

Memorandum

Date: October 8, 2022

From: Division of Food Ingredients
Toxicology Review Branch-Team 2 (HFS-255)

To: Division of Food Ingredients
Regulatory Review Branch-Team 2 (HFS-255)
Attention: Stephanie Hice, Ph.D.

Subject: Final Toxicology Memorandum. **CAP 0C0316:** Hogan Lovells US LLP on behalf of GNT USA, Inc: Petition for the use of spirulina (*Arthrospira platensis*) extract as a color additive in various foods. Submissions dated January 28, 2020 and July 31, 2020.

1. Introduction

GNT USA, Inc. (GNT), through its agent Hogan Lovells US LLP, has submitted a Color Additive Petition (CAP 0C0316) proposing an amendment to 21 CFR 73.530 to allow for the safe use of spirulina extract, the water extract of a powdered form of blue-green cyanobacterium *A. platensis* (also known as *Spirulina platensis*) in the following food categories at levels consistent with good manufacturing practices: alcoholic beverages (with less than 20% alcohol-by-volume content)¹, non-alcoholic beverages, dairy product alternatives (identified as non-dairy yogurt alternatives, non-dairy frozen desserts, and non-dairy puddings), seasoning mixes (unheated), condiments and sauces, dips, and salad dressings.

The purpose of this memorandum is to summarize the Division of Food Ingredients (DFI) Toxicology's safety review of spirulina extract and phycocyanins (the major coloring components) as a color additive for the above proposed food uses.

2. Dietary Exposure Estimates for Spirulina Extract and its Major Coloring Components, Phycocyanins

¹ In its proposed amendment to § 73.530, the petitioner excluded beer from alcoholic beverages (with less than 20% alcohol-by-volume content). On January 25, 2022, the petitioner subsequently expanded the scope of its petition to include beer. Therefore, we consider beer to be within the scope of the amended regulation as an alcoholic beverage with less than 20% alcohol-by-volume content.

DFI Chemistry amended the petitioner's dietary exposure estimates for spirulina extract and for phycocyanins, the coloring component of this color additive, to include additional food codes for beer.² For spirulina extract, the 90th percentile estimates of eaters-only dietary exposure from the new proposed food uses in the U.S. population 2+ years of age were estimated to be 31 g/person (p)/d with the highest 90th percentile eaters-only estimate to be among adults 19+ years of age at 33 g/p/d. For the phycocyanins, the highest 90th percentile estimate of dietary exposure from the new proposed food uses in the U.S. population was estimated to be 0.7 g/p/d among adults 19+ years of age. The proposed new uses of spirulina extract are not expected to change the upper bound cumulative estimated daily intake (CEDI) for the phycocyanins from all spirulina-based color additives and ingredients, which is 1.14 g/p/d.³

3. Previous Regulatory Decisions for Spirulina Powder, Spirulina Extract and its Major Coloring Components, Phycocyanins

FDA has previously reviewed the safety of spirulina powder (i.e., dried biomass of *A. platensis*), spirulina extract (prepared from spirulina powder), and the phycocyanins, the major coloring component of both spirulina powder and spirulina extract in the following submissions: GRNs 000127, 000394, 000417, 000424 and CAPs 2C0293, 2C0297, 4C0300, and 6C0306. FDA has also established an acceptable daily intake (ADI) for phycocyanins.

3.1. Previous Generally Recognized as Safe (GRAS) Notices for Spirulina (Table 1)

Table 1: GRAS Notices for Spirulina and Spirulina Extract

GRN (Year)	Subject of the GRAS Notice	Food Uses	Notifier's Exposure Estimate	FDA Response
000127 (2003)	Dried biomass of <i>A. platensis</i>	Food bars, powdered nutritional drinks, popcorn, and as a condiment in salads and pasta (0.5 – 3 g/serving)	6 g biomass/p/d High consumer	No questions letter (October 6, 2003)
000394 (2011)	Dried biomass of <i>A. platensis</i>	Grape juice, blackberry juice, lime juice, low calorie fruit drinks, and low calorie vegetable juice drinks at a level of 0.3%, medical foods at a level of 1.25%	2.95 g biomass/p/d 90 th Percentile	No questions letter (June 4, 2012)
000417 (2012)	Dried biomass of <i>A. platensis</i>	Beverages and beverage bases, breakfast cereals, fresh fruits and fruit juices, frozen dairy desserts and mixes, grain products and pastas, milk products, plant protein products, processed fruit and fruit juices, processed vegetables and vegetable juices, snack foods, soft candy, and soups and soup mixes at a level of 0.5-3 g/serving	1-3 g biomass/p/d	No questions letter (August 10, 2012)

² Marla Swain to Stephanie Hice, October 7, 2022, CAP 0C0316: Hogan Lovells US LLP, on behalf of GNT USA, Inc: Petition for the use of spirulina (*Arthrospira platensis*) extract as a color additive in various foods. Submissions dated January 28, 2020, June 29, 2020, July 31, 2020, and January 25, 2022

³ Initially estimated in GRN 000424 as the EDI and later, in CAP 2C0293 and other spirulina extract CAPs, as the CEDI.

