U.S. FOOD & DRUG ADMINISTRATION
Human Foods Program

Susan S. Cho, Ph.D.
AceOne RS, Inc.
5903 Hampton Forest Way
Fairfax, VA 22030

Re: GRAS Notice No. GRN 001186

Dear Dr. Cho:

The Food and Drug Administration (FDA, we) is responding to GRN 001186, which you submitted on behalf of Runke Bioengineering (Fujian) Co., Ltd. (Runke) on April 3, 2024, and which we filed on May 10, 2024. In response to our questions, we received additional information on August 28 and September 1, 2024, regarding Runke's microbial testing and lot distribution.[1]

The subject of the notice is fungal oil (≥38% arachidonic acid ARA from *Mortierella alpina* "FJRK-MA01" (*M. alpina* oil) for use as an ingredient in cow milk-, goat milk-, soy-, amino acid-, and extensively hydrolyzed protein-based, non-exempt infant formula for term infants and exempt infant formula for pre-term and low birthweight infants, at a level of 0.75% and 0.50% (w/w) of total fatty acids for term and pre-term or low-birthweight infants, respectively, and in combination with a safe and suitable source of docosahexaenoic acid (DHA) at a ratio ranging from 2:1 to 1:1 of ARA to DHA. The notice provides food-grade specifications for *M. alpina* oil, including a specification for *Cronobacter* spp. The notice also provides testing results for these specifications. The notice informs us of Runke's view that these uses of *M. alpina* oil are GRAS through scientific procedures.

FDA's regulation, specifically 21 CFR 170.230(c), establishes requirements in Part 2 of a GRAS notice for specifications for food-grade materials. The method of manufacture and specifications identified in a GRAS notice are relevant to the identity of the substance and are an important component of the safety assessment when establishing the safe conditions of use in food.[2] In Part 2 of its GRAS Notice, Runke established a specification for *Cronobacter* spp. of "not detectable" in 10 g of *M. alpina* oil. During

[1] Based on Runke's responses and other information available to us, we determined that the subject of this notice was not used in infant formula in the U.S.
[2] *See e.g.,* Reponses to comments 23 and 40, Substances Generally Recognized as Safe, Final Rule. 81 Fed. Reg. 54960, 54978-79 and 54984. (October 17, 2016); "Guidance for Industry: Assessing the Effects of Significant Manufacturing Process Changes, Including Emerging Technologies, on the Safety and Regulatory Status of Food Ingredients and Food Contact Substances, Including Food Ingredients that Are Color Additives," http://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/ucm300661.htm, 2014.

our evaluation of GRN 001186, we identified a safety issue with the *M. alpina* oil produced by Runke, specifically a positive test result for *Cronobacter* spp. in one of the batch analyses, presented in the notice. The positive test result indicates that, among other safety concerns described below, the notice's food-grade specification for *Cronobacter* spp. in this ingredient has not been met. Because of the positive test result, and because the subject of this notice, *M. alpina* oil, is to be free of *Cronobacter* spp. and the batch analyses indicate otherwise, the notice does not provide a sufficient basis to conclude that *M. alpina* oil produced by Runke is GRAS under the conditions of its intended uses.

As explained below, we have questions regarding the safety of this ingredient due to the known contamination of one batch, and potential contamination of other batches, with *Cronobacter* spp. Contamination of this ingredient would make it harmful under its conditions of intended use. Therefore, our evaluation is limited to the contamination of this ingredient and the impact of such contamination on its notified use.[3]

Contamination of *M. alpina* oil Produced by Runke

In the notice, Runke provides food-grade specifications for *M. alpina* oil, including a specification for *Cronobacter* spp. (not detected in 10 g). In the notice, Runke lists the analytical method for testing *Cronobacter* spp. as the International Organization for Standardization (ISO) method 22964:2017 and reports that three non-consecutive batches of *M. alpina* oil meet the specification for *Cronobacter* spp.

Runke also provides Certificates of Analyses (COAs) demonstrating measured values for food-grade specifications, as well as the composition of *M. alpina* oil from three non-consecutive batches. Runke only tested three batches of *M. alpina* oil, but submitted COAs from five separate dates (December 2021, February 2022, April 2022, July 2022, and January 2023).

In the notice, Runke reports that all three batches had no detectable *Cronobacter* spp. as measured by ISO method 22964:2017; the April 2022 COA for all three batches reports the same information. However, we found an inconsistency in the December 2021 COA provided by Runke, where all three batches were tested for *Cronobacter* spp.[4]

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[3] Our safety concerns are limited to *M. alpina* oil as manufactured by Runke. We are not questioning the safety of ARA-containing *M. alpina* oil as reviewed by FDA in other GRNs. ARA-containing *M. alpina oil*, in combination with a safe and suitable source of DHA, was the subject of GRNs 000041, 000080, 000094, 000326, 000730, 000963, and 001115. We evaluated these notices and responded in letters dated May 17, 2001, December 11, 2001, April 18, 2006, February 16, 2011, March 30, 2018, October 19, 2021, and September 18, 2023, respectively, stating that we had no questions at that time regarding the notifiers' GRAS conclusions.

[4] We note that the December 2021 COAs refer to *Enterobacter sakazakii*; this organism was renamed to *Cronobacter* as reported in Iversen, C., et al. *Cronobacter* gen. nov., a new genus to accommodate the biogroups of *Enterobacter sakazakii*, and proposal of *Cronobacter sakazakii* gen. nov., comb. nov., *Cronobacter malonaticus* sp. nov., *Cronobacter turicensis* sp. nov., *Cronobacter muytjensii* sp. nov., *Cronobacter dublinensis* sp. nov., *Cronobacter genomospecies* 1, and of three subspecies, *Cronobacter dublinensis* subsp. *dublinensis* subsp. nov., *Cronobacter dublinensis* subsp. *lausannensis* subsp. nov. and

using a modified method from Chapter 29 of the FDA Bacteriological Analytical Manual (BAM);[5] the method modifications were not provided. For one batch, the December 2021 COA reports a *Cronobacter* spp. value of 2.3 most probable number (MPN)/10 mL.

These inconsistencies in reported values for *Cronobacter* spp. raise concerns about potential contamination of this ingredient manufactured in Runke's facility and appear to show that Runke did not perform corrective actions when a positive result in a batch was identified.

The information provided by Runke shows that when the December 2021 COA identified *Cronobacter* spp. in one batch of the *M. alpina* oil, the company used a different analytical method and retested the same batch, as shown in the April 2022 COA. This retest was performed with ISO Method 22964:2017, which may perform differently from the FDA BAM method. Runke only reported the negative *Cronobacter* spp. value from the April 2022 COA in the body of the notice and did not mention the positive result, which was included in an appendix. However, retesting is not an appropriate mechanism to ensure compliance when batch results do not meet specifications. In response to our question sent to Runke on August 27, 2024, as to why the *Cronobacter* spp.-positive batch was retested with a different method, Runke responded that the reason was that the original results were reported in a volumetric unit (mL) rather than a unit of mass (g). This explanation is not adequate; the microorganism was present in the sample, regardless of whether it was reported in a volumetric or mass unit.

Given that Runke's ingredient is intended for use in infant formula for healthy and pre-term/low-birthweight infants, a positive *Cronobacter* spp. result in any batch of *M. alpina* oil intended for this use could be harmful to these vulnerable populations. Infant formula containing such an ingredient could be contaminated with *Cronobacter* spp.; infant formula contaminated with *Cronobacter* spp. would be adulterated under 21 U.S.C. 342(a)(1). In FDA's letter issued to the infant formula industry in March 2023, which outlines a strategy to prevent future *Cronobacter* spp. illnesses associated with contaminated powdered infant formula, the Agency emphasized the importance of preventing *Cronobacter* spp. contamination at all stages of the supply chain, and requested that companies voluntarily notify the Agency if a product sample is found to be positive for *Cronobacter* spp.[6] Therefore, the potential for contamination with *Cronobacter* spp. raises safety questions about the intended use of this ingredient.

Cronobacter dublinensis subsp. *lactaridi* subsp. nov. *International journal of systematic and evolutionary microbiology*. DOI 10.1099/ijs.0.65577-0

[5] FDA's BAM presents the Agency's preferred laboratory procedures for microbiological analyses of foods and cosmetics. See: https://www.fda.gov/food/laboratory-methods-food/bacteriological-analytical-manual-bam.

[6] FDA Calls for Enhanced Safety Measures in Letter to Powdered Infant Formula Industry | FDA https://www.fda.gov/food/cfsan-constituent-updates/fda-calls-enhanced-safety-measures-letter-powdered-infant-formula-industry

Conclusions

Based on the information that you provided on behalf of Runke, as well as other information available to us, we have questions regarding the safety of this ingredient produced by Runke due to known and potential contamination with *Cronobacter* spp. In addition, the notice demonstrates that specifications for the ingredient were not met. Therefore, the notice does not provide a sufficient basis for a conclusion of GRAS status for the intended use of *M. alpina* oil manufactured by Runke. Additionally, infant formula containing such an ingredient could be contaminated with *Cronobacter* spp.; infant formula contaminated with *Cronobacter* spp. would be adulterated under 21 U.S.C. 342(a)(1).

In accordance with 21 CFR 170.275(b)(2), the text of this letter responding to GRN 001186 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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Director
Division of Food Ingredients
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