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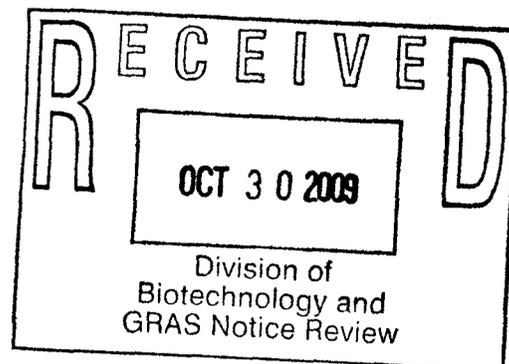
**AJINOMOTO CORPORATE SERVICES LLC**

1120 Connecticut Avenue, N.W., Suite 1010, Washington, D.C. 20036-3953, U.S.A.

Tel: (202) 457-0284 Fax: (202) 457-0107

October 30, 2009

Document Receiving  
U.S. Food and Drug Administration  
5100 Paint Branch Parkway  
College Park, MD 20740



Attention:  
Dr. Robert L. Martin, Deputy Director  
Division of Biotechnology and GRAS Notice Review  
Office of Food Additive Safety  
Center for Food Safety and Applied Nutrition  
Mail code: HFS-255  
Building: CPK 2 Room 2045

RE: GRAS Exemption Claim Notification – L –Leucine

Dear Dr. Martin:

Attached please find three copies of a GRAS Exemption Claim Notification for L-leucine that has been determined to be Generally Recognized As Safe by scientific procedures. This communication is a letter of transmittal for that submission.

If you have any questions, please do not hesitate to contact me.

Personal regards,

(b) (6)

Robert G. Bursey, Ph.D.  
President, Ajinomoto Corporate Services, LLC.

Attachments 3

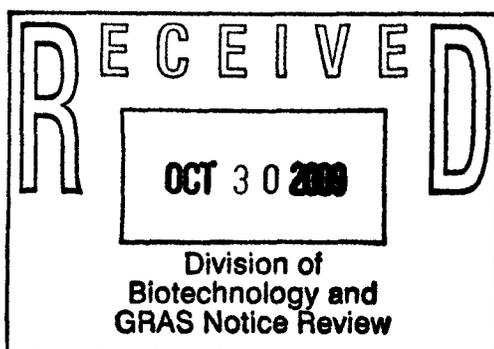
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# GRAS EXEMPTION CLAIM FOR L-LEUCINE

## Summary of Data Concerning the Safety and GRAS Determination of L-Leucine for Use as a Food Ingredient

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

**Submitted by:** Ajinomoto Aminoscience, LLC  
4020 Ajinomoto Drive  
Raleigh, NC  
U.S.A. 27610



October 27, 2009

000003

# GRAS EXEMPTION CLAIM FOR L-LEUCINE

## Table of Contents

	Page
EXECUTIVE SUMMARY .....	i
I. GRAS EXEMPTION CLAIM .....	1
I.A Claim of Exemption from the Requirement for Premarket Approval Pursuant to Proposed 21 CFR §170.36(c)(1) [62 FR 18938 (17 April 1997)] .....	1
I.B Name and Address of Notifier .....	1
I.C Common Name of the Notified Substance .....	2
I.D Conditions of Intended Use in Food and Consumer Exposure .....	2
I.D.1 Current Regulatory Status and Background Dietary Intake of L-Leucine .....	2
I.D.2 Intended Use of L-Leucine and Levels of Use in Foods .....	2
I.D.3 Estimated Dietary Consumption of L-Leucine Based upon Intended Food Uses .....	3
I.E Basis for the GRAS Determination .....	4
I.F Availability of Information .....	5
II. DETAILED INFORMATION ABOUT THE IDENTITY OF THE SUBSTANCE .....	5
II.A Identity .....	5
II.A.1 Chemical Description .....	5
II.B Method of Manufacture .....	6
II.C Specifications for Food Grade Material .....	8
II.C.1 Product Specifications and Analysis .....	8
II.C.2 Stability .....	9
III. SELF-LIMITING LEVELS OF USE .....	10
IV. BASIS FOR GRAS DETERMINATION .....	10
IV.A Introduction .....	10
IV.B Absorption, Distribution, Metabolism, and Elimination .....	11
IV.B.1 Absorption and Distribution .....	11
IV.B.2 Metabolism .....	11
IV.B.3 Excretion .....	14
IV.C Toxicological Studies .....	14
IV.C.1 Repeated Dose Studies .....	14
IV.C.2 Reproductive and Developmental Toxicity Studies .....	15
IV.C.3 Genotoxicity and Carcinogenicity Studies .....	16
IV.D Human Studies .....	17
IV.E Other Considerations .....	18
IV.E.1 Safety of the Fermentation Microorganism .....	18
IV.E.2 Potential Antagonism of Other Amino Acids .....	18

## L-LEUCINE GRAS NOTIFICATION

IV F	Summary and Basis for GRAS Conclusion .....	21
V.	REFERENCES.....	23

### List of Appendices

APPENDIX I	EXPERT PANEL REPORT REGARDING THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF L-LEUCINE FOR USE IN FOODS
APPENDIX II	METHODS OF ANALYSES
APPENDIX III	BATCH ANALYSES RESULTS
APPENDIX IV	STABILITY DATA

### List of Tables and Figures

Table I.D.2-1	Summary of the Individual Proposed Food-Uses and Use-Levels for L-Leucine in the U.S.....	3
Table II.B-1	List of Processing Aids Used in the Manufacture of L-Leucine .....	8
Table II.C.1-1	Product Specifications and Analytical Methods for L-Leucine .....	9
Table IV.B.2-1	BCKDH Activity in Selected Rodent and Human Tissues .....	13
Table IV.E.2-1	Summary of the Estimated Daily Intake of Protein in Identified Consumers of Foods Intended for Weight Reduction in which Leucine is Proposed for Use <i>versus</i> the U.S. Population (2005- 2006 NHANES Data) ..	20
Table IV.E.2-2	Summary of the Estimated Daily per Kilogram Body Weight Intake of Protein in Identified Consumers of Foods Intended for Weight Reduction in Which Leucine is Proposed for Use <i>versus</i> the U.S Population (2005-2006 NHANES Data).....	20
Figure II.B-1	Schematic Overview of the Manufacturing Process for L-Leucine .....	7
Figure IV.B.2-1	BCAA Catabolic Pathway (reproduced from Brosnan and Brosnan, 2006) ..	12

## GRAS EXEMPTION CLAIM FOR L-LEUCINE

### EXECUTIVE SUMMARY

Ajinomoto Aminoscience LLC (Ajinomoto) intends to market food-grade L-leucine ( $\geq 98.5\%$  purity) as a food ingredient for use at levels up to 3.0 g/serving in selected food categories, milk and non-milk based meal replacements, sports and isotonic beverages, vitamin enhanced waters, and meal replacement bars. The exposure to L-leucine from the proposed food uses in the total U.S. population is estimated to be 1.9 g/day at the mean, and 4.1 g/day at the 90<sup>th</sup> percentile (28 and 64 mg/kg body weight/day, respectively). L-Leucine is a branched-chain amino acid (BCAA) that is present in protein. Ajinomoto's L-leucine is Generally Recognized as Safe (GRAS) for use as a food ingredient because:

- It is a high-purity, well-characterized ingredient that is manufactured in accordance with current Good Manufacturing Practice (cGMP) and meets appropriate food-grade specifications;
- Its safety for its intended use in foods and beverages is supported by published studies conducted specifically with Ajinomoto's L-leucine product. These pivotal studies include (i) a 90-day toxicity rat study, in which a no-observed-adverse-effect level (NOAEL) of 5% mixed into the diet was determined (equivalent to 3.3 or 3.8 g/kg body weight/day for male and female rats respectively, in addition to the amount present in the basal diet), and (ii) a reproductive and developmental study wherein the highest dose administered by oral gavage, 1,000 mg/kg body weight/day, was established as the NOAEL for female reproductive function and embryo-fetal development;
- Additional published animal and human studies conducted with L-leucine further corroborate its safety. These include (i) studies on metabolism that indicate that the metabolic capacity for L-leucine is high and metabolism can be induced to deal with excess L-leucine intake; (ii) studies in healthy human subjects wherein no adverse events have been reported by subjects consuming 45 mg L-leucine/kg body weight/day (2.7 g/day in a 60 kg individual) for a 6-week period or by subjects consuming 7.5 g of supplemental L-leucine/day for a period of 3 months; and (iii) studies in human subjects with various disease states wherein no adverse effects are reported to be associated with repeated oral administration of up to 60 g/day of BCAAs (providing 24 g L-leucine);
- Its safety is further supported by the regular dietary consumption of L-leucine as a component of protein, and by the permitted uses of L-leucine in food in the U.S.;

## L-LEUCINE GRAS NOTIFICATION

- An independent Expert Panel comprised of scientists with expertise and training in the evaluation of the safety of food ingredients has critically examined the safety database concerning L-leucine and concluded based on scientific procedures that L-leucine is GRAS and safe for its intended use; and
- The data supporting the safety of L-leucine are available in the published literature.

The manufacturing process for L-Leucine involves fermentation using a bacterial strain derived from *Escherichia coli* (*E. coli*) K-12 by a series of selection steps using conventional mutagenic techniques and amplification of the genes encoding L-leucine biosynthetic enzymes from *E. coli*. *E. coli* K-12 is considered to be non-pathogenic and non-toxicogenic. Subsequent steps involved in the manufacturing process for L-leucine consist of filtration, decolorization, concentration, crystallization, separation, and step-wise purification to consistently produce a product that meets food-grade specifications. Non-consecutive batch analyses confirm the consistency of the product and reproducibility of the manufacturing process.

The weight of evidence clearly supports the safety of L-leucine, when produced in accordance with cGMP to a food-grade specification, for its intended use as a food ingredient.

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## GRAS EXEMPTION CLAIM FOR L-LEUCINE

### I. GRAS EXEMPTION CLAIM

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

L-Leucine has been determined by Ajinomoto Aminoscience, LLC (hereafter Ajinomoto) 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 its intended use in food, among experts qualified by scientific training and expertise. Therefore, the use of L-leucine in food as described below is exempt from the requirement of premarket approval.

