



Jim Lassiter
Rejimus, Inc.
600 W Santa Ana Blvd, Suite 1100
Santa Ana, CA 92701

Re: GRAS Notice No. GRN 001182

Dear Mr. Lassiter:

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 001182. We received the notice that you submitted on behalf of Hangzhou Viablif Biotech Co, Ltd. (Hangzhou Viablif Biotech) on February 26, 2024, and filed it on May 20, 2024. Hangzhou Viablif Biotech submitted amendments to the notice on October 7, 2024, November 4, 2024, December 5, 2024, and December 23, 2024, that revised the specifications and clarified the intended uses, manufacturing process, dietary exposure, and elements of the safety narrative.

The subject of the notice is hydroxytyrosol for use as an antioxidant in various foods and at the maximum use levels specified in Table 1.¹ The notice informs us of Hangzhou Viablif Biotech's view that these uses of hydroxytyrosol are GRAS through scientific procedures.

Table 1. Intended food uses and maximum use levels of hydroxytyrosol

Food use	Maximum use level (mg/serving²)	Maximum use level (mg/kg)
Butter, margarine, oil, and shortening	5	333
Salad dressing	5	167
Mayonnaise and mayonnaise-type sandwich spreads	5	333
Ready-to-drink sports drinks, "energy" drinks, milk-based meal replacements, flavored water, and fruit flavored drinks	5	20
Fruit juices, fruit nectars, and vegetable juices	5	20

Hangzhou Viablif Biotech describes hydroxytyrosol as a slightly yellow viscous liquid. Hangzhou Viablif Biotech states that hydroxytyrosol is a naturally occurring phenolic

¹ Hangzhou Viablif Biotech states that hydroxytyrosol is not intended for use in infant formula, foods for infants and young children, products under the jurisdiction of the United States Department of Agriculture, or in foods for which standards of identity preclude its use.

² Based on the Reference Amounts Customarily Consumed (RACC) per eating occasion.

antioxidant found in olives and olive oil. Hydroxytyrosol is designated by the CAS Registry No. 10597-60-1 and has the molecular formula C₈H₁₀O₃.

Hangzhou Viablife Biotech describes the method of manufacture for hydroxytyrosol, produced by fermentation of a culture of non-toxicogenic, non-pathogenic *Escherichia coli* BL21 (DE3) VBL112 expressing a gene for phenol hydroxylase from *Geobacillus thermoglucosidasius*. *E. coli* BL21 (DE3) VBL112 is fermented to a desired cell density and cells are then harvested by centrifugation and washed to remove any components of the media. Following this, the cells are broken by homogenization to yield phenol hydroxylase, the enzyme that converts tyrosol to hydroxytyrosol. The homogenate is adsorbed on an activated macroporus resin and mixed with water. The pH is adjusted to neutral followed by the addition of tyrosol. After the conversion of tyrosol is complete, citric acid is added to terminate the reaction. The phenol hydroxylase, other proteins, and impurities are removed by centrifugation. The liquid filtrate is purified by extraction with water and then evaporated to yield hydroxytyrosol. Hangzhou Viablife Biotech states that all raw materials and processing aids are food grade and are used in accordance with existing U.S. regulations or are GRAS for their intended uses, and that the manufacturing process follows current good manufacturing practices. Hangzhou Viablife Biotech states that hydroxytyrosol does not contain any of the nine major allergens.

Hangzhou Viablife Biotech provides specifications for hydroxytyrosol that include assay (>99%), tyrosol (<0.5%), total protein (<200 mg/kg), loss on drying (<1%), lead (<0.1 mg/kg), arsenic (<0.1 mg/kg), mercury (<0.05 mg/kg), cadmium (<0.1 mg/kg), and limits for microorganisms, including *Salmonella* serovars (negative in 25 g). Hangzhou Viablife Biotech provides results from the analyses of five non-consecutive batches to demonstrate that hydroxytyrosol can be manufactured to meet the specifications. Hangzhou Viablife Biotech states that hydroxytyrosol is stable for at least 39 weeks at 25 °C and 47% relative humidity based on an accelerated stability study.

Hangzhou Viablife Biotech estimates the dietary exposure to hydroxytyrosol from the intended uses based on food consumption data from the 2017-2020 National Health and Nutrition Examination Survey (NHANES). Hangzhou Viablife Biotech estimates the eaters-only dietary exposure to hydroxytyrosol to be 8.4 mg/person (p)/day (d) at the mean and 17.8 mg/p/d at the 90th percentile for the U.S. population aged 2 years and older. Hangzhou Viablife Biotech states that the intended uses are substitutional for current uses of hydroxytyrosol and as such there would be no increase in the cumulative dietary exposure to hydroxytyrosol reported in GRNs 000726, 000876, and 000978.³

Hangzhou Viablife Biotech discusses published and unpublished data and information on either high purity (>99%) hydroxytyrosol and/or olive extract containing hydroxytyrosol to support the safety of hydroxytyrosol at the intended uses based on a

³ The subjects of GRNs 000726, 000876, and 000978 are phenolic preparation from olive fruit (≥40% hydroxytyrosol), hydroxytyrosol, and hydrolyzed aqueous olive pulp extract (3-4.5% hydroxytyrosol), respectively. We evaluated these notices and responded in letters dated February 28, 2018, January 21, 2020, and December 10, 2021, respectively, stating that we had no questions at that time regarding the notifiers' GRAS conclusions.