000424 (2012)	c-c- phycocyanin- enriched water extract of the cyanobacterium <i>Arthrospira maxima</i> or <i>A. platensis</i>	All foods except infant formulas and foods under USDA's jurisdiction at levels up to a maximum of 0.250 g/serving	2.28 g phycocyanin water extract/p/d (1.14 g/p/d phycocyanin) equivalent to 5.7 g of spirulina biomass/p/d	No questions letter (December 6, 2012)
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3.2 Previous Color Additive Petitions (CAP) for Spirulina Extract

3.2.1 Color Additive Petition 2C0293

In 2011, the FDA received the first color additive petition for the use of spirulina extract as a color additive. Mars, Inc., through its agent, Keller and Heckman, LLP, submitted a CAP for the use of Spirulina Blue in candy and chewing gum at levels consistent with good manufacturing practice (GMP). Spirulina Blue is a blue color additive extracted and concentrated from dried *A. platensis* cyanobacterium. The petitioner used the common name “spirulina” or “spirulina powder” to describe the dried product from which the color additive was made. FDA’s review of the safety information was captured in a series of Division of Petition Review (DPR) toxicological interim memoranda dated July 18, 2012, September 14, 2012, September 26, 2012, September 27, 2012, September 28, 2012, and October 1, 2012. In a final toxicology memorandum,⁴ DPR Toxicology summarizes the important toxicological conclusions from their review. In particular, the memorandum states that a 21-month chronic oral rat toxicity study published by Chamorro et al., 1988 was identified as the pivotal safety study on which to evaluate the safety of Spirulina Blue as a color additive. In this study, spirulina powder, as the test article, was mixed into the feed. Based on this study, DPR Toxicology established 30% spirulina powder in the feed, the highest concentration tested, as a No-Effect Level (NOEL) for spirulina powder. As reported by the petitioner, this concentration is equivalent to 15 g spirulina powder/kg body weight (bw)/d or 900 g spirulina powder/p/d for a 60-kg person. Additionally, the memorandum states that based on a phycocyanin content of 12-20% in spirulina powder, the NOEL for spirulina powder also supports a No-Effect range⁵ for phycocyanin. The memorandum refers to an upper bound CEDI of 1.14 g/p/d for phycocyanins from the proposed uses of Spirulina Blue as a color additive as well as its uses in foods as described in GRN 000424 and concluded to be GRAS.⁶ Based on the NOEL-CEDI comparison, DPR Toxicology concluded that there was an adequate margin of safety between the NOEL and the CEDI for phycocyanins. Overall, DPR Toxicology concluded that there were no questions or concerns regarding the safety of Spirulina Blue (phycocyanins) for its proposed use as a color additive in candy and chewing gum.

⁴ Gladys Erives to Felicia Ellison, October 2, 2012, CAP 2C0293: Final Toxicology Review Memorandum; Safety Review of *Arthrospira (A.) platensis* cyanobacterium (Spirulina, Spirulina Blue)

⁵ Corresponding to 1.8-3.0 g phycocyanins/kg bw/d or 108-180 g phycocyanins/p/d based on the 12-20% phycocyanin content (as noted in this memorandum). These numbers were not calculated in the Final Toxicology memorandum for CAP 2C0293

⁶ Daniel Folmer to F. Ellison, May 31, 2012, CAP 2C0293: Keller and Heckman, on behalf of Mars Inc. Submissions of 10/7/2011 and 4/11/2012. Petition for the use of Spirulina Blue as a color additive in candy and chewing gum at levels consistent with food manufacturing practices (GMP)

3.2.2 Color Additive Petition 2C0297

In 2012, Hogan Lovells US LLP, on behalf of GNT, submitted a CAP proposing that the FDA's color additive regulation be amended to allow for the safe use of spirulina extract in the following categories of food products: colored confectioneries, ice cream and frozen desserts, dessert coatings and toppings, beverage mixers and powders, yogurts, custards, and puddings and ready-to-eat cereals. The DPR Toxicology review memorandum summarizes the conclusions of DPR Toxicology and contains a comprehensive inventory of the spirulina publications received by FDA at that time.⁷ DPR Chemistry concluded^{8,9} that the previously estimated dietary exposure to phycocyanins (1.14 g/p/d) represented the upper bound cumulative exposures from all spirulina products, including ones from the proposed food uses in CAP 2C0297. The DPR Toxicology review memorandum⁷ stated that based on all the available safety information for spirulina, a typical range of 12-20% phycocyanin content for spirulina, and a 100-fold safety factor (to address uncertainty in extrapolating chronic animal data to humans), the CEDI for the phycocyanins did not exceed a likely ADI range of 1.0 to 1.8 g/p/d. The memorandum concluded that there are no safety concerns for the proposed uses of the color additive when it is used up to either the calculated CEDI for its phycocyanin content or for the spirulina powder form.