Signed,

---

Robert G. Bursey, Ph.D.  
President  
Ajinomoto Corporate Services LLC

10/30/09  
Date

#### I.B Name and Address of Notifier

Robert G. Bursey, Ph.D.  
President

Ajinomoto Corporate Services LLC  
1120 Connecticut Avenue, N.W., Suite 1010  
Washington, District of Columbia 20036

Telephone: 202-457-0284  
Facsimile: 202-457-0107  
Email: [BurseyB@ajiusa.com](mailto:BurseyB@ajiusa.com)

## **L-LEUCINE GRAS NOTIFICATION**

### **I.C Common Name of the Notified Substance**

The common name of the notified substance is L-leucine

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

#### **I.D.1 Current Regulatory Status and Background Dietary Intake of L-Leucine**

L-Leucine, meeting the specifications established by the Food Chemicals Codex (FCC), is permitted for use as a special dietary and nutritional additive that is intended to significantly improve the biological quality of the total protein in a food containing naturally occurring primarily-intact protein that is considered a significant dietary protein source (21 CFR 172.320) (U.S. FDA, 2009). The amount of L-leucine added for nutritive purposes plus the amount naturally present in free and combined (as protein) form is not to exceed 8.8% by weight of the total protein of the finished food. L-Leucine also may be used as a lubricant in the manufacture of aspartame or neotame tablets for sweetening hot beverages at levels not to exceed 3.5% of the tablet weight (21 CFR 172.804; 21 CFR 172.829) (U.S. FDA, 2009). Additionally, L-Leucine is deemed to be GRAS by the Flavor and Extract Manufacturers' Association (FEMA) under specific conditions of use.

L-Leucine occurs in the diet as a component of protein. Dairy products are a particularly rich source of L-leucine (USDA, 2009). The mean and 90<sup>th</sup> percentile total population background dietary intakes of L-leucine in the U.S. are estimated to be 6.08 and 8.90 g/person/day, respectively (IOM, 2005). The highest intakes of L-leucine are reported in men ages 51 to 70 years (14.1 g/person/ day at the 90<sup>th</sup> percentile) (IOM, 2005).

#### **I.D.2 Intended Use of L-Leucine and Levels of Use in Foods**

Ajinomoto intends to market L-Leucine as a food ingredient in the United States in selected food categories, including milk and non-milk based meal replacements, sports and isotonic beverages, vitamin enhanced waters, and meal replacement bars. L-Leucine will be added to foods at levels that provide 0.5 to 3 g L-leucine/serving. The intended food uses and use levels are presented in Table I.D.2-1.

## L-LEUCINE GRAS NOTIFICATION

**Table I.D.2-1 Summary of the Individual Proposed Food-Uses and Use-Levels for L-Leucine in the U.S.**

Food Category	Proposed Food Uses	Use-Level (g of Added L-Leucine/Serving)	RACC (g or mL)	Use-Level (% of Added L-Leucine)
Beverages and Beverage Bases	Non-Milk based Meal Replacements	1.5 to 3.0	240	0.625 to 1.25
	Sports and Isotonic Beverages	0.5	240	0.208
	Vitamin Enhanced Waters	1.0	240	0.416
Grain Products and Pastas	Meal Replacement Bars	3.0	40	7.50
Milk Products	Milk-Based Meal Replacements	1.5 to 3.0	240	0.625 to 1.25

RACC = Reference Amounts Customarily Consumed Per Eating Occasion (refers to the amount of the food or beverage product consumed)

### I.D.3 Estimated Dietary Consumption of L-Leucine Based upon Intended Food Uses

Estimates for the intake of L-leucine were based on the proposed food-uses and use-levels in conjunction with food consumption data included in the National Center for Health Statistics' (NCHS) 2005-2006 National Health and Nutrition Examination Surveys (NHANES) (CDC, 2006; USDA, 2009)

Approximately 10% of the total U.S. population was identified as consumers of added L-leucine from the proposed food-uses (843 actual users identified). Male teenagers reported the greatest percentage of users of any population group at 18.2%. Consumption of these types of foods by the total U.S. population resulted in estimated mean all-person and all-user intakes of added L-leucine of 217 mg/person/day (3 mg/kg body weight/day) and 1,863 mg/person/day (28 mg/kg body weight/day), respectively. The 90<sup>th</sup> percentile all-person and all-user intakes of added L-leucine from all proposed food-uses by the total population were 382 mg/person/day (6 mg/kg body weight/day) and 4,088 mg/person/day (64 mg/kg body weight/day), respectively.

On an individual population basis, the greatest mean all-person and all-user intakes of added L-leucine on an absolute basis were determined in male teenagers at 328 mg/person/day and female adults at 2,532 mg/person/day, respectively. Infants displayed the lowest mean all-person and all-user intakes of added L-leucine on an absolute basis, of 38 (3 mg/kg body weight/day) and 598 mg/person/day (49 mg/kg body weight/day), respectively. On a body weight basis, the highest mean all-person and all-user intakes of added L-leucine occurred in male teenagers (5 mg/kg body weight/day) and infants (49 mg/kg body weight/day), respectively.

When heavy consumers (90<sup>th</sup> percentile) were assessed, all-person and all-user intakes of added L-leucine from all proposed food-uses also were determined to be greatest in male teenagers (1,195 mg/person/day) and male adults (5,920 mg/person/day), respectively. The lowest 90<sup>th</sup> percentile all-user intakes occurred in infants at 1,405 mg/person/day, respectively.

## L-LEUCINE GRAS NOTIFICATION

on an absolute basis. On a body weight basis, infants were determined to have the greatest all-user 90<sup>th</sup> percentile intakes of added L-leucine at 134 mg/kg body weight/day, respectively.

### I.E Basis for the GRAS Determination

Pursuant to 21 CFR §170.35, L-leucine has been determined by Ajinomoto to be GRAS on the basis of scientific procedures (U.S. FDA, 2009). This determination is based on the views of experts who are qualified by scientific training and experience to evaluate the safety of L-leucine as a component of food [see Appendix I, **EXPERT PANEL REPORT REGARDING THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF L-LEUCINE FOR USE IN FOODS**].

At the request of Ajinomoto, an Expert Panel (the "Panel") of independent scientists, qualified by their relevant national and international experience and scientific training to evaluate the safety of food ingredients, was specially convened on June 23, 2009 to conduct a critical and comprehensive evaluation of the available pertinent data and information, and determine whether the intended uses of L-leucine as a food ingredient are safe and suitable and would be GRAS based on scientific procedures.

The Panel consisted of the following qualified scientific experts: John Fernstrom, Ph.D. (University of Pittsburgh School of Medicine), Donald Layman, Ph.D. (University of Illinois), Ian Munro, Ph.D. (Cantox Health Sciences International), and William Waddell, M.D. (University of Louisville).

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

L-leucine is GRAS based on scientific procedures for its intended use as a food ingredient; therefore, it is excluded from the definition of a food additive and thus may be marketed and sold for its intended purpose in the United States without the promulgation of a food additive regulation under 21 CFR.

## L-LEUCINE GRAS NOTIFICATION

### I.F Availability of Information

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

Robert G. Bursey, Ph.D.  
President

Ajinomoto Corporate Services LLC  
1120 Connecticut Avenue, N.W., Suite 1010  
Washington, District of Columbia 20036

Telephone: 202-457-0284  
Facsimile: 202-457-0107  
Email: [BurseyB@ajiusa.com](mailto:BurseyB@ajiusa.com)

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

## II. DETAILED INFORMATION ABOUT THE IDENTITY OF THE SUBSTANCE

### II.A Identity

#### II.A.1 Chemical Description

Ajinomoto's L-leucine product comprises not less than 98.5% L-leucine. L-Leucine occurs as white crystals or a crystalline powder with a slightly bitter taste. L-Leucine is freely soluble in formic acid, sparingly soluble in water, and relatively insoluble in ethanol. The solubility of L-leucine in water at varying temperatures, expressed in units of g/100 g of H<sub>2</sub>O are 2.38 at 20°C, 2.63 at 40°C, and 3.19 at 60°C. L-Leucine dissolves in dilute hydrochloric acid.

**Common or Usual Name:** L-Leucine

**Chemical Name:** (2S)-2-amino-4-methylpentanoic acid; L-2-amino-4-methylvaleric acid

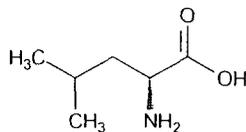
**Chemical Abstracts Service (CAS) Number:** 61-90-5

**Empirical Formula and Formula Weight:** C<sub>6</sub>H<sub>13</sub>NO<sub>2</sub>

**Molecular Weight:** 131.17 g/mol

## L-LEUCINE GRAS NOTIFICATION

### Structural Formula:



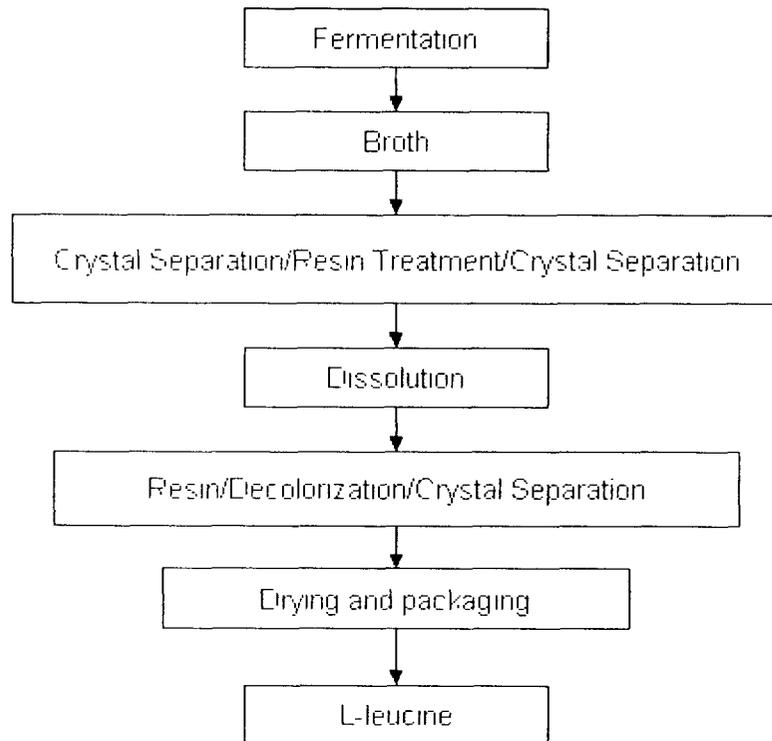
### II.B Method of Manufacture

L-Leucine is obtained *via* fermentation using a bacterial strain derived from *Escherichia coli* (*E. coli*) K-12 by a series of selection steps using conventional mutagenic techniques and amplification of *E. coli* K-12 genes that encode L-leucine biosynthetic enzymes. No foreign DNA is introduced to the bacterial strain.