scientific literature search conducted through January 2024. Hangzhou Viablife Biotech discusses data and information previously reviewed by FDA from GRAS notices 000600⁴ and 000876³ for high purity hydroxytyrosol, and from 000726³ and 000978³ for olive extracts that contain varying concentrations of hydroxytyrosol.⁵ Hangzhou Viablife Biotech summarizes the results from multiple published acute and 90-day repeat dose oral toxicity studies in rodents, using test articles containing 2.4-99% hydroxytyrosol, including one teratogenicity study using olive pulp extract (OPE) containing approximately 2.4% hydroxytyrosol. Hangzhou Viablife Biotech considers a published 90-day subchronic repeat-dose oral toxicity study in rats with the test article containing high purity hydroxytyrosol as pivotal in their safety determination, where no adverse effects were observed at doses up to 50 mg/kg bw/d. Further, Hangzhou Viablife Biotech discusses an additional published study in which no adverse effects were reported after the administration of 691 mg olive extract containing 35% hydroxytyrosol (equivalent to 250 mg/kg bw/d of hydroxytyrosol). Based on the results of a published teratogenicity study in rats, Hangzhou Viablife Biotech concludes that hydroxytyrosol is unlikely to be a reproductive or developmental toxicant at doses up to 2,000 mg/kg bw/d of OPE (equivalent to 48 mg/kg bw/d of hydroxytyrosol, the highest dose tested). Additionally, Hangzhou Viablife Biotech concludes that, based on the results of multiple published *in vitro* and *in vivo* genetic toxicity tests (i.e., a bacterial reverse mutation assay, three *in vitro* chromosomal aberration assays, and two *in vivo* micronucleus assays), hydroxytyrosol is non-genotoxic and non-clastogenic. Hangzhou Viablife Biotech also states that the results of multiple published human studies with olive oil containing varying levels of hydroxytyrosol did not reveal any adverse effects.

Based on the totality of the data and information, Hangzhou Viablife Biotech concludes that hydroxytyrosol is GRAS under the intended conditions of use.

Standards of Identity

In the notice, Hangzhou Viablife Biotech states its intention to use hydroxytyrosol 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 hydroxytyrosol bear any nutrient content or health claims on the label or in labeling,

⁴ The subject of GRN 000600 is hydroxytyrosol. We evaluated this notice and responded in a letter dated May 13, 2016, stating that we had no questions at that time regarding the notifier's GRAS conclusion.

⁵ Hangzhou Viablife Biotech notes that hydroxytyrosol is a commonly consumed constituent of olives and olive oil.

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 (NCE). The Office of Pre-Market Additive Safety (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.

Under section 403(k) of the FD&C Act, a food is misbranded if it contains any chemical preservative, unless the label states that fact. Under section 403(i)(2) of the FD&C Act, a food is misbranded unless its label bears the common or usual name of each ingredient. Hangzhou Viablife Biotech's intended use of hydroxytyrosol constitutes use as a preservative. Therefore, the ingredient statement on labels of food products containing hydroxytyrosol must comply with the labeling regulations implemented in sections 403(k) and 403(i)(2) of the FD&C Act. For example, 21 CFR 101.22(j) requires that the label of a food with an added chemical preservative must declare both the common or usual name of the ingredient and a separate description of its function. Further, food that is subjected to any form of preservation, except as provided in 21 CFR 101.95(c), may not be labeled as "fresh." Questions related to food labeling should be directed to ONFL in the NCE.

Potential Requirement for a Color Additive Petition

There is no GRAS provision for color additives. In the notice, Hangzhou Viablife Biotech describes hydroxytyrosol as yellow. As such, the use of hydroxytyrosol in food products may constitute a color additive use under section 201(t)(1) of the FD&C Act and FDA's implementing regulations in 21 CFR Part 70. Under section 201(t)(1) and 21 CFR 70.3(f), a color additive is a material that is a dye, pigment, or other substance made by a synthetic process or similar artifice, or is extracted, isolated, or otherwise derived from a vegetable, animal, mineral, or other source. Under 21 CFR 70.3(g), a material that otherwise meets the definition of a color additive can be exempt from that definition if it is used (or is intended to be used) solely for a purpose or purposes other than coloring. Our response to GRN 001182 is not an approval for use as a color additive nor is it a finding of the Secretary of the Department of Health and Human Services within the meaning of section 721(b)(4) of the FD&C Act. Questions about color additives should be directed to the Division of Food Ingredients in OPMAS.

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 Hangzhou Viablife Biotech's notice concluding that hydroxytyrosol 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 hydroxytyrosol. Accordingly, our response should not be construed to be a statement that foods containing hydroxytyrosol, if introduced or delivered for introduction into

interstate commerce, would not violate section 301(l).

Conclusions

Based on the information that Hangzhou Viablife Biotech provided, as well as other information available to FDA, we have no questions at this time regarding Hangzhou Viablife Biotech's conclusion that hydroxytyrosol is GRAS under its intended conditions of use. This letter is not an affirmation that hydroxytyrosol 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 001182 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

Susan J.
Carlson -S

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