U.S. FOOD & DRUG ADMINISTRATION

Human Foods Program

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

Re: GRAS Notice No. GRN 001270

Dear Ms. Si:

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 001270. We received the GRAS notice dated May 7, 2025 that you submitted on behalf of Gene III Biotechnology Co., Ltd. (Gene III) and filed it on July 8, 2025. We received amendments to the notice on September 15, 2025, and October 3, 2025, that clarified the manufacturing process, potential allergenicity of the enzymes, specifications, stability, dietary exposure, intended uses, and safety information.

The subject of the notice is L-ergothioneine produced by *Escherichia coli* K-12 MG1655 expressing enzymes encoded by the genes from *Neurospora crassa* and *Mycobacterium smegmatis* MC2 155 (ergothioneine) for use as an ingredient at the maximum use levels as specified in Table 1, excluding use in infant formula and products under the jurisdiction of the United States Department of Agriculture. The notice informs us of Gene III's view that these uses of ergothioneine are GRAS through scientific procedures.

Table 1. Proposed Uses for Ergothioneine.

Food Category	**Food Uses**	**Maximum Use Level (mg/100 g)**
Cakes, Cookies, and Pastries	Sweet rolls, cinnamon buns, pan dulce, croissants, brioche, cakes, cupcakes, cookies, pies, cobblers, fritters, cream puffs, eclairs, turnovers, pastries, Danish pastry, doughnuts, breakfast tarts, graham crackers, funnel cake	34.6
Nutrition Bars and Cereal Bars	Cereal bars, granola bars, nutrition bars, breakfast bars	75
Coffee	Coffee, coffee substitutes, chicory beverage, cereal	16.7

Food Category	**Food Uses**	**Maximum Use Level (mg/100 g)**
	beverage	
Tea	Tea, tea/lemonade juice drinks	16.7
Fruit Drinks and Ades	Coconut water, fruit nectars, fruit juice drinks, lemonade, fruit punch, fruit flavored drinks, slush drinks	12.5
Carbonated Soft Drinks	Soft drinks, diet sodas, tonic water, carbonated water, flavored carbonated water	16.7
Candy Containing Chocolate	Chocolate covered almonds/nuts, caramel chocolate candy, milk chocolate, dark chocolate, chocolate chips, fudge, chocolate covered raisins/marshmallows, toffee	100
Sports and "Energy" Drinks	Sports drinks, "energy" drinks, electrolyte solutions, fluid replacement drinks	8.3
Nutritional Beverages	Nutritional drinks and shakes, ready-to-drink nutritional products	25
Protein and Nutritional Powders	Nutritional powder mixes, protein powders	150
Candy not Containing Chocolate	Carob chips, hard candy, gummy candy, lollipops, caramel, butterscotch, marshmallows, nougat, peanut brittle, licorice, cotton candy, fruit snacks, taffy, toffee, mints, sugar-coated nuts, fondant, fruit leather, chewing gum	100

Gene III provides information on the identity and composition of ergothioneine, describing it as a white powder containing ≥ 99.5% ergothioneine. Ergothioneine is a derivative of the amino acid histidine (thiolhistidine) with a molecular formula of $C_9H_{15}N_3O_2S$, a mass of 229.30 Daltons, and is identified by the CAS Number 497-30-3. Ergothioneine occurs naturally in foods such as mushrooms, offal, cereals, and some varieties of black and red beans (*Phaseolus vulgaris*).

Gene III describes the production organism used in the manufacture of ergothioneine. Ergothioneine is manufactured from histidine by biosynthesis with enzymes Egt1, EgtD and EgtE produced by fermentation of a genetically engineered *Escherichia coli* CCTCC M2023894. Gene III states that the production strain *E. coli* CCTCC M2023894 is non-

pathogenic, non-toxigenic and non-antibiotic resistant. Gene III describes that the production microorganism is host strain *E. coli* K-12 MG1655 carrying a plasmid for expression of the three synthesized genes: *egt1* from *Neurospora crassa*, and *egtD* and *egtE* from *Mycobacterium smegmatis*. Gene III states that the production strain has been deposited in the China Center for Type Culture Collection (CCTCC) under deposit number M2023894. Gene III states that the host strain *E. coli* K-12 MG1655 has safely been used in the manufacturing of ergothioneine, as documented in GRN 000734[1]. Gene III confirms that the proteins encoded by the inserted genes into the expression plasmid do not pose a safety concern.

Gene III describes the manufacture of ergothioneine by fermentation of a pure culture under controlled conditions. The fermentation is terminated by heat treatment (95 °C for 25-30 minutes) once the desired ergothioneine concentration is reached. The mixture is then purified through plate and frame filtration, ultrafiltration, nanofiltration, cation exchange chromatography, and eluted with aqueous ammonia. The eluent is concentrated, decolorized with activated carbon, ethanol is added, crystallized, and then the crystals collected via centrifugation. The crystals are then vacuum dried and sieved to obtain the final product. Gene III states that all raw materials and processing aids used in the production of ergothioneine are food grade and are used in accordance with existing U.S. regulations, are GRAS for their respective uses, or are the subject of an effective food contact notification.