During the fermentation process, pH conditions (6.0 to 8.0) are maintained by adding ammonia. Once fermentation is complete, the pH of the broth is adjusted to below 5.0 using H<sub>2</sub>SO<sub>4</sub> solution and the broth is sterilized by heating to 120°C for not less than 10 minutes. The microbial cells in the fermented broth are removed by filtration with a microfiltration system and the filtrate undergoes decolorization, resin treatment, and concentration. During this step, the pH of the solution is adjusted to 3 to 5 with NaOH solution, and a defoaming agent is added. Crystallization occurs through cooling and the crystals are separated through centrifugation. The crystals are dissolved in water and the pH is adjusted to below 5 with H<sub>2</sub>SO<sub>4</sub> solution. Next, the solution undergoes decolorization, resin treatment, and concentration. During this step, the pH of the solution is adjusted to below 6. The resulting crystal slurry is cooled, and then centrifuged to separate the crystals. The separated crystals are dried at a temperature of 100 to 150°C to obtain the L-leucine product. A schematic diagram of the manufacturing process for L-leucine product is provided in Figure II.B-1.

## L-LEUCINE GRAS NOTIFICATION

Figure II.B-1 Schematic Overview of the Manufacturing Process for L-Leucine



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

## L-LEUCINE GRAS NOTIFICATION

**Table II.B-1 List of Processing Aids Used in the Manufacture of L-Leucine**

<b>Processing Aids Used in the Manufacture of L-Leucine</b>	<b>Function/Manufacturing Step(s) at which Processing Aid is Used</b>	<b>Reference to Appropriate Use in Food Method of Analysis</b>
Ammonia	pH control agent	No specific regulations pertaining to ammonia, however, various ammonium salts are recognized as direct food substances affirmed as GRAS (21 CFR § 184 1133, 21 CFR § 184 1135, 21 CFR § 184 1137, 21 CFR § 184 1138, 21 CFR § 184 1139, 21 CFR § 184 1140, 21 CFR § 184 1141a, 21 CFR § 184 1141b; 21 CFR § 184 1143, 21 CFR § 184 1296, 21 CFR § 184 1311, 21 CFR § 184 1545; 21 CFR § 184 1588)
Sulfuric Acid (H <sub>2</sub> SO <sub>4</sub> )	pH control agent	21 CFR §184 1095
Sodium hydroxide (NaOH)	pH control agent	21 CFR § 184 1763
Activated carbon	Decolorization	No specific regulations pertaining to activated carbon, however, it is an accepted processing aid in food oil manufacture (GRAS Notice No GRN 000138)
Filters (Microfiltration systems)	Various separation steps throughout processing	21 CFR § 177 2910
Resins	Resin treatment	Permitted under 21 CFR § 173 25
Anti-foaming agent	Added after resin treatment	Permitted under 21 CFR § 173 340

CFR = Code of Federal Regulation, cGMP = current good manufacturing practice, GRAS = Generally Recognized as Safe

## II.C Specifications for Food Grade Material

### II.C.1 Product Specifications and Analysis

The product specifications for L-leucine are presented in Table II.C.1-1, and include physical and chemical specifications. Analyses of representative, non-consecutive lots demonstrated compliance with final product physical and chemical specifications. Refer to Appendix II for methods of analyses.

## L-LEUCINE GRAS NOTIFICATION

**Table II.C.1-1 Product Specifications and Analytical Methods for L-Leucine**

Specification Parameter	Specification	Method of Analysis <sup>a</sup>
<b>Identification Parameters</b>		
Assay	98.5 to 101.0%	AJI TEST 14
pH	5.5 to 7.0	AJI TEST 33
State of solution (transmittance)	Clear and colorless (NLT 98.0%)	AJI TEST 2
<b>Chemical Impurity Specification Parameters</b>		
Chloride (Cl)	NMT 0.020%	AJI TEST 3
Ammonium (NH <sub>4</sub> )	NMT 0.02%	AJI TEST 4
Sulfate (SO <sub>4</sub> )	NMT 0.03%	AJI TEST 5
Iron	NMT 10 ppm	AJI TEST 6
Heavy metals (as lead)	NMT 10 ppm	AJI TEST 7
Related substances <sup>b</sup>	Conforms	AJI TEST 9
Lead (Pb)	NMT 2 ppm	FCC lead limit test
<b>Specific Tests on Identity</b>		
Loss on drying	NMT 0.2%	AJI TEST 11
Specific rotation, $[\alpha]_D^{20}$	+14.6 to +16.5°	AJI TEST 1
Residue on ignition (sulfated)	NMT 0.1%	AJI TEST 13

NMT = not more than, ppm = parts per million

<sup>a</sup> Details of the analytical methods are presented in Appendix II.

<sup>b</sup> Conforms indicates that less than 4 impurity spots (including other amino acids) are detected on thin layer chromatogram and not more than 2% of total impurities are found

L-Leucine is produced under pharmaceutical manufacturing conditions and cGMP, undergoing several filtration steps and a final drying step creating an unfavorable environment to support microbial growth. The contamination risk of microorganisms such as *Salmonella* and *E. coli* would be low. Furthermore, total microorganism screening with a limit of not more than (NMT) 500 CFU/g is performed. If the growth is detected over the limit, the growth is plated and undergoes identification testing.

Several lots of the manufactured product were analyzed to confirm that the manufacturing process produced a consistent product within the physical and chemical parameters of the product specifications. Certificates of analysis for 5 non-consecutive lots of L-leucine are presented in Appendix III.

### II.C.2 Stability

The stability of the L-leucine ingredient that is the subject of the GRAS determination (food-grade L-leucine) has been assessed under accelerated conditions (40 ± 2°C and 75 ± 5% relative humidity) and was shown to be stable for at least 6 months. Furthermore, Ajinomoto's food-grade L-leucine has been tested under ambient conditions (25 ± 2°C and 60 ± 5% relative

## L-LEUCINE GRAS NOTIFICATION

humidity) and was shown to be stable for at least 4 years. Samples were tested for the following parameters: specific rotation, state of solution, ammonium, related substances, loss on drying, purity, and pH. Based on these results, the shelf life of L-leucine was determined to be 4 years.

Stability data for the 6-month study (under accelerated conditions) and 4-year study (under ambient conditions) are presented in Appendix IV.

### III. SELF-LIMITING LEVELS OF USE

The use of L-leucine is limited by its slightly bitter taste. Thus, the use of L-leucine in foods at upper use-levels is largely self-limiting based on its organoleptic properties.

## IV. BASIS FOR GRAS DETERMINATION

### IV.A Introduction

The determination that L-leucine is GRAS is on the basis of scientific procedures. The exposure to L-leucine from the proposed food uses is estimated to be 1.9 g/day at the mean, and 4.1 g/day at the 90<sup>th</sup> percentile. The safety of L-leucine is based on the database of published toxicological and human studies, including studies conducted with Ajinomoto's L-leucine product. In a 13-week rat toxicity study, the highest level studied for Ajinomoto's L-leucine product, 5% mixed into the diet, was established as the no-observed-adverse-effect level (NOAEL). This NOAEL corresponded to a daily intake of approximately 3,333 or 3,835 mg L-leucine/kg body weight/day for female and male rats, respectively, in addition to the amount provided by the basal diet. In a reproductive and developmental study conducted on Ajinomoto's L-leucine product, the highest dose administered by oral gavage, 1,000 mg/kg body weight/day, was established as the NOAEL for female reproductive function and embryo-fetal development. Sakai *et al.* (2004) also demonstrated the capacity of rats to catabolize large amounts of dietary L-leucine. Oxidation of dietary L-leucine only reached a plateau phase at 10% in the diet and a supplemental L-leucine dose of 8,900 mg/kg body weight/day was determined to correspond to the maximal oxidation rate.

The information in the human studies corroborates the safety information provided from animal studies. In healthy subjects, no adverse events have been reported by subjects consuming 45 mg L-leucine/kg body weight/day (equivalent to 2.7 g/day in a 60 kg individual) for a 6-week period. In a recently published study, healthy elderly men supplemented with 2.5 g of L-leucine at each main meal (*i.e.*, 7.5 g/day) for a period of 3 months without any reported adverse effects (Verhoeven *et al.*, 2009). Furthermore, Scarna *et al.* (2003) has reported that no adverse effects are associated with repeated oral administration of up to 60 g/day of branched-chain amino acids (BCAAs) (providing 24 g of L-leucine) in bipolar subjects. The body of evidence on

## L-LEUCINE GRAS NOTIFICATION

oral administration of L-leucine in humans indicates that it can be consumed in considerable amounts without adverse effects. BCAA antagonism would not be expected from the amount of L-leucine estimated to be consumed, as there have been no long-term effects on plasma isoleucine and valine levels following L-leucine consumption at levels similar to the estimated intakes from the proposed food uses.

The weight of the available scientific evidence supports the safety of the proposed uses of Ajinomoto's L-leucine [see Appendix I, **EXPERT PANEL REPORT REGARDING THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF L-LEUCINE FOR USE IN FOODS**].

### IV.B Absorption, Distribution, Metabolism, and Elimination

#### IV.B.1 Absorption and Distribution

L-leucine absorbed from the GI tract into the enterocytes as either free amino acids or as constituents of peptides. Following absorption into enterocytes, L-leucine is transported to the liver, where a portion of the L-leucine is used for protein synthesis (PDRNS, 2008). The remaining L-leucine is distributed via the systemic circulation to various tissues of the body (PDRNS, 2008).

BCAAs and other large neutral amino acids (LNAAs) compete for transport across membranes including the blood-brain barrier; however, there is no evidence in animal studies of neurotoxic effects following long-term L-leucine consumption, nor have there been reports of neurotoxicity in humans receiving oral L-leucine or BCAA supplementation. Although plasma concentrations of most neutral amino acids fluctuate daily due to changes in food consumption, protein synthesis, and hormonal activity, these changes are not accompanied by equal changes in brain amino acid levels under most physiological conditions. For example, altering the protein content of diets fed to rats resulting in serum L-leucine levels that varied by 7-fold led to changes in brain L-leucine concentrations of less than 2-fold (Glaeser *et al.*, 1983; Peters and Harper, 1985). Neurological effects resulting from L-leucine consumption under the intended conditions of use would not be expected because brain function is protected from the large variation in nutrients consumed as a result of the diversity in human diets and dietary intakes.

