), m (0/1), period pain (2 fatigue from ove • All judged no corr with test food.	
RPCT, DB High normal BP Control: 47.1 ± 1.7 years (mean) GABA: 46.4 ± 1.7 years (mean)	Control: 21M, 33F GABA: 21 M, 33F	0 or 12.3 [as 100 mL GABA-containing fermented milk[d]]	12 weeks	• SBP decreased 8 weeks after GABA intake (~5%; $p<0.01$) *vs.* control. A maximum decrease of ~7% ($p<0.001$) relative to controls was reported by the end of Week 12. • DBP decreased after 12 weeks of GABA intake (~5%; $p<0.05$) *vs*. control.	• Symptoms includ diarrhea (1/5), cold skin itching (1/1), constipation (0/1 body tired-feeling • All judged no corr with test food.	

Table IV.E-1 Human Studies Evaluating the Supplemental Use of GABA or GABA-Enriched Foods

Study Design/ Health Status/ Age	Number of Subjects/ Group	GABA Dose (mg/day) [Delivery Matrix]	Duration of GABA Intake	Major Findings	Reported Side Effect (Incidence GABA/ Control)	
				• No significant changes in pulse, body weight, hematological and blood chemistry tests, urinalysis.		
GABA-Enriched Agaricus blazei Murill						
DB, open, comparative, crossover test Mild hypertension Group 1: 45.6 ± 12.2 years (mean) Group 2: 46.4 ± 15.4 years (mean)	14M;	0 or 25 [GABA enriched *Agaricus blazei* in capsules[e]]	4 weeks	• SBP (-2%; $p<0.05$) and DBP (-3%; $p<0.05$) decreased in treatment group relative to baseline beginning at Weeks 2 and 3, respectively. • No significant changes in biochemical and hematological tests.	• Not reported.	
GABA-Enriched Soy Sauce						
RPCT, DB, parallel study High normal BP or mild hypertension Regular soy: 48.9 ± 9.1 years (mean) Low-salt soy: 48.1 ± 9.7 years (mean) GABA-enriched low-salt soy sauce: 48.9 ± 8.9 years (mean)	Regular soy: 28M, 24F Low-salt soy: 31M, 25F GABA-enriched low-salt soy sauce: 27M, 26F	0 or 120 [as 8 mL low-salt soy sauce]	12 weeks	• SBP decreased (~4.6 mm Hg, $p<0.05$) after GABA intake *vs*. regular soy sauce, without significant changes in DBP. • No significant changes in pulse, heart rate, blood and urine tests.	• Symptoms included diarrhea (2/1/2), pain (2/2/1), ecz (1/0/0); all judged correlation with te • Dizziness/poor appetite/nausea blood pressure ri subject in the GABA salt group: origin high BP and rise on 81st day after Judged no correl test food.	

Table IV.E-1 Human Studies Evaluating the Supplemental Use of GABA or GABA-Enriched Foods

Study Design/ Health Status/ Age	Number of Subjects/ Group	GABA Dose (mg/day) [Delivery Matrix]	Duration of GABA Intake	Major Findings	Reported Side Effect (Incidence GABA/ Control)	
GABA-Enriched Cheese						
RPCT, DB intervention Mild hypertension Placebo: 43.2±4.0 years (mean) GABA: 45.8±3.6 years (mean)	Control: 12M; GABA: 11M	0.12 [control] or 16 [GABA-enriched cheese[g]]	12 weeks	• SBP decreased in both groups (-2.7 and -3.9% for the placebo and treated groups, respectively, p<0.05) at 12 weeks *vs*. baseline measures; however, there were no significant differences between groups. • No significant changes were observed in DBP for either group. • No AE on heart rate.	• Increase in HDL in the placebo gr (p=0.002) *vs*. bas • No other AE repor	
GABA-Enriched Rice Germ						
RPCT, DB, crossover design Sleeplessness, depression and autonomic disorder during the menopausal or pre-senile period 49.4 ± 11.7 years (mean)	20F	0 or 26.4 [GABA fortified rice germ powder; placebo was powdered white rice[h]]	8 weeks	• Improvement in blood vessel motor nerve disorder (p<0.01), sleeplessness (p<0.01), emotional disorder (p<0.01), depression (p<0.01), dizziness (p<0.05), fatigability (p<0.01), arthritic pain (p<0.01), headache and heavy-headed (p<0.01) and digestive symptoms (p<0.05). • SBP (-5%; p<0.05) and DBP (-8%; p<0.05) decreased *vs*. baseline (measured only in 6 patients with borderline or essential hypertension). • No significant effect on blood cell components, liver function and electrolyte balance *vs*. baseline (parameters measured for only 9 patients).	• No AE reported.	

AE adverse events; BP = blood pressure; DB double blind study; DBP = diastolic blood pressure; F = females; GABA = gamma-amin growth hormone; HDL high-density lipoprotein; M males; min = minutes; NR not reported; PCT placebo controlled trial; RPCT = rando controlled trial; SBP systolic blood pressure; *vs*. = *versus*; WBC = white blood cell.

[a] The GABA-containing fermented milk was produced using 2 strains of lactic acid bacteria (*L. casei* and *L. lactis)* that are capable of conv GABA.
[b] Subjects that completed the study.
[c] The test product used in the study was produced from skim milk fermented with *L. casei* and *L. lactis*. The placebo test article was prepare to the skim milk.
[d] The GABA-enriched fermented milk was prepared using *L. casei* and *L. lactis*. The placebo test article was prepared using skim milk pow to contain a similar amount of lactic acid as the GABA milk such that both products were indistinguishable in all sensory aspects.
[e] The test article was produced by adding the *Agaricus* fruit body to water and allowing it to self-digest (original article in Japanese, exact mea the English translation) for 17 hours at 50°C. The end product contained 2.7% GABA and the freeze-dried powder was added to opaque study. Each GABA capsule contained 6.25 mg GABA. Placebo capsules were manufactured to contain a similar compositional content w
[f] Incidence of side effects noted as GABA-enriched low-salt soy group/low-salt soy group/ regular soy group.
[g] GABA-enriched cheese was produced with a starter of GABA-producing *Lactococcus lactis* ssp.
[h] GABA fortified rice germ was produced by taking rice germ from the bran by milling Sasanishiki rice and then de-fatting with n-hexane and enrichment with GABA (exact English translation of the original Japanese article unclear).

F. GABA and Blood Pressure Effects

It may be concluded from a critical evaluation of the data summarized above that daily consumption of GABA is associated with modest hypotensive effects that are limited to hypertensive or high-normal blood pressure subjects. This response does not appear to be dose-dependent, and the maximal effect, although statistically significant in many studies, is modest (*i.e.*, maximum decreases of 5 to 7% when compared to changes in the control subjects, and maximum reductions of 12% when compared to baseline measures). This hypotensive effect was limited to hypertensive individuals or individuals with high-normal blood pressure as studies in normotensive individuals at low (12 to 70 mg/person/day for 8 to 12 weeks, respectively) and high doses (5 to 18 g/person/day for 1 to 4 days) of GABA did not affect blood pressure (Cavagnini *et al.*, 1980a,b; Kimura *et al.*, 2002; Tanaka *et al.*, 2009). In addition, most studies utilized GABA fermented beverages or other GABA-supplemented foods as the test article, and it is therefore unclear if slight reductions in blood pressure reported in hypertensive subjects are attributed to GABA. The effect of GABA on blood pressure in hypertensive subjects appeared to be time-dependent and typically required at least 2 weeks of continual daily use before significant reductions were observed. The slight reductions in blood pressure towards normal levels that were reported in hypertensive subjects consuming GABA, as reported above, are commonly observed in association with the consumption of other foods that have long history of safe consumption. For example, comparable effects on blood pressure have been recently reported in mild-hypertensive individuals consuming polyphenol rich dark chocolate (Taubert *et al.*, 2003, 2007; Grassi *et al.*, 2005) or fish oil (Dickenson *et al*., 2006).

G. GABA and Growth Hormone Secretion

The use of GABA in dietary supplements is frequently targeted to the body building market, with purported claims of increasing growth hormone (GH) secretion. Serving sizes of 3 to 10 g of GABA are usually recommended for this effect. Evidence of increased GH secretion in humans following the oral administration of GABA has been reported in the literature. In studies reported by Cavagnini *et al.* (1980a,b), single oral doses of 5 g GABA were associated with rapid increases in GH plasma levels, which peaked at 3 hours post-dosing. Maximum plasma levels were increased by approximately 5-fold, with GH concentrations returning to baseline within 180 minutes. The administration of 18 g GABA daily for 4 days to 8 healthy female subjects caused a significant blunting of the release of GH implying that GH secretion by GABA may be down-regulated by high-dose and consecutive daily administration.

Powers *et al.* (2008) reported that the consumption of a large dose (3 g) of GABA resulted in significant increases in circulating levels of GH in a randomized, double-blind, placebo-controlled, crossover study in 11 resistance-trained men (18 to 30 years). Subjects consumed either 3 g of GABA or sucrose placebo, which was then followed by resting or resistance exercise sessions. Fasting venous blood samples were taken at time 0, 15, 30, 45, 60, 75, and

90 minutes post-GABA or placebo administration. GABA consumption resulted in an increase in peak serum concentrations of both immunoreactive (ir) and immunofunctional (if) GH levels by approximately 4-fold (p<0.05). ifGH and irGH area under the curve values (AUC; t 0 to 90 minutes) also were increased by approximately 3-fold over the 90-minute period (p<0.05 for irGH only); however, the increases in irGH and ifGH serum concentrations observed following GABA consumption were significantly less than those observed following exercise, where increases in peak concentrations of ifGH and irGH were increased by 16-fold above the levels in resting subjects consuming the placebo. Similarly, AUC values were increased 14-fold relative to those reported in subjects at rest.

The ability to increase GH secretion is not unique to GABA; similar increases in plasma GH levels were reported in association with exercise and with the consumption of high doses of arginine, an amino acid routinely consumed in the diet (Paddon-Jones *et al.*, 2004; Collier *et al*., 2005; Kanaley, 2008). For example, as reported by Collier *et al.* (2005), the consumption of 5, 9, or 13 grams of arginine resulted in significant increases in GH AUC values by as much as 3-fold at the highest dose.

Based on a critical analysis of the information summarized, a threshold for GABA-induced increases in GH secretion could not be determined. GABA-induced increases in GH appear to be limited to the consumption of large doses of GABA (>3 g; 34 mg/kg body weight). The increases that have been reported appear large (3- to 5-fold); however, they are clearly within the normal physiological range, and the increases are well below those observed in the same subjects during exercise (Powers *et al.*, 2008). A transient induction and rapid clearance (return to baseline) of GH from the plasma is observed following GABA consumption in humans (Cavagnini *et al.*, 1980a,b). Thus, it is highly unlikely that GABA, when used as an ingredient in food under the intended conditions of use described herein, will stimulate the release of GH.

H. Information Pertaining to the Safety of the Source Organism

Description of the Source Organism, L. hilgardii K-3

GABA is produced from a fermentation process using *L. hilgardii* K-3. The taxonomic classification of *L. hilgardii* K-3 is presented below:

Taxonomic Classification of *L. hilgardii* K3:

Kingdom:	Bacteria
Subkingdom:	Bacteria
Phylum:	*Firmicutes*
Class:	*Bacilli*
Order:	*Lactobacillales*
Family:	*Lactobacillaceae*
Genus:	*Lactobacillus*
Species:	*Lactobacillus hilgardii*
Strain:	K-3

During a screening process of various lactic acid bacteria, PFI identified *L. hilgardii* K-3 to be a significant GABA-producing strain, which due to its high glutamate decarboxylase metabolic capacity, is capable of converting large amounts of glutamate to GABA during large scale fermentation. The bacterial strain was originally isolated from kimchi, which are fermented Korean pickles. *L. hilgardii* K-3 is not genetically modified and is a bacterial strain from the Lactobacillaceae family of the genus Lactobacillus.

The *L. hilgardii* K-3 is a non-motile, non-spore forming and non-acid-fast microaerophilic gram-positive organism. The phenotypic characteristics of the organism are presented in Table IV.H-1. The organism is a facultative anaerobe, and the optimal cultivation temperature is 25°C. The organism undergoes extensive quality control procedures (phenotypic growth characteristics) to ensure the purity and phenotypic stability of the organism.

Table IV.H-1 Phenotypic Characteristics of *Lactobacillus hilgardii* K-3

Characteristic	Analytical Result
Cultivation temperature	25°C (MRS agar medium)
Cell morphology	Bacillus, including elongated type
Gram stain	Positive
Cytoid spore	Negative
Mobility	Negative
Cell morphology	Circular, smooth entire fringe, low convex, creamy white
Growth temperature	NT
Catalase	Negative
Oxidase	Positive
Oxidative-fermentative test	Positive
Result of identification	*Lactobacillus*

NT normal temperature

Use of Lactobacillus hilgardii in the Production of Foods

The presence of *L. hilgardii* in foods produced by fermentation is well-established and the organism has a long history of consumption. For example, *L. hilgardii* was originally isolated and characterized from California wine by Douglas and Cruess in 1936 (Douglas and Cruess, 1936). A number of lactic acid bacteria, including various strains of *L. hilgardii*, are responsible for malolactic fermentation, an important step in wine making. Several strains of *L. hilgardii* have been isolated from a number of fermented grape sources used in the production of wine, as well as from tibi grains and sugar beets (Sohier *et al.*, 1999; StrainInfo, 2015). Rodas *et al.* (2005) isolated 178 bacterial strains from a microbiological survey of 32 grape musts and wine samples belonging to the Utiel-Requena and Juilla Origin Denominations in Spain. The authors discovered that the majority (~40%) of *Lactobacilli* in wine belong to the *L. hilgardii* species. Baruzzi *et al*. (2000) also identified *L. hilgardii* as a strain associated with ricotta cheese fermentation; up to 10% of the total microflora in the cheese during the ripening process were identified as *L. hilgardii* after 7 months of ripening.

Safety of L. hilgardii in Foods

Information relevant to the safety of *L. hilgardii K-3* for use in the manufacture of GABA was obtained from the general literature since no existing U.S. regulations permitting the use of *L. hilgardii* in foods or in the production of foods have been identified and the use of *L. hilgardii* in the production of foods has not been addressed by the FDA. A summary of the information used to establish the safety and suitability and the general recognition of safety of the proposed use of *L. hilgardii* K-3 in the manufacture of PFI's GABA is presented below.

Organisms from the genus *Lactobacillus* are Gram-positive, non-spore-forming rods or coccobacilli. To date, 136 species and 27 subspecies of *Lactobacillus* have been identified (Euzéby, 2014). The bacteria are strictly fermentive organisms and can be aerotolerant or anaerobic, aciduric or acidophilic, and display complex nutritional requirements (*i.e.,* requirements for carbohydrates, amino acids, peptides, fatty acid esters, salts, nucleic acid derivatives, and vitamins) (Bernardeau *et al.*, 2006). Due to their fermentation capabilities, *lactobacilli* have been used to alter the texture and or flavor of a large variety of foods (*e.g.*, beer, wine, cheese, yogurt, cured meats, *etc*.) for well over a millennia. More recently, *lactobacilli* have received increased popularity in association with their use as probiotic foods. In 2000-2001, a workshop that included panel members with expertise in clinical practice, microbiology, intestinal microecology, pathogenicity, nutrition, toxicology, and public health was convened to discuss the safety of *lactobacilli* and *bifidobacteria* used as probiotics in foods, and to review criteria for the evaluation of the safety of new probiotics (Borriello *et al.*, 2003). It was reported that cases of infection due to *lactobacilli* and *bifidobacteria* are rare and that increased consumption of probiotic products containing *lactobacilli* and *bifidobacteria* has not led to increases in these infections in consumers (Borriello *et al.*, 2003).

The safety of the *Lactobacillus* genus has also been extensively evaluated by Bernardeau *et al.* (2006) and the authors stated that "no cases of collective food borne disease have been reported in healthy people or farm animals following the ingestion of food or feed containing *Lactobacilli*". This view is also supported in an earlier review by Adams (1999) regarding the safety of industrial lactic acid bacteria. Adams (1999) concluded that based on the long history of consumption, in conjunction with the current available epidemiological, clinical and acute toxicity data, that lactic acid bacteria commonly occurring in fermented foods and used in probiotics are safe and that "*the available evidence does not indicate any significant health risk posed by ingested lactic acid bacteria*". Based on these and other reviews, it may be concluded that the use of lactobacilli in food production is safe and generally recognized as safe based on a long history of use in food (Holzapfel *et al.*, 1998; Vanderhoof and Young, 1998; Adams, 1999; Saarela *et al.*, 2002; Borriello *et al.*, 2003; Picard *et al.*, 2005; Boyle *et al.*, 2006; Bernardeau *et al.,* 2006).

Generation of Bacterial By-Products and Relevance to the Toxicity of PFI's GABA

Biogenic Amines

The *Lactobacillus* genus is non-pathogenic and non-toxigenic; however, some species of *Lactobacillus*, including *L. hilgardii*, are capable of producing biogenic amines such as tyramine, histamine, putricene, and phenylethylamine. The presence of biogenic amines in wine, cider, cheeses, and cured meats due to the presence of *Lactobacillus* is common (Suzzi and Gardini, 2003; Ferreira and Pinho, 2006; Garai *et al.*, 2006; Landete *et al.*, 2007), and generally does not result in adverse effects or toxicity. Although rare, toxicity due to high levels of biogenic amines (usually histamine) has been reported to occur in healthy individuals, and symptoms generally involve headache, palpitations, flushing, and to a lesser extent nausea, diarrhea, and erythema (Becker *et al.*, 2001; Ohnuma *et al.*, 2001; Miki *et al.*, 2005). For histamine toxicity, the hazardous intake level is believed to be ≥50 mg/100 g of food (Lehane and Olley, 2000), although this threshold is likely lower in subjects with genetic or drug-induced impairment of biogenic amine metabolism due to deficiencies in, or the inhibition of, enzymes catalyzing their decarboxylation. Costantini *et al.* (2006) examined 133 strains of bacteria isolated from musts and wine from various regions in Italy to identify the bacteria strain(s) responsible for biogenic amine (histidine, tyramine, and putrescine) production. Only 1 of 11 *L. hilgardii* strains was identified as expressing histidine decarboxylase mRNA (*hdc*), the gene encoding the enzyme responsible for the conversion of histidine to histamine. This strain also was shown to be capable of histamine production. All *L. hilgardii* strains were negative for genes encoding ornithine decarboxylase and tyrosine decarboxylase, the genes encoding the enzymes responsible for putrescine and tyramine respectively. In contrast, Landete *et al.* (2005) investigated the ability of 136 bacteria strains isolated from wine to determine which strains were responsible for histamine production, and determined that all 4 *L. hilgardii* strains were histamine producers. In addition, of the bacteria isolated and determined to be histamine

producers, *L. hilgardii* and *Pediococcus parvulus,* were identified as the principle bacteria responsible for the occurrence of histamine in wine. Under optimal experimental conditions (plate assay incubation at 28°C for 2 days), histamine production for *L. hilgardii* isolated from wine ranged between 39 to 235 mg/L.

Moreno-Arribas *et al.* (2000) isolated a number of bacteria from tyramine-containing wines and discovered that the primary tyramine producer was *Lactobacillus brevis*, and no *L. hilgardii* strains were detected in the tyramine-containing wine. The authors also investigated a large number of commercially available (isolated from various fermented grape products) lactic acid bacteria strains for their tyramine producing capacity and discovered that only 1 strain in 17 *L. hilgardii* strains could produce tyramine. Similar observations for tyramine production by *L. hilgardii* and *L. brevis* were reported by Landete *et al.* (2007) who identified 2 strains from 8 strains of *L. hilgardii* as producers of tyramine and phenylethylamine, and average production of these biogenic amines under optimal growth conditions ranged from 120 to 535 mg/L.

Although certain strains of *L. hilgardii* can produce a variety of biogenic amines, contamination of PFI's GABA in a manner that would be toxicologically relevant is unlikely. A detailed compositional analysis of PFI's GABA ingredient was performed (see Section II.C, Table II.C-3) indicating that the level of free amino acid contamination is low, and with the exception of glutamic acid, all amino acids are below 0.5%, and many of the biogenic amine precursors (histidine, tyrosine, and phenylalanine) were detected at a concentration of ≤0.04%. In addition, PFI has tested their GABA ingredient for the presence of histamine using HPLC analysis and confirmed that the product is histamine-free (analytical results of histamine analysis are presented in Appendix C; Attachment C-2).

