



Steven Overgaard
GRAS Associates, LLC
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Suite 412
Bonita Springs, FL 34134

Re: GRAS Notice No. GRN 000734

Dear Mr. Overgaard:

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 000734. We received the notice you submitted on behalf of Blue California on September 28, 2017, and filed it on November 8, 2017. We received an amendment to the notice on December 21, 2017. In the amendment, Blue California clarifies the intended use for the substance.

The subject of the notice is ergothioneine for use as an ingredient in cakes, cookies, and pastries (including granola bars); coffee; tea; fruit drinks and ades; carbonated soft drinks; and candy-containing chocolates, at a level of 5 mg ergothioneine/serving. The notice informs us of Blue California's view that this use of ergothioneine is GRAS through scientific procedures.

Blue California provides information about the identity and composition of ergothioneine. Blue California describes ergothioneine as a derivative of the amino acid histidine 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 naturally occurs in several foods that are part of the diet, such as mushrooms, red and black beans, cereal, garlic, and offal.

Blue California describes the method of manufacture for ergothioneine, which is produced through a fermentation process utilizing a modified strain of *Escherichia coli* K12, which is nonpathogenic and nontoxicogenic.¹ The parental strain is *E. coli* K12 MG1655. Blue California cloned four genes into *E. coli* MG1655 to construct the production strain; these genes encode proteins that convert histidine to ergothioneine.² Blue California describes growing the production strain in a fermenter under controlled

¹ We note our conclusion that this organism is non-toxicogenic and non-pathogenic (55 FR 10932 at 10934, March 23, 1990).

² These four genes include a formylglycine-generating enzyme-like protein (EgtB), a glutamine amidotransferase (EgtC), a histidine methyltransferase (EgtD), and a pyridoxal 5-phosphate binding protein (EgtE).

conditions in a fermentation media without allergenic ingredients. When mid-exponential phase is reached, the genes for ergothioneine production are induced and the fermenter is held until the desired concentration of ergothioneine is reached. The supernatant from the *E. coli* culture is collected by centrifugation and then passed through an ion-exchange resin. Ergothioneine is removed from the eluent by crystallization. Crystals are dissolved in water, decolorized with activated charcoal, then dried and crushed into a fine powder. This powder contains $\geq 98\%$ ergothioneine. It is then blended with maltodextrin to formulate the final product containing 5% ergothioneine. Blue California states that the raw materials used in the manufacture of ergothioneine are food-grade materials and are used in accordance with applicable U.S. regulations.

Blue California provides specifications for ergothioneine. These specifications include ergothioneine ($\geq 5\%$), loss on drying ($\leq 6\%$), cadmium (≤ 1 mg/kg), mercury (≤ 1 mg/kg), arsenic (≤ 1 mg/kg), lead (≤ 1 mg/kg), and microbial limits. The analyses of three non-consecutive batches demonstrate that ergothioneine can be produced to meet these specifications.

Blue California estimates the dietary exposure to ergothioneine. Blue California intends to use ergothioneine in cakes, cookies, and pastries (including granola bars); coffee; tea; fruit drinks and ades; carbonated soft drinks; and candy containing chocolates, at a use level of 5 mg ergothioneine/serving. Using USDA survey data on daily consumption of various foods, Blue California estimates a mean dietary exposure of 16.79 mg ergothioneine per day (d) for all individuals. Blue California also reports estimates for specific subpopulations. For the adult subpopulation (70-kg body weight (bw)), the dietary exposures were 0.24 mg/kg bw/d at the mean and twice that amount, 0.48 mg/kg bw/d, at the 90th percentile.

Blue California notes that ergothioneine is naturally present in several foods and that the consumption of mushrooms, beans, offal, and oat meal are the main background dietary sources of ergothioneine. Blue California cites a published study that estimated the background dietary exposures of ergothioneine in the U.S. population as 0.153 mg/kg bw/d for adult consumers and as 0.299 mg/kg bw/d for adolescent consumers.

Blue California combines these background exposures with the estimated exposures that would result from the intended uses to obtain a total dietary exposure of ergothioneine. In adults, the combined dietary exposure of ergothioneine from the intended uses (0.48 mg/kg bw/d) plus the background diet (0.153 mg/kg bw/d) results in a total ergothioneine exposure of 0.633 mg/kg bw/d. In the subpopulation of children 3 to 10 years old, the combined exposure from the intended uses (1.55 mg/kg bw/d) plus from the background diet (0.299 mg/kg bw/d) would result in a total ergothioneine exposure of 1.849 mg/kg bw/d.

Blue California discusses published and unpublished studies regarding the safety of ergothioneine. In a published acute toxicity study in rats, ergothioneine exhibits low oral toxicity. In published 14-day and 28-day toxicity studies in rats, no adverse effects were reported at 662 mg/kg bw/d in males and 721 mg/kg bw/d in females and 500 mg/kg

bw/d in both sexes, respectively, at the highest doses tested. In a published 90-day study in rats no adverse effects were reported at up to 800 mg/kg bw/d in both sexes. In a published combined subchronic (13-week) and reproductive toxicity study in rats, no adverse effects were reported at 615 mg/kg bw/d in males and 721 mg/kg bw/d in females, the highest level tested in each sex.

Blue California describes two published studies and one unpublished study where *in vitro* bacterial reverse mutation assays showed that ergothioneine is not mutagenic. A published *in vitro* chromosome aberration assay and an *in vivo* mammalian erythrocyte micronucleus test showed that ergothioneine showed no clastogenic or mutagenic activity.

Blue California also discusses a published randomized, placebo-controlled, double-blinded study where no adverse effects were reported in healthy male volunteers after ingestion of up to 25 mg ergothioneine/person/d (approximately 0.36 mg/kg bw/day) for seven days.

Blue California includes the statement of a panel of individuals (Blue California's GRAS panel). Based on its review, Blue California's GRAS panel concluded that ergothioneine is safe under the conditions of its intended use.

On the basis of the information presented in the notice, Blue California concludes that ergothioneine is GRAS under the conditions of its intended use.

Standards of Identity

In the notice, Blue California states its intention to use ergothioneine 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 as nutrient content claims and health claims). The notice raises a potential issue under these labeling provisions. In the notice, Blue California cites a study that describes ergothioneine as having an effect on kidney function. If products containing ergothioneine bear any nutrient content or health claims on the label or in labeling, such claims are the subject to the applicable requirements and are under the purview of the Office of Nutrition and Food Labeling (ONFL) in the Center for Food Safety and Applied Nutrition. The Office of Food 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(II) of the FD&C Act

Section 301(II) 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(II)(1)-(4) applies. In our evaluation of Blue California's notice concluding that ergothioneine is GRAS under its intended conditions of use, we did not consider whether section 301(II) 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(II).

Conclusions

Based on the information that Blue California provided, as well as other information available to FDA, we have no questions at this time regarding Blue California'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 000734 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,
**Michael A.
Adams -S**

Dennis M. Keefe, Ph.D.
Director
Office of Food Additive Safety
Center for Food Safety
and Applied Nutrition

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