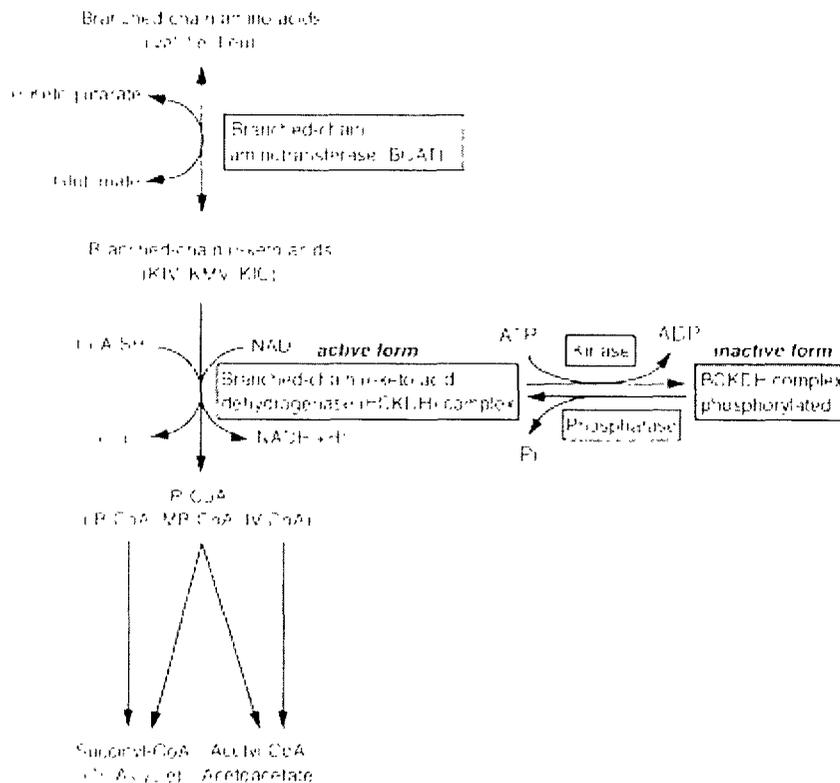
#### IV.B.2 Metabolism

In humans, the majority of L-leucine metabolism occurs in the skeletal muscle (PDRNS, 2008). L-Leucine undergoes reversible transamination, catalyzed by branched-chain aminotransferase (BCAT), to yield *alpha*-ketoisocaproate (KIC) (IOM, 2005). This product then undergoes an irreversible oxidative decarboxylation by branched-chain *alpha*-ketoacid dehydrogenase (BCKDH) to produce isovaleryl CoA (IV-CoA), an acyl CoA derivative (Harper *et al.*, 1984; IOM, 2005). This undergoes further metabolism through several steps, eventually resulting in the

## L-LEUCINE GRAS NOTIFICATION

production of acetoacetate and acetyl CoA, which can be used as a source of energy *via* the pathways of oxidative metabolism (Harper *et al.*, 1984; IOM, 2005, PDRNS, 2008) The catabolic pathway is presented in Figure IV.B.2-1.

**Figure IV.B.2-1 BCAA Catabolic Pathway (reproduced from Brosnan and Brosnan, 2006)**



As shown in Figure IV.B.2-1, BCKDH activity is regulated by phosphorylation and dephosphorylation. Phosphorylation of BCKDH by BCKDH kinase inhibits its activity; this is reversed by dephosphorylation by BCKDH phosphatase. BCKDH kinase is inhibited by KIC and other branched-chain keto acids (*i.e.*, the substrates for BCKDH), allowing for enhanced BCAA metabolism when they are present in excess amounts and decreased metabolism when they need to be conserved.

The findings that reserve BCKDH can be activated by dephosphorylation are important, particularly for tissues with low basal BCKDH activity. BCKDH in skeletal muscle has been reported to be only 7% active (dephosphorylated) in rats (Suryawan *et al.*, 1998) and less than

## L-LEUCINE GRAS NOTIFICATION

5% active<sup>1</sup> in mice (Joshi *et al.*, 2006) (Table IV B.2-1), indicating that the skeletal muscle of rodents has a considerable reserve for BCAA metabolism since the majority of the BCKDH complex is present in the inactive (phosphorylated) form. Moreover, the skeletal muscle accounts for 35 to 45% of the total body weight and therefore, any small change in the degree of BCKDH activation would have a marked effect on BCAA metabolism (Harper *et al.*, 1984). In contrast, liver BCKDH is already 88% active (dephosphorylated) in rodents (Table IV.B.2-1) and thus activity is already near-maximal (*i.e.*, it cannot be increased to a significant extent). In humans, both skeletal muscle and liver BCKDH are less than 30% active; therefore, metabolism of BCAA in these tissues can be greatly increased over basal levels to deal with excess BCAA.

The activities of BCKDH in various rodent and human tissues are presented in Table IV.B.2-1.

**Table IV.B.2-1 BCKDH Activity in Selected Rodent<sup>a</sup> and Human Tissues**

Tissue	% Active in Rodent <sup>b</sup>	% Active in Human <sup>b</sup>
Skeletal muscle	5 to 7	26
Liver	88	28
Kidney	76	14
Brain	77	59

<sup>a</sup> All rodent tissue measurements were conducted in rats except for skeletal muscle measurements, which were conducted in rats (7% active) and mice (<5% active)

<sup>b</sup> % activity was calculated as follows: actual (basal activity)/total activity x 100%, where total activity was measured after full activation of the enzyme by phosphatase treatment

When considering overall body distribution of BCAA metabolic enzymes, it has been shown that the majority of BCAT activity in rats and humans occurs in the skeletal muscle (81.6 and 65.4%, respectively), whereas the distribution of BCKDH activity differs between species (Suryawan *et al.*, 1998). When BCKDH is fully activated (*i.e.*, through treatment with phosphatase), skeletal muscle contributes to 66% of total BCKDH activity in humans but only to 29% of that in rats. Sixty percent of total BCKDH activity in rats occurs in the liver. Therefore, the *alpha*-ketoacid produced from transamination in the skeletal muscle of rats must be transported to the liver *via* the circulation for oxidation (potentially exposing all organs to it), while in humans, both transamination and oxidation occur primarily in the skeletal muscle.

The overall capacity for metabolizing dietary L-leucine is high, as demonstrated in a rat study in which oxidation of dietary L-leucine only reached a plateau phase at 10% (w/w) in the diet and a supplemental L-leucine dose of 8.9 g/kg body weight/day was determined to correspond to the maximal oxidation rate (Sakai *et al.*, 2004). Moreover, as discussed above, reserve metabolic

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<sup>1</sup> Percentage activity was calculated by dividing the actual (basal) activity by the total activity (measured after full activation of the enzyme by phosphatase treatment), and multiplying this result by 100%.

## L-LEUCINE GRAS NOTIFICATION

capacity can be activated, allowing for enhanced catabolism of L-leucine when present in excess quantities (Brosnan and Brosnan, 2006).

Suryawan *et al.* (1998) suggested that studies in rodents may underestimate the potential toxicity of BCAAs in humans because total activities of BCAT and BCKDH were determined to be higher in rats than in humans (respective enzyme capacities determined for the skeletal muscle, kidney, liver, brain, and stomach+intestine tissues were summed), however, the findings of toxicological studies conducted in rats, in which no adverse effects attributable to L-leucine were reported at levels of 5% (w/w) in the diet (equivalent to approximately 3 g/kg body weight/day), are corroborated by human studies that included oral administration of L-leucine at doses ranging from 7 to 200 mg/kg body weight/day, including 2 long-term studies in which no compound-related adverse effect were reported following daily consumption of 2.7 and 7.5 g L-leucine (Crowe *et al.*, 2006, Verhoeven *et al.*, 2009). Based on the safe consumption of supplemental L-leucine at high doses in humans, it is evident that humans have the capacity to metabolize large quantities of exogenous L-leucine; therefore, despite the differences between rats and humans in metabolic capacity, toxicological studies in rats are considered to be relevant to safety in humans.

### IV.B.3 Excretion

L-Leucine is degraded by deamination (*i.e.*, removal of the amino group). The amino portions are captured as urea *via* the Krebs-Henseleit cycle in the liver and urea is excreted in urine by the kidneys (IOM, 2005). The nitrogen-free portion (deaminated amino acid) enters the metabolic pathway and forms acetyl CoA, which can be used as a source of energy *via* the pathways of oxidative metabolism.

## IV.C Toxicological Studies

### IV.C.1 Repeated Dose Studies

A published subchronic (13-week) toxicity study of Ajinomoto's L-leucine product was conducted in male and female Sprague-Dawley rats (Tsubuku *et al.*, 2004). This study was conducted in compliance with the *Good Laboratory Practice Standards for Safety Studies on Drugs* (Notification N. 313, March 31, 1982) and *Guidelines for Toxicity Studies Required for Applications for Approval to Manufacture (Import) Drugs* (Ordinance N.1, Article N.24, September 11, 1989). Groups of 12 male and 12 female 6-week-old rats were provided a standard diet (CRF-1) supplemented with 0, 1.25, 2.5, or 5% (w/w) L-leucine *ad libitum* for a period of 13 weeks (designated as control and low-, mid-, and high-dose groups, respectively). A 5-week recovery period was included wherein 6 rats/sex randomly selected from the control and high-dose groups were provided the control diet. The average intakes of L-leucine for the low-, mid-, and high-dose groups during the study were 832.6, 1,660, and 3,332.9 mg/kg body weight/day for male rats and, 961.0, 1,904.8, and 3,835.2 mg/kg body weight/day for female

## L-LEUCINE GRAS NOTIFICATION

rats, respectively. The amount provided by the control CRF-1 diet was not provided. Unless selected for the recovery period, all animals were killed after 13 weeks of treatment while animals in the recovery groups were killed after the recovery period (*i.e.*, at Week 18).

No deaths or compound-related clinical signs of toxicity were observed during the experimental and recovery periods. The body weights of all treatment animals were comparable to the control group throughout the study. Although feed consumption for males of the mid-dose group was significantly increased at Days 45 and 59 relative to the control group, changes at other time-points were not observed and the total diet consumption at the end of the treatment period was not significantly different between groups. No significant differences in water consumption were noted among the groups. Ophthalmologic examination at the end of the administration period did not reveal any test substance-related effects. No significant differences in quantitative and qualitative urinalysis parameters were observed between control and treatment groups and no compound-related changes in hematology and blood chemistry endpoints were observed. The adrenal gland absolute weight of mid-dose group female rats was significantly increased relative to the control group, however, this change was not considered toxicologically significant as it was not dose-dependent. At the end of the recovery period, the pituitary absolute weight of the high-dose females was significantly increased; however, this finding was not considered toxicologically significant since no difference was noted at the end of the 13-week treatment period. No other significant differences in organ weights were reported and no compound-related macroscopic and microscopic findings were observed. The NOAEL for L-leucine was determined to be 5% in the diet (approximately 3,333 and 3,835 mg/kg body weight/day in males and females, respectively), the highest dose tested in this study.

