



Stella Si
Anchor Center for Certification
No. 1295 Chuan Qiao Road,
Building 2, Suite 302
Pudong, Shanghai 201206
CHINA

Re: GRAS Notice No. GRN 001311

Dear Stella Si:

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 001311. We received the notice you submitted on behalf of Zhejiang Zhenyuan Biotech Co., Ltd (Zhejiang Zhenyuan) on December 15, 2025, and filed it on March 13, 2026. Zhejiang Zhenyuan submitted amendments to the notice on June 01, 2026, July 01, 2026, August 03, 2026, and August 31, 2026, providing additional information and clarifications regarding the intended uses, specifications, dietary exposure, manufacturing process, analytical methods, and safety narrative.

The subject of the notice is L-theanine produced by *Escherichia coli* (*E. coli*) CICC 11189s (L-theanine) for use as an ingredient in the food categories and at the maximum levels as shown in Table 1, excluding use in infant formula, foods for infants and toddlers, and in products under the jurisdiction of the United States Department of Agriculture. The notice informs us of Zhejiang Zhenyuan's view that these uses of L-theanine are GRAS through scientific procedures.

Table 1. Intended food categories and maximum use levels for L-theanine	
Food Category	Maximum Use Level (mg/g)
Fruit flavored drinks, juices, and nectars (excluding 100% juice)	1
Frozen fruit juice bars	1
Sports drinks	1
Non-herbal teas	1
Enhanced and flavored water	1
Cereal, granola, breakfast, nutrition, and cookie bars	5
Ice cream and frozen yogurt bars containing chocolate	5
Hard candy and cough drops; chocolate covered nuts; soft candy	8
Chewing gum	83.3

Table 1. Intended food categories and maximum use levels for L-theanine	
Food Category	Maximum Use Level (mg/g)
Mints	125

Our use of the term, “L-theanine,” in this letter is not our recommendation of that term as an appropriate common or usual name for declaring the substance in accordance with FDA’s labeling requirements. Under 21 CFR 101.4, each ingredient must be declared by its common or usual name. In addition, 21 CFR 102.5 outlines general principles to use when establishing common or usual names for nonstandardized foods. Issues associated with labeling and the common or usual name of a food ingredient are under the purview of the Office of Nutrition and Food Labeling (ONFL) in the Nutrition Center for Excellence (NCE). The Office of Pre-Market Additive Safety (OPMAS) did not consult with ONFL regarding the appropriate common or usual name for “L-theanine.”

Zhejiang Zhenyuan provides information about the chemical identity and composition of L-theanine. L-theanine, or γ -glutamylethylamide (CAS Reg. No. 3081-61-6), is a non-protein amino acid, and the primary free amino acid found in tea. Zhejiang Zhenyuan describes L-theanine as a white, odorless, crystalline powder with a slightly sweet taste, a molecular formula of $C_7H_{14}N_2O_3$, and a molecular weight of 174.20 g/mol.

Zhejiang Zhenyuan describes the production organism, *E. coli* CICC 11189s, used in the manufacturing of L-theanine. This organism is genetically engineered from the parent strain, *E. coli* K12 W3110 (ATCC 27325) to produce L-theanine. Zhejiang Zhenyuan constructed the production organism through targeted genome editing using Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-Cas9 technology to introduce the T7 RNA polymerase (*T7 RNAP*) gene originating from the donor organism *E. coli* phage T7, and the *gmas* gene sourced from the donor organism *Paracoccus aminovorans*. Zhejiang Zhenyuan states that the gene insertions were verified by whole genome sequencing and that the production organism does not contain any antibiotic resistance genes. Zhejiang Zhenyuan states that *E. coli* CICC 11189s is non-pathogenic and non-toxicogenic and is deposited in the China Center of Industrial Culture Collection (CICC) and designated as CICC 11189s.

Zhejiang Zhenyuan describes the method of manufacture of L-theanine. L-theanine is produced through fermentation of a pure culture of *E. coli* CICC 11189s under controlled conditions. After fermentation, the culture broth is heated to eliminate any viable microorganisms and subjected to a series of purification steps: microfiltration, diafiltration, ultrafiltration, and a second diafiltration step. This filtrate is then subjected to resin decolorization, cation exchange chromatography, and activated carbon column decolorization. The purified solution is then vacuum concentrated and cooled to induce crystallization of L-theanine. The crystals are collected by centrifugation, washed with ethanol, centrifuged again, and dried to yield the final product. Zhejiang Zhenyuan states that L-theanine is manufactured in accordance with current good manufacturing practices and that all raw materials and processing aids are food grade and are approved for their respective uses in accordance with appropriate

U.S. regulations, are GRAS for their intended use, or are the subject of an effective food contact notification. Zhejiang Zhenyuan states that no components of the culture medium are allergens or are derived from allergenic sources.

Zhejiang Zhenyuan provides specifications for L-theanine that include identification by infrared (IR) spectrum (matching with the reference standard), melting range (205-206 °C), assay (98.0 – 102.0%, dry basis), loss on drying (< 1%), residue on ignition (< 0.2%), optical rotation (7.7 – 8.5°), pH (5.0 – 6.0), chloride (< 210 mg/kg), ethanol (< 500 mg/kg), arsenic (< 0.01 mg/kg), lead (< 0.01 mg/kg), mercury (< 0.002 mg/kg), cadmium (< 0.002 mg/kg), and microorganisms, including *Salmonella* (absent in 25 g) and *Escherichia coli* (≤ 10 most probable number (MPN)/g). Zhejiang Zhenyuan provides the results from the analyses of three non-consecutive batches to demonstrate that L-theanine can be manufactured to meet the specifications. Zhejiang Zhenyuan states that L-theanine has a shelf-life of 24 months when stored at 30 °C/65% relative humidity.