Ethyl Carbamate and Citrulline

Lactic acid bacteria (*Lactobacillus buchneri, Oenococcus oeni,* and *L. hilgardii*) are often used to develop flavors in wine *via* a process called malolactic fermentation, a stage of wine-making that is performed subsequent to alcoholic fermentation during wine production. Periodically, malolactic fermentation is associated with the formation of the mutagenic compound, ethyl carbamate in a process that is assumed to occur in association with L-arginine catabolism (Mira De Orduña *et al.*, 2001). Lactic acid bacteria that express significant levels of arginine deaminase are believed to catalyze this process *via* the formation of L-citrulline. The L-citrulline is then released from the bacteria where the compound slowly reacts with the ethanol present in the wine producing ethyl carbamates. Given the slow production of ethyl carbamate from citrulline in wine, studies specifically demonstrating ethyl carbamate production by *L. hilgardii* are not available; however, a number of experiments have shown that some strains of the organism can actively degrade L-arginine to citrulline (Arena *et al.*, 2002; Azevedo *et al.*, 2002; Rodríguez *et al.*, 2007), and wines produced using these strains could be susceptible to ethyl carbamate production if significant concentrations of L-arginine remain following alcoholic fermentation. It is unclear if the strain of *L. hilgardii* used in the production of GABA (*L. hilgardii*

K-3) expresses a functional arginine deaminase enzyme; however, a significant source of arginine is not present during GABA fermentation, nor is ethanol used during the manufacture of GABA; thus, the formation of ethyl carbamate during GABA production is not expected. This hypothesis is supported by studies conducted by PFI at the Japanese Food Research Laboratory, in which neither ethyl carbamate nor citrulline were detected in the final product at limits of detection of 0.01 and 200 ppm, respectively[9]. The results of the analyses are presented in Appendix C, Attachment C-3 and C-4, respectively.

Additionally, the absence of viable *L. hilgardii* K-3 in the final product is ensured by the use of appertization and microfiltration (0.65 µm) during the manufacturing process, and analytical data confirm the absence of protein in the final product (see Section II.C). Therefore, the use of *L. hilgardii K-3* in the production of GABA for use in food is safe based on the following: *L. hilgardii K-3* strain was isolated from kimchi, which has been and is consumed in the human diet; *L. hilgardii* has a long history of safe consumption due to its presence in fermented foods; *L. hilgardii* is the most prevalent species of lactic acid bacteria identified in wine, and the absence of strain specific metabolites of toxicological relevance is supported by the absence of histamine, ethyl carbamate, and citrulline in GABA produced by PFI.

I. Summary and Basis for GRAS

PFI intends to market GABA as a food ingredient in the U.S. for use at a level of 100 mg GABA/serving (*i.e*., 0.04 to 0.67%) in various foods, such as snack bars, breakfast cereals, processed cheese, yoghurts, chewing gum, hard and soft candies and chocolate, as well as beverages and beverage bases including carbonated, energy, flavored, powdered and sports drinks, flavored milk and milk drinks, and coffee and tea.

PFI's GABA (not less than 80% purity) is manufactured by a fermentation process that utilizes *L. hilgardii* K-3 to catalyze the conversion of L-glutamate to GABA. The product is manufactured consistent with cGMP and the final GABA ingredient meets appropriate food-grade specifications. GABA can also be diluted 4-fold with food-grade starch to produce a product with not less than 20% purity (GABA 20). Batch analyses of 3 non-consecutive lots of GABA and 3 non-consecutive lots of GABA 20 demonstrate that the final products are consistently produced in accordance with the established specifications. Stability studies have shown that GABA 20 is stable for up to 27 months under ambient conditions and the shelf life was set at 2 years. Additionally, in aqueous solution dissolved at a level of 5%, GABA was stable under conditions of elevated temperatures and varied pH.

L. hilgardii is a non-toxicogenic and non-pathogenic bacteria that is present in a number of commonly consumed foods (*e.g.*, cheese, wine, port, and brandy). Although select members of *L. hilgardii* are known producers of biogenic amines, analytical data confirm the absence of

[9] Certificate of Analysis for citrulline indicates that the detection limit was set at 0.02 g/100 g due to the existence of highly-concentrated amino acid.

histamine in PFI's product. Furthermore, precursor amino acids (histidine, tyrosine, and phenylalanine) required for the synthesis of biogenic amines by microorganisms were not detected in the fermentation media at significant levels (≤0.04%). Although some strains of *L. hilgardii* have been shown to synthesize the mutagenic compound ethyl carbamate, the results of analytical data confirm the absence of this compound in PFI's GABA. Likewise, since ethyl carbamate also can form spontaneously in the presence of alcohol, analytical studies conducted by PFI confirm the absence of citrulline in the final product, supporting the use of PFI's GABA in alcoholic beverages. Thus, these findings support the safety of the use of *L. hilgardii* K-3 in the production of GABA.

GABA exists naturally in a wide variety of foods typical of the North American diet, and thus has a long-history of safe consumption by humans. Based on published estimates of GABA in food, in conjunction with *per capita* consumption data (Stofberg and Grundschober, 1987), estimated intakes of GABA in the U.S. from its natural occurrence were determined to be 136 mg/person/day. The all-user total population consumption of GABA from the proposed uses in food and beverages were estimated to result in a mean intake of 429 mg/person/day (6.6 mg/kg body weight/day) and a 90th percentile all-user intake of 811 mg/person/day (12.4 mg/kg body weight/day). The intended use-level of PFI's GABA (100 mg/serving) is within the range of GABA present in GABA-enriched foods marketed in Japan, which may contain as much as 280 mg of GABA per serving. The estimated intakes from the proposed uses are also within the recommended intakes of GABA from its inclusion in a number of dietary supplement products (typically around 750 mg/day and can be as high as 1,500 to 5,000 mg/day). Based on the estimated exposure to GABA from its natural occurrence in food, combined mean and 90th percentile all-user intakes of GABA from background sources and exposure under the proposed uses was estimated to be 565 and 947 mg/person/day, respectively. Intake estimates on an absolute basis were highest in male adults with mean and 90th percentile all-user intakes of 535 and 978 mg/person (6.2 and 11.2 mg/kg body weight respectively). However, on a body weight basis, infants and children were estimated to have the highest GABA intakes (all-user consumption) relative to body weight (*i.e.*, for infants, 11.1 and 23.1 mg/kg body weight/day for mean and 90th percentile exposures, respectively, and for children, 9.9 and 18.5 mg/kg body weight/day for mean and 90th percentile, respectively). Since PFI does not intend to market GABA for use in products likely to be consumed by infants or children, it is expected that the actual exposure to GABA in this group will be minimal.

GABA is a charged molecule, and rodent studies indicate that the compound displays poor bioavailability when administered orally (van Gelder and Elliott, 1958). The daily consumption of GABA (80 mg/person) for a period of 8 to 12 weeks did not result in increased plasma GABA levels, suggesting that GABA is either poorly bioavailable in humans and/or is rapidly metabolized following consumption (Matsubara *et al*., 2002; Kajimoto *et al*., 2004a). Similar pharmacokinetics exist among various laboratory animal species (*e.g.,* rats, cats, and rabbits) and exogenous GABA systemically administered is rapidly cleared from the blood (*i.e.*,

20-minute half-life in rodents) without evidence of GABA bioaccumulation or tissue retention (van Gelder and Elliott, 1958; Hespe *et al*., 1969). In rodents, the liver is the primary metabolic site for GABA (Ferenci *et al.*, 1988) and the catabolism of GABA occurs exclusively *via* GABA transaminase for use as a carbon source in the TCA cycle resulting in CO_2 as the primary waste product (Patel *et al.*, 2005). In humans, pharmacokinetic studies conducted in 1 subject with hepatic impairment suggest that GABA is rapidly metabolized in the liver (Tower, 1960).

The permeation of GABA across the blood-brain barrier is highly limited and not significantly affected by the dose of GABA (Roberts *et al.*, 1958; van Gelder and Elliott, 1958; Kuriyama and Sze, 1971; Oldendorf, 1971; Frey and Löscher, 1980; Krantis, 1984; Al-Sarraf, 2002; Al-Awadi *et al.*, 2006). Exogenous GABA does not accumulate in the brain following systemic exposure because the efflux rate of GABA across the BBB is greater than its permeation rate and the high GABA transaminase activity in the CSF (Kuriyama and Sze, 1971; Kakee *et al.*, 2001). The publicly available data provide no evidence to suggest that GABA can cross the BBB in mature healthy animals in sufficient quantities to either increase GABA concentrations in the brain, or induce inhibitory effects in the brain. General recognition of this is supported by the EPA who reviewed the safety of human exposure to GABA, and concluded that "GABA does not cross the blood-brain barrier" (U.S. EPA, 2001).

Animal studies have demonstrated that GABA is of low oral toxicity and any effects induced were unremarkable. An acute toxicity study conducted with PFI's GABA resulted in no toxicity in rats at a single oral dose of 1,000 mg/kg body weight (JFLR, 2002 [unpublished]). Sauchi *et al*. (2009) reported a NOAEL of 200 mg/kg body weight/day, the only dose tested, for PFI's GABA when administered to male and female Wistar rats in the diet over a 28-day period. Likewise, no toxicologically relevant effects were reported in a 90-day study in Sprague-Dawley rats that received PFI's GABA by oral gavage at doses up to 2,500 mg/kg body weight/day (Takeshima *et al.*, 2014). Two repeated-dose studies conducted with GABA-containing fermented milk reported that exposure to GABA was well-tolerated at doses up to 5 mg/kg body weight/day (Kato *et al.*, 2005). No chronic studies with GABA or PFI's GABA were identified in the literature search. Tower (1960) reviewed unpublished reports of chronic toxicity studies conducted in rats and dogs and concluded that the chronic consumption of GABA at doses up to 1 g/kg body weight/day for prolonged periods did not elicit signs of toxicity. Chronic studies in rats, dogs and monkeys receiving GVG, an inhibitor of GABA transaminases and GABA turnover, failed to elicit signs of peripheral neuropathy (Gibson *et al*., 1990).

A number of published human studies containing relevant hematological and/or clinical chemistry and safety endpoints, and adverse event monitoring demonstrated that repeat consumption of GABA is well-tolerated by humans consuming GABA in repeat-dose quantities of up to 120 mg/person/day for up to 12 weeks or 250 mg/day for up to 30 days. Additionally, acute exposure to high doses of GABA (5 to 18 g/day for up to 4 days), were reported to be well-tolerated with minor side-effects limited to transient shortness of breath, and slight burning

sensation in the throat lasting a few minutes after GABA ingestion. To date, no evidence of severe adverse events has been reported in the literature. The consumption of large doses of GABA (0.8 g/kg body weight/day corresponding to 56 g/day for a 70 kg individual) for 3 months to 2 years has also been reported to be well-tolerated with no evidence of severe adverse events in a study involving subjects with various neurophysiological disorders (Tower, 1960).

GABA consumption has been reported to be associated with reduced blood pressure in hypertensive subjects; however, there is no clear dose-response relationship for this effect, and the maximal effect, although statistically significant in many studies, is modest relative to controls (*i.e.*, maximum decreases of 5 to 7%) and baseline values (*i.e.*, maximum reduction of 12%). In addition, the effect also appears to be limited to hypertensive subjects and individuals with high-normal blood pressure as GABA studies in normotensive individuals at low (10 to 70 mg/person/day for 8 to 12 weeks) and high doses (5 to 18 g/day for 1 to 4 days) did not induce significant changes to blood pressure (Cavagnini *et al.,* 1980a,b; Kimura *et al.,* 2002; Tanaka *et al.*, 2009). Significant deleterious decreases in blood pressure in association with GABA consumption have not been reported. Modest reductions in blood pressure in hypertensive subjects is not uncommon with various foods, and comparable reductions in blood pressure limited to hypertensive subjects has been well established in association with the consumption of dark chocolate and fish oil. Furthermore, the GABA-associated reductions in blood pressure also appeared to be time-dependent and typically required at least 2 weeks of repeat consumption before significant reductions are observed.

GABA also has been reported to affect GH production when consumed in quantities (5 to 18 g/day) that are well in excess of the proposed uses (Cavagnini *et al.*, 1980a,b; Powers *et al.*, 2008); however, these affects are within the normal physiological range observed in response to exercise, and are comparable to those reported following arginine consumption (Paddon-Jones *et al.*, 2004; Collier *et al.*, 2005; Kanaley, 2008; Powers *et al.*, 2008). Moreover, GH-induced responses to high concentrations of GABA appear to be rapidly desensitized during repeat exposures (Gamel-Didelon *et al.*, 2003). There is currently no evidence indicating that GABA induced GH production is of physiological significance or of toxicological concern.

The weight of the available scientific evidence supports the safety of the proposed uses of GABA. The oral bioavailability of GABA is low and clearance is rapid. Moreover, an extensive body of evidence has shown that GABA has a low capacity for permeation through the BBB that does not lead to significant accumulation of GABA in the CNS. GABA is of low oral toxicity in rats. The cumulative estimated intake of GABA for the total population at the 90th percentile from all proposed food uses and background dietary sources is 947 mg/day, equivalent to 14.6 mg/kg body weight/day for a 65 kg individual. Furthermore, humans have a long-history of safe exposure to quantities of GABA in the diet from the consumption of a wide variety of foods. A number of human clinical studies have also demonstrated that GABA supplements and GABA-enriched foods are well-tolerated with only modest reductions on blood pressure in

hypertensive individuals that were not considered pathogenic, while similar changes in blood pressure were not observed in normotensive individuals.

In conclusion, based on the above totality of evidence approach, and the consensus of a qualified Expert Panel, GABA produced from the non-toxicogenic, non-pathogenic microorganism *L. hilgardii* K-3, meeting appropriate food-grade specifications and manufactured consistent with cGMP, is GRAS based on scientific procedures for its intended conditions of use as described herein and therefore is exempt from pre-market approval (Section 409 of the Federal Food, Drug and Cosmetic Act).

V. References

Adams MR (1999). Safety of industrial lactic acid bacteria. J Biotechnol 68(2/3):171-178.

Akatsu T (2000). Studies on the development of food processing (Part 1) quality analysis of kimchi on the market. Rep Tochigi Prefectural Inst Ind Technol pp. 29-34 [Incl. Engl.

Al-Awadi M, Pavlik A, Al-Sarraf H (2006). Increased brain uptake and brain to blood efflux transport of 14C-GABA in spontaneously hypertensive rats. Life Sci 79(9):847-853.

Al-Sarraf H (2002). Transport of 14(C)-γ}-aminobutyric acid into brain, cerebrospinal fluid and choroid plexus in neonatal and adult rats. Brain Res Dev Brain Res 139(2):121-129.

Arena ME, Manca de Nadra MC, Munoz R (2002). The arginine deiminase pathway in the wine lactic acid bacterium *Lactobacillus hilgardii* X1B: structural and functional study of the *arcABC* genes. Gene 301(1/2):61-66.

Azevedo Z, Couto JA, Hogg T (2002). Citrulline as the main precursor of ethyl carbamate in model fortified wines inoculated with *Lactobacillus hilgardii*: a marker of the levels in a spoiled fortified wine. Lett Appl Microbiol 34(1):32-36.

Baruzzi F, Morea M, Matarante A, Cocconcelli PS (2000). Changes in the *Lactobacillus* community during Ricotta forte cheese natural fermentation. J Appl Microbiol 89(5):807-814.

Becker K, Southwick K, Reardon J, Berg R, Maccormack JN (2001). Histamine poisoning associated with eating tuna burgers. JAMA 285(10):1327-1330.

Bernardeau M, Guguen M, Vernoux JP (2006). Beneficial lactobacilli in food and feed: long-term use, biodiversity and proposals for specific and realistic safety assessments. FEMS Microbiol Rev 30(4):487-513.

Bianchi L, Della Corte L, Tipton KF (1999). Simultaneous determination of basal and evoked output levels of aspartate, glutamate, taurine and 4-aminobutyric acid during microdialysis and from superfused brain slices. J Chromatogr B Biomed Sci Appl 723(1&2):47-59.

Borriello SP, Hammes WP, Holzapfel W, Marteau P, Schrezenmeir J, Vaara M et al. (2003). Safety of probiotics that contain lactobacilli or bifidobacteria. Clin Infect Dis 36(6):775-780.

Boyle RJ, Robins-Browne RM, Tang ML (2006). Probiotic use in clinical practice: What are the risks? Am J Clin Nutr 83(6):1256-1264.

Cavagnini F, Benetti G, Invitti C, Ramella G, Pinto M, Lazza M et al. (1980a). Effect of γ-aminobutyric acid on growth hormone and prolactin secretion in man: influence of pimozide and domperidone. J Clin Endocrinol Metab 51(4):789-792.

Cavagnini F, Invitti C, Pinto M, Maraschini C, Di Landro A, Dubini A et al. (1980b). Effect of acute and repeated administration of gamma aminobutyric acid (GABA) on growth hormone and prolactin secretion in man. Acta Endocrinol (Copenh) 93(2):149-154.

Cavagnini F, Pinto M, Dubini A, Invitti C, Cappelletti G, Polli EE (1982). Effects of gamma aminobutyric acid (GABA) and muscimol on endocrine pancreatic function in man. Metabolism 31(1):73-77.

CDC (2014). *National Health and Nutrition Examination Survey (NHANES): 2011-2012*. Hyattsville (MD): Centers for Disease Control and Prevention (CDC), National Center for Health Statistics (NCHS). Available at: http://wwwn.cdc.gov/nchs/nhanes/search/nhanes11_12.aspx [Page last updated: October 31, 2014].

ChemIDplus (2015). Gamma-aminobutyric acid [JAN] [GABA]. RN: 56-12-2. In: *ChemIDplus Advanced*. Bethesda (MD): National Library of Medicine (NLM). Available at: http://chem.sis.nlm.nih.gov/chemidplus/ [Last accessed January 6, 2015].

Collier SR, Casey DP, Kanaley JA (2005). Growth hormone responses to varying doses of oral arginine. Growth Horm IGF Res 15(2):136-139.

Costantini A, Cersosimo M, Del Prete V, Garcia-Moruno E (2006). Production of biogenic amines by lactic acid bacteria: screening by PCR, thin-layer chromatography, and high-performance liquid chromatography of strains isolated from wine and must. J Food Prot 69(2):391-396.

Dickinson HO, Mason JM, Nicolson DJ, Campbell F, Beyer FR, Cook JV et al. (2006). Lifestyle interventions to reduce raised blood pressure: a systematic review of randomized controlled trials. J Hypertens 24(2):215-223.

Douglas HC, Cruess WV (1936). A Lactobacillus from California wine: *Lactobacillus hilgardii*. Food Res 1(2):113-119.

Euzéby JP [Founder] (2014). Genus *Lactobacillus*. In: *List of Prokaryotic Names with Standing in Nomenclature*. [Update of original article: Int J Syst Evol Microbiol, 1997; 47(2):590-592]. List of Prokaryotic names with Standing in Nomenclature (LPSN). Available at: http://www.bacterio.cict.fr/l/lactobacillus.html [Curator from 1 July 2013: A.C. Parte].

Ferenci P, Stehle T, Ebner J, Schmid R, Haussinger D (1988). Uptake and catabolism of γ-aminobutyric acid by the isolated perfused rat liver. Gastroenterology 95(2):402-407.

Ferreira IM, Pinho O (2006). Biogenic amines in Portuguese traditional foods and wines. J Food Prot 69(9):2293-2303.

Fox PF, editor (1995). [GABA]. In: *Cheese: Chemistry, Physics and Microbiology: Vol. 2: Major Cheese Groups, 2nd edition*. San Diego (CA) / London, Engl.: Elsevier Academic Press, pp. 233, 528.

Frey HH, Löscher W (1980). Cetyl GABA: effect on convulsant thresholds in mice and acute toxicity. Neuropharmacology 19(2):217-220.