Results from additional rodent studies on L-leucine, which were not conducted primarily to assess safety but included measurements of some safety-related endpoints, support the safety of L-leucine under the intended conditions of use. In these studies, L-leucine was consumed in the diet or drinking water at levels ranging from 0.1 to 14 g/kg body weight/day for 10 days to 14 weeks (Lynch *et al.*, 2002; Matsuzaki *et al.*, 2005; Donato *et al.*, 2006; Sugawara *et al.*, 2007; Zhang *et al.*, 2007). In one study, body weight gain was significantly lower in rats fed diets containing 15 or 30% (w/w) L-leucine (equivalent to approximately 11 and 14 g/kg body weight/day, respectively) compared to control animals; however, such effects did not occur at levels up to 10% (equivalent to approximately 7 g/kg body weight/day).

### IV.C.2 Reproductive and Developmental Toxicity Studies

In a published reproductive and developmental toxicity study conducted in compliance with the *Standard for Conduct of Non-Clinical Studies on Safety of Drugs* (Ordinance No. 21 of the Japanese Ministry of Health and Welfare, 1997) and the *Revision of the Guideline for Reproductive and Developmental Toxicity Studies of Drugs* (Notification No. 316 of the

## L-LEUCINE GRAS NOTIFICATION

Japanese Ministry of Health and Welfare, 1997), 20 pregnant female Sprague-Dawley SPF rats per group, 11 to 12 weeks old, were administered 0 (control), 300, or 1,000 mg L-leucine (provided by Ajinomoto)/kg body weight/day from gestational days (GDs) 7 to 17 by oral gavage (Mawatari *et al* , 2004). Rats were fed a standard solid feed (NMF), which typically contains 29.1% protein, *ad libitum* (Nagura *et al* , 1996). The presence of a vaginal plug or sperm on a vaginal smear was designated as GD 0. Fetal examinations were conducted immediately following Caesarean section on GD 20.

No deaths and no changes in the general condition of the dams were reported in any of the groups. Maternal body weights did not significantly differ among groups, while feed intake was higher in the high-dose group compared to the control group on GDs 14 and 18. The authors noted that increased feed intake in the high-dose group likely resulted from the large doses of L-leucine, which "promoted the consumption of other essential amino acids, leading to a negative nitrogen balance (Harper *et al.*, 1984; May *et al.*, 1991) compensated for by the enhanced feed intake." Macroscopic examination of the major intra-thoracic or intra-abdominal viscera and tissues, as well as intra-uterine analysis conducted during Caesarean section, did not reveal any differences among groups.

Fetal body weights and abnormalities (external, visceral, and skeletal) were not significantly different between the treated and control groups. Additionally, there were no changes in gender ratio and no gender-specific alterations in fetal body weight and fetal abnormalities. The NOAEL for female reproductive function and embryo-fetal development was concluded to be 1,000 mg/kg body weight, the highest dose assessed in the study.

Matsueda and Niiyama (1982) also conducted 2 experiments that examined the potential effect of excess amino acids on maintenance of pregnancy and fetal growth; however, as the pregnant rats were not provided with adequate protein in the diet (6% casein), the relevance of the study findings to the safety of L-leucine under the intended conditions of use is highly limited.

### IV.C.3 Genotoxicity and Carcinogenicity Studies

In a mutagenesis assay conducted by Sargentini and Smith (1986), L-leucine at a concentration of 2 mM (234 µg/mL) was not mutagenic in *E. coli* strains *uvrB*, *uvrB umu C*, and *uvrB LexA* in the absence of metabolic activation. In another study, L-leucine at concentrations of 10, 50, or 100 mg/mL produced slight, yet significant increases in sister chromatid exchanges (SCEs) in human lymphocytes compared to a control group (Xing and Na, 1996). The authors suggested that the elevated frequencies of SCE were considered to be metabolic rather than genotoxic responses, since the effect was not dose-dependent.

Two carcinogenicity studies were identified wherein rats exposed to low doses of N-butyl-N (4-hydroxybutyl) nitrosamine (BHBN), a known initiator of urinary bladder carcinoma, were given

## L-LEUCINE GRAS NOTIFICATION

diets supplemented with 2 or 4% (w/w) L-leucine (approximately 1,000 and 2,000 mg/kg body weight/day, respectively (U.S. FDA, 1993) for 40 or 60 weeks (Kakizoe *et al.*, 1983; Nishio *et al.*, 1986). Increased incidence and number of carcinomas were reported in the group treated with BHBN and L-leucine compared to the group treated with BHBN alone; however, no lesions in the urinary bladder were reported in the group receiving diets supplemented with 2 or 4% L-leucine without BHBN. These findings are of limited relevance in the safety evaluation of L-leucine because tumor promotion is a complex, incompletely understood phenomenon and it should be noted that tumor promotion studies were neither designed nor have been validated for the purposes of hazard assessment (Kraus *et al.*, 1995).

Wakshlag *et al.* (2006) assessed the ability of BCAAs to augment or diminish cell growth in neoplastic cell lines. Three canine cell lines (osteosarcoma, bronchoalveolar carcinoma, and Madine-Darby kidney cells) were incubated with media containing 0 (control), 5, 10, 50, or 100 mM of leucine, isoleucine, or valine and cell proliferation after 48 hours of treatment was analyzed. All the BCAAs significantly diminished cell growth in all 3 cell lines at the highest concentration tested (100 mM). At lower concentrations of 10 and 50 mM, leucine suppressed proliferation the most relative to valine and isoleucine. No significant differences in cell proliferation were noted at the lowest concentration studied (5 mM). The results of the study suggest that BCAA treatment does not potentiate growth of neoplastic cells and in fact, may diminish neoplastic cell proliferation at high concentrations.

### IV.D Human Studies

A limited number of studies examining oral L-leucine supplementation in humans were identified in the scientific literature (Swendseid *et al.*, 1965; Mero *et al.*, 1997, 2009; Pitkänen *et al.*, 2003; Crowe *et al.*, 2006; Verhoeven *et al.*, 2009). The majority of the studies did not include assessment of safety-related endpoints, but rather focused on the effects of acute and prolonged dietary intake of L-leucine in individuals undergoing exercise training; however, the information in the human studies corroborates the safety information provided from animal studies.

In healthy subjects, no adverse events have been reported by subjects consuming 45 mg L-leucine/kg body weight/day (equivalent to 2.7 g/day in a 60 kg individual) for a 6-week period (Crowe *et al.*, 2006). In a recent study, healthy elderly men were supplemented with 2.5 g of L-leucine at each main meal (*i.e.*, 7.5 g/day) for a period of 3 months without any reported adverse effects (Verhoeven *et al.*, 2009). Furthermore, Scarna *et al.* (2003) has reported that no adverse effects are associated with repeated oral administration of up to 60 g/day of BCAAs (providing 24 g/day L-leucine) in bipolar subjects. The body of evidence on oral administration of L-leucine in humans indicates that it can be consumed in considerable amounts without adverse effects.

## L-LEUCINE GRAS NOTIFICATION

### IV.E Other Considerations

#### IV.E.1 Safety of the Fermentation Microorganism

*E. coli* K-12 was first isolated in 1922 and has been widely utilized for genetic experiments for over 50 years (Gorbach, 1978). It is a Gram-negative, rod-shaped bacterium that is impaired in most, if not all, of the characteristics of a pathogenic microorganism (Gorbach, 1978)

Results from several human studies demonstrate the inability of *E. coli* K-12 to colonize the intestine (Anderson, 1975; Gorbach, 1978; Smith, 1978, Levy *et al.*, 1980). Similar findings were reported in studies conducted on various laboratory animals, including mice, rats, chickens, pigs, and calves (Curtiss, 1978; Freter, 1978; Levy *et al.*, 1980; Muth *et al.*, 1993). Studies in humans also indicate that plasmids in *E. coli* K-12 are not transferred to other microorganisms within the intestinal flora (Anderson, 1978; Smith, 1978; Levy *et al.*, 1980)

In the U.S., chymosin preparation derived, *via* fermentation, from a nonpathogenic and nontoxic strain of *E. coli* K-12 containing the prochymosin gene, is permitted for use as a direct food substance affirmed as GRAS (21 CFR 184.1685) (U.S. FDA, 2009).

The FDA indicated that it had no questions in response to a GRAS Notice for *alpha*-cyclodextrin (GRN 000155), produced using an enzyme obtained from a recombinant strain of *E. coli* K-12, harboring the cyclodextringlycosyltransferase gene of *Klebsiella oxytoca* (U.S. FDA, 2004)

#### IV.E.2 Potential Antagonism of Other Amino Acids

In animals, L-Leucine has been shown to have antagonistic effects on other amino acids in the presence of a low protein diet (9% casein). This is evidenced by decreased plasma levels of various amino acids, including BCAAs and their metabolites (keto acids) in rats or pigs administered L-leucine (orally *via* the diet or by gavage or by infusion) at levels providing 104 to 5,000 mg/kg body weight (Block and Harper, 1984; Escobar *et al.*, 2007). In an infusion study conducted in fasted 7- or 26-day-old pigs, the co-infusion of 52 mg/kg body weight/hour of L-leucine with a replacement amino acid mixture (without L-leucine) for 2 hours largely prevented the decreases in plasma amino acid levels induced by L-leucine administration alone (Escobar *et al.*, 2007).

Findings from some early studies also indicate that oral L-leucine supplementation (1.0 to 5% L-leucine in the diet but not 0.5%) in rats fed a low-protein diet (9% casein) resulted in growth depression (Harper *et al.*, 1954, 1955; Benton *et al.*, 1956; Rogers *et al.*, 1967). The growth-suppressing effects of L-leucine could be largely or fully prevented by the addition of amino acids (isoleucine, valine, phenylalanine, tryptophan, and threonine) or protein to the diet

## L-LEUCINE GRAS NOTIFICATION

Results from more recently published, long-term studies conducted to assess the safety of oral L-leucine have demonstrated a lack of effect on growth when supplementary L-leucine was given at levels of up to approximately 6 g/kg body weight/day in conjunction with adequate dietary protein for periods ranging from 10 days to 13 weeks (Lynch *et al.*, 2002; Tsubuku *et al.*, 2004; Donato *et al.*, 2006; Sugawara *et al.*, 2007; Zhang *et al.*, 2007). Although a significant decrease in body weight was observed in Fischer rats given diets containing 15 or 30% L-leucine (equivalent to 11,250, or 14,167 mg/kg body weight/day, respectively) in comparison to an unsupplemented (control) diet, body weights were unaffected in rats receiving 1.5, 5, or 10% L-leucine (equivalent to 1,071, 3,690, or 7,183 mg/kg body weight/day, respectively) in the diet compared the control diet (Matsuzaki *et al.*, 2005). Similarly, Sakai *et al.* (2004) reported growth suppression in Fischer rats fed diets supplemented with 15% L-leucine (approximately 12.4 g/kg body weight; duration not reported); however, no statistically significant changes in body weight were reported when rats were fed diets supplemented with 10% L-leucine (approximately 8.2 g/kg body weight). The levels of L-leucine that induced growth suppression in these studies are far higher than the estimated intakes of L-leucine from the proposed food uses (all-user total population intakes of 28 and 64 mg/kg body weight/day at the mean and 90<sup>th</sup> percentile, respectively).