Zhejiang Zhenyuan provides estimates of dietary exposure to L-theanine from the intended uses, from background levels in non-herbal teas, and from the combined dietary exposure. Zhejiang Zhenyuan estimates the eaters-only dietary exposure to L-theanine from the intended uses combined with background levels in non-herbal teas to be 1,242 mg/person/day (18 mg/kg body weight/day) at the 90th percentile for the U.S. population aged 2 years and older using food consumption data from the 2021–2023 National Health and Nutrition Examination Survey (NHANES). Zhejiang Zhenyuan states that the intended uses will be substitutional to prior uses of L-theanine, and therefore, there will be no increase in the cumulative dietary exposure to L-theanine.

Zhejiang Zhenyuan discusses the safety of the intended use of L-theanine and notes that L-theanine has been the subject of previous GRAS notices (GRNs 000209¹, 000338², and 000501³). For the current GRAS notice, Zhejiang Zhenyuan conducted additional unpublished studies, including genotoxicity studies and a 90-day subchronic toxicity study in rats. The notifier also conducted a literature search through May 2026 and did not identify any information that would contradict its GRAS conclusion.

Zhejiang Zhenyuan describes published studies examining the absorption, distribution, metabolism, and excretion of L-theanine, as well as published and unpublished acute, subacute, chronic, and chronic oral toxicity studies in rats and mice. Zhejiang Zhenyuan further describes the pivotal 13-week dietary toxicity study by Borzelleca et al. (2006), which showed no treatment-related adverse effects at the highest dose tested, 4,000 mg/kg body weight/day. The notifier also included a summary of human clinical trials

¹ The subject of GRN 000209 is L-theanine. We evaluated this notice and responded in a letter dated February 5, 2007, stating that we had no questions at that time regarding the notifier's GRAS conclusion.

² The subject of GRN 000338 is L-theanine extracted from tea leaves (*Camellia sinensis*). We evaluated this notice and responded in a letter dated November 5, 2010, stating that we had no questions at that time regarding the notifier's GRAS conclusion.

³ The subject of GRN 000501 is L-theanine (chemically synthesized). We evaluated this notice and responded in a letter dated July 30, 2014, stating that we had no questions at that time regarding the notifier's GRAS conclusion.

demonstrating that L-theanine is safe and well-tolerated in healthy individuals and clinical populations at doses up to 900 mg/day for durations up to 12 weeks, with no serious adverse events reported.

Zhejiang Zhenyuan notes that L-theanine was found to be non-mutagenic and non-genotoxic in published and unpublished studies. Bioinformatic allergenicity assessments showed no significant sequence homology or structural similarity to known allergens, indicating low allergenic potential.

Based on the totality of information discussed in the notice, Zhejiang Zhenyuan concludes that L-theanine is GRAS under the conditions of its intended use.

Standards of Identity

In the notice, Zhejiang Zhenyuan states its intention to use L-theanine in several food categories, including foods for which standards of identity exist, located in Title 21 of the Code of Federal Regulations. We note that an ingredient that is lawfully added to food products may be used in a standardized food only if it is permitted by the applicable standard of identity.

Potential Labeling Issues

Under section 403(a) of the Federal Food, Drug, and Cosmetic Act (FD&C Act), a food is misbranded if its labeling is false or misleading in any way. Section 403(r) of the FD&C Act lays out the statutory framework for labeling claims characterizing a nutrient level in a food or the relationship of a nutrient to a disease or health-related condition (also referred to as nutrient content claims and health claims). If products containing L-theanine bear any nutrient content or health claims on the label or in labeling, such claims are subject to the applicable requirements and are under the purview of ONFL in NCE. OPMAS did not consult with ONFL on this issue or evaluate any information in terms of labeling claims. Questions related to food labeling should be directed to ONFL.

Section 301(ll) of the FD&C Act

Section 301(ll) of the FD&C Act prohibits the introduction or delivery for introduction into interstate commerce of any food that contains a drug approved under section 505 of the FD&C Act, a biological product licensed under section 351 of the Public Health Service Act, or a drug or a biological product for which substantial clinical investigations have been instituted and their existence made public, unless one of the exemptions in section 301(ll)(1)-(4) applies. In our evaluation of Zhejiang Zhenyuan's notice concluding that L-theanine is GRAS under its intended conditions of use, we did not consider whether section 301(ll) or any of its exemptions apply to foods containing L-theanine. Accordingly, our response should not be construed to be a statement that foods containing L-theanine, if introduced or delivered for introduction into interstate commerce, would not violate section 301(ll).

Conclusions

Based on the information that Zhejiang Zhenyuan provided, as well as other information available to FDA, we have no questions at this time regarding Zhejiang Zhenyuan's conclusion that L-theanine is GRAS under its intended conditions of use. This letter is not an affirmation that L-theanine is GRAS under 21 CFR 170.35. Unless noted above, our review did not address other provisions of the FD&C Act. Food ingredient manufacturers and food producers are responsible for ensuring that marketed products are safe and compliant with all applicable legal and regulatory requirements.

In accordance with 21 CFR 170.275(b)(2), the text of this letter responding to GRN 001311 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

SUSAN J.

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Susan J. Carlson, Ph.D.

Director

Division of Food Ingredients

Office of Pre-Market Additive Safety

Office of Food Chemical Safety, Dietary
Supplements, and Innovation

Human Foods Program