Gamel-Didelon K, Kunz L, Fohr KJ, Gratzl M, Mayerhofer A (2003). Molecular and physiological evidence for functional γ-aminobutyric acid (GABA)-C receptors in growth hormone-secreting cells. J Biol Chem 278(22):20192-20195.

Garai G, Duenas MT, Irastorza A, Martin-Alvarez PJ, Moreno-Arribas MV (2006). Biogenic amines in natural ciders. J Food Prot 69(12):3006-3012.

Gibson JP, Yarrington JT, Loudy DE, Gerbig CG, Hurst GH, Newberne JW (1990). Chronic toxicity studies with vigabatrin, a GABA-transaminase inhibitor. Toxicol Pathol 18(2):225-238.

Grassi D, Necozione S, Lippi C, Croce G, Valeri L, Pasqualetti P et al. (2005). Cocoa reduces blood pressure and insulin resistance and improves endothelium-dependent vasodilation in hypertensives. Hypertension 46(2):398-405.

Hayakawa K, Ueno. Y, Kawamura S, Taniguchi R, Oda K (1997). Production of γ-aminobutyric acid by lactic acid bacteria. Seibutsu-Kogaku Kaishi 75(4):239-244.

Hespe W, Roberts E, Prins H (1969). Autoradiographic investigation of the distribution of [^{14}C]GABA in tissues of normal and aminooxyacetic acid-treated mice. Brain Res 14(3):663-671.

Holzapfel WH, Haberer P, Snel J, Schillinger U, Huis in't Veld JH (1998). Overview of gut flora and probiotics. Int J Food Microbiol 41(2):85-101

Horie N, Yokoyama Y, Kojima H, Takeshima K, Masuda K, Kim M et al. (2011). Long-term human safety test of orally administered GABA. Yakuri To Chiryo [Basic Pharmacol Ther] 39(7):665-669.

Inoue K, Shirai T, Ochiai H, Kasao M, Hayakawa K, Kimura M et al. (2003). Blood-pressure-lowering effect of a novel fermented milk containing γ-aminobutyric acid (GABA) in mild hypertensives. Eur J Clin Nutr 57(3):490-495.

JFRL (2002) [unpublished]. *Acute Toxicity Test [Confidential]*. (Experimental Report No. 202061563-001). Prepared by Tokyo, Japan: Japan Food Research Laboratories (JFRL) for Pharma Foods International Co., Ltd. (PFI).

Kajimoto O, Hirata H, Nishimura A (2003). Hypotensive action of novel fermented milk containing γ-aminobutyric acid (GABA) in subjects with mild or moderate hypertension. Kenko Eiyo Shokuhin Kenkyu [J Nutr Food] 6(2):51-64 [Incl. Engl. Translat].

Kajimoto O, Ueno H, Nagata Y, Yabune M, Kajimoto Y (2004a). Hypotensive effects of tablet containing γ-aminobutyric acid (GABA) on high normal blood pressure and mild hypertensive subjects. Yakuri To Chiryo [Basic Pharmacol Ther] 32(12):929-944 [Engl. Translat.].

Kajimoto O, Hirata H, Nakagawa S, Kajimoto Y, Hayakawa K, Kimura M (2004b). [Hypotensive effect of fermented milk containing γ-aminobutyric acid (GABA) in subjects with high normal blood pressure]. Nihon Shokuhin Kagaku Kogakkaishi 51(2):79-86 [Incl. Engl. Translat].

Kakee A, Takanaga H, Terasaki T, Naito M, Tsuruo T, Sugiyama Y (2001). Efflux of a suppressive neurotransmitter, GABA, across the blood-brain barrier. J Neurochem 79(1):110-118.

Kanaley JA (2008). Growth hormone, arginine and exercise. Curr Opin Clin Nutr Metab Care 11(1):50-54.

Kato I, Shimizu S, Kobayashi T, Kado S, Kojima K, Miura K et al. (2005). Single-dose, 1-month repeated dose, and 3-month repeated dose oral toxicity studies of fermented milk containing γ-aminobutyric acid (GABA) in rodents. Yakuruto Kenkyujo Kenkyu Hokokushu (24):43-66 [Engl. Translation].

Kimura M, Chounan O, Takahashi R, Ohashi A, Arai Y, Hayakawa K et al. (2002). Effects of fermented milk containing γ-aminobutyric acid (GABA) on normal adult subjects. Jpn J Food Chem 9(1):1-6 [Incl. Engl. Translation].

Krantis A (1984). The involvement of GABA-transaminase in the blood-brain barrier to radiolabelled GABA. Acta Neuropathol 64(1):61-67.

Kuriyama K, Sze PY (1971). Blood-brain barrier to H(3)-γ-aminobutyric acid in normal and amino oxyacetic acid-treated animals. Neuropharmacology 10(1):103-108.

Landete JM, Ferrer S, Pardo I (2005). Which lactic acid bacteria are responsible for histamine production in wine. J Appl Microbiol 99(3):580-586.

Landete JM, Pardo I, Ferrer S (2007). Tyramine and phenylethylamine production among lactic acid bacteria isolated from wine. Int J Food Microbiol 115(3):364-368.

Lehane L, Olley J (2000). Histamine fish poisoning revisited. Int J Food Microbiol 58(1/2):1-37

Loiudice R, Impembo M, Laratta B, Villari G, Voi AL, Siviero P et al. (1995). Composition of San Marzano tomato varieties. Food Chem 53(1):81-89.

Lowe IP, RobinS E, Eyerman GS (1958). The fluorometric measurement of glutamic decarboxylase and its distribution in brain. J Neurochem 3(1):8-18.

Matsubara F, Ueno H, Tadano K, Suyama T, Imaizumi K, Suzuki T et al. (2002). Effects of GABA supplementation on blood pressure and safety in adults with mild hypertension. Yakuri To Chiryo [Basic Pharmacol Ther] 30(11):963-972 [Engl. Translation].

Matumoto Y, Ohno K, Hiraoka Y (1997). Studies on the utilization of functional food materials containing high levels of gamma-aminobutyric acid (Part 1). Contents of gamma-aminobutyric acid and its precursor glutamic acid in vegetables and fruit. Rep Ehime Prefectural Inst Ind Technol (35):97-100.

MHLW (2000). B. General tests; Heavy metals limit test; Loss on drying; Microbial limit tests. In: *Japan's Specifications and Standards for Food Additives, 7th edition*. Tokyo, Japan: Ministry of Health, Labor and Welfare, Japan (MHLW) and Translated by Japan Food Chemical Research Foundation. Available at: http://www.ffcr.or.jp/zaidan/FFCRHOME.nsf/pages/spec.stand.fa [2000, Last updated: 2006/11/30].

MHLW [2007a]. *[Summary of Japan National Health and Nutrition Examination Survey 2005 (including a flash report of Japan National Health and Nutrition Examination Survey 2006)]*. Tokyo, Japan: Ministry of Health, Labour and Welfare (MHLW). Available at: http://www.mhlw.go.jp/houdou/2007/05/h0516-3.html.

MHLW [2007b]. *[Report of Japan National Health and Nutrition Examination Survey 2005]*. Tokyo, Japan: Ministry of Health, Labour and Welfare (MHLW). Available at: http://www.mhlw.go.jp/bunya/kenkou/eiyou07/01.html.

Miki M, Ishikawa T, Okayama H (2005). An outbreak of histamine poisoning after ingestion of the ground saury paste in eight patients taking isoniazid in tuberculous ward. Intern Med 44(11):1133-1136.

Mira De Orduña R, Patchett ML, Liu SQ, Pilone GJ (2001). Growth and arginine metabolism of the wine lactic acid bacteria *Lactobacillus buchneri* and *Oenococcus oeni* at different pH values and arginine concentrations. Appl Environ Microbiol 67(4):1657-1662.

Moreno-Arribas V, Torlois S, Joyeux A, Bertrand A, Lonvaud-Funel A (2000). Isolation, properties and behaviour of tyramine-producing lactic acid bacteria from wine. J Appl Microbiol 88(4):584-593.

Murcia MA, López-Ayerra B, Martínez-Tomé M, García-Carmona F (2001). Effect of industrial processing on amino acid content of broccoli. J Sci Food Agric 81(14):1299-1305.

Nácher A, Polache A, Moll-Navarro MJ, Plá-Delfina JM, Merino M (1994). Intestinal absorption pathway of γ-aminobutyric acid in rat small intestine. Biopharm Drug Dispos 15(5):359-371.

Nakamura T, Hasegawa T, Ueno S, Kumamoto S, Matsubayashi T, Ando Y et al. (2000). [Effect of γ-aminobutyric acid-rich Chlorella on blood pressure in mildly hypertensive subjects]. Yakuri To Chiryo [Basic Pharmacol Ther] 28(6):529-533.

Nakamura K, Nara K, Noguchi T, Oshiro T, Koga H (2006). [Contents of γ-aminobutyric acid (GABA) in potatoes and processed potato products]. Nihon Shokuhin Kagaku Kogakkaishi 53(9):514-517.

Nomura M, Kimoto H, Someya Y, Furukawa S, Suzuki I (1998). Production of γ-aminobutyric acid by cheese starters during cheese ripening. J Dairy Sci 81(6):1486-1491.

OECD (1987). Acute oral toxicity. In: *OECD Guidelines for the Testing of Chemicals*. (OECD Guideline no 401) [Adopted February 24, 1987]. Paris, France: Organisation for Economic Co-operation and Development (OECD). Available at: http://www.oecd-ilibrary.org/environment/test-no-401-acute-oral-toxicity_9789264040113-en;jsessionid=21q861k9hfaiu.x-oecd-live-01 [**Deleted 17 December 2002**].

OECD (1998a). *OECD Principles of Good Laboratory Practice (as Revised in 1997)*. (OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring, no 1 - ENV/MC/CHEM(98)17). Paris, France: Organisation for Economic Co-operation and Development (OECD), Environment Directorate, Chemicals Group and Management Committee, OECD Environmental Health and Safety Publications. Available at: http://www.oecd.org/officialdocuments/displaydocumentpdf/?cote_env/mc/chem(98)17&doclanguage_en.

OECD (1998b). Repeated dose 90-day oral toxicity study in rodents. In: *OECD Guidelines for the Testing of Chemicals*. (OECD Guideline no 408) [Updated & Adopted 21st September 1998]. Paris, France: Organisation for Economic Co-operation and Development (OECD). Available at: http://www.oecd-ilibrary.org/environment/test-no-408-repeated-dose-90-day-oral-toxicity-study-in-rodents_9789264070707-en;jsessionid=1m6bvwspup322.delta.

Ohnuma S, Higa M, Hamanaka S, Matsushima K, Yamamuro W (2001). An outbreak of allergy-like food poisoning. Intern Med 40(8):833-835.

Okada T, Sugishita T, Murakami T, Murai H, Saikusa T, HorinoT et al. (2000). Effect of the defatted rice germ enriched with GABA for sleeplessness, depression, autonomic disorder by oral administration. Nihon Shokuhin Kagaku Kogakkaishi 47(8):596-603 [Incl. Engl. Translat.].

Oldendorf WH (1971). Brain uptake of radiolabeled amino acids, amines, and hexoses after arterial injection. Am J Physiol 221(6):1629-1639.

Osawa T, Goto T, Gonda M, Oyama W, Yasutake N, Onoue M (2005). Mutagenicity test of gamma-aminobutyric acid-containing fermented milk "GABA-10" and "GABA-25" using DNA repair system. Yakuruto Kenkyujo Kenkyu Hokokushu 24:67-72 [Engl. Translation].

Oshima Y, Kasahara A, Shibata M, Mogi M (1965). [Studies on DL-alpha-lipoic acid derivatives. II. On the Pharmacological activities of 4-(DL-alpha-lipamido)butyric acid (LABA)]. Yakugaku Zasshi 85(5):463-468. Cited In: ChemIDplus, 2014.

Paddon-Jones D, Borsheim E, Wolfe RR (2004). Potential ergogenic effects of arginine and creatine supplementation [Includes discussion by Joseph Loscalzo]. J Nutr 134(10, Suppl.):2888S-2894S, 2895S.

Patel AB, De Graaf RA, Mason GF, Rothman DL, Shulman RG, Behar KL (2005). The contribution of GABA to glutamate/glutamine cycling and energy metabolism in the rat cortex *in vivo*. Proc Natl Acad Sci USA 102(15):5588-5593.

Picard C, Fioramonti J, Francois A, Robinson T, Neant F, Matuchansky C (2005). Review article: bifidobacteria as probiotic agents — physiological effects and clinical benefits. Aliment Pharmacol Ther 22(6):495-512.

Pouliot-Mathieu K, Gardner-Fortier C, Lemieux S, St-Gelais D, Champagne CP, Vuillemard JC (2013). Effect of cheese containing gamma-aminobutyric acid-producing lactic acid bacteria on blood pressure in men. PharmaNutrition 1(4):141-148.

Powers ME, Yarrow JF, McCoy SC, Borst SE (2008). Growth hormone isoform responses to GABA ingestion at rest and after exercise. Med Sci Sports Exerc 40(1):104-110.

Qume M, FowlerLJ (1996). Effects of chronic oral treatment with GABA-transaminase inhibitors on the GABA system in brain, liver, kidney, and plasma of the rat. Biochem Pharmacol 52(9):1355-1363.

Roberts E, Baxter CF (1958). Metabolic studies of gamma-aminobutyric acid. Neurology 8(Suppl. 1):77-78.

Roberts E, Lowe I.P, Guth L, Jeline B (1958). Distribution of gamma-aminobutyric acid and other amino acids in nervous tissue of various species. J Exp Zool 138(2):313-328. Cited In: Roberts, 1984.

Rodas AM, Ferrer S, Pardo I (2005). Polyphasic study of wine *Lactobacillus* strains: taxonomic implications. Int J Syst Evol Microbiol 55(1):197-207.

Rodríguez H, de las Rivas B, Muñoz R (2007). Efficacy of *recA* gene sequence analysis in the identification and discrimination of *Lactobacillus hilgardii* strains isolated from stuck wine fermentations. Int J Food Microbiol 115(1):70-78.

Saarela M, Mättö J, Mattila-Sandholm T (2002). Safety aspects of *Lactobacillus* and *Bifidobacterium* species originating from human oro-gastrointestinal tract or from probiotic products. Microb Ecol Health Dis 14(4):233-240.

Sauchi Y, Hisaizumi K, Akao M, Kim M, Yoshikuni Y (2009). Toxicity of PG2K20 for 28 days administration in rats. Yakuri To Chiryo [Basic Pharmacol Ther] 37(3):173-179.

Schafer DF, Fowler JM, Jones EA (1981). Colonic bacteria: a source of gamma-aminobutyric acid in the blood. Proc Soc Exp Biol Med 167(3):301-303. Cited In: Ferenci *et al.*, 1988.

Shimada M, Hasegawa T, Nishimura C, Kan H, Kanno T, Nakamura T et al. (2009). Anti-hypertensive effect of γ-aminobutyric acid (GABA)-rich *Chlorella* on high-normal blood pressure and borderline hypertension in placebo-controlled double blind study. Clin Exp Hypertens 31(4):342-354.

Shimizu S, Shibata N, Yamaguchi O (1959). Effect and application of γ-aminobutyric acid. Chiryo 41:959-966. Cited In: Kato *et al.*, 2005.

Sohier D, Coulon J, Lonvaud-Funel A (1999). Molecular identification of *Lactobacillus hilgardii* and genetic relatedness with *Lactobacillus brevis*. Int J Syst Bacteriol 49(3):1075-1081.

Steward FC, Thompson JF, Dent CE (1949). γ-aminobutyric acid: a constituent of the potato tuber? Science 110(2861):439-440.

Stofberg J, Grundschober F (1987). Consumption ratio and food predominance of flavoring materials (Third cumulative series). Perfum Flavor 12(3):27-68.

StrainInfo (2015). NCIMB 8040: *Lactobacillus hilgardii*. In: *StrainInfo Bioportal*. Gent, Belgium: StrainInfo, Department of Applied Mathematics and Computer Science, Ghent University. Available at: http://www.straininfo.net/strainPassport.action?sort_sequenceLength&dir_desc&cultureId=13761 [Last accessed: January 6, 2015].

Suzzi G, Gardini F (2003). Biogenic amines in dry fermented sausages: a review. Int J Food Microbiol 88(1):41-54.

Takeshima K, Yamatsu A, Yamashita Y, Watabe K, Horie N, Masuda K et al. (2014). Subchronic toxicity evaluation of γ-aminobutyric acid (GABA) in rats. Food Chem Toxicol 68:128-134.

Tanaka H, Watanabe K, Ma M, Hirayama M, Kobayashi T, Oyama H et al. (2009). The effects of γ-aminobutyric acid, vinegar, and dried bonito on blood pressure in normotensive and mildly or moderately hypertensive volunteers. J Clin Biochem Nutr 45(1):93-100.

Taubert D, Berkels R, Roesen R, Klaus W (2003). Chocolate and blood pressure in elderly individuals with isolated systolic hypertension. JAMA 290(8):1029-1030.

Taubert D, Roesen R, Lehmann C, Jung N, Schömig E (2007). Effects of low habitual cocoa intake on blood pressure and bioactive nitric oxide: a randomized controlled trial. JAMA 298(1):49-60.

Tower DB (1960). The administration of gamma-aminobutyric acid to man: systematic effects and anticonvulsant action. In: Roberts E, Baxter CF, Van Harrveld A, Wiersma CAG, Adehy WR, Killam KF, editors. *Inhibition in the Nervous System and Gamma-Aminobutyric Acid: Proceedings of an International Symposium*, May 22-24, 1959, Duarte, Calif. New York (NY): Permagon Press, pp. 562-578.

U.S. ATTTB (2014). Part 24—Wine. Section §24.246—Materials authorized for the treatment of wine and juice. In: *U.S. Code of Federal Regulations (CFR). Title 27—Alcohol, Tobacco Products and Firearms (Alcohol and Tobacco Tax and Trade Bureau, Department of the Treasury)*. Washington (DC): U.S. Government Printing Office (GPO). Available at: http://www.gpo.gov/fdsys/browse/collectionCfr.action?collectionCode=CFR.

U.S. EPA (2001). L-Glutamic acid and gamma aminobutyric acid; exemptions from the requirement of a tolerance; Final rule (40 CFR Part 180) [OPP–301136; FRL–6785–6; RIN 2070–AB78]. Fed Regist (US) 66(120):33195-33198. Available at: http://www.gpo.gov/fdsys/pkg/FR-2001-06-21/pdf/01-15615.pdf#page=1.

U.S. FDA (1993). Appendix I. Table 14. Conversion table for test chemical treatment doses used in PAFA. In: *Priority Based Assessment of Food Additives (PAFA) Database*. Washington (DC): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety and Applied Nutrition (CFSAN), p. 58. Available at: http://www.fda.gov/ohrms/DOCKETS/DOCKETS/95s0316/95s-0316-Rpt0272-36-Appendix-D-Reference-F-FDA-vol205.pdf [Last accessed Mar. 14, 2014].

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. Available at: http://www.gpo.gov/fdsys/pkg/FR-1997-04-17/pdf/97-9706.pdf.

U.S. FDA (2002). *Agency Response Letter GRAS Notice No. GRN 000087 [Composite Filtration Media (Diatomaceous Earth and Perlite), Submitted by Lompoc (CA): World Minerals, Inc*.]. College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety & Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: http://www.accessdata.fda.gov/scripts/fcn/fcnDetailNavigation.cfm?rpt_grasListing_id_87 [Feb. 26, 2002].

U.S. FDA (2013). *[Letter to Mr. Sachiko Caesura, Tokyo, Japan: Kirin Kyowa Foods Company Ltd. Inspection of manufacturing of PharmaGABA (No objectionable conditions observed)]*. (Establishment Inspection Report FEI: 2000002537). College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety and Applied Nutrition (CFSAN).

U.S. FDA (2014). *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.gpo.gov/fdsys/browse/collectionCfr.action?collectionCode=CFR.