Based on the results from animal studies that indicated leucine-induced growth suppression (attributed to antagonism of other amino acids) does not occur in the presence of adequate protein, daily protein intakes for the users of the L-leucine products under the proposed conditions of use were calculated. In particular, protein intakes for the users of foods intended for weight reduction were compared to those in the total U.S. population. Protein intakes were shown to be similar between the groups, indicating that consumers of the foods intended for weight reduction proposed to contain added L-leucine will consume adequate levels of protein in the diet. Results of these analyses are presented in Tables IV.E.2-1 and IV.E.2-2 below.

## L-LEUCINE GRAS NOTIFICATION

**Table IV.E.2-1 Summary of the Estimated Daily Intake of Protein in Identified Consumers of Foods Intended for Weight Reduction in which Leucine is Proposed for Use versus the U.S. Population (2005-2006 NHANES Data)**

Population Group	Age Group (Years)	# of Users	Protein Consumption in Identified Consumers of Foods Intended for Weight Reduction (g)		Protein Consumption in the U.S. Population (g)	
			Mean	90 <sup>th</sup> Percentile	Mean	90 <sup>th</sup> Percentile
Infants	0 to 2	4	54	59	48	66
Children	3 to 11	10	68	94	70	103
Female Teenagers	12 to 19	9	83	95	72	124
Male Teenagers	12 to 19	13	198	313	103	169
Female Adults	20 and Up	89	80	125	79	123
Male Adults	20 and Up	53	131	219	112	169
Total Population	All Ages	178	101	153	93	148

**Table IV.E.2-2 Summary of the Estimated Daily per Kilogram Body Weight Intake of Protein in Identified Consumers of Foods Intended for Weight Reduction in Which Leucine is Proposed for Use versus the U.S. Population (2005-2006 NHANES Data)**

Population Group	Age Group (Years)	# of Users	Protein Consumption in Identified Consumers of Foods Intended for Weight Reduction (mg/kg)		Protein Consumption in the U.S. Population (mg/kg)	
			Mean	90 <sup>th</sup> Percentile	Mean	90 <sup>th</sup> Percentile
Infants	0 to 2	4	4,032	5,125	3,912	5,812
Children	3 to 11	10	2,752	4,534	2,330	3,659
Female Teenagers	12 to 19	9	1,316	1,494	1,226	1,968
Male Teenagers	12 to 19	13	3,076	5,131	1,571	2,598
Female Adults	20 and Up	89	1,201	1,794	1,145	1,781
Male Adults	20 and Up	53	1,553	2,561	1,315	2,185
Total Population	All Ages	178	1,442	2,743	1,492	2,649

In humans, study findings indicate that generally, oral L-leucine supplementation does not chronically deplete circulating BCAA levels at the levels expected to be consumed from the proposed food uses. In a 6-week study in which subjects received 45 mg L-leucine/kg body weight/day (equivalent to 2.7 g/day in a 60 kg adult), no significant differences in isoleucine or valine levels were noted between pre- and post-supplementation (Crowe *et al.*, 2006). Pitkänen *et al.* (2003) reported that decreases in post- versus pre-exercise serum isoleucine and valine concentrations were significantly greater following the consumption of 100 or 200 mg L-leucine/kg body weight (equivalent to 6 or 12 g in a 60 kg individual) in comparison to placebo

## L-LEUCINE GRAS NOTIFICATION

ingestion prior to intense exercise sessions. In another study, a bolus oral dose of L-leucine (2 or 10 g) resulted in decreased plasma isoleucine and valine levels 2 and 4 hours after consumption compared to baseline values (Swendseid *et al.*, 1965); however, no control group was included in this study. The effects observed in the Pitkänen *et al.* (2003) and Swendseid *et al.* (1965) studies were likely to be transient, as long-term effects on isoleucine and valine levels were not observed in the 6-week study reported by Crowe *et al.* (2006). Moreover, the proposed food uses that will provide the largest bolus doses of L-leucine (non-milk based and milk-based meal replacement beverages and meal replacement bars; 3 g/serving) also will contain additional sources of protein, and results from animal studies indicate that L-leucine-induced growth suppression (attributed to antagonism of other amino acids) does not occur in the presence of adequate protein. Moreover, as demonstrated in Tables IV.E.2-1 and IV.E.2-2, daily protein intakes in the identified users of foods in which L-leucine is proposed for use (particularly foods intended for weight reduction) are similar to daily protein intakes in the total U.S. population, indicating that adequate protein will be consumed. Based on the overall results of the above-described studies, BCAA antagonism would not be expected from the amount of L-leucine estimated to be consumed.

In a 3-month study, the fasting plasma valine levels of 15 healthy elderly men supplemented with 2.5 g of L-leucine at each main meal (*i.e.*, 7.5 g/day) decreased significantly by approximately 25% (*vs.* basal) within 2 weeks of treatment (Verhoeven *et al.*, 2009). The study authors indicated that the clinical significance of this decline in plasma valine concentrations "remains debatable" because the plasma concentrations did not decline further after 2 weeks and remained within a normal physiologic range. Plasma isoleucine levels did not change significantly over the course of the study. No adverse effects were reported in this study. The dose of L-leucine that resulted in a decrease in valine concentrations in this study is higher than the estimated intakes of L-leucine from the proposed food uses (all-user total population intakes of 1.9 and 4.1 g/day at the mean and 90<sup>th</sup> percentile, respectively).

### IV.F Summary and Basis for GRAS Conclusion

The GRAS determination for the use of L-leucine as a food ingredient is based on scientific procedures. L-Leucine is proposed for use as an ingredient in milk and non-milk based meal replacements, sports and isotonic beverages, vitamin enhanced waters, and meal replacement bars, at levels of up to 3.0 g/serving. Under the intended conditions of use, the estimated all-user mean and 90<sup>th</sup> percentile intakes of supplemental L-leucine in the total population are 1.9 and 4.1 g/day, respectively (28 and 64 mg/kg body weight/day, respectively).

L-leucine is produced in accordance with cGMP and meets appropriate food-grade specifications. L-Leucine is produced *via* fermentation using a bacterial strain derived from *E. coli* K-12 by a series of selection steps using conventional mutagenic techniques and amplification of the genes encoding L-leucine biosynthetic enzymes from *E. coli*. Following

## L-LEUCINE GRAS NOTIFICATION

fermentation, the crude L-leucine crystals that are produced undergo numerous purification processes, including decolorization, concentration, crystallization, ion exchange, and filtration. These processes remove all fermentation media components and processing aids, resulting in a highly pure ( $\geq 98.5\%$  L-leucine) final product. Ajinomoto has established chemical and microbiological specifications consistent with other food-grade materials. Lots sample are routinely evaluated to verify compliance with the specifications.

The safety of L-leucine under the intended conditions of use is supported by the available toxicological and human studies, including studies conducted with Ajinomoto's L-leucine product (detailed in Sections IV.B through IV.F). In a 13-week rat toxicity study, the highest level studied for Ajinomoto's L-leucine product, 5% mixed into the diet, was established as the NOAEL. This NOAEL corresponded to a daily intake of 3,333 or 3,835 mg L-leucine/kg body weight/day for female and male rats, respectively, in addition to the amount provided by the basal diet. In a reproductive and developmental study conducted on Ajinomoto's L-leucine product, the highest dose administered by oral gavage, 1,000 mg/kg body weight/day, was established as the NOAEL for female reproductive function and embryo-fetal development. Sakai *et al.* (2004) also demonstrated the metabolic capacity of rats to catabolize large amounts of dietary L-leucine. Oxidation of dietary L-leucine only reached a plateau phase at 10% in the diet and a supplemental L-leucine dose of 8,900 mg/kg body weight/day was determined to correspond to the maximal oxidation rate.

The information in the human studies corroborates the safety information provided from animal studies. In healthy subjects, no adverse events were reported by subjects consuming 45 mg L-leucine/kg body weight/day (equivalent to 2.7 g/day in a 60 kg individual) for a 6-week period. In a more recent investigation, healthy elderly men supplemented with 2.5 g of L-leucine at each main meal (*i.e.*, 7.5 g/day) for a period of 3 months without any reported adverse effects (Verhoeven *et al.*, 2009). Furthermore, no adverse effects have been associated with repeated oral administration of up to 60 g/day of BCAAs (providing 24 g L-leucine) in bipolar subjects (Scarna *et al.*, 2003). The body of evidence on oral administration of L-leucine in humans indicates that it can be consumed in considerable amounts without adverse effects. BCAA antagonism would not be expected from the amount of L-leucine estimated to be consumed, as there have been no long-term effects on plasma isoleucine and valine levels following L-leucine consumption at levels similar to the estimated intakes from the proposed food uses.

The data provided support the conclusion that the consumption of added L-leucine under the intended conditions of use would not be expected to produce adverse effect in consumers.

The Expert Panel convened on behalf of Ajinomoto, independently and collectively, critically evaluated the data and information summarized above and concluded that the proposed uses of L-leucine, produced consistently with cGMP and meeting appropriate food grade specifications described herein, are safe and suitable. Furthermore, the Expert Panel unanimously concluded

## L-LEUCINE GRAS NOTIFICATION

that the intended uses of L-leucine are Generally Recognized as Safe (GRAS) based on scientific procedures. It is also Ajinomoto's opinion that other qualified and competent scientists reviewing the same publicly available toxicological and safety information would reach the same conclusion. Therefore, Ajinomoto has concluded that L-leucine is GRAS under the intended conditions of use on the basis of scientific procedures. L-Leucine is GRAS based on scientific procedures for its proposed uses in food; therefore, it is excluded from the definition of a food additive and thus may be marketed and sold for the uses designated above in the U.S. without the promulgation of a food additive regulation under 21 CFR

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## L-LEUCINE GRAS NOTIFICATION

Table of CFR Sections Referenced (Title 21—Food and Drugs)		
Part	Section §	Section Title
170—Food additives	170.35	Affirmation of generally recognized as safe (GRAS) status
172—Food additives permitted for direct addition to food for human consumption	172.320	Amino acids
	172.804	Aspartame
	172.829	Neotame
184—Direct food substances affirmed as generally recognized as safe	184.1685	Rennet (animal-derived) and chymosin preparation (fermentation-derived)

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**APPENDIX I**

**EXPERT PANEL REPORT REGARDING THE GENERALLY RECOGNIZED AS  
SAFE (GRAS) STATUS OF L-LEUCINE FOR USE IN FOODS**

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# **EXPERT PANEL CONSENSUS STATEMENT REGARDING THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF L-LEUCINE FOR USE IN FOODS**

June 23, 2009

## **INTRODUCTION**

At the request of Ajinomoto Co., Inc. (Ajinomoto), an Expert Panel (the "Panel") of independent scientists, qualified by their relevant national and international experience and scientific training to evaluate the safety of food ingredients, was specially convened to conduct a critical and comprehensive evaluation of the available pertinent data and information, and determine whether the intended uses of L-leucine as a food ingredient are safe and suitable and would be Generally Recognized as Safe (GRAS) based on scientific procedures. The Panel consisted of: Dr. John Fernstrom, Ph.D. (University of Pittsburgh School of Medicine), Dr. Donald Layman, Ph.D. (University of Illinois), Dr. Ian Munro, Ph.D. (Cantox Health Sciences International), and Dr. William Waddell, M.D. (University of Louisville).

The Panel, independently and collectively, critically examined a comprehensive package of scientific information and data on L-leucine from the literature and other published sources through June 2009 compiled by Cantox Health Sciences International (Cantox) and Ajinomoto. In addition, the Panel evaluated other information deemed appropriate or necessary. The information evaluated by the Panel included details pertaining to the method of manufacture and product specifications, supporting analytical data, intended use-levels in specified food products, consumption estimates for all intended uses, and a comprehensive assessment of the available scientific literature pertaining to the safety of L-leucine.

Following independent, critical evaluation of such data and information, the Panel convened on 23 June 2009 and unanimously concluded that the intended uses in traditional foods described herein for L-leucine, meeting appropriate food-grade specifications as described in the supporting dossier [Documentation Supporting the Evaluation of L-Leucine as Generally Recognized as Safe (GRAS) for Use as a Food Ingredient] and manufactured according to current Good Manufacturing Practice (cGMP), are safe and suitable and GRAS based on scientific procedures. A summary of the basis for the Panel's conclusion is provided below.

## SUMMARY

L-Leucine is 1 of 9 amino acids that cannot be endogenously synthesized and thus is an essential amino acid. It is one of 3 branched-chain amino acids (BCAAs), along with isoleucine and valine. L-Leucine is permitted for use as a special dietary and nutritional additive (21 CFR 172.320). The amount of L-leucine added for nutritive purposes plus the amount naturally present in free and combined (as protein) form is not to exceed 8.8% by weight of the total protein of the finished food. L-Leucine also may be used as a lubricant in the manufacture of aspartame or neotame tablets for sweetening hot beverages at levels not to exceed 3.5% of the tablet weight (21 CFR 172.804; 21 CFR 172.829). Additionally, L-Leucine is deemed to be GRAS by the Flavor and Extract Manufacturers' Association (FEMA). L-Leucine occurs in the diet as a component of protein. Dairy products are a particularly rich source of L-leucine (USDA, 2008). The mean and 90<sup>th</sup> percentile total population background dietary intakes of L-leucine in the U.S. are estimated to be 6.08 and 8.90 g/person/day, respectively (IOM, 2005). The highest intakes of L-leucine are reported in men ages 51 to 70 years (14.1 g/person/day at the 90<sup>th</sup> percentile) (IOM, 2005).

L-Leucine is manufactured by Ajinomoto *via* fermentation using a bacterial strain derived from *Escherichia coli* K-12 by a series of selection steps using conventional mutagenic techniques and amplification of the genes encoding L-leucine biosynthetic enzymes from *E. coli*. *E. coli* K-12 is considered to be non-pathogenic and non-toxicogenic. L-leucine is manufactured in accordance with current Good Manufacturing Practice and meets appropriate food-grade specifications.

L-Leucine is intended for use as an ingredient in non-milk and milk based meal replacements, sports and isotonic beverages, vitamin enhanced waters, and meal replacement bars, at levels of up to 3.0 g/serving. Estimates for the intake of added L-leucine were based on the proposed food-uses and use-levels in conjunction with food consumption data included in the National Center for Health Statistics' (NCHS) 2005-2006 National Health and Nutrition Examination Surveys (NHANES) (CDC, 2006; USDA, 2009). The total population daily exposure to added L-leucine from the proposed food uses is estimated to be 1.9 g/day at the mean, and 4.1 g/day at the 90<sup>th</sup> percentile.

The safety of L-leucine is supported by the available toxicological and human studies, including studies conducted with Ajinomoto's L-leucine product. In a 13-week rat toxicity study, the highest level studied for Ajinomoto's L-leucine product, 5% mixed into the diet, was established as the no-observed-adverse-effect level (NOAEL). This NOAEL corresponded to a daily intake of 3,333 or 3,835 mg L-leucine/kg body weight/day for female and male rats, respectively, in addition to the amount provided by the basal diet. In a reproductive and developmental study conducted on Ajinomoto's L-leucine product, the highest dose administered by oral gavage, 1,000 mg/kg body weight/day, was established as the NOAEL for female reproductive function

000037

and embryo-fetal development. Sakai *et al* (2004) also demonstrated the metabolic capacity of rats to catabolize large amounts of dietary L-leucine. Oxidation of dietary L-leucine only reached a plateau phase at 10% in the diet and a supplemental L-leucine dose of 8,900 mg/kg body weight/day was determined to correspond to the maximal oxidation rate.

The information in the human studies corroborates the safety information provided from animal studies. In healthy subjects, no adverse events have been reported by subjects consuming 45 mg L-leucine/kg body weight/day (equivalent to 2.7 g/day in a 60 kg individual) for a 6-week period. Furthermore, no adverse effects have been associated with chronic oral administration of up to 60 g/day of BCAAs (providing 24 g L-leucine) in individuals with various disease states including phenylketonuria, hepatic cirrhosis, and neurological diseases. The body of evidence examined by the Panel indicates that consumption of added L-leucine under the intended conditions of use would not be expected to produce adverse effects in consumers.

## CONCLUSION

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

We further conclude that the intended uses of L-leucine, meeting appropriate food-grade specifications presented in the supporting dossier and produced consistent with current GMP, 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)

John Fennstrom, Ph.D.  
University of Pittsburgh School of Medicine

06/23/09  
Date

(b) (6)

Donald Layman, Ph.D.  
University of Illinois

6/23/09  
Date

(b) (6)

Ian Munro, Ph.D.  
Cantox Health Sciences International

6/23/09  
Date

(b) (6)

William Waddell, M.D.  
University of Louisville

6/23/09  
Date

000039

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**APPENDIX II**  
**METHODS OF ANALYSES**

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Pages 000042 - 000050 have been removed in accordance with copyright laws. Please see appended bibliography list of the references that have been removed from this request.

**CERTIFICATE OF ANALYSIS**

**ANALYTICAL RESULTS OF:** L-LEUCINE

**LOT N°:** 08 0061 M 04 CA J82LH009

ITEM	LIMIT	RESULT
Description	Conforms	Conforms
Identification (FT-IR)	Conforms	Conforms
Specific rotation (D-line, 20°) - HCl	+14.6 to +16.5°	+15.3
State of solution ( Transmittance )	NLT 98.0%	NLT 98.0%
State of solution	Clear & colorless	Clear & colorless
Chloride (Cl)	NMT 0.020%	NMT 0.020%
Ammonium (NH <sub>4</sub> )	NMT 0.02%	NMT 0.02%
Sulfate (SO <sub>4</sub> )	NMT 0.03%	NMT 0.03%
Iron (Fe)	NMT 10 ppm	NMT 10 ppm
Heavy metals (Pb)	NMT 10 ppm	NMT 10 ppm
Related substances	Conforms	Conforms
Loss on drying	NMT 0.2%	0.0%
Residue on ignition ( Sulfated )	NMT 0.1%	0.0%
Assay	98.5 to 101.0%	100.1%
pH	5.5 to 7.0	5.8

We certify the quality of this product conforms to USP Residual Solvents requirements.  
 We certify the quality of this product conforms to USP and EP.  
 This material conforms to FCC (5<sup>th</sup> edition) specification (Lead NMT 5 ppm).

**Manufacturing Date:** July 12, 2008  
**Retest Date:** July 10, 2012

(b) (6)

Olivier Zanetta Filho  
 QA Manager  
 LA Plant

000051

# CERTIFICATE OF ANALYSIS

ANALYTICAL RESULTS OF: L-LEUCINE  
 LOT N<sup>o</sup>: 08 0060 M 04 CA JSZ HCCS

ITEM	LIMIT	RESULT
Description	Conforms	Conforms
Identification (FT-IR)	Conforms	Conforms
Specific rotation (D-L.me.20 <sup>o</sup> ) - HCl	-14.6 to +16.5 <sup>o</sup>	+15.3 <sup>o</sup>
State of solution ( Transmittance )	NLT 98.0%	NLT 98.0%
State of solution	Clear & colorless	Clear & colorless
Chloride (Cl)	NMT 0.020%	NMT 0.020%
Ammonium (NH <sub>4</sub> )	NMT 0.02%	NMT 0.02%
Sulfate (SO <sub>4</sub> )	NMT 0.03%	NMT 0.03%
Iron (Fe)	NMT 10 ppm	NMT 10 ppm
Heavy metals (Pb)	NMT 10 ppm	NMT 10 ppm
Related substances	Conforms	Conforms
Loss on drying	NMT 0.2%	0.0%
Residue on ignition ( Sulfated )	NMT 0.1%	0.0%
Assay	98.5 to 101.0%	100.0%
pH	5.5 to 7.0	5.8

We certify the quality of this product conforms to USP Residual Solvents requirements.  
 We certify the quality of this product conforms to USP and EP.  
 This material conforms to FCC (5<sup>th</sup> edition) specification (Lead NMT 5 ppm).

Manufacturing Date: July 11, 2008  
 Retest Date: July 09 2012

(b) (6)

Olivier Zanella Filho  
 QA Manager  
 LA Plant

000052

## CERTIFICATE OF ANALYSIS

ANALYTICAL RESULTS OF: L-LEUCINE  
 LOT N<sup>o</sup>: 08 0059 M 04 CA 58021002

ITEM	LIMIT	RESULT
Description	Conforms	Conforms
Identification (FT-IR)	Conforms	Conforms
Specific rotation (D-Line, 20°) -- HCl	+14.6 to +16.5°	+15.3
State of solution ( Transmittance )	NLT 98.0%	NLT 98.0%
State of solution	Clear & colorless	Clear & colorless
Chloride (Cl)	NMT 0.020%	NMT 0.020%
Ammonium (NH <sub>4</sub> )	NMT 0.02%	NMT 0.02%
Sulfate (SO <sub>4</sub> )	NMT 0.03%	NMT 0.03%
Iron (Fe)	NMT 10 ppm	NMT 10 ppm
Heavy metals (Pb)	NMT 10 ppm	NMT 10 ppm
Related substances	Conforms	Conforms
Loss on drying	NMT 0.2%	0.0%
Residue on ignition ( Sulfated )	NMT 0.1%	0.0%
Assay	98.5 to 101.0%	100.4%
pH	5.5 to 7.0	5.9

We certify the quality of this product conforms to USP Residual Solvents requirements.

