U.S. FOOD & DRUG ADMINISTRATION
Human Foods Program

Lei Zhang
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Chengdu, Sichuan Province
CHINA

Re: GRAS Notice No. GRN 001230

Dear Mr. Zhang:

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 001230. We received Sichuan Ingia Biosynthetic Co., Ltd. (Sichuan Ingia)'s notice on October 22, 2024 and filed it on February 7, 2025. Sichuan Ingia submitted amendments to the notice on May 2, 2025 and May 12, 2025, that clarified the production strain's identity, manufacturing process, specifications, intended uses, dietary exposure, and safety information.

The subject of the notice is vanillin preparation produced by *Escherichia coli* BL21(DE3) SI-VAN1 (vanillin preparation) for use as a flavoring agent in baked goods and baking mixes; alcoholic beverages; non-alcoholic beverages and beverage bases; breakfast cereals; chewing gum; confections and frostings; fat and oils; frozen dairy desserts; gelatins, puddings, and fillings; hard candy; milk products; snack foods; soft candy; sweet sauces, toppings and syrups at levels ranging from 0.0095 g to 0.1 g per 100 g.[1] The notice informs us of Sichuan Ingia's view that these uses of vanillin preparation are GRAS through scientific procedures.

Our use of the term, "vanillin preparation," 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 non-standardized 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 of Excellence. The Office of Pre-Market Additive Safety did not consult with ONFL regarding the appropriate common or usual name for "vanillin preparation."

Sichuan Ingia describes vanillin preparation as a white to yellow powder containing ≥ 98% vanillin ($C_8H_8O_3$, CAS Registry Number 121-33-5). Sichuan Ingia states that the fermentation-derived vanillin is structurally and

chemically identical to vanillin found in vanilla extract from the *Vanilla planifolia* bean.

Sichuan Ingia describes the method of manufacture of vanillin preparation, which results from the enzymatic reaction of rice-derived ferulic acid in the presence of phenolic acid decarboxylase (PAD) and carotenoid oxygenase (CSO2) produced by fermentation of genetically engineered *E. coli* BL21(DE3) SI-VAN1. Sichuan Ingia describes that the production microorganism *E. coli* BL21(DE3) SI-VAN1 is a strain of *E. coli* BL21(DE3) carrying a plasmid for expression of genes encoding PAD and CSO2 from *Bacillus pumilus* and *Caulobacter segnis*, respectively. Sichuan Ingia states that *E. coli* BL21(DE3) SI-VAN1 is non-pathogenic and non-toxigenic. Sichuan Ingia states that *E. coli* BL21(DE3) has safely been used in the production of other ingredients that have been concluded to be GRAS for their intended uses (e.g., GRNs 000485, 000571, 000876, 000921, 000922, 000923, 000925, 001014, 001015, and 001016).[2] Sichuan Ingia conducts phenotypic and genotypic analysis of *E. coli* BL21(DE3) SI-VAN1 and confirms that it does not generate undesirable secondary metabolites during fermentation.

Sichuan Ingia states that the manufacture of vanillin preparation starts with the production of enzymes PAD and CSO2 by fermentation of *E. coli* BL21(DE3) SI-VAN1 under controlled conditions. After fermentation, the bacteria cells are isolated by centrifugation and resuspended in sodium phosphate buffer containing ferulic acid as a precursor and ferrous sulfate as an iron source. Ferulic acid is first converted to a 4-vinlyguiacol intermediate by PAD. The 4-vinlyguiacol intermediate is then oxidized by CSO2 to produce vanillin. Following the bioconversion, the reaction mixture is heated and centrifuged. The resulting supernatant is acidified, heated, decolorized with activated carbon, and filtered. The filtered solution is further purified using an adsorption resin column. The absorbed vanillin is then recovered using ethanol, and the ethanol eluate is concentrated and resuspended in ethanol for crystallization. The resulting crystals are centrifuged, sieved, and vacuum-dried to yield the final product. Sichuan Ingia states that vanillin preparation is manufactured in accordance with current good manufacturing practices and that all raw materials and processing aids are food-grade and are used in accordance with applicable U.S. regulations, are GRAS for the intended uses, or are subjects of effective food contact notifications.

Sichuan Ingia provides specifications for vanillin preparation that include vanillin content (≥ 98 %), loss on drying (≤ 0.5%), residue on ignition (≤ 0.05%), melting point (≤ 83 °C), and limits for ethanol (≤ 1000 mg/kg), methanol (≤ 200 mg/kg), lead (≤ 0.1 mg/kg), mercury (≤ 0.1 mg/kg), arsenic (≤ 0.1 mg/kg), cadmium (≤ 0.1 mg/kg), and microorganisms. Sichuan Ingia provides the results from the analyses of five non-consecutive batches to demonstrate that vanillin preparation can be manufactured to meet these specifications. Based on results from stability studies, Sichuan Ingia concludes that vanillin preparation is stable for six months when stored at 40 ^{0}C and 75% relative humidity, packaged in low-density polyethylene.

Based on food consumption data from the 2017-2018 National Health and Nutrition Examination Survey (NHANES), Sichuan Ingia estimates the eaters-only dietary exposure to vanillin based on the current uses of synthetic vanillin[3] for the U.S. population aged 2 years or older to be 173 mg/person (p)/d (2.51 mg/kg bodyweight (bw)/d) at the mean and 326 mg/p/d (4.93 mg/kg bw/d) at the 90th percentile. Sichuan Ingia indicates that the intended uses of vanillin preparation are substitutional to the uses of synthetic vanillin currently permitted under 21 CFR §182.60 and therefore, there will be no increase in the total dietary exposure to vanillin.

Sichuan Ingia discusses the long history of vanilla bean and vanilla extract consumption and use in foods, of which vanillin is a characteristic constituent. Sichuan Ingia notes the safety of their starting material (ferulic acid) by discussing its bioconversion to vanillin by the production organism *E. coli* BL21(DE3) SI-VAN1, and states that any remaining ferulic acid is removed from the vanillin preparation by column absorption chromatography and provides analytical data to support this conclusion.

Sichuan Ingia reviews published safety data and information sourced from a comprehensive search of the scientific literature. These data are comprised toxicology studies, including absorption, distribution, metabolism and excretion studies, genotoxicity and mutagenicity studies, and acute, sub-chronic, and chronic oral toxicity studies on relatively pure vanillin (≥ 98%). From these data, Sichuan Ingia reports that vanillin is primarily excreted in urine as intact vanillin, vanillyl alcohol, vanillic acid, or their respective glucuronidate and sulfate metabolites. Sichuan Ingia also discusses toxicology data pivotal to their safety determination, which includes a 2-year repeated dose oral toxicity study in rats that concludes no adverse effects in test animals receiving dietary vanillin (up to 20,000 ppm, equivalent to approximately 1,000 mg/kg bw/d, the highest dose tested) were observed that could be attributed to the test article. Further, Sichuan Ingia notes that this study served as the basis for a Joint FAO/WHO Expert Committee on Food Additives acceptable daily intake level determination for vanillin, which supports the current GRAS conclusion. Sichuan Ingia reports that some literature suggests that vanillin consumption may positively impact glycemic response in consumers but reports that dietary exposure from the intended uses are magnitudes below the levels reported to elicit this biological response in rats, and therefore, no significant effects on glycemic response or insulin sensitivity are expected in consumers.

Based on the totality of data and information, Sichuan Ingia concludes that vanillin preparation produced through bioconversion is GRAS for its intended uses.

Standards of Identity

In the notice, Sichuan Ingia states its intention to use vanillin preparation 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.

Allergen Labeling

The FD&C Act requires that the label of a food that is or contains an ingredient that contains a "major food allergen" declare the allergen's presence (section 403(w)). The FD&C Act defines a "major food allergen" as one of nine foods or food groups (i.e., milk, eggs, fish, Crustacean shellfish, tree nuts, peanuts, wheat, soybeans, and sesame) or a food ingredient that contains protein derived from one of those foods. Vanillin preparation may require labeling under the FD&C Act because the fermentation media may contain protein derived from milk. Questions about petitions or notifications for exemptions from the food allergen labeling requirements should be directed to the Division of Food Ingredients in the Office of Pre-Market Additive Safety. Questions related to food labeling in general should be directed to ONFL.

Potential Requirement for a Color Additive Petition

There is no GRAS provision for color additives. In the notice, Sichuan Ingia notes that vanillin preparation has color. As such, the use of vanillin preparation 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 001230 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 the Office of Pre-Market Additive Safety.

Section 301(ll) of the Federal Food, Drug, and Cosmetic Act (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 Sichuan Ingia's notice concluding that vanillin preparation 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 vanillin preparation. Accordingly, our response should not be construed to be a statement that foods containing vanillin preparation, if introduced or delivered for introduction into interstate commerce, would not violate section 301(ll).

Conclusions

Based on the information that Sichuan Ingia provided, as well as other information available to FDA, we have no questions at this time regarding Sichuan Ingia's conclusion that vanillin preparation is GRAS under its intended conditions of use. This letter is not an affirmation that vanillin preparation 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 001230 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

Susan J. Carlson -S

Digitally signed by Susan J. Carlson -S
Date: 2025.06.11 10:46:03 -04'00'

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

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1. Sichuan Ingia states that vanillin preparation is not intended for use in infant formula, infant food products, or in products under the jurisdiction of the United States Department of Agriculture.
2. Beta-galactosidase enzyme preparation, 2'-fucosyllactose, hydroxytyrosol, 3'-sialyllactose sodium salt, 6′-sialyllactose sodium salt, lacto-N-tetraose, 3-fucosyllactose, 2′-fucosyllactose, 3′-sialyllactose sodium salt, and 6′-sialyllactose sodium salt are the subject of GRNs 000485, 000571, 000876, 000921, 000922, 000923, 000925, 001014, 001015, and 001016, respectively. We evaluated these notices and responded in letters dated April 15, 2014, November 6, 2015, January 21, 2020, October 30, 2020, April 23, 2021, February 2, 2021, February 8, 2021, July 15, 2022, July 15, 2022, and July 15, 2022, respectively, stating that we had no questions at that time regarding each notifier's GRAS conclusion.
3. Synthetic vanillin is the subject of 21 CFR 182.60. Sichuan Ingia indicates that the current uses of synthetic vanillin covered under this regulation include use in meat products. However, the uses of vanillin preparation proposed by Sichuan Ingia in this GRN do not include use in meat products. However, when estimating the dietary exposure to vanillin, Sichuan Ingia included the use in meat products to reflect the dietary exposure to vanillin for the current uses covered under 21 CFR 182.60.