Gene III provides specifications for ergothioneine that include ergothioneine content (≥99.5%) and limits for ethanol (≤500 mg/kg), loss on drying (≤0.5%), lead (<0.1 mg/kg), arsenic (<0.1 mg/kg), cadmium (<0.1 mg/kg), mercury (<0.1 mg/kg), and microorganisms. Gene III provides the results from the analyses of five non-consecutive batches to demonstrate that ergothioneine can be produced to meet these specifications. Gene III states that ergothioneine is stable for one year at 30 ± 2 °C and 65 ± 5% relative humidity.

Gene III estimates an eaters-only dietary exposure to ergothioneine from the proposed uses to be 140 mg/person(p)/d (1.95 mg/kg body weight (bw)/d) at the mean and 275 mg/p/d (3.73 mg/kg bw/d) at the 90th percentile for the U.S. population aged 2 years or older using food consumption data from the 2017-2020 pre-pandemic National Health and Nutrition Examination Survey (NHANES).[2] Gene III cited the same published study as in GRN 000734[1] and GRN 001191[3] that estimated the background dietary exposure to ergothioneine (i.e., from mushrooms, beans, meat organs, and oatmeal) in the U.S. population to be 0.153 mg/kg bw/d for adult consumers and 0.299 mg/kg bw/d for adolescent consumers. Gene III estimated the cumulative dietary exposure to

[1] Ergothioneine was the subject of GRN 000734. We evaluated this notice and responded in a letter dated May 7, 2018, stating that we had no questions at the time regarding the notifier's GRAS conclusion.
[2] Gene III used the NHANES food codes for gelatin as a surrogate for gummy candies. We note that there are NHANES food codes that represent gummy candies/fruit snacks. We further note that the dietary exposure is essentially the same if the NHANES food codes for fruit snacks are included and the gelatin food codes are excluded.
[3] Ergothioneine was the subject of GRN 001191. We evaluated this notice and responded in a letter dated January 30, 2025, stating that we had no questions at the time regarding the notifier's GRAS conclusion.

ergothioneine based on all uses combined with the background diet (0.299 mg/kg bw/d) to be 4.05 mg/kg bw/d at the 90th percentile for the U.S. population aged 2 years or older.[4] Gene III reported that the highest cumulative dietary exposure would be in children 2 to 5 years old where the maximum cumulative dietary exposure from the proposed uses (5.24 mg/kg bw/day) plus from the background diet would result in a cumulative dietary exposure to ergothioneine of 5.54 mg/kg bw/d.

Gene III conducted a literature search through April 2025 and discussed published studies regarding the safety of ergothioneine. They reported a published acute toxicity study in rats indicating that ergothioneine exhibits low oral toxicity. Gene III discusses a 90-day study in rats that examined reproductive and developmental toxicity. This study reports that the no observed adverse effect levels (NOAELs) of L-ergothioneine as 615 mg/kg bw/day and 721 mg/kg bw/day in male and female rats, respectively (highest dose level tested). Gene III discusses another publication with 28-day and 90-day toxicity studies in rats, stating that the NOAEL was reported as 800 mg/kg bw/day.

Gene III describes several publications that assessed the toxicity of ergothioneine using *in vitro* methods. In these publications, the notifier reports that Ames assays demonstrated that ergothioneine is not mutagenic. Chromosomal aberration and micronucleus assays indicated that ergothioneine showed no clastogenic or mutagenic activity.

Gene III also discusses several published human clinical studies with ergothioneine. In one study that was randomized, placebo-controlled, and double-blinded, no adverse effects were reported in healthy male volunteers after ingestion of up to 25 mg ergothioneine /p/d for seven days. A study conducted in senior adults also reported no adverse events when 25 mg of ergothioneine was administered three times per week for one year.

Based on the information presented in the notice, Gene III concludes that ergothioneine is GRAS under the conditions of its intended use.

Standards of Identity

In the notice, Gene III states its intention to use ergothioneine in several food categories, including foods for which standards of identity exist, located in Title 21 of the CFR. 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

[4] We note that Gene III included food codes for fruit juice in their cumulative dietary exposure estimate. This use was withdrawn during the review of GRN 001191. We further note that the dietary exposure is essentially the same when the fruit juice food codes are excluded.

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 ergothioneine 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 the Office of Nutrition and Food Labeling (ONFL) in the Nutrition Center of Excellence. The Office of Pre-Market Additive Safety 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 Gene III's notice concluding that ergothioneine 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 ergothioneine. Accordingly, our response should not be construed to be a statement that foods containing ergothioneine, if introduced or delivered for introduction into interstate commerce, would not violate section 301(ll).

Conclusions

Based on the information that Gene III provided, as well as other information available to FDA, we have no questions at this time regarding Gene III's conclusion that ergothioneine is GRAS under its intended conditions of use. This letter is not an affirmation that ergothioneine 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 001270 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

Susan J. Carlson -S

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