We certify the quality of this product conforms to USP and EP.

This material conforms to FCC (5<sup>th</sup> edition) specification (Lead NMT 5 ppm).

Manufacturing Date: July 10, 2008  
 Retest Date: July 08, 2012

(b) (6)

Olivier Zanella Filho  
 QA Manager  
 LA Plant

000053

# CERTIFICATE OF ANALYSIS

ANALYTICAL RESULTS OF: L-LEUCINE  
 LOT N<sup>o</sup>: 08 0042 M 04 CA

J 822 H006

ITEM	LIMIT	RESULT
Description	Conforms	Conforms
Identification (FT-IR)	Conforms	Conforms
Specific rotation (D-Line, 20 <sup>o</sup> ) - HCl	+14.6 to +16.5 <sup>o</sup>	+15.3 <sup>o</sup>
State of solution ( Transmittance )	NLT 98.0%	NLT 98.0%
State of solution	Clear & colorless	Clear & colorless
Chloride (Cl)	NMT 0.020%	NMT 0.020%
Ammonium (NH <sub>4</sub> )	NMT 0.02%	NMT 0.02%
Sulfate (SO <sub>4</sub> )	NMT 0.03%	NMT 0.03%
Iron (Fe)	NMT 10 ppm	NMT 10 ppm
Heavy metals (Pb)	NMT 10 ppm	NMT 10 ppm
Related substances	Conforms	Conforms
Loss on drying	NMT 0.2%	0.0%
Residue on ignition ( Sulfated )	NMT 0.1%	0.0%
Assay	98.5 to 101.0%	100.0%
pH	5.5 to 7.0	5.9

We certify the quality of this product conforms to USP Residual Solvents requirements.

We certify the quality of this product conforms to USP and EP.

This material conforms to FCC (5<sup>th</sup> edition) specification (Lead NMT 5 ppm).

ORAL

Manufacturing Date: June 23, 2008  
 Retest Date: June 21, 2012

(b) (6)

Olivier Zanella Filho  
 QA Manager  
 LA Plant

000054

# CERTIFICATE OF ANALYSIS

ANALYTICAL RESULTS OF: L-LEUCINE  
 LOT N<sup>o</sup>: 08 0047 M 04 CA 3822 H005

ITEM	LIMIT	RESULT
Description	Conforms	Conforms
Identification (FT-IR)	Conforms	Conforms
Specific rotation (D-1 mc.20 <sup>o</sup> ) - HCl	+ 14.6 to +16.5 <sup>o</sup>	+15.3 <sup>o</sup>
State of solution ( Transmittance )	NLT 98.0%	NLT 98.0%
State of solution	Clear & colorless	Clear & colorless
Chloride (Cl)	NMT 0.020%	NMT 0.020%
Ammonium (NH <sub>4</sub> )	NMT 0.02%	NMT 0.02%
Sulfate (SO <sub>4</sub> )	NMT 0.03%	NMT 0.03%
Iron (Fe)	NMT 10 ppm	NMT 10 ppm
Heavy metals (Pb)	NMT 10 ppm	NMT 10 ppm
Related substances	Conforms	Conforms
Loss on drying	NMT 0.2%	0.0%
Residue on ignition ( Sulfated )	NMT 0.1%	0.0%
Assay	98.5 to 101.0%	99.9%
pH	5.5 to 7.0	5.9

We certify the quality of this product conforms to USP Residual Solvents requirements.

We certify the quality of this product conforms to USP and EP.

This material conforms to FCC (5<sup>th</sup> edition) specification (Lead NMT 5 ppm).

Manufacturing Date: June 28, 2008  
 Retest Date: June 26, 2012

(b) (6)

Olivia Zapella Filho  
 QA Manager  
 LA Plant

000055

BioLink Life Sciences, Inc.  
250 Quade Drive  
Cary, NC 27513

## Certificate of Analysis

4 September 2009

Title: Determination of Lead Content

PROTOCOL NO.: BLS-2009-0006	COMPANY: Ajinomoto Aminoscience Inc.	PRODUCT: L-Leucine	
LOT NO.: See Below	STORAGE/PACKAGING: Room Temperature/Clear, Plastic Bags		
Lot	Method	Specification	Results
J822H007	USP <251>; Lead	Report Results	< 1 ppm
J822H016			< 1 ppm
J822H017			< 1 ppm
J822H019			< 1 ppm
J822H020			< 2 ppm

All data generated at BioLink Life Sciences and described in this report were collected under the direction of the Study Director and were reviewed for compliance to Good Manufacturing Practices (21 CFR Part 210 and 211). This report accurately reflects the raw data that has been stored in the archives of BioLink Life Sciences, Inc.

(b) (6)

Walter C. Holberg, Study Director

*04 Sept 2009*

(b) (6)

Deanna J. Nelson, Quality Management

*4 Sept 2009*

000056

**APPENDIX IV**  
**STABILITY DATA**

000057

**Accelerated Stability Study (40°C/75%-RH)**

Parameter	Specification	Lot number 080026M04CA					Lot number 080027M04CA					Lot number 080028M04CA				
		Initial	1 month	2 months	3 months	6 months	Initial	1 month	2 month	3 months	6 months	Initial	1 month	2 months	3 months	6 months
Specific Rotation-HCl 6M(0)	+14.9 a +16.0°	15.3	15.4	15.4	15.4	15.5	15.4	15.4	15.5	15.4	15.4	15.4	15.4	15.5	15.6	15.5
State of Solution (transmittance) (%) - H <sub>2</sub> O	Conforms	Conforms	Conforms	Conforms	Conforms	Conforms	Conforms	Conforms	Conforms	Conforms	Conforms	Conforms	Conforms	Conforms	Conforms	Conforms
	≥98.0%	99.9	99.9	100.0	100.1	99.9	99.9	99.8	100.0	99.9	99.9	100.0	99.9	100.0	100.0	100.0
Loss on Drying (%)	≤ 0.20%	0.01	0.02	0.01	0.02	0.02	0.00	0.01	0.01	0.02	0.02	0.01	0.02	0.01	0.02	0.01
Purity (%)	98.5 a 101.0%	100.3	100.2	100.3	100.4	100.1	100.1	100.0	100.4	100.3	100.0	100.2	100.2	100.2	100.0	100.0
pH	H <sub>2</sub> O 5.5 a 6.5	5.8	5.8	5.9	5.9	5.9	5.8	5.9	5.9	6.0	5.9	5.9	5.8	5.9	5.9	6.0
Ammonium (%)	≤0.02%	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02
Related Substances (%)	Other amino acids total ≤2.0%	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0

000058

<b>Table A.IV-1 Stability Analysis for L-Leucine (Lot 501FK26)</b>						
<b>Specification Parameter</b>		<b>Period</b>				
<b>Test</b>	<b>Specification</b>	<b>0 year</b>	<b>1 year</b>	<b>2 years</b>	<b>3 years</b>	<b>4 years</b>
<b>Identification Parameters</b>						
L-leucine (%)	98.5-101.0	100.2	100.2	100.0	100.1	100.1
pH	5.5-6.5	6.0	6.1	6.0	6.1	6.1
State of solution (transmittance) (%)	NLT 98.0	99.9	98.9	99.9	99.9	99.9
<b>Chemical Impurity Specification Parameters</b>						
Ammonium (%)	NMT 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02
<b>Specific Tests on Identity</b>						
Loss on drying (%)	NMT 0.20	0.01	0.01	0.02	0.02	0.03
Specific rotation $[\alpha]_D^{20}$	+14.9 to +16.0	+15.5	+15.5	+15.4	+15.4	+15.4
Related substances (%)	NMT 2.00	Conforms	Conforms	Conforms	Conforms	Conforms

NLT = not less than, NMT = not more than

<b>Table A.IV-2 Stability Analysis for L-Leucine (Lot 501FK34)</b>						
<b>Specification Parameter</b>		<b>Period</b>				
<b>Test</b>	<b>Specification</b>	<b>0 year</b>	<b>1 year</b>	<b>2 years</b>	<b>3 years</b>	<b>4 years</b>
<b>Identification Parameters</b>						
L-leucine (%)	98.5-101.0	100.0	100.0	99.7	100.1	100.1
pH	5.5-6.5	6.0	6.0	6.0	6.0	6.1
State of solution (transmittance) (%)	NLT 98.0	99.9	99.5	99.9	99.9	99.9
<b>Chemical Impurity Specification Parameters</b>						
Ammonium (%)	NMT 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02
<b>Specific Tests on Identity</b>						
Loss on drying (%)	NMT 0.20	0.01	0.02	0.02	0.01	0.01
Specific rotation $[\alpha]_D^{20}$	+14.9 to +16.0	+15.5	+15.5	+15.6	+15.5	+15.5
Related substances (%)	NMT 2.00	Conforms	Conforms	Conforms	Conforms	Conforms

NLT = not less than, NMT = not more than

000059

<b>Table A.IV-3 Stability Analysis for L-Leucine (Lot 501FK36)</b>						
<b>Specification Parameter</b>		<b>Period</b>				
<b>Test</b>	<b>Specification</b>	<b>0 year</b>	<b>1 year</b>	<b>2 years</b>	<b>3 years</b>	<b>4 years</b>
<b>Identification Parameters</b>						
L-leucine (%)	98.5-101.0	99.9	99.8	99.5	100.0	100.3
pH	5.5-6.5	6.0	6.1	6.0	6.1	6.1
State of solution (transmittance) (%)	NLT 98.0	99.9	99.8	99.9	99.9	99.9
<b>Chemical Impurity Specification Parameters</b>						
Ammonium (%)	NMT 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02
<b>Specific Tests on Identity</b>						
Loss on drying (%)	NMT 0.20	0.01	0.01	0.02	0.01	0.03
Specific rotation $[\alpha]_D^{20}$	+14.9 to +16.0	+15.4	+15.6	+15.5	+15.4	+15.5
Related substances (%)	NMT 2.00	Conforms	Conforms	Conforms	Conforms	Conforms

NLT = not less than, NMT = not more than

000060

SUBMISSION END

000061

## *Reference List for Industry Submission, GRN 000308*

<i>Pages</i>	<i>Author</i>	<i>Title</i>	<i>Publish Date</i>	<i>Publisher</i>	<i>BIB_Info</i>
000042 - 000050	NA	Analysis of Amino Acids	NA	Amino Acid Handbook	NA

*NA- Not applicable*

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