3.2.3 Color Additive Petition 4C0300

In 2014, Colorcon, Inc. submitted a CAP proposing that the FDA's color additive regulation be amended to allow for the safe use of spirulina extract as a color additive in coating formulations applied to dietary supplement and drug tablets and capsules at levels consistent with GMP. The petitioner did not submit any new toxicological studies but included in the safety package a list of publications and an updated safety narrative. DPR Chemistry determined that the incremental increase in exposure to phycocyanins (1.8% increase) from the use of spirulina extract containing up to 28% phycocyanin for their proposed uses would not significantly contribute to the previously determined upper bound exposure of 1.14 g/p/d for phycocyanins.¹⁰ DPR Toxicology concluded that the publications submitted by the petitioner did not present any new information on spirulina extract and phycocyanins that would indicate a safety concern at levels lower than the previously derived NOEL. Thus, DPR Toxicology had no questions or concerns regarding the proposed use of spirulina extract as a color additive in coating formulations applied to dietary supplements and drug product tablets and capsules at levels consistent with GMP when used up to the calculated CEDI for its phycocyanin content.¹¹

⁷ Jin-Young Park to Felicia Ellison, November 1, 2013, Color Additive Petition (CAP) 2C0297: *Spirulina* Concentrate (or Water Extract from *Spirulina Platensis*) as a Color Additive in Foods – Toxicology Review

⁸ Hyoung Lee to Raphael Davy, January 15, 2013, CAP 2C0297: Hogan Lovells US LLP, on behalf of GNT USA, Inc. submissions of 6/8/2012, 9/6/12, and 9/9/12. Petition for the use of Spirulina Concentrate as a blue color in various foods (Related to GRN 000127)

⁹ Hyoung Lee to Raphael Davy, May 7, 2013, CAP 2C0297: Hogan Lovells US LLP, on behalf of GNT USA, Inc. Submission of 4/18/13. Petition for the Use of Spirulina Concentrate as a blue color in various foods (Related to GRN 000127) – Update of exposure estimates

¹⁰ Hyoung Lee to Molly Harry, January 30, 2015, CAP 4C0300: Colorcon, Inc. Petition to amend 21 CFR 73.530 for the listing of spirulina extract as a color additive for use in coating formulations applied in dietary supplement and drug tablets and capsules. Submissions of 9/3/14, 10/6/14, and 12/1/14

¹¹ Tina Walker to Molly Harry, April 2, 2015, Color Additive Petition (CAP) 4C0300 – To provide for the safe use of Spirulina extract as a color additive in coating formulations applied to dietary supplement and drug tablets.

3.2.4 Color Additive Petition 6C0306

In 2016, Exponent, on behalf of McCormick & Company, Inc. submitted a CAP proposing that the FDA's color additive regulation be amended to allow for the safe use of Linablue G1 spirulina extract (manufactured by DIC LIFETEC Co., Ltd, Japan) as a color additive to seasonally color the shells of hard-boiled eggs (Easter eggs). The Office of Cosmetics and Colors Division of Color Technology Team noted¹² that the Linablue G1 spirulina extract is equivalent to the spirulina extract that was approved under 21 CFR 73.530(a)(1). DPR Chemistry estimated that the 90th percentile estimates of dietary exposure to phycocyanins from the proposed food uses in the U.S. population aged 2+ was 0.0019 g/p/d,¹³ a negligible increase to the previously determined CEDI of 1.14 g/p/d. In the final toxicology review memorandum¹⁴ DPR Toxicology considered the previous ADI determination of 1.0 to 1.8 g/p/d for phycocyanins, a search of recently published literature on spirulina extract and phycocyanins, a negligibly increased exposure to phycocyanins from the subject petition and concluded that the requested proposed use of spirulina extract did not pose a safety concern to human health at the proposed use level. Additionally, in two addendums to the final toxicology review memorandum,^{15,16} DPR Toxicology briefly reviewed previous conclusions regarding the potential allergenicity of spirulina extract and/or phycocyanins as color additives in food. DPR Toxicology concluded that they were not aware of any new relevant information that would alter the conclusion that spirulina extract as a color additive for both current uses and the proposed use in food presents an insignificant allergy risk for the general population.

4. DFI Toxicology's Safety Evaluation for Spirulina Extract and its Major Coloring Components, Phycocyanins

In the product safety narrative (see petition, Volume I, Section D), the petitioner stated that previous safety determinations made by FDA for CAP 2C0293, CAP 2C0297, CAP 4C0300, and CAP 6C0306 were incorporated by reference. Additionally, the petitioner discussed the results of an updated literature search from July 1, 2016 to November 8, 2019, in which three studies (Bigagli et al., 2017; Hernández-Lepe et al., 2018; Hernández-Lepe et al., 2019) were identified by the petitioner as relevant to the safety of spirulina.¹⁷ After reviewing these three studies, the petitioner concluded that they did not reveal any significant new toxicological effects and would not alter the conclusions of FDA's previous reviