



Ms. Amy Mozingo
GRAS Associates, LLC
11810 Grand Park Ave, Suite 500
North Bethesda, MD 20852

Re: GRAS Notice No. GRN 001191

Dear Ms. Mozingo:

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 001191. We received the notice that you submitted on behalf of Shanghai EGT Synbio Group Co., LTD (EGT Synbio) on May 10, 2024, and filed it on July, 17 2024. EGT Synbio submitted amendments to the notice on October 11, 2024, and November 4, 2024, clarifying information about the results of the batch analyses, the ergothioneine synthases used, the manufacturing process, intended uses, dietary exposure, and the host strain.

The subject of the notice is ergothioneine produced by *Escherichia coli* BL-21 (DE3) expressing ergothioneine synthases from *Schizosaccharomyces pombe* (ergothioneine) for use as an ingredient in cakes, cookies, and pastries; granola bars; coffee; tea; fruit drinks and ades; carbonated soft drinks; and candy containing chocolate, at a level of 5 mg per serving.¹ The notice informs us of EGT Synbio's view that these uses of ergothioneine are GRAS through scientific procedures.

EGT Synbio provides information about the identity and composition of ergothioneine. EGT Synbio describes ergothioneine as a white crystal of $\geq 99.9\%$ purity. Ergothioneine is a derivative of the amino acid histidine (thiohistidine) 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*).

EGT Synbio describes the method of manufacture of ergothioneine. Ergothioneine is manufactured from hercynine by biosynthesis with ergothioneine synthases EGT1 and EGT2 produced by fermentation of two *E. coli* BL-21 (DE3) strains "EGT1(DE3)" and "EGT2(DE3)". EGT Synbio describes that the production strains are *E. coli* BL-21 (DE3) carrying their respective plasmids pET28a-EGT1 and pET28a-EGT2 for expression of

¹ EGT Synbio states that ergothioneine is not intended for use in infant formula, products under the jurisdiction of the United States Department of Agriculture, or in food where standards of identity preclude its use.

genes encoding the ergothioneine synthases from *Schizosaccharomyces pombe*. EGT Synbio states that the production strains *E. coli* BL-21 (DE3) strains “EGT1(DE3)” and “EGT2(DE3)” are maintained by EGT Synbio. When the bioconversion is complete, hydrochloric acid is added to denature and precipitate the enzymes. The mixture is then purified through centrifugation and ion exchange chromatography, filtered, and eluted with sodium hydroxide. The eluent is concentrated, ethanol is added, and the ergothioneine is crystallized by cooling. The crystals are then dried and sieved to obtain the final product. EGT Synbio states that ergothioneine is manufactured in accordance with good manufacturing practices and that all raw materials and processing aids are food grade and used in accordance with applicable U.S. regulations.

EGT Synbio states that host strain *E. coli* BL-21 (DE3) is non-pathogenic and non-toxicogenic. EGT Synbio states that *E. coli* BL-21 (DE3) has safely been used in the production of other ingredients that have been concluded to be GRAS for their intended uses. EGT Synbio confirms that whole genome sequencing of the *E. coli* BL-21 (DE3) revealed no genes for invasion, adhesion or enterotoxins. Each of the two production strains carry a kanamycin resistance plasmid. However, EGT Synbio states that the production strains are removed from the final ergothioneine product.

EGT Synbio provides specifications for ergothioneine that include purity ($\geq 99.9\%$) and limits for total residual solvents (≤ 200 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. EGT Synbio provides the results from the analyses of five non-consecutive batches to demonstrate that ergothioneine can be produced to meet these specifications.

EGT Synbio estimates an eaters-only dietary exposure to ergothioneine from the proposed uses to be 13.4 mg/person(p)/d (0.19 mg/kg body weight (bw)/d) at the mean and 25.8 mg/p/d (0.36 mg/kg bw/d) at the 90th percentile for the U.S. population aged 2 years or older using food consumption data from the 2017-2018 National Health and Nutrition Examination Survey (NHANES). EGT Synbio indicates that the intended uses are substitutional for those in GRN 000734² and there would be no increase in the cumulative dietary exposure to ergothioneine from the proposed uses. This would include dietary exposure from background sources in the diet (i.e., from mushrooms, beans, organ meat, and oatmeal).

EGT Synbio discusses published acute toxicity, 14-day, 28-day, and 90-day studies. In a published 90-day study, no adverse effects were reported at up to 800 mg/kg bw/d in male and female rats. A published combined subchronic (13 week) and reproductive toxicity study in rats reported no adverse effects at 615 mg/kg bw/d in males and 721 mg/kg bw/d in females, the highest level tested in each sex. EGT Synbio describes unpublished studies that showed that ergothioneine was not mutagenic or genotoxic. EGT Synbio includes a summary table of genotoxicity studies conducted on ergothioneine that were previously discussed in GRN 000734 and incorporated into this

² The subject of GRN 000734 is ergothioneine. We evaluated GRN 000734 and responded in a letter dated May 7, 2018, stating that we had no questions at that time regarding the notifier’s GRAS conclusion.

notice. EGT Synbio discusses a randomized, placebo-controlled, double-blinded study conducted in healthy males that ingested up to 25 mg of ergothioneine daily for seven days. No adverse effects were observed, and the subjects did not report any adverse effects. EGT Synbio conducted a literature search through March 2024 and did not identify any information that would contradict its GRAS conclusion.

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

Standards of Identity

In the notice, EGT Synbio 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 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 EGT Synbio'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 EGT Synbio provided, as well as other information

available to FDA, we have no questions at this time regarding EGT Synbio'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 001191 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

**Susan J.
Carlson -S**

Digitally signed by Susan
J. Carlson -S

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