Table of CFR Sections Referenced (Title 21—Food and Drugs)

Part	§	Section Title
101—Food labeling	101.12	Reference amounts customarily consumed per eating occasion
170—Food additives	170.3	Definitions
	170.30	Eligibility for classification as generally recognized as safe (GRAS)
172—Food additives permitted for direct addition to food for human consumption	172.320	Amino acids
	172.854	Polyglycerol esters of fatty acids
	172.892	Food starch-modified
	172.896	Dried yeasts
175—Indirect food additives: Adhesives and components of coatings	175.300	Resinous and polymeric coatings
177—Indirect food additives: Polymers	177.1520	Olefin polymers
	177.1655	Polysulfone resins
	177.2260	Filters, resin-bonded
	177.2910	Ultra-filtration membranes

Table of CFR Sections Referenced (Title 21—Food and Drugs)		
Part	**§**	**Section Title**
182—Substances generally recognized as safe	182.1	Substances that are generally recognized as safe
184—Direct food substances affirmed as generally recognized as safe	184.1857	Corn sugar

USDA (2014). *What We Eat in America: National Health and Nutrition Examination Survey (NHANES): 2011-2012.* Riverdale (MD): U.S. Department of Agriculture (USDA). Available at: http://www.ars.usda.gov/Services/docs.htm?docid=13793#release [Last Modified: 10/2/2014].

van Gelder NM, Elliott KAC (1958). Disposition of γ-aminobutyric acid administered to mammals. J Neurochem 3(2):139-143.

Vanderhoof JA, Young RJ (1998). Use of probiotics in childhood gastrointestinal disorders. J Pediatr Gastroenterol Nutr 27(3):323-332.

Waagepetersen HS, Sonnewald U, Schousboe A (1999). The GABA paradox: multiple roles as metabolite, neurotransmitter, and neurodifferentiative agent. J Neurochem 73(4):1335-1342.

Watanabe T, Kawashita A, Ishi S, Mazumder TK, Naigai S, Tsuji K et al. (2002). [Antihypertensive effect of γ-aminobutyric acid-enriched *Agaricus blazei* on mild hypertensive human subjects]. Nihon Shokuhin Kagaku Kogakkaishi 50(4):167-173 [Incl. Engl. Translation].

Yamakoshi J, Shiojo R, Nakagawa S, Ogihaara T (2006). Hypotensive effects and safety of less-salt soy sauce containing γ-aminobutyric acid (GABA) on high-normal blood pressure and mild hypertensive subjects. Yakuri To Chiryo [Basic Pharmacol Ther] 34(6):691-709 [Including English Translation].

APPENDIX A

EXPERT PANEL CONSENSUS STATEMENT CONCERNING THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF GABA FOR USE AS AN INGREDIENT IN FOOD

Expert Panel Report Concerning the Generally Recognized as Safe (GRAS) Status of *gamma*-Aminobutyric Acid (GABA) for Use as a Food Ingredient

February 3, 2015

Pharma Foods International Co. Ltd. (hereafter 'PFI') intends to market *gamma*-aminobutyric acid (GABA) as an ingredient in foods and beverages in the United States (U.S.) at a maximum use level of 100 mg/serving (0.04 to 0.67%). PFI's GABA is manufactured by a fermentation process that utilizes *Lactobacillus hilgardii* K-3 to catalyze the conversion of L-glutamate to GABA. In 2008, PFI submitted a Generally Recognized as Safe (GRAS) notification to the U.S. Food and Drug Administration (FDA) for their GABA ingredient (GRN No 000257; U.S. FDA, 2008). However, while there was a general consensus that GABA was safe under the intended conditions of use, the notification was withdrawn on the basis that the pivotal data provided within the notice, and relied upon to establish safety, was not generally available. Accordingly, rodent toxicity studies conducted using PFI's GABA are now available in the peer-reviewed literature. Additionally, PFI has also expanded the conditions of use of GABA from those determined to be GRAS in 2008.

PFI convened an Expert Panel of independent scientists, qualified by their relevant national and international experience and scientific training to evaluate the safety of food ingredients, to conduct a critical and comprehensive evaluation of the available and pertinent data and information, and to determine whether, under the conditions of intended use as an ingredient in foods and beverages, GABA would be GRAS based on scientific procedures. The Panel consisted of the below-signed qualified scientific experts: Professor Joseph F. Borzelleca, Ph.D. (Virginia Commonwealth University School of Medicine), Professor Stephen L. Taylor, Ph.D. (University of Nebraska), and Professor John A. Thomas, Ph.D. (Indiana University School of Medicine).

The Expert Panel, independently and collectively, critically evaluated a supporting dossier **[Documentation Supporting the Generally Recognized as Safe (GRAS) Status of *gamma*-Aminobutyric Acid (GABA) For Use as a Food Ingredient]**, which included information pertaining to the method of manufacture and product specifications, analytical data, stability data, conditions of intended use in specified food and beverage products, consumption estimates for all intended uses, and a comprehensive assessment of the available scientific literature through January 2015 on the safety of GABA. Data and information to support the safety *Lactobacillus hilgardii* K-3 were also provided.

Following an independent and critical evaluation of the data and information, the Expert Panel convened by teleconference on February 3, 2015. Upon review and discussion of the data and

information presented in the dossier, the Expert Panel concluded that under the conditions of intended use as described herein, GABA, meeting appropriate food-grade specifications as described in the supporting dossier, and manufactured in accordance with current Good Manufacturing Practices (cGMP), is safe and suitable and GRAS based on scientific procedures. A summary of the basis for the Expert Panel's conclusions are provided in the section that follows.

Summary and Basis for GRAS Determination

GABA is an endogenous compound that functions as the primary inhibitory neurotransmitter in the central nervous system. As such, high concentrations of GABA are present in the brain and circulate in the plasma at readily detectable levels (Song *et al.,* 2005). GABA is also naturally present in a number of commonly consumed foods (*e.g*., melons, potatoes, tomatoes, *etc*.) and GABA-enriched foods have been routinely consumed in some countries (*e.g*., Japan). In the U.S., GABA is available as an ingredient in a number of dietary supplements.

PFI's GABA (not less than 80% purity) is manufactured consistent with cGMP and appropriate food-grade specifications by a fermentation process that utilizes *Lactobacillus hilgardii* K-3 to catalyze the conversion of L-glutamate to GABA. Initially, the pre-fermentation broth is prepared by adding the appropriate substrates to water inside a sealed fermentation vessel. This is followed by sterilization and cooling of the broth prior to the addition of the *L*. *hilgardii* K-3 culture for a series of fermentation steps. Upon completion of fermentation, the organism is inactivated by appertization prior to undergoing a sequence of concentration and filter sterilization steps to produce a highly-concentrated GABA solution absent of the source organism, *L. hilgardii* K-3. GABA is then precipitated by spray-drying, followed by two consecutive steps using a magnetic bar to remove any particulate impurities in the powder. The GABA powder is then sifted before being stored in aluminum pouches. Further dilution with FLO-MAX™ 8 Starch produces a reformulated product with a purity of not less than 20% GABA (GABA 20).

The Expert Panel reviewed analytical data for 3 non-consecutive lots of PFI's GABA along with 3 non-consecutive lots of GABA 20 and concluded that the manufacturing process produced a consistent product that conforms to the established food grade specifications. Additionally, the compositional analysis of one lot of GABA was conducted and the data demonstrated that the GABA product consists of a minimum of 80% GABA, with lesser amounts of moisture (1.9%), ash (3.4%), and lipids (≤1%), as well as a small amount of carbohydrates such as dietary fiber (0.5%) and saccharides (0.2%). Sodium chloride accounts for approximately 90% of the total ash. No minerals were present at levels of toxicological concern. Amino acids resulting from the fermentation media comprised 7% of the final ingredient in which glutamic acid represented approximately 4.7% while the remainder of the identified amino acids were individually present at levels below 0.5%. Since free amino acids and dipeptides accounted for 100% of the total acid-hydrolyzed amino acids identified, it may be concluded that PFI's GABA product is free of

protein contamination. Under ambient conditions, the GABA 20 formulation is stable for up to 27 months. On the basis of the stability data, the shelf-life of GABA was set at 2 years. GABA was also found to be stable as a 5% solution under varied pH conditions ranging from 2 to 6 and elevated temperatures of 100 to 120°C.

GABA exists naturally at low levels in a wide variety of foods and has a well-established history of consumption in humans from the background diet. Many lactic acid bacteria can convert glutamate to GABA and, as such, other foods are likely to contain appreciable amounts of GABA as a result of the processing methods used (*e.g.*, lactic acid-fermented foods such as cured meats and cheeses). Therefore, substantial background exposure to GABA is expected from a typical North American diet. Based on published estimates of GABA content in foods, and *per capita* consumption data (Stofberg and Grundschober, 1987), average intakes of GABA in the U.S. from its natural occurrence were estimated to be 136 mg/person/day. Using Japanese survey data (NIHN, 2006), GABA exposure was estimated to be 80.20 mg/person/day from natural sources. GABA has also been added to a number of food products in Japan for more than 20 years at levels of up to 280 mg/serving without any reported significant adverse effects on human health.

GABA is also incorporated into a number of dietary supplement products available in the U.S. market with typical product-specific daily recommended intakes of 750 mg GABA/person/day. Doses as high as 1,500 to 5,000 mg GABA/person/day have also been reported for some products, without recommended durations for use, nor are any specific precautions provided.

For the total US population, the consumption of GABA under the intended conditions of use was estimated to result in an all-user mean intake of 429 mg/day (6.6 mg/kg body weight/day) and a 90th percentile intake of 811 mg/person/day (12.4 mg/kg body weight/day). On an absolute basis, intake estimates were highest in male adults with mean intakes of 535 mg/person/day (6.2 mg/kg body weight/day) and 90th percentile intakes of 978 mg/person/day (11.2 mg/kg body weight). In contrast, infants and children were estimated to have the highest all-user GABA intakes relative to body weight (*i.e.*, for infants, 11.1 and 23.1 mg/kg body weight/day for the mean and 90th percentile exposures, respectively; for children, 9.9 and 18.5 mg/kg body weight/day for the mean and 90th percentile exposures, respectively). Since GABA is not intended for use in infant or children's foods, it is expected that the actual exposure to GABA in these groups will be minimal. Based on the estimated exposure to GABA from its natural occurrence in food and its proposed conditions of use, the cumulative exposure to GABA would result in combined mean and 90th percentile all-user intakes of 565 and 947 mg/person/day, respectively.

As a charged molecule, GABA has a poor bioavailability following oral administration to rodents (van Gelder and Elliott, 1958). Likewise, healthy human subjects consuming 80 mg GABA per day for 8 to 12 weeks failed to show an increase in plasma GABA levels (Matsubara *et al*., 2002; Kajimoto *et al*., 2004a). The pharmacokinetic profile in laboratory animals (*e.g.,* rats,

cats, and rabbits) are similar wherein clearance of systemically administered (*i.e.,* intraperitoneal or intravenous administration) GABA from the blood is rapid (*i.e.*, 20-minute half-life in rodents) without evidence of GABA bioaccumulation (van Gelder and Elliott, 1958; Hespe *et al*., 1969). The liver is the primary metabolic site for GABA in rodents (Ferenci *et al.*, 1988) and the catabolism of GABA occurs exclusively *via* GABA transaminase for use as a carbon source in the tricarboxylic acid cycle (TCA) and results in CO_2 as the primary waste product (Patel *et al.*, 2005). A pharmacokinetic study conducted in a single human subject with hepatic impairment demonstrated that GABA is rapidly metabolized in the liver (Tower, 1960). Thus, it may be concluded that GABA is utilized as an energy source by the body and metabolized primarily by the liver to innocuous compounds.

The permeation of GABA across the blood-brain barrier (BBB) is highly limited and not substantially influenced by dose (Roberts *et al.*, 1958; van Gelder and Elliott, 1958; Kuriyama and Sze, 1971; Oldendorf, 1971; Frey and Löscher, 1980; Krantis, 1984; Al-Sarraf, 2002; Al-Awadi *et al.*, 2006). Following systemic exposure, GABA does not accumulate in the brain due to the efflux rate of GABA across the BBB being greater than its permeation rate and the high GABA transaminase activity in the cerebrospinal fluid (CSF) (Kuriyama and Sze, 1971; Kakee *et al.*, 2001). It has been suggested that GABA entry into the brain may be even more restricted in higher order mammals, as levels of GABA in the CSF were undetectable 30 minutes following the intravenous administration of high doses of GABA (200 mg/kg body weight) to monkeys (van Gelder and Elliott, 1958). This is in contrast to rodents, in which small amounts of GABA have been shown to enter the brain. Thus, based on a critical review of the literature, it may be concluded that it is unlikely that GABA can cross the BBB in mature healthy animals under the intended conditions of use in sufficient quantities to either increase GABA concentrations or induce inhibitory effects in the brain.

GABA is of low oral toxicity in rats and mice. A single oral dose of 1,000 mg GABA/kg body weight in rats failed to elicit any signs of toxicity (JFRL, 2002). When administered as a dietary admixture to male and female Wistar rats for 28 consecutive days, PFI's GABA did not elicit any signs of toxicity and the no-observed-adverse-effect level (NOAEL) was 200 mg/kg body weight/day, the only dose tested (Sauchi *et al.*, 2009). Likewise, in a 90-day rat study conducted in accordance with Good Laboratory Practice (GLP) and the Organisation for Economic Co-operation and Development (OECD) guidelines (OECD TG 408; OECD, 1998), no toxicologically relevant effects were reported in male and female Sprague-Dawley (Crl:CD (SD)) rats that received PFI's GABA by gavage at doses of 0, 500, 1,250, or 2,500 mg/kg body weight/day and the NOAEL was determined to be 2,500 mg/kg body weight/day, the highest dose tested (Takeshima *et al.*, 2014).

While no chronic studies of GABA were identified, anecdotal reports of chronic oral administration of GABA to rats and dogs were without evidence of toxicity (Tower, 1960). Use of γ-vinyl-GABA, a GABA transaminase inhibitor to elevate the plasma and tissue levels of

GABA in rats, dogs, and monkeys did not induce compound-related neuropathy when administered for a period of up to 1 year in rats and dogs and a period of up to 6 years in monkeys (Gibson *et al.*, 1990). In regards to genotoxicity, only one study consisting of a Rec assay (*Bacillus subtilis* strains H17 (Rec+) and M45 (Rec-)) was identified to assess the potential mutagenicity of GABA-containing fermented milk in the presence and absence of metabolic activation, in which no evidence of mutagenicity was reported (Osawa *et al.*, 2005).

Published human studies containing relevant hematological and/or clinical chemistry and safety endpoints and adverse event monitoring demonstrated that the consumption of GABA in supplements or GABA-enriched foods is well-tolerated at doses up to 120 mg/person/day for up to 12 weeks or 250 mg/day for up to 30 days. The consumption of 5 to 18 g GABA/day for up to 4 days was also reported to be well-tolerated; minor side-effects were limited to transient shortness of breath and a slight burning sensation in the throat lasting a few minutes after GABA ingestion (Cavagnini *et al*., 1980a,b). Likewise, the consumption of 56 g GABA/day for up to 2 years was reported to be well-tolerated in subjects with various neurophysiological disorders (Tower, 1960). No evidence of severe adverse events associated with the consumption of GABA was identified in the published literature.

In more than 300 hypertensive or high-normal blood pressure subjects, GABA consumption has been reported to be associated with a modest reduction in blood pressure (Nakamura *et al.*, 2000; Matsubara *et al.*, 2002; Watanabe *et al.*, 2002; Inoue *et al.*, 2003; Kajimoto *et al.*, 2003, 2004a,b; Yamakoshi *et al.*, 2006; Shimada *et al.*, 2009; Tanaka *et al.*, 2009; Pouliot-Mathieu *et al.*, 2013). However, no clear dose-response relationship has been identified for this hypotensive effect, and the maximal effect, although statistically significant in many studies, is modest relative to controls (*i.e.*, maximum decreases of 5 to 7%) and baseline values (*i.e.*, maximum reduction of 12%). In contrast, hypotensive effects were not reported in normotensive individuals consuming 10 to 70 mg/person/day for 8 to 12 weeks or 5 to18 g/day for 1 to 4 days (Cavagnini *et al.,* 1980a,b; Kimura *et al.,* 2002; Tanaka *et al.*, 2009). Moreover, significant deleterious hypotensive effects in association with GABA consumption have not been reported and the GABA-associated hypotensive effects appear to be time-dependent, typically requiring at least 2 weeks of repeat consumption before significant reductions are observed. Modest reductions in blood pressure in hypertensive subjects are not uncommon with various foods including polyphenol rich dark chocolate and fish oil.

Human studies on GABA, in doses >3 g/day, have also been reported to transiently increase the production of growth hormone (GH) (Cavagnini *et al.*, 1980a,b; Powers *et al.*, 2008). The increased production is within the normal physiological range reported in response to exercise, and are comparable to those reported following arginine consumption (Paddon-Jones *et al.*, 2004; Collier *et al.*, 2005; Kanaley, 2008; Powers *et al.*, 2008). Moreover, GH-induced responses to GABA appear to be rapidly desensitized during repeat exposures (Gamel-Didelon

et al., 2003). Thus, it may be concluded that neither the hypotensive effects nor the increase in GH production induced by GABA are physiologically or toxicologically significant in humans.

The Expert Panel also critically reviewed the available information on the source organism, *L. hilgardii* K-3. The specific *L. hilgardii* K-3 strain used in the production of PFI's GABA was originally isolated from kimchi (Korean pickles) and is not genetically modified. While no regulatory provisions have been identified for the use of *L. hilgardii* in food in the U.S., *L. hilgardii* is a non-toxicogenic and non-pathogenic bacteria species with a substantial history of use in food production, and is present in a number of commonly consumed foods (*e.g.*, cheese, wine, port, and brandy). Due to their fermentation capabilities, *lactobacilli* have been used to produce a large variety of foods (*e.g.*, beer, wine, cheese, yogurt, cured meats, *etc.*) for well over a millennium. More recently, *lactobacilli* have received increased popularity in association with their use as probiotic foods. Nevertheless, *L. hilgardii* is not expected to be present in the final GABA product as a result of the appertization and filtration steps conducted during the manufacturing process employed by PFI. While select members of the *lactobacillus* species are known producers of biogenic amines, histamine was not detected in PFI's final GABA product (limit of detection of 0.5 mg/100g). Furthermore, precursor amino acids (histidine, tyrosine, and phenylalanine) required for the synthesis of biogenic amines by microorganisms are present at insignificant concentrations in the fermentation media (≤0.04%). Some strains of *L. hilgardii* have also been reported to synthesize the mutagenic compound ethyl carbamate; however, analytical data confirm the absence of this compound in PFI's GABA product (limit of detection of 0.01 ppm). Likewise, given that ethyl carbamate can form spontaneously in the presence of alcohol and citrullliline, analytical studies conducted by PFI confirm the absence of citrulline in the final product (limit of detection of 200 ppm[1]). Thus, in consideration that the final GABA product is absent of any by-products derived from the source organism, no safety concerns are anticipated with the use of *L. hilgardii* K-3 in the production of GABA.

Overall, the weight of the scientific evidence supports the safety of the proposed uses of PFI's GABA. The oral bioavailability of GABA is low and clearance is rapid in rats (and presumably humans). An extensive body of evidence demonstrates that GABA does not accumulate in the CNS, as exogenous GABA has a low capacity for permeation through the BBB due to a high efflux rate relative to the permeation rate and an abundance of GABA transaminase activity within the CSF. The oral toxicity of GABA in rodents is low. On the basis of a 90-day rat study conducted on PFI's GABA ingredient, a NOAEL of 2,500 mg/kg body weight/day (the highest dose tested) was established, to which no remarkable changes were observed. Additionally, humans have a long-history of safe consumption of GABA in the diet originating from a wide variety of foods. A number of human studies have also demonstrated that GABA supplements and GABA-enriched foods are well-tolerated. Thus, in consideration of the available data and

[1] Certificate of Analysis for citrulline indicates that the detection limit was set at 0.02 g/100 g due to the existence of highly-concentrated amino acid.

that the intake of GABA from all proposed food uses and background dietary sources would result in a cumulative 90th percentile all-user intake of 947 mg/day (14.6 mg/kg body weight/day for a 65 kg individual) for the total population, the intake of GABA from the proposed conditions of use is reasonably expected to be safe.

CONCLUSION

We, the Expert Panel, have independently and collectively, critically evaluated the information and data summarized above and conclude that the intended uses of GABA as an ingredient in foods and beverages at levels providing up to 100 mg/serving, meeting appropriate food-grade specifications and produced consistent with current Good Manufacturing Practices (cGMP), are Generally Recognized as Safe (GRAS) based on scientific procedures.

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

(b) (6)

20 February 2015
Date

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

23 February 2015
Date

Stephen L. Taylor, Ph.D.
Professor
Department of Food Science and Technology,
University of Nebraska

02/26/2015
Date

John A. Thomas, Ph.D.
Professor
Department of Pharmacology and Toxicology,
Indiana University School of Medicine

REFERENCES

Al-Awadi M, Pavlik A, Al-Sarraf H (2006). Increased brain uptake and brain to blood efflux transport of 14C-GABA in spontaneously hypertensive rats. Life Sci 79(9):847-853.

Al-Sarraf H (2002). Transport of 14(C)-γ-aminobutyric acid into brain, cerebrospinal fluid and choroid plexus in neonatal and adult rats. Brain Res Dev Brain Res 139(2):121-129.

Cavagnini F, Benetti G, Invitti C, Ramella G, Pinto M, Lazza M et al. (1980a). Effect of γ-aminobutyric acid on growth hormone and prolactin secretion in man: influence of pimozide and domperidone. J Clin Endocrinol Metab 51(4):789-792.

Cavagnini F, Invitti C, Pinto M, Maraschini C, Di Landro A, Dubini A et al. (1980b). Effect of acute and repeated administration of gamma aminobutyric acid (GABA) on growth hormone and prolactin secretion in man. Acta Endocrinol (Copenh) 93(2):149-154.

Collier SR, Casey DP, Kanaley JA (2005). Growth hormone responses to varying doses of oral arginine. Growth Horm IGF Res 15(2):136-139.

Ferenci P, Stehle T, Ebner J, Schmid R, Haussinger D (1988). Uptake and catabolism of γ-aminobutyric acid by the isolated perfused rat liver. Gastroenterology 95(2):402-407.

Frey HH, Löscher W (1980). Cetyl GABA: effect on convulsant thresholds in mice and acute toxicity. Neuropharmacology 19(2):217-220.

Gamel-Didelon K, Kunz L, Fohr KJ, Gratzl M, Mayerhofer A (2003). Molecular and physiological evidence for functional γ-aminobutyric acid (GABA)-C receptors in growth hormone-secreting cells. J Biol Chem 278(22):20192-20195.

Gibson JP, Yarrington JT, Loudy DE, Gerbig CG, Hurst GH, Newberne JW (1990). Chronic toxicity studies with vigabatrin, a GABA-transaminase inhibitor. Toxicol Pathol 18(2):225-238.

Hespe W, Roberts E, Prins H (1969). Autoradiographic investigation of the distribution of [^{14}C]GABA in tissues of normal and aminooxyacetic acid-treated mice. Brain Res 14(3):663-671.

Inoue K, Shirai T, Ochiai H, Kasao M, Hayakawa K, Kimura M et al. (2003). Blood-pressure-lowering effect of a novel fermented milk containing γ-aminobutyric acid (GABA) in mild hypertensives. Eur J Clin Nutr 57(3):490-495.

JFRL (2002) [unpublished]. *Acute Toxicity Test [Confidential]*. (Experimental Report No. 202061563-001). Prepared by Tokyo, Japan: Japan Food Research Laboratories (JFRL) for Pharma Foods International Co., Ltd. (PFI).

Kajimoto O, Hirata H, Nishimura A (2003). Hypotensive action of novel fermented milk containing γ-aminobutyric acid (GABA) in subjects with mild or moderate hypertension. Kenko Eiyo Shokuhin Kenkyu [J Nutr Food] 6(2):51-64 [includes English translation].

Kajimoto O, Ueno H, Nagata Y, Yabune M, Kajimoto Y (2004a). [Hypotensive effects of tablet containing γ-aminobutyric acid (GABA) on high normal blood pressure and mild hypertensive subjects. Yakuri To Chiryo [Basic Pharmacol Ther] 32(12):929-944 [English translation].

Kajimoto O, Hirata H, Nakagawa S, Kajimoto Y, Hayakawa K, Kimura M (2004b). [Hypotensive effect of fermented milk containing γ-aminobutyric acid (GABA) in subjects with high normal blood pressure]. Nihon Shokuhin Kagaku Kogakkaishi 51(2):79-86 [includes English translation].

Kakee A, Takanaga H, Terasaki T, Naito M, Tsuruo T, Sugiyama Y (2001). Efflux of a suppressive neurotransmitter, GABA, across the blood-brain barrier. J Neurochem 79(1):110-118.

Kanaley JA (2008). Growth hormone, arginine and exercise. Curr Opin Clin Nutr Metab Care 11(1):50-54.

Kimura M, Chounan O, Takahashi R, Ohashi A, Arai Y, Hayakawa K et al. (2002). Effects of fermented milk containing γ-aminobutyric acid (GABA) on normal adult subjects. Jpn J Food Chem 9(1):1-6 [includes English translation].

Krantis A (1984). The involvement of GABA-transaminase in the blood-brain barrier to radiolabelled GABA. Acta Neuropathol 64(1):61-67.

Kuriyama K, Sze PY (1971). Blood-brain barrier to H(3)-γ-aminobutyric acid in normal and amino oxyacetic acid-treated animals. Neuropharmacology 10(1):103-108.

Matsubara F, Ueno H, Tadano K, Suyama T, Imaizumi K, Suzuki T et al. (2002). Effects of GABA supplementation on blood pressure and safety in adults with mild hypertension. Yakuri To Chiryo [Basic Pharmacol Ther] 30(11):963-972 [English translation].

Nakamura T, Hasegawa T, Ueno S, Kumamoto S, Matsubayashi T, Ando Y et al. (2000). [Effect of γ-aminobutyric acid-rich Chlorella on blood pressure in mildly hypertensive subjects]. Yakuri To Chiryo [Basic Pharmacol Ther] 28(6):529-533.

NIHN [2006]. *[Japan National Health and Nutrition Examination Survey 2005]*. Tokyo, Japan: National Institute of Health and Nutrition. English surveys available at: http://www0.nih.go.jp/eiken/english/research/project_nhns.html.

OECD (1998). Repeated dose 90-day oral toxicity study in rodents. In: *OECD Guidelines for the Testing of Chemicals*. (OECD Guideline no 408) [Updated Adopted 21st September 1998]. Paris, France: Organisation for Economic Co-operation and Development (OECD). Available at: http://www.oecd-ilibrary.org/environment/test-no-408-repeated-dose-90-day-oral-toxicity-study-in-rodents_9789264070707-en;jsessionid=1m6bvwspup322.delta.

Oldendorf WH (1971). Brain uptake of radiolabeled amino acids, amines, and hexoses after arterial injection. Am J Physiol 221(6):1629-1639.

Osawa T, Goto T, Gonda M, Oyama W, Yasutake N, Onoue M (2005). Mutagenicity test of gamma-aminobutyric acid-containing fermented milk "GABA-10" and "GABA-25" using DNA repair system. Yakuruto Kenkyujo Kenkyu Hokokushu 24:67-72 [English translation].

Paddon-Jones D, Borsheim E, Wolfe RR (2004). Potential ergogenic effects of arginine and creatine supplementation [Includes discussion by Joseph Loscalzo]. J Nutr 134(10, Suppl.):2888S-2894S, 2895S.

Patel AB, De Graaf RA, Mason GF, Rothman DL, Shulman RG, Behar KL (2005). The contribution of GABA to glutamate/glutamine cycling and energy metabolism in the rat cortex *in vivo*. Proc Natl Acad Sci USA 102(15):5588-5593.

Pouliot-Mathieu K, Gardner-Fortier C, Lemieux S, St-Gelais D, Champagne CP, Vuillemard JC (2013). Effect of cheese containing gamma-aminobutyric acid-producing lactic acid bacteria on blood pressure in men. PharmaNutrition 1(4):141-148.

Powers ME, Yarrow JF, McCoy SC, Borst SE (2008). Growth hormone isoform responses to GABA ingestion at rest and after exercise. Med Sci Sports Exerc 40(1):104-110.

Roberts E (1984). The γ-aminobutyric acid (GABA) system and hepatic encephalopathy. Hepatology 4(2):342-345.

Roberts E, Lowe I.P, Guth L, Jeline B (1958). Distribution of gamma-aminobutyric acid and other amino acids in nervous tissue of various species. J Exp Zool 138(2):313-328. Cited In: Roberts, 1984.

Sauchi Y, Hisaizumi K, Akao M, Kim M, Yoshikuni Y (2009). Toxicity of PG2K20 for 28 days administration in rats. Yakuri To Chiryo [Basic Pharmacol Ther] 37(3):173-179.

Song Y, Shenwu M, Dhossche DM, Liu Y-M (2005). A capillary liquid chromatographic/tandem mass spectrometric method for the quantification of γ-aminobutyric acid in human plasma and cerebrospinal fluid. J Chromatogr B 814(2):295-302.

Stofberg J, Grundschober F (1987). Consumption ratio and food predominance of flavoring materials (Third cumulative series). Perfum Flavor 12(3):27-68.

Takeshima K, Yamatsu A, Yamashita Y, Watabe K, Horie N, Masuda K et al. (2014). Subchronic toxicity evaluation of γ-aminobutyric acid (GABA) in rats. Food Chem Toxicol 68:128-134.

Tanaka H, Watanabe K, Ma M, Hirayama M, Kobayashi T, Oyama H et al. (2009). The effects of γ-aminobutyric acid, vinegar, and dried bonito on blood pressure in normotensive and mildly or moderately hypertensive volunteers. J Clin Biochem Nutr 45(1):93-100.

Tower DB (1960). The administration of gamma-aminobutyric acid to man: systematic effects and anticonvulsant action. In: Roberts E, Baxter CF, Van Harrveld A, Wiersma CAG, Adehy WR, Killam KF, editors. *Inhibition in the Nervous System and Gamma-Aminobutyric Acid: Proceedings of an International Symposium*, May 22-24, 1959, Duarte, Calif. New York (NY): Permagon Press, pp. 562-578.

U.S. FDA (2008). *Agency Response Letter GRAS Notice No. GRN 000257 [gamma-Amino butyric acid, Kyoto, Japan: Pharma Foods International Co., Ltd.].* College Park (MD): U.S. Food and Drug Administration (U.S. FDA), CenHter for Food Safety Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: http://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set GRASNotices&id=257 [Dec. 22, 2008].

van Gelder NM, Elliott KAC (1958). Disposition of γ-aminobutyric acid administered to mammals. J Neurochem 3(2):139-143.

Watanabe T, Kawashita A, Ishi S, Mazumder TK, Naigai S, Tsuji K et al. (2002). [Antihypertensive effect of γ-aminobutyric acid-enriched *Agaricus blazei* on mild hypertensive human subjects]. Nihon Shokuhin Kagaku Kogakkaishi 50(4):167-173 [Includes English translation].

Yamakoshi J, Fukuda S, Satoh T, Tsuji R, Saito M, Obata A et al. (2007). Antihypertensive and natriuretic effects of less-sodium soy sauce containing γ-aminobutyric acid in spontaneously hypertensive rats. Biosci Biotechnol Biochem 71(1):165-173.

APPENDIX B

CERTIFICATES OF ANALYSIS

- Attachment B-1: Certificates of Analysis of GABA
- Attachment B-2: Certificates of Analysis for GABA 20

2014/5/19

Pharma Foods International Co., Ltd.
1-49 Goryo-Ohara, Nishikyo-ku,
Kyoto 615-8245, Japan
Phone : +81-75-394-8605
FAX : +81-75-394-8889

SPECIFICATION

Commodity: PharmaGABA™
Shelf Life : 2 years from manufacturing date

	Specification	Methods
Appearance	light yellow to light brown powder	Visual appearance
GABA	more than 80.0%	Amino acid analysis with HPLC
Moisture	less than 5.0%	Heated-air drying at normal pressure method
Ash	less than 15.0%	Direct ashing method
Arsenic	less than 2μg/g	DDTC-Ag method
Heavy Metals	less than 10μg/g	Sodium sulfide colorimetry
Lead	less than 0.5μg/g	Atomic absorption method
Total Aerobic Counts	less than 1000 CFU/g	Methods established by Food Hygiene Guidance (Edited by Japan Food Hygiene Association)
Yeast and Mold	less than 300 CFU/g	Methods established by Food Hygiene Guidance (Edited by Japan Food Hygiene Association)
Coliform / *E. coli*	negative	Methods established by Food Hygiene Guidance (Edited by Japan Food Hygiene Association)

※Storage temperature 15℃ or less

(b) (6)

Production Control Div.

2014/6/9

Pharma Foods International Co., Ltd.
1-49 Goryo-Ohara,Nishikyo-ku,
Kyoto 615-8245 Japan
Phone : +81-75-394-8605
F A X : +81-75-394-8889

CERTIFICATE OF ANALYSIS

Commodity: PharmaGABA™
Lot No.: 2J10
Manufacturing Date: 2012/10/10
Measurement Date: 2012/10/26
Shelf-Life: 2 years from manufacturing date

	Specification	Results
Appearance	light yellow to light brown powder	conformed
GABA	more than 80.0%	87.2%
Moisture	less than 5.0%	2.8%
Ash	less than 15.0%	3.0%
Arsenic	less than 2μg/g	less than 2μg/g
Heavy Metals	less than 10μg/g	less than 10μg/g
Lead	less than 0.5μg/g	less than 0.5μg/g
Total Aerobic Counts	less than 1000CFU/g	20CFU/g
Yeast and Mold	less than 300CFU/g	less than 10CFU/g
Coliform / *E. coli*	negative	negative

(b) (6)

Production Control Div.

2014/6/9

Pharma Foods International Co., Ltd.
1-49 Goryo-Ohara,Nishikyo-ku,
Kyoto 615-8245 Japan
Phone : +81-75-394-8605
F A X : +81-75-394-8889

CERTIFICATE OF ANALYSIS

Commodity: PharmaGABA™
Lot No.: 4B06
Manufacturing Date: 2014/2/6
Measurement Date: 2014/2/14
Shelf-Life: 2 years from manufacturing date

	Specification	Results
Appearance	light yellow to light brown powder	conformed
GABA	more than 80.0%	84.6%
Moisture	less than 5.0%	3.0%
Ash	less than 15.0%	3.1%
Arsenic	less than 2μg/g	less than 2μg/g
Heavy Metals	less than 10μg/g	less than 10μg/g
Lead	less than 0.5μg/g	less than 0.5μg/g
Total Aerobic Counts	less than 1000CFU/g	less than 10CFU/g
Yeast and Mold	less than 300CFU/g	less than 10CFU/g
Coliform / *E. coli*	negative	negative

(b) (6)

Production Control Div.

2014/6/2

PHARMA FOODS INTERNATIONAL CO.,LTD.

Pharma Foods International Co., Ltd.
1-49 Goryo-Ohara,Nishikyo-ku,
Kyoto 615-8245 Japan
Phone : +81-75-394-8605
F A X : +81-75-394-8889

CERTIFICATE OF ANALYSIS

Commodity: PharmaGABA™
Lot No.: 4E21
Manufacturing Date: 2014/5/21
Measurement Date: 2014/6/2
Shelf-Life: 2 years from manufacturing date

	Specification	Results
Appearance	light yellow to light brown powder	conformed
GABA	more than 80.0%	89.8%
Moisture	less than 5.0%	3.0%
Ash	less than 15.0%	3.1%
Arsenic	less than 2μg/g	less than 2μg/g
Heavy Metals	less than 10μg/g	less than 10μg/g
Lead	less than 0.5μg/g	less than 0.5μg/g
Total Aerobic Counts	less than 1000CFU/g	30 CFU/g
Yeast and Mold	less than 300CFU/g	less than 10CFU/g
Coliform / *E. coli*	negative	negative

(b) (6)

Production Control Div.

2014/8/5

Pharma Foods International Co., Ltd.
1-49 Goryo-Ohara, Nishikyo-ku,
Kyoto 615-8245, Japan
Phone : +81-75-394-8605
FAX : +81-75-394-8889

SPECIFICATION

Commodity: Pharma GABA 20-D
Shelf Life : 2 years from manufacturing date

	Specification	Methods
Appearance	white to light yellow powder	Visual appearance
GABA	more than 20.0%	Amino acid analysis with HPLC
Moisture	less than 10.0%	Heated-air drying at normal pressure method
Ash	less than 18.0%	Direct ashing method
Arsenic	less than 2μg/g	DDTC-Ag method
Heavy Metals	less than 10μg/g	Sodium sulfide colorimetry
Total Aerobic Counts	less than 1000 CFU/g	Methods established by Food Hygiene Guidance (Edited by Japan Food Hygiene Association)
Yeast and Mold	less than 300 CFU/g	Methods established by Food Hygiene Guidance (Edited by Japan Food Hygiene Association)
Coliform / *E. coli*	negative	Methods established by Food Hygiene Guidance (Edited by Japan Food Hygiene Association)

Storage temperature 25℃ or less

(b) (6)

Production Control Div.

PHARMA FOODS INTERNATIONAL CO.,LTD.

2014/7/28

Pharma Foods International Co., Ltd.
1-49 Goryo-Ohara,Nishikyo-ku,
Kyoto 615-8245 Japan
Phone : +81-75-394-8605
F A X : +81-75-394-8889

CERTIFICATE OF ANALYSIS

Commodity:	PharmaGABA 20-D
Lot No.:	6A31
Manufacturing Date:	2006/1/31
Measurement Date:	2006/2/14
Shelf-Life:	2 years from manufacturing date

	Specification	Results
Appearance	white to light yellow powder	conformed
GABA	more than 20.0%	22.9%
Moisture	less than 10.0 %	4.8%
Ash	less than 18.0 %	15.0%
Arsenic	less than 2 μg/g	less than 2 μg/g
Heavy Metals	less than 10 μg/g	less than 10 μg/g
Total Aerobic Counts	less than 1000 CFU/g	less than 10 CFU/g
Yeast and Mold	less than 300 CFU/g	less than 10 CFU/g
Coliform / *E. coli*	negative	negative

(b) (6)

Production Control Div.

2014/7/28

Pharma Foods International Co., Ltd.
1-49 Goryo-Ohara,Nishikyo-ku,
Kyoto 615-8245 Japan
Phone : +81-75-394-8605
F A X : +81-75-394-8889

CERTIFICATE OF ANALYSIS

Commodity:	**PharmaGABA 20-D**
Lot No.:	**6B07**
Manufacturing Date:	**2006/2/7**
Measurement Date:	**2006/2/27**
Shelf-Life:	**2 years from manufacturing date**

	Specification	Results
Appearance	white to light yellow powder	conformed
GABA	more than 20.0%	22.9%
Moisture	less than 10.0 %	5.6%
Ash	less than 18.0 %	14.7%
Arsenic	less than 2 μg/g	less than 2 μg/g
Heavy Metals	less than 10 μg/g	less than 10 μg/g
Total Aerobic Counts	less than 1000 CFU/g	10 CFU/g
Yeast and Mold	less than 300 CFU/g	less than 10 CFU/g
Coliform / *E. coli*	negative	negative

(b) (6)

Production Control Div.

PHARMA FOODS INTERNATIONAL CO.,LTD.

2014/7/28

Pharma Foods International Co., Ltd.
1-49 Goryo-Ohara,Nishikyo-ku,
Kyoto 615-8245 Japan
Phone : +81-75-394-8605
F A X : +81-75-394-8889

CERTIFICATE OF ANALYSIS

Commodity:	**PharmaGABA 20-D**
Lot No.:	**7F15**
Manufacturing Date:	**2007/6/15**
Measurement Date:	**2007/6/26**
Shelf-Life:	**2 years from manufacturing date**

	Specification	Results
Appearance	**white to light yellow powder**	**conformed**
GABA	**more than 20.0%**	**21.2%**
Moisture	**less than 10.0 %**	**4.8%**
Ash	**less than 18.0 %**	**13.1%**
Arsenic	**less than 2 μg/g**	**less than 2 μg/g**
Heavy Metals	**less than 10 μg/g**	**less than 10 μg/g**
Total Aerobic Counts	**less than 1000 CFU/g**	**less than 10 CFU/g**
Yeast and Mold	**less than 300 CFU/g**	**less than 10 CFU/g**
Coliform / *E. coli*	**negative**	**negative**

(b) (6)

Production Control Div.

APPENDIX C

ANALYSES RELATED TO THE SOURCE ORGANISM

- Attachment C-1: Analysis of *L. hilgardii* K-3
- Attachment C-2: Analysis of Histamine
- Attachment C-3: Analysis of Ethylcarbamate
- Attachment C-4: Analysis of Citrulline

Specific-Primer PCR analysis report

Identification No.	SIID16618-01
Date this report was written	March 30, 2015
Client	PHARMA FOODS INTERNATIONAL CO., LTD.

CONFIDENTIAL

Confirmation

1. This report is authorized and approved by the scientist responsible on behalf of TechnoSuruga Laboratory Co., Ltd.
2. Unless otherwise divert to research presentation (prepublication paper) or patent description, when you wish to duplicate all or part of this report and provide to third parties, this shall not be reproduced without prior approval of TechnoSuruga Laboratory Co., Ltd.
3. No prior agreement of TechnoSuruga Laboratory Co., Ltd. is necessary for reproduction if the name of the company is specified as the data source.

TechnoSuruga Laboratory Co., Ltd.
330 Nagasaki Shimizu-ku Shizuoka-shi, Shizuoka 424-0065, Japan
TEL: (+81)54-349-6155 FAX: (+81)54-349-6121

Sample Information

Sample code	SIID	Date culture received	Source of sample
PharmaGABA Lot 7B09	16618-01	March 11, 2015	Lactic acid fermented extract

Notes

PURPOSE

In order to investing whether *Lactobacillus* species exists in a sample or not, *Lactobacillus* species Specific-Primer-PCR analysis was performed.

METHODS

1. DNA extraction
   - Method: MORA-EXTRACT kit (Kyokuto Pharmaceutical, Tokyo)

2. PCR
   - Primer set: LactoR'F[1)] – LBFR[1)] (16S rRNA gene targeted *Lactobacillus* species-specific primer set)
   - PCR condition: 3 step PCR on a Rotor-Gene™ Q (QIAGEN) cycler using SYBR® *Premix Ex Tag*™ II (TaKaRa Bio, Shiga)

RESULTS

PCR detection using *Lactobacillus* Species-Specific-Primer

PCR amplification was performed with extracted DNA from the sample. The *Lactobacillus* species-specific sequences was amplified using PCR method with *Lactobacillus* species-specific-primer set, Lacto R'F-LBFR. The estimated fragment size of amplification product was about 360 base pairs (bp) when this primer set was used. The presence of PCR product (amplicon) was checked by agarose gel electrophoresis. The 16S rRNA gene sequence fragment of *Lactobacillus* species was not amplified (Figure 1).

Figure 1. Agarose gel image of PCR product (*Lactobacillus* species-specific-primer set Lacto R'F-LBFR, Arrow, the fragment size of PCR product bout 360 bp in size.

M: size marker (100bp ladder), 1:16381-03.

CONCLUSION

Table 1. The result of *Lactobacillus* species-specific-primer PCR

SIID	*Lactobacillus* spp.
16618-01 (PharmaGABA Lot. 7B09)	–

+: PCR amplicon was observed

-: PCR amplicon was not observed.

Specific-Primer-PCR analysis was performed to investigate whether *Lactobacillus* species exists in this sample or not. From the result mentioned above, 16S rRNA gene fragment of the *Lactobacillus* species was not amplified using sample DNA for PCR by *Lactobacillus* species-specific primer set. Therefore, it was considered that the *Lactobacillus* species did not exist or lower than the detection limit of PCR in this sample.

REFERENCES

1) SONGJIDA (P.), NAKAYAMA (J.), TATEYAMA (A.), TANAKA (S.), TSUBOUCHI (M.), KIYOHARA (C.), SHIRAKAWA (T.), and SONOMMOTO (K.): Differences in developing intestinal microbiota between allergic and non-allergic infants; a pilot study in JAPAN. *Biosci. Biotechnol, Biochem.*, 2007, **71**, 2338-2342.

This analysis report was translated by Pharma Foods International., Co., Ltd., based on the test results provided by TechnoSuruga Laboratory Co., Ltd. We, Pharma Foods International Co., Ltd. hereby confirm that the translation and original test results are consistent.

Date: March 31, 2015

(b) (6)

Translated by Kyung Hwa Moon

Deputy Manager, Sales Department, Global Strategy Group

Pharma Foods International Co., Ltd.

Specific-Primer PCR analysis report

Identification No.	SIID16381-02
Date this report was written	March 9, 2015
Client	PHARMA FOODS INTERNATIONAL CO., LTD.

CONFIDENTIAL

Confirmation

1. This report is authorized and approved by the scientist responsible on behalf of TechnoSuruga Laboratory Co., Ltd.
2. Unless otherwise divert to research presentation (prepublication paper) or patent description, when you wish to duplicate all or part of this report and provide to third parties, this shall not be reproduced without prior approval of TechnoSuruga Laboratory Co., Ltd.
3. No prior agreement of TechnoSuruga Laboratory Co., Ltd. is necessary for reproduction if the name of the company is specified as the data source.

TechnoSuruga Laboratory Co., Ltd.
330 Nagasaki Shimizu-ku Shizuoka-shi, Shizuoka 424-0065, Japan
TEL: (+81)54-349-6155 FAX: (+81)54-349-6121

Sample Information

Sample code	SIID	Date culture received	Source of sample
PharmaGABA Lot 4B06	16381-02	February 6, 2015	–

Notes

PURPOSE

In order to investing whether *Lactobacillus* species exists in a sample or not, *Lactobacillus* species Specific-Primer-PCR analysis was performed.

METHODS

1. DNA extraction
   - Method: MORA-EXTRACT kit (Kyokuto Pharmaceutical, Tokyo)

2. PCR
   - Primer set: LactoR'F[1)] – LBFR[1)] (16S rRNA gene targeted *Lactobacillus* species-specific primer set)
   - PCR condition: 3 step PCR on a Rotor-Gene™ Q (QIAGEN) cycler using SYBR® *Premix Ex Tag*™ II (TaKaRa Bio, Shiga)

RESULTS

PCR detection using *Lactobacillus* Species-Specific-Primer

PCR amplification was performed with extracted DNA from the sample. The *Lactobacillus* species-specific sequences was amplified using PCR method with *Lactobacillus* species-specific-primer set, Lacto R'F-LBFR. The estimated fragment size of amplification product was about 360 base pairs (bp) when this primer set was used. The presence of PCR product (amplicon) was checked by agarose gel electrophoresis. The 16S rRNA gene sequence fragment of *Lactobacillus* species was not amplified (Figure 1).

Figure 1. Agarose gel image of PCR product (*Lactobacillus* species-specific-primer set Lacto R'F-LBFR, Arrow, the fragment size of PCR product bout 360 bp in size.

M: size marker (100bp ladder), 1:16381-03.

CONCLUSION

Table 1. The result of *Lactobacillus* species-specific-primer PCR

SIID	*Lactobacillus* spp.
16381-02 (PharmaGABA Lot. 4B06)	–

+: PCR amplicon was observed

-: PCR amplicon was not observed.

Specific-Primer-PCR analysis was performed to investigate whether *Lactobacillus* species exists in this sample or not. From the result mentioned above, 16S rRNA gene fragment of the *Lactobacillus* species was not amplified using sample DNA for PCR by *Lactobacillus* species-specific primer set. Therefore, it was considered that the *Lactobacillus* species did not exist or lower than the detection limit of PCR in this sample.

REFERENCES

1) SONGJIDA (P.), NAKAYAMA (J.), TATEYAMA (A.), TANAKA (S.), TSUBOUCHI (M.), KIYOHARA (C.), SHIRAKAWA (T.), and SONOMMOTO (K.): Differences in developing intestinal microbiota between allergic and non-allergic infants; a pilot study in JAPAN. *Biosci. Biotechnol, Biochem.*, 2007, **71**, 2338-2342.

This analysis report was translated by Pharma Foods International., Co., Ltd., based on the test results provided by TechnoSuruga Laboratory Co., Ltd. We, Pharma Foods International Co., Ltd. hereby confirm that the translation and original test results are consistent.

Date: March 31, 2015

(b) (6)

Translated by Kyung Hwa Moon
Deputy Manager, Sales Department, Global Strategy Group
Pharma Foods International Co., Ltd.

Specific-Primer PCR analysis report

Identification No.	SIID16381-03
Date this report was written	March 20, 2015
Client	PHARMA FOODS INTERNATIONAL CO., LTD.

CONFIDENTIAL

Confirmation

1. This report is authorized and approved by the scientist responsible on behalf of TechnoSuruga Laboratory Co., Ltd.
2. Unless otherwise divert to research presentation (prepublication paper) or patent description, when you wish to duplicate all or part of this report and provide to third parties, this shall not be reproduced without prior approval of TechnoSuruga Laboratory Co.,Ltd.
3. No prior agreement of TechnoSuruga Laboratory Co., Ltd. is necessary for reproduction of results if the name of the company is specified as the data source.

Scientist Responsible
印 20

TechnoSuruga Laboratory Co., Ltd.
330 Nagasaki Shimizu-ku Shizuoka-shi, Shizuoka 424-0065, Japan
TEL: (+81)54-349-6155 FAX: (+81)54-349-6121

Sample Information

Sample code	SIID	Date culture received	Source of sample
PharmaGABA Lot.4E21	16381-03	February 6, 2015	—

Notes

PURPOSE

In order to investigate whether *Lactobacillus* species exists in a sample or not, *Lactobacillus* species Specific-Primer-PCR analysis was performed.

METHODS

1. DNA extraction

・ Method	MORA-EXTRACT kit (Kyokuto Pharmaceutical, Tokyo)

2. PCR

・ Primer set	LactoR'F[1)] - LBFR[1)] (16S rRNA gene targeted *Lactobacillus* species-specific primer set)
・ PCR condition	3 step PCR on a Rotor-Gene™ Q (QIAGEN) cycler using SYBR® *Premix Ex Taq*™ II (TaKaRa Bio, Shiga)

RESULTS

PCR detection using *Lactobacillus* Species-Specific-Primer

PCR amplification was performed with extracted DNA from the sample. The *Lactobacillus* species-specific sequence was amplified using PCR method with *Lactobacillus* species-specific-primer set, LactoR'F - LBFR. The estimated fragment size of amplification product was about 360 base pairs (bp) when this primer set was used. The presence of PCR product (amplicon) was checked by agarose gel electrophoresis. The 16S rRNA gene sequence fragment of the *Lactobacillus* species was not amplified (Figure 1).

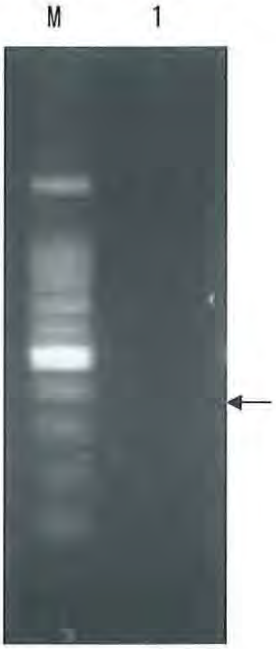


Figure 1. Agarose gel image of PCR product (*Lactobacillus* species-specific-primer set LactoR'F - LBFR, Arrow, the fragment size of PCR product about 360 bp in size. M: size marker (100bp ladder), 1: 16381-03.

CONCLUSION

Table 1. The result of *Lactobacillus* species-specific-primer PCR

SIID	*Lactobacillus* spp.
16389-03 (PharmaGABA Lot. 4E21)	−

+: PCR amplicon was observed,

−: PCR amplicon was not observed

Specific-Primer-PCR analysis was performed to investigate whether *Lactobacillus* species exists in this sample or not. From the result mentioned above, 16S rRNA gene fragment of the *Lactobacillus* species was not amplified using sample DNA for PCR by *Lactobacillus* species-specific-primer set. Therefore, it was considered that the *Lactobacillus* species did not existed or lowers than the detection limit of PCR in this sample.

REFERENCES

1) SONGJINDA (P.), NAKAYAMA (J.), TATEYAMA (A.), TANAKA (S.), TSUBOUCHI (M.), KIYOHARA (C.), SHIRAKAWA (T.) and SONOMOTO (K.): Differences in developing intestinal microbiota between allergic and non-allergic infants: a pilot study in Japan. *Biosci. Biotechnol. Biochem.*, 2007, **71**, 2338-2342.

特異プライマーPCR 解析報告書

登録番号　SIID16381-02
日　　付　2015 年 3 月 9 日
顧 客 名　株式会社ファーマフーズ
担当部署　研究開発部
担当者名　主任研究員　渡部　和哉　様

極秘資料

本報告書の使用にあたっての確認事項

1. 本報告書は株式会社テクノスルガ・ラボ 技術責任者による承認済みです。
2. 研究発表（論文投稿）や特許明細書への転用を除き、本報告書の一部または全てを複製して第三者へ転用する場合には、株式会社テクノスルガ・ラボの事前の合意が必要となります。
3. 第三者への転用物に「株式会社テクノスルガ・ラボによる試験結果」の旨を明記した場合、株式会社テクノスルガ・ラボの事前の合意は不要となります。

技術担当
印

株式会社テクノスルガ・ラボ 技術部
〒424-0065　静岡県静岡市清水区長崎 330 番地
TEL：054-349-6155　FAX：054-349-6121

検体情報

検体名	SIID	受取日	検体の種類
PharmaGABA Lot.4B06	16381-02	2015 年 2 月 6 日	―

備考

目的

Lactobacillus 属が検体中に存在するかどうかについて特異プライマーを用いた PCR 法による検出を行います。

方法

1. DNA 抽出
   - 抽出方法　MORA-EXTRACT（極東製薬、東京）

2. PCR
   - 使用プライマー　LactoR'F[1] - LBFR[1] (*Lactobacillus* 属 16S rDNA)
   - PCR 条件　SYBR® *Premix Ex Taq*™ II (TaKaRa Bio, Shiga)
     Rotor-Gene™ Q (QIAGEN) による 3step PCR

結果

図 1. *Lactobacillus* 属特異プライマーによる PCR 増幅産物のアガロースゲル電気泳動像.
レーン番号 1：SIID16381-02、M：サイズマーカー（100 bp）

まとめ

対象菌群	SIID16381-02
Lactobacillus 属	－

＋：PCR 増幅，－：PCR 非増幅

上記の結果、*Lactobacillus* 属を対象とした特異プライマーPCR 産物が得られませんでした（図 1：レーン番号 1)。このことから、検体中には *Lactobacillus* 属のゲノム DNA が存在しない、あるいは検出限界以下であると考えられます。

補足

本報告書に関する技術的なご質問等につきましては、株式会社テクノスルガ・ラボ 技術部までお問合わせください。

引用文献

1) SONGJINDA (P.), NAKAYAMA (J.), TATEYAMA (A.), TANAKA (S.), TSUBOUCHI (M.), KIYOHARA (C.), SHIRAKAWA (T.) and SONOMOTO (K.): Differences in developing intestinal microbiota between allergic and non-allergic infants: a pilot study in Japan. *Biosci. Biotechnol. Biochem.*, 2007, **71**, 2338-2342.

特異プライマーPCR 解析報告書

登録番号　SIID16618-01
日　　付　2015 年 3 月 30 日
顧 客 名　株式会社ファーマフーズ
担当部署　研究開発部
担当者名　主任研究員　渡部　和哉　様

極秘資料

本報告書の使用にあたっての確認事項

1. 本報告書は株式会社テクノスルガ・ラボ 技術責任者による承認済みです。
2. 研究発表（論文投稿）や特許明細書への転用を除き、本報告書の一部または全てを複製して第三者へ転用する場合には、株式会社テクノスルガ・ラボの事前の合意が必要となります。
3. 第三者への転用物に「株式会社テクノスルガ・ラボによる試験結果」の旨を明記した場合、株式会社テクノスルガ・ラボの事前の合意は不要となります。

技術担当
印

株式会社テクノスルガ・ラボ 技術部
〒424-0065　静岡県静岡市清水区長崎 330 番地
TEL：054-349-6155　FAX：054-349-6121

検体情報

検体名	SIID	受取日	検体の種類
PharmaGABA Lot.7B09	16618-01	2015 年 3 月 11 日	乳酸菌発酵エキス

備考

目的

Lactobacillus 属が検体中に存在するかどうかについて特異プライマーを用いた PCR 法による検出を行います。

方法

1. DNA 抽出
   - 抽出方法　MORA-EXTRACT（極東製薬、東京）

2. PCR
   - 使用プライマー　LactoR'F[1] - LBFR[1] (*Lactobacillus* 属 16S rDNA)
   - PCR 条件　SYBR® *Premix Ex Taq*™ II (TaKaRa Bio, Shiga)
     Rotor-Gene™ Q (QIAGEN) による 3step PCR

結果

図 1. *Lactobacillus* 属特異プライマーによる PCR 増幅産物のアガロースゲル電気泳動像.
レーン番号 1：SIID16618-01、M：サイズマーカー（100 bp）

まとめ

対象菌群	SIID16618-01
Lactobacillus 属	－

＋：PCR 増幅，－：PCR 非増幅

上記の結果、*Lactobacillus* 属を対象とした特異プライマーPCR 産物が得られませんでした（図 1：レーン番号 1）。このことから、検体中には *Lactobacillus* 属のゲノム DNA が存在しない、あるいは検出限界以下であると考えられます。

補足

本報告書に関する技術的なご質問等につきましては、株式会社テクノスルガ・ラボ 技術部までお問合わせください。

引用文献

1） SONGJINDA (P.), NAKAYAMA (J.), TATEYAMA (A.), TANAKA (S.), TSUBOUCHI (M.), KIYOHARA (C.), SHIRAKAWA (T.) and SONOMOTO (K.): Differences in developing intestinal microbiota between allergic and non-allergic infants: a pilot study in Japan. *Biosci. Biotechnol. Biochem.*, 2007, **71**, 2338-2342.

Japan Food Research Laboratories

分析試験成績書

第207060355-001号
2007年(平成19年)06月18日

依 頼 者 株式会社 ファーマフーズ

検 体 名 ﾌｧｰﾏｷﾞｬﾊﾞ80
(Lot.070209A)

財団法人
日本食品分析センター
東京本部 〒151-0062 東京都渋谷区元代々木町52番1号
大阪支所 〒564-0051 大阪府吹田市豊津町3番1号
名古屋支所 〒460-0011 名古屋市中区大須4丁目5番13号
九州支所 〒812-0034 福岡市博多区下呉服町1番12号
多摩研究所 〒206-0025 東京都多摩市永山6丁目11番10号
千歳研究所 〒066-0052 北海道千歳市文京2丁目3番
彩都研究所 〒567-0085 大阪府茨木市彩都あさぎ7丁目4番41号

2007年(平成19年)06月05日当センターに提出された上記検体について分析試験した結果は次のとおりです。

分析試験結果

分析試験項目	結果	検出限界	注	方法
ﾋｽﾀﾐﾝ	検出せず	0.5 mg/100g		高速液体ｸﾛﾏﾄｸﾞﾗﾌ法

以 上

Japan Food Research Laboratories

Authorized by the Japanese Government

52-1 Motoyoyogi-cho, Shibuya-ku, Tokyo 151-0062, Japan

No. 207060355-001

June 18, 2007

CERTIFICATE OF ANALYSIS

Client: PHARMA FOODS INTERNATIONAL CO., LTD.
1-49 Goryo-Ohara, Nishikyo-ku, Kyoto 615-8245, JAPAN

Sample name: PharmaGABA Lot.070209A

Received date: June 05, 2007

This is to certify that the following result(s) have been obtained from our analysis on the above-mentioned sample(s) submitted by the client.

Test Result(s)

Test Item	Result	QL	N	M
Histamine	Not detected	0.5 mg / 100g		1

QL: Quantitation limit N: Notes M: Method

Method

1: HPLC

This test result was translated by Pharma Foods International Co., Ltd. Based on the analysis results provided by Japan Food Research Laboratories and we, Pharma Foods International Co., Ltd. hereby confirm that the translation and analysis result are consistent.

Date: 24 March 2015

(b) (6)

Translated by Kyung Hwa Moon

Deputy Manager, Sales Department, Global Strategy Group

Pharma Foods International Co., Ltd.

Japan Food Research Laboratories
Authorized by the Japanese Government
52-1 Motoyoyogi-cho, Shibuya-ku, Tokyo 151-0062, Japan http://www.jfrl.or.jp/

No. 15010612001-05 1/1
February 09, 2015

CERTIFICATE OF ANALYSIS

Client: PHARMA FOODS INTERNATIONAL CO., LTD.
1-49 Goryo-Ohara, Nishikyo-ku, Kyoto 615-8245, JAPAN

Sample name: PharmaGABA Lot. 4B06

Received date: February 02, 2015

This is to certify that the following result(s) have been obtained from our analysis on the above-mentioned sample(s) submitted by the client.

Test Result(s)

Test Item	Result	QL	N	M
Histamine	Not detected	0.5 mg/100g		1

QL: Quantitation limit N: Notes M: Method

Method
1:HPLC

JAPAN FOOD RESEARCH LABORATORIES

(b) (6)

Michiyo Horiuchi
Principal Investigator

Feb. 26, 2015
Date

Japan Food Research Laboratories
Authorized by the Japanese Government
52-1 Motoyoyogi-cho, Shibuya-ku, Tokyo 151-0062, Japan http://www.jfrl.or.jp/

No. 15010612002-05 1/1
February 09, 2015

CERTIFICATE OF ANALYSIS

Client: PHARMA FOODS INTERNATIONAL CO., LTD.
1-49 Goryo-Ohara, Nishikyo-ku, Kyoto 615-8245, JAPAN

Sample name: PharmaGABA Lot. 4E21

Received date: February 02, 2015

This is to certify that the following result(s) have been obtained from our analysis on the above-mentioned sample(s) submitted by the client.

Test Result(s)

Test Item	Result	QL	N	M
Histamine	Not detected	0.5 mg/100g		1

QL: Quantitation limit N: Notes M: Method

Method
1:HPLC

JAPAN FOOD RESEARCH LABORATORIES

(b) (6)

Michiyo Horiuchi
Principal Investigator

Feb. 26, 2015
Date

一般財団法人
日本食品分析センター

Japan Food Research Laboratories

分析試験成績書

第208021242-001号
2008年(平成20年)02月21日

依 頼 者 株式会社 ファーマフーズ

検 体 名 Pharma GABA
(Lot.7B09)

財団法人
日本食品分析センター
東京本部 〒151-0062 東京都渋谷区元代々木町52番1号
大阪支所 〒564-0051 大阪府吹田市豊津町3番1号
名古屋支所 〒460-0011 名古屋市中区大須4丁目5番13号
九州支所 〒812-0034 福岡市博多区下呉服町1番12号
多摩研究所 〒206-0025 東京都多摩市永山6丁目11番10号
千歳研究所 〒066-0052 北海道千歳市文京2丁目3番
彩都研究所 〒567-0085 大阪府茨木市彩都あさぎ7丁目4番41号

2008年(平成20年)02月15日当センターに提出された上記検体について分析試験した結果は次のとおりです。

分析試験結果

分析試験項目	結果	検出限界	注	方法
ｶﾙﾊﾞﾐﾝ酸ｴﾁﾙ(ｳﾚﾀﾝ)	検出せず	0.01 ppm		ｶﾞｽｸﾛﾏﾄｸﾞﾗﾌｰ質量分析法

以 上

Japan Food Research Laboratories

Authorized by the Japanese Government
52-1 Motoyoyogi-cho, Shibuya-ku, Tokyo 151-0062, Japan

No. 208021242-001
February 21, 2008

CERTIFICATE OF ANALYSIS

Client: PHARMA FOODS INTERNATIONAL CO., LTD.
1-49 Goryo-Ohara, Nishikyo-ku, Kyoto 615-8245, JAPAN

Sample name: PharmaGABA Lot.7B09

Received date: February 15, 2008

This is to certify that the following result(s) have been obtained from our analysis on the above-mentioned sample(s) submitted by the client.

Test Result(s)

Test Item	Result	QL	N	M
Ethyl carbamate	Not detected	0.01 ppm		1

QL: Quantitation limit N: Notes M: Method

Method

1: Gas chromatography-mass spectrometory

This test result was translated by Pharma Foods International Co., Ltd. Based on the analysis results provided by Japan Food Research Laboratories and we, Pharma Foods International Co., Ltd. hereby confirm that the translation and analysis result are consistent.

Date: 24 March 2015

(b) (6)

Translated by Kyung Hwa Moon
Deputy Manager, Sales Department, Global Strategy Group
Pharma Foods International Co., Ltd.

Japan Food Research Laboratories
Authorized by the Japanese Government
52-1 Motoyoyogi-cho, Shibuya-ku, Tokyo 151-0062, Japan http://www.jfrl.or.jp/

No. 15010612001-04 1/1
February 09, 2015

CERTIFICATE OF ANALYSIS

Client: PHARMA FOODS INTERNATIONAL CO., LTD.
1-49 Goryo-Ohara, Nishikyo-ku, Kyoto 615-8245, JAPAN

Sample name: PharmaGABA Lot. 4B06

Received date: February 02, 2015

This is to certify that the following result(s) have been obtained from our analysis on the above-mentioned sample(s) submitted by the client.

Test Result(s)

Test Item	Result	QL	N	M
Ethyl carbamate	Not detected	0.01 ppm		1

QL: Quantitation limit N: Notes M: Method

Method
1:Gas chromatography-mass spectrometry

JAPAN FOOD RESEARCH LABORATORIES

(b) (6)

Michiyo Horiuchi
Principal Investigator

Feb. 26, 2015
Date

Japan Food Research Laboratories
Authorized by the Japanese Government
52-1 Motoyoyogi-cho, Shibuya-ku, Tokyo 151-0062, Japan http://www.jfrl.or.jp/

No. 15010612002-04 1/1
February 09, 2015

CERTIFICATE OF ANALYSIS

Client: PHARMA FOODS INTERNATIONAL CO., LTD.
1-49 Goryo-Ohara, Nishikyo-ku, Kyoto 615-8245, JAPAN

Sample name: PharmaGABA Lot. 4E21

Received date: February 02, 2015

This is to certify that the following result(s) have been obtained from our analysis on the above-mentioned sample(s) submitted by the client.

Test Result(s)

Test Item	Result	QL	N	M
Ethyl carbamate	Not detected	0.01 ppm		1

QL: Quantitation limit N: Notes M: Method

Method
1:Gas chromatography-mass spectrometry

JAPAN FOOD RESEARCH LABORATORIES

(b) (6)

Michiyo Horiuchi
Principal Investigator

Feb. 26, 2015
Date

Japan Food Research Laboratories
Authorized by the Japanese Government
52-1 Motoyoyogi-cho, Shibuya-ku, Tokyo 151-0062, Japan http://www.jfrl.or.jp/

No. 15010612002-06 1/1
February 13, 2015

CERTIFICATE OF ANALYSIS

Client: PHARMA FOODS INTERNATIONAL CO., LTD.
1-49 Goryo-Ohara, Nishikyo-ku, Kyoto 615-8245, JAPAN

Sample name: PharmaGABA Lot. 4E21

Received date: February 02, 2015

This is to certify that the following result(s) have been obtained from our analysis on the above-mentioned sample(s) submitted by the client.

Test Result(s)

Test Item	Result	QL	N	M
Free citrulline	Not detected	0.01 g/100g		1

QL: Quantitation limit N: Notes M: Method

Method
1:Amino acid analyzer method

JAPAN FOOD RESEARCH LABORATORIES

(b) (6)

Michiyo Horiuchi
Principal Investigator

Feb. 26, 2015
Date

一般財団法人
日本食品分析センター

Japan Food Research Laboratories

分析試験成績書

第209010538-001号
2009年(平成21年)01月23日

依 頼 者 株式会社 ファーマフーズ

検 体 名 Pharma GABA™
(Lot.7B09)

財団法人
日本食品分析センター
東京本部 〒151-0062 東京都渋谷区元代々木町52番1号
大阪支所 〒564-0051 大阪府吹田市豊津町3番1号
名古屋支所 〒460-0011 名古屋市中区大須4丁目5番13号
九州支所 〒812-0034 福岡市博多区下呉服町1番12号
多摩研究所 〒206-0025 東京都多摩市永山6丁目11番10号
千歳研究所 〒066-0052 北海道千歳市文京2丁目3番
彩都研究所 〒567-0085 大阪府茨木市彩都あさぎ7丁目4番41号

2009年(平成21年)01月09日当センターに提出された上記検体について分析試験した結果は次のとおりです。

分析試験結果

分析試験項目	結果	検出限界	注	方法
遊離シトルリン	検出せず	0.02 g/100g	1	アミノ酸自動分析法

注1. 検出限界は高濃度のアミノ酸が存在するため,0.02 g/100gとした。

以 上

Japan Food Research Laboratories

Authorized by the Japanese Government
52-1 Motoyoyogi-cho, Shibuya-ku, Tokyo 151-0062, Japan

No. 20901538-001
January 23, 2009

CERTIFICATE OF ANALYSIS

Client: PHARMA FOODS INTERNATIONAL CO., LTD.
1-49 Goryo-Ohara, Nishikyo-ku, Kyoto 615-8245, JAPAN

Sample name: PharmaGABA™ Lot.7B09

Received date: January 9, 2009

This is to certify that the following result(s) have been obtained from our analysis on the above-mentioned sample(s) submitted by the client.

Test Result(s)

Test Item	Result	QL	N	M
Free citrulline	Not detected	0.02 g/100g		1

QL: Quantitation limit N: Notes M: Method

Method

1: Amino acid analyzer method

This test result was translated by Pharma Foods International Co., Ltd. Based on the analysis results provided by Japan Food Research Laboratories and we, Pharma Foods International Co., Ltd. hereby confirm that the translation and analysis result are consistent.

Date: 24 March 2015

(b) (6)

Translated by Kyung Hwa Moon
Deputy Manager, Sales Department, Global Strategy Group
Pharma Foods International Co., Ltd.

Japan Food Research Laboratories
Authorized by the Japanese Government
52-1 Motoyoyogi-cho, Shibuya-ku, Tokyo 151-0062, Japan http://www.jfrl.or.jp/

No. 15010612001-06 1/1
February 13, 2015

CERTIFICATE OF ANALYSIS

Client: PHARMA FOODS INTERNATIONAL CO., LTD.
1-49 Goryo-Ohara, Nishikyo-ku, Kyoto 615-8245, JAPAN

Sample name: PharmaGABA Lot. 4B06

Received date: February 02, 2015

This is to certify that the following result(s) have been obtained from our analysis on the above-mentioned sample(s) submitted by the client.

Test Result(s)

Test Item	Result	QL	N	M
Free citrulline	Not detected	0.01 g/100g		1

QL: Quantitation limit N: Notes M: Method

Method
1:Amino acid analyzer method

JAPAN FOOD RESEARCH LABORATORIES

(b) (6)

Michiyo Horiuchi
Principal Investigator

Feb. 26, 2015
Date

SUBMISSION END

From: Rosenfeld, Leah
To: "n-tani@pharmafoods.co.jp"
Subject: Acknowledgement of the filing of GRN 000595 on GABA
Date: Wednesday, September 16, 2015 11:45:00 AM
Attachments: Acknowledgment_Letter_GRN000595.pdf

Dear Ms. Horie,

Please find attached a digital copy of our letter acknowledging receipt and filing of your GRAS notice on GABA.

Sincerely,
Leah Rosenfeld

Leah Rosenfeld, Ph.D.
Consumer Safety Officer
Division of Biotechnology and GRAS Notice Review
Office of Food Additive Safety
U.S. Food and Drug Administration
Tel: 240-402-1386
Email:Leah.Rosenfeld@fda.hhs.gov

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
College Park, MD 20740-3835

September 16, 2015

Noriko Horie
Pharma Foods International Co., Ltd.
1-49 Goryo-Ohara, Nishikyo-ku,
Kyoto 615-8245
JAPAN

Re: GRAS Notice No. GRN 000595

Dear Ms. Horie:

The Food and Drug Administration (FDA) has received the notice, dated August 3, 2015, that you submitted on in accordance with the agency's proposed regulation, proposed 21 CFR 170.36 (62 FR 18938; April 17, 1997; Substances Generally Recognized as Safe (GRAS); the GRAS proposal). FDA received this notice on August 6, 2015, filed it on August 27, 2015, and designated it as GRN No. 000595.

The subject of the notice is *gamma*-aminobutyric acid (GABA). The notice informs FDA of the view of Pharma Foods International Co., Ltd. that GABA is GRAS, through scientific procedures, for use as an ingredient in snack bars, carbonated drinks, energy drinks, flavored drinks, powdered drinks, sports drinks, flavored milk and milk drinks, yogurts, vegetable juices, breakfast cereals, processed cheese, chewing gum, coffee, tea, hard candies, soft candies, and chocolates at a level of 100 mg per serving which is equivalent to 0.0416–0.667%.

In accordance with proposed 21 CFR 170.36(f), a copy of the information in this notice that conforms to the information in the GRAS exemption claim (proposed 21 CFR 170.36(c)(1)) is available for public review and copying at www.fda.gov/grasnoticeinventory. If you have any questions about the notice, contact me at Leah.Rosenfeld@fda.hhs.gov.

Sincerely yours,

Leah Rosenfeld -S

Digitally signed by Leah Rosenfeld -S
DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, cn=Leah Rosenfeld -S, 0.9.2342.19200300.100.1.1=2000734748
Date: 2015.09.16 11:40:45 -04'00'

Leah Rosenfeld, PhD
Division of Biotechnology and
GRAS Notice Review
Center for Food Safety
and Applied Nutrition

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
College Park, MD 20740-3835

February 26, 2016

Noriko Horie
Pharma Foods International Co., Ltd.
1-49 Goryo-Ohara, Nishikyo-ku,
Kyoto 615-8245
JAPAN

Re: GRAS Notice No. GRN 000595

Dear Ms. Horie:

The Food and Drug Administration (FDA) has received the notice, dated August 3, 2015, that you submitted on in accordance with the agency's proposed regulation, proposed 21 CFR 170.36 (62 FR 18938; April 17, 1997; Substances Generally Recognized as Safe (GRAS); the GRAS proposal). FDA received this notice on August 6, 2015, filed it on August 27, 2015, and designated it as GRN No. 000595.

The subject of the notice is *gamma*-aminobutyric acid (GABA). The notice informs FDA of the view of Pharma Foods International Co., Ltd. that GABA is GRAS, through scientific procedures, for use as an ingredient in snack bars, carbonated drinks, energy drinks, flavored drinks, powdered drinks, sports drinks, flavored milk and milk drinks, yogurts, vegetable juices, breakfast cereals, processed cheese, chewing gum, coffee, tea, hard candies, soft candies, and chocolates at a level of 100 mg per serving which is equivalent to 0.0416–0.667%.

In accordance with proposed 21 CFR 170.36(f), a copy of the information in this notice that conforms to the information in the GRAS exemption claim (proposed 21 CFR 170.36(c)(1)) is available for public review and copying at www.fda.gov/grasnoticeinventory. If you have any questions about the notice, contact me at Leah.Rosenfeld@fda.hhs.gov.

Sincerely yours,

Leah Rosenfeld, PhD
Division of Biotechnology and
GRAS Notice Review
Center for Food Safety
and Applied Nutrition

Hard copy cc: GRN 000595 (1 copy)
Filename: gn0595ak
R/D:HFS-255:LRsenfeld:9/4/15
Init:HFS-255:SCarlson:9/16/15
F/T:HFS-255:LRosenfeld:2/26/16

From:	Rosenfeld, Leah
To:	n-tani@pharmafoods.co.jp; Ashley Roberts (ashley.roberts@intertek.com)
Cc:	Carlson, Susan
Subject:	Summary of information from yesterday"s teleconference on GRN 000595 (GABA)
Date:	Tuesday, October 27, 2015 4:01:00 PM

Dear Ms. Horie and Dr. Roberts,

Thank you for taking the time to have a teleconference with us. We have summarized below the major issues that were discussed yesterday. Our review team believes that additional experimental data, which we were unable to find, are required to adequately address issues raised and thus not addressable within two-week timeframe. We have other items that would need to be addressed in our review, but these could be done in a shorter time span, and we have not included them in this e-mail. We would be happy to share the additional items with you, but for the moment, we'd like to focus on the main two points below. After reviewing the information below, please share your thoughts.

For your records, at the meeting from FDA were:
Leah Rosenfeld
Susan Carlson
Kotaro Kaneko
Tim Twaroski
Aydin Orstan
Alison Edwards
Lane Highbarger

Would you please provide the spelling of the names of the PFI representatives on the phone?

Pharma Foods International's safety determination largely rests on several points, including the history of safe consumption of foods and supplements, both natural and processed, which contain GABA; as well as GABA's presence as a naturally occurring bioactive compound in animals.

The safety determinations based on experimental data rely on two critical points:
1) oral ingestion of GABA results in low absorption into the blood stream
2) GABA does not cross the blood-brain barrier (BBB).

These two critical assumptions are not yet validated in several subpopulations that are expected to consume the foods proposed by the notifier for its intended uses. There appear to be critical data gaps that exist to support their GRAS determination under the conditions of their intended use.

We note that foods ingredients need to be safe for their intended use, which includes all consumers of those foods containing that ingredient.
PFI states on p. 4 of the notice that "GABA is not to be used or marketed in infant and children's food products." The intended uses include "snack bars, breakfast cereals, processed cheese, chewing gum, yogurts, hard and soft candies, and chocolate as well as beverages and beverage bases including carbonated, energy, flavored, powdered and sports drinks, flavored milk and milk drinks," many of which are consumed by young children and pregnant women. Since the notifier's exposure calculation included these populations, the safety determination needs to include information that supports safe consumption by these populations for the intended uses.

I) **Lack of data (i.e. developmental and reproductive toxicity studies and ADME) that support safety in pregnant or lactating women and in young children**

i. As is stated on p.24, no ADME study has been done in humans. Given potential

interspecies differences that complicate extrapolation of absorption data obtained in the rat to humans (Cao et al. 2006; DeSesso and Jacobson 2001), there is currently insufficient data to support the conclusion that oral ingestion of GABA does not result in appreciable systemic circulation in humans.

ii. During development, GABA switches from depolarizing (stimulatory) to hyperpolarizing (inhibitory) during gestation [postnatally in rodents; (Ben-Ari et al. 1989)]. Thus, GABA has an important role in neuronal growth and synapse formation and therefore has a much more complex role in developing fetus compared to its role in adults (Ben-Ari 2002; Kirmse et al. 2015; Kwon et al. 2014).

iii. GABA transporter (BGT-1) is expressed in the placenta (Kitano et al. 2004; Rasola et al. 1995). Therefore, GABA in the maternal circulation could potentially cross the placental barrier to affect fetal development.

iv. Existing data suggest that transport mechanisms to support BBB function is still developing in the late fetal/neonatal stages (Blanchette and Daneman 2015; Hagan and Ben-Zvi 2015; Saunders, Liddelow, and Dziegielewska 2012). On pg. 25, the notifier describes a study by Kakee et al. (2001) which showed "the efflux rate of GABA through BBB of rats exceeded influx by approximately 16-fold", strongly suggesting that transport mechanisms play a key role in excluding GABA from the brain. Therefore, BBB data of a particular substance obtained in the adult cannot necessarily be directly extrapolated to the developing fetus. Consistent with this hypothesis, Al-Sarraf has shown that ^{14}C-GABA uptake in the immature rats is roughly twice that of the adult (Al-Sarraf 2002).

Although there are no specific adverse effects of GABA in pregnant or lactating women and in children reported in the literature, there clearly exists a data gap to effectively assess safety of orally ingested GABA in this vulnerable subpopulation, related to data on uptake across the gastrointestinal tract, crossing the blood brain barrier in fetuses and young children, and whether this causes adverse effects.

II) **<u>Reported effects on the pituitary gland</u>**

i. BBB does not exist in the circumventricular zone, which includes the pituitary gland; therefore GABA in the bloodstream will have direct access to this so-called "master gland". This is supported by several studies that showed that parenterally introduced ^{14}C-GABA specifically accumulates in the pituitary and median eminence (Hespe, Roberts, and Prins 1969; Kuroda et al. 2000).

ii. GABA receptors are known to be expressed in the lactotrophs (prolactin secreting cells) and somatotrophs in the anterior pituitary (Duvilanski et al. 2000), and GABA was shown to alter prolactin secretion in human anterior pituitary tissue (Grandison et al. 1982). In contrast to normally inhibitory action of GABA, Zemkova et al. showed that GABA has a depolarizing effect on secretory pituitary cells (Zemkova et al. 2008). Given the existence of GABA transport system in the rat anterior pituitary gland (Duvilanski et al. 1994), circulatory GABA could effectively modulate its function (Kreft and Zorec 2008).

iii. GABA was shown to induce GH secretion in a dose-dependent manner in neonatal rats (Acs et al. 1987). These authors note that GABA-dependent responsiveness gradually decreased in the second and third week post-natally, suggesting that newborn rats are more sensitized to GABA stimulation of GH secretion compared to older rats.

These data suggest that orally administered GABA could affect pituitary and hypothalamic function. The notifier dismisses the effects of GABA on GH based on high doses that appeared to be required for the effect in adults; however, the notifier also states on p. 42 that "… a threshold for GABA-induced increases in GH secretion could not be determined."

Thus, no study has been undertaken to examine dose-effects or whether such high doses are required to elicit pituitary/hypothalamic response in children and pregnant/lactating women. Given such an important role both organs play during pregnancy, lactation, reproduction, and development, we were unable to find sufficient data to evaluate the safety of chronic oral exposure of GABA to these susceptible population groups. Therefore, there appears to be insufficient data to conclude, as stated by the notifier on p. 42, that "it is highly unlikely that GABA, when used as an ingredient in food under the intended conditions of use … will stimulate the release of GH."

Please let me know if you have additional questions.

Sincerely,

Leah Rosenfeld

Leah Rosenfeld, Ph.D.
Consumer Safety Officer
Division of Biotechnology and GRAS Notice Review
Office of Food Additive Safety
U.S. Food and Drug Administration
Tel: 240-402-1386
Email:Leah.Rosenfeld@fda.hhs.gov

REFERNCES

Acs, Z., B. Szabo, G. Kapocs, and G. B. Makara. 1987. 'gamma-Aminobutyric acid stimulates pituitary growth hormone secretion in the neonatal rat. A superfusion study', Endocrinology, 120: 1790-8.

Al-Sarraf, H. 2002. 'Transport of 14C-gamma-aminobutyric acid into brain, cerebrospinal fluid and choroid plexus in neonatal and adult rats', Brain Res Dev Brain Res, 139: 121-9.

Ben-Ari, Y. 2002. 'Excitatory actions of gaba during development: the nature of the nurture', Nat Rev Neurosci, 3: 728-39.

Ben-Ari, Y., E. Cherubini, R. Corradetti, and J. L. Gaiarsa. 1989. 'Giant synaptic potentials in immature rat CA3 hippocampal neurones', J Physiol, 416: 303-25.

Blanchette, M., and R. Daneman. 2015. 'Formation and maintenance of the BBB', Mech Dev.

Cao, Xianhua, Seth T. Gibbs, Lanyan Fang, Heather A. Miller, Christopher P. Landowski, Ho-Chul Shin, Hans Lennernas, Yanqiang Zhong, Gordon L. Amidon, Lawrence X. Yu, and Duxin Sun. 2006. 'Why is it challenging to predict intestinal drug absorption and oral bioavailability in human using rat model', Pharmaceutical research, 23: 1675-86.

DeSesso, J. M., and C. F. Jacobson. 2001. 'Anatomical and physiological parameters affecting gastrointestinal absorption in humans and rats', Food Chem Toxicol, 39: 209-28.

Duvilanski, B. H., R. Perez, A. Seilicovich, M. Lasaga, M. C. Diaz, and L. Debeljuk. 2000. 'Intracellular distribution of GABA in the rat anterior pituitary. An electron microscopic autoradiographic study', Tissue Cell, 32: 284-92.

Duvilanski, B., A. Seilicovich, L. Debeljuk, M. Lasaga, M. C. Diaz, and D. Pisera. 1994. 'GABA transport and subcellular distribution in the rat anterior pituitary gland', Neuroendocrinology, 59: 183-8.

Grandison, L., F. Cavagnini, R. Schmid, S. C. Invitti, and A. Guidotti. 1982. 'gamma-Aminobutyric acid- and benzodiazepine-binding sites in human anterior pituitary tissue', J Clin Endocrinol Metab, 54: 597-601.

Hagan, N., and A. Ben-Zvi. 2015. 'The molecular, cellular, and morphological components of blood-brain barrier development during embryogenesis', Semin Cell Dev Biol, 38: 7-15.

Hespe, W., E. Roberts, and H. Prins. 1969. 'Autoradiographic investigation of the distribution of [14C]GABA in tissues of normal and aminooxyacetic acid-treated mice', Brain Res, 14: 663-71.

Kakee, A., H. Takanaga, T. Terasaki, M. Naito, T. Tsuruo, and Y. Sugiyama. 2001. 'Efflux of a suppressive neurotransmitter, GABA, across the blood-brain barrier', Journal of neurochemistry, 79: 110-8.

Kirmse, K., M. Kummer, Y. Kovalchuk, O. W. Witte, O. Garaschuk, and K. Holthoff. 2015. 'GABA depolarizes immature neurons and inhibits network activity in the neonatal neocortex in vivo', Nat Commun, 6: 7750.

Kitano, T., H. Iizasa, I. W. Hwang, Y. Hirose, T. Morita, T. Maeda, and E. Nakashima. 2004. 'Conditionally immortalized syncytiotrophoblast cell lines as new tools for study of the blood-placenta barrier', Biol Pharm Bull, 27: 753-9.

Kreft, M., and R. Zorec. 2008. 'Anterior pituitary cells excited by GABA', J Physiol, 586: 3023-4.

Kuroda, E., M. Watanabe, T. Tamayama, and M. Shimada. 2000. 'Autoradiographic distribution of radioactivity from (14)C-GABA in the mouse', Microsc Res Tech, 48: 116-26.

Kwon, S. H., D. Scheinost, C. Lacadie, J. Benjamin, E. H. Myers, M. Qiu, K. C. Schneider, D. L. Rothman, R. T. Constable, and L. R. Ment. 2014. 'GABA, resting-state connectivity and the developing brain', Neonatology, 106: 149-55.

Rasola, A., L. J. Galietta, V. Barone, G. Romeo, and S. Bagnasco. 1995. 'Molecular cloning and functional characterization of a GABA/betaine transporter from human kidney', FEBS Lett, 373: 229-33.

Saunders, N. R., S. A. Liddelow, and K. M. Dziegielewska. 2012. 'Barrier mechanisms in the developing brain', Front Pharmacol, 3: 46.

Zemkova, H. W., I. Bjelobaba, M. Tomic, H. Zemkova, and S. S. Stojilkovic. 2008. 'Molecular, pharmacological and functional properties of GABA(A) receptors in anterior pituitary cells', J Physiol, 586: 3097-111.

From:	子
To:	Rosenfeld, Leah; Carlson, Susan
Cc:	Ashley Roberts (ashley.roberts@intertek.com); Winnie Y Ng Intertek; 金正元
Subject:	Summary of information from yesterday"s teleconference on GRN 000595 (GABA) (GRN 000595(GABA)
Date:	Tuesday, November 10, 2015 7:03:23 AM
Attachments:	Letter of Withdrawal(PharmaFoods).pdf

Dear Dr. Rosenfeld and review team,

Regarding our application for GRAS, we feel we need time to conduct further studies and we would like to withdraw it.
We will continue to make efforts to address the concerns raised by you, eventually for our re-application in the future.

We believe Ashley at Intertek will arrange a meeting with you sooner or later.
We will truly appreciate it if you could let Intertek and us know what studies need to be done.

Best regards,
Noriko Horie

****
～みんなにやさしいバイオの夢～
株式会社ファーマフーズ
営業部 谷 典子

〒615-8245
京都市西京区御陵大原1番地49
TEL：075-394-8610（営業部）
FAX：075-394-0009（営業部）
e-mai n-tani@pharmafoods.co.jp
URL http://www.pharmafoods.co.jp/
ギャバ・ストレス研究センター http://www.gabastress.jp/
****

PHARMA FOODS INTERNATIONAL CO.,LTD.
1-49 Goryo-Ohara, Nishikyo-ku,
Kyoto 615-8245 Japan
PHONE : +81-75-394-8605
FAX : +81-75-394-8889

2015/11/10

Letter of Withdrawal

Dear Dr. Rosenfeld and review team,

We would like to withdraw our application for GRAS on PharmaGABA as we need more time to conduct further studies in order to address the concerns you and your team have raised.

Sincerely,

Noriko Horie
Director of Sales
Pharma Foods International Co., Ltd.

DEPARTMENT OF HEALTH & HUMAN SERVICES Public Health Service

Food and Drug Administration
College Park, MD 20740-3835

Noriko Horie
Pharma Foods International Co., Ltd.
1-49 Goryo-Ohara, Nishikyo-ku,
Kyoto 615-8245
JAPAN

Re: GRAS Notice No. GRN 000595

Dear Ms. Horie:

The Food and Drug Administration (FDA) is responding to the notice, dated August 3, 2015, that you submitted in accordance with the agency's proposed regulation, proposed 21 CFR 170.36 (62 FR 18938; April 17, 1997; Substances Generally Recognized as Safe (GRAS); the GRAS proposal). FDA received the notice on August 6, 2015, filed it on September 1, 2015, and designated it as GRAS Notice No. GRN 000595.

The subject of the notice is *gamma*-aminobutyric acid (GABA). The notice informs FDA of the view of Pharma Foods International Co., Ltd. that GABA is GRAS, through scientific procedures, for use as an ingredient in snack bars, carbonated drinks, energy drinks, flavored drinks, powdered drinks, sports drinks, flavored milk and milk drinks, yogurts, vegetable juices, breakfast cereals, processed cheese, chewing gum, coffee, tea, hard candies, soft candies, and chocolates at a level of 100 mg per serving which is equivalent to 0.0416–0.667%.

In a letter sent to us by e-mail dated November 10, 2105, you withdrew your notice. Given your letter, we ceased to evaluate your GRAS notice, effective November 10, 2015, the date that we received your letter.

In accordance with proposed 21 CFR 170.36(f), a copy of the text of this letter responding to GRN 000595, as well as a copy of the information in this notice that conforms to the information in the GRAS exemption claim (proposed 21 CFR 170.36(c)(1)), is available for public review and copying via the FDA home page at http://www.fda.gov/grasnoticeinventory.

Sincerely,

Antonia Mattia -S

Digitally signed by Antonia Mattia -S
DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, cn=Antonia Mattia -S, 0.9.2342.19200300.100.1.1=1300071580
Date: 2015.11.12 10:29:57 -05'00'

Antonia Mattia, Ph.D.
Director
Division of Biotechnology
and GRAS Notice Review
Office of Food Additive Safety
Center for Food Safety
and Applied Nutrition

Hard copy cc: **GRN 000595** (1 copy)
Electronic mail cc: PBeckerman (GCF-1)
HFS-200 (DKeefe, MAdams)
HFS-255 (AMattia, RFChanderbhan, MDiNovi, SCarlson, RIMerker, SWestBarnette, PMGaynor, AOrstan, KKaneko, LHighbarger, LShepherd)
HFS-850 (CAssar)

gn0595doc

Filename: gn0595doc
R/D:HFS-255:LRosenfeld:11/10/15
Init:HFS-255:SCarlson:11/10/15
F/T:LRosenfeld:11/10/15

NAME	ELECTRONIC SIGN-OFF	ACTING?
Leah Rosenfeld, PhD Consumer Safety Officer	Leah Rosenfeld -S Digitally signed by Leah Rosenfeld -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, cn=Leah Rosenfeld -S, 0.9.2342.19200300.100.1.1=2000734748 Date: 2015.11.10 12:46:32 -05'00'	☐
Susan Carlson Supervisory Consumer Safety Officer	Susan J. Carlson -S Digitally signed by Susan J. Carlson -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, 0.9.2342.19200300.100.1.1=200 0419015, cn=Susan J. Carlson -S Date: 2015.11.10 15:09:50 -05'00'	☐
Antonia Mattia Director, Division of Biotechnology & GRAS Notice Review	Antonia Mattia -S Digitally signed by Antonia Mattia -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, cn=Antonia Mattia -S, 0.9.2342.19200300.100.1.1=130007 1580 Date: 2015.11.12 10:29:25 -05'00'	☐

From:	Rosenfeld, Leah
To:	"Ashley Roberts Intertek"; ???
Cc:	Winnie Y Ng Intertek; ???; Carlson, Susan
Subject:	RE: Withdrawal of GRN 000595 (GABA) - additional questions identified
Date:	Monday, December 21, 2015 5:21:00 PM

Dear Dr. Roberts,

Per your request from Nov. 12, 2015, please find below a summary of the other issues identified during our review of the GRAS notice No. 000595 on GABA. Please be mindful that there may be additional issues that we had not identified when we stopped our review.

Sincerely,

Leah Rosenfeld

Additional issues and questions are as follows:

1. **Effects of GABA on the enteric nervous system (ENS) and the immune system:** There is a report of GABA having secretory and motility effects on the gastrointestinal (GI) tract through the ENS (Auteri et al., 2015). Additionally, another report demonstrates that GABA has immunomodulatory effects in the gut immune system (Jin et al., 2013). Thus, orally administered GABA would be expected to function as an effective neurotransmitter to modulate GI function (Sudo, 2014). For example, Li et al. (2012) found that not only is GABAergic signaling operational in the gut, but also its activity is upregulated in allergic diarrhea; moreover, the incidence of allergic diarrhea is reduced when the signaling is blocked. Furthermore, GABA signaling is involved in modulating macrophage function such that GABA agonists may compromise macrophage function during infection (Sanders et al., 2013). Given the developmental importance of gut microflora with respect to allergenicity and oral tolerance in infants, the effects of oral exposure of GABA to lactating women and young children needs to be considered. Given all of the points above, we would expect a revised notice to include a discussion of the potential for effects of GABA from within the GI tract with respect to the safety of the intended uses.

2. **Lack of data to alleviate concerns regarding the effects of GABA on patients taking blood pressure medication:** On pg. 41, the notifier discusses studies that indicate effects of GABA supplementation on lowering blood pressure. Although the studies report modest effects on subjects with higher than normal blood pressure, neither this study nor any other study has been cited that examine the effects of GABA on hypertensive individuals who are taking blood pressure medication. Approximately 30% of the United States population has high blood pressure[1], many of whom are likely taking medication to control blood pressure. The notice should include a discussion of considerations regarding any additive effects that GABA might have on blood pressure reduction or how GABA might interact with angiotensin-converting enzyme inhibitors, beta-blockers, and other types of blood pressure medication.

3. **The notifier discusses two 28-day oral toxicity studies in rats; we were unable to access an English translation of the study published by Kato et al (2005).** A revised notice should include a certified English translation of the Kato et al. study.

4. **Pharmokinetic similarities between GABA and Baclofen [ß-(4-chlorophenyl)-γ-amino**

butyric acid, an approved GABA derivative drug]: The notifier's safety narrative states that GABA does not appreciably cross the blood-brain-barrier (BBB). It should be noted that Baclofen also does not appreciably cross the BBB and appears to have similar pharmacokinetic properties as GABA with respect to the BBB.

a. Deguchi et al. (1995) conclude: "The restricted distribution of baclofen in the brain ISF (interstitial fluid) may be ascribed to the efficient efflux from the brain through the BBB …" This conclusion was essentially the same conclusion reached by [(Kakee et al., 2001), cited by the notifier], "The BBB acts as the efflux pump for GABA to reduce the brain interstitial fluid concentration."

Given the results and conclusions of these two studies, it is not unreasonable to assume that the pharmacokinetic properties of baclofen and GABA are similar. In light of this, any adverse effects reported for baclofen become relevant to the safety of GABA. There are adverse effects of baclofen reported in the literature: "Baclofen and pregnancy: birth defects and withdrawal symptoms" (Anonymous, 2015). A revised notice should include a discussion of the relevance of these adverse effects reported for Baclofen to the safety of GABA for pregnant women.

5. **Clarification of test articles cited in the notice:** Is PharmaGABA, cited in the subchronic study in Takeshima et al. (2014) the same with respect to composition and purity as the GABA preparation (GABA and GABA 20) that is the subject in the notice?

6. **Citations or source of information cited within the Expert Panel report:** On pg. 3 of the Expert Panel report, it states: "GABA has also been added to a number of food products in Japan for more than 20 years at levels of up to 280 mg/serving without any reported significant adverse effects on human health." A revised notice should include a citation to the source of this information.

7. **Exposure estimate considerations:** We note that GABA is sold as dietary supplement. A revised notice should include a discussion and estimate of the expected cumulative dietary exposure to GABA from all diet sources, including dietary supplements.

8. **Fermentation media composition:** In a revised notice, there should be a statement as to whether or not there are allergens present in the fermentation media.

9. **Definition of infants, by age:** On Page 23, the term "infants" is used for children aged 0-2. FDA has defined infants as children aged 0-1. In a revised notice, the age grouping for infants should be clarified with respect to FDA's definition.

10. **Specification for arsenic:** In the table on page 9 (Table II.C-1) there is no separate specification for arsenic. There is an arsenic specification in Appendix B, where the Certificates of Analysis are present. In a revised notice an arsenic specification is needed within Table II.C-1.

References:

Anonymous (2015). Baclofen and pregnancy: birth defects and withdrawal symptoms. Prescrire Int *24*, 214.

Auteri, M., Zizzo, M.G., and Serio, R. (2015). GABA and GABA receptors in the gastrointestinal tract: from motility to inflammation. Pharmacol Res *93*, 11-21.

Deguchi, Y., Inabe, K., Tomiyasu, K., Nozawa, K., Yamada, S., and Kimura, R. (1995). Study on brain interstitial fluid distribution and blood-brain barrier transport of baclofen in rats by microdialysis. Pharm Res *12*, 1838-1844.

Jin, Z., Mendu, S.K., and Birnir, B. (2013). GABA is an effective immunomodulatory molecule. Amino Acids *45*, 87-94.

Kakee, A., Takanaga, H., Terasaki, T., Naito, M., Tsuruo, T., and Sugiyama, Y. (2001). Efflux of a suppressive neurotransmitter, GABA, across the blood-brain barrier. J Neurochem *79*, 110-118.

Kato, I., Shimizu, S., Kobayashi, T., Kado, S., Kojima, K., Miura, K., and al., e. (2005). Single-dose, 1-month repeated dose, and 3-month repeated dose oral toxicity studies of fermented milk containing g-aminobutyric acid (GABA) in rodents. Yakuruto Kenkyujo Kenkyu Hokokushu *24*, 43-66.

Li Y, Xiang YY, Lu WY, Liu C, Li J (2012) A novel role of intestine epithelial GABAergic

signaling in regulating intestinal fluid secretion. Am J Physiol Gastrointest Liver Physiol 303

(4):G453–G460

Sanders, R.D., Godlee, A., Fujimori, T., Goulding, J., Xin, G., Salek-Ardakani, S., Snelgrove, R.J., Ma, D., Maze, M., and Hussell, T. (2013). Benzodiazepine augmented gamma-amino-butyric acid signaling increases mortality from pneumonia in mice. Crit Care Med *41*, 1627-1636.

Sudo, N. (2014). Microbiome, HPA axis and production of endocrine hormones in the gut. Adv Exp Med Biol *817*, 177-194.

Takeshima, K., Yamatsu, A., Yamashita, Y., Watabe, K., Horie, N., Masuda, K., and Kim, M. (2014). Subchronic toxicity evaluation of gamma-aminobutyric acid (GABA) in rats. Food Chem Toxicol *68*, 128-134.

---

[1] http://www.cdc.gov/bloodpressure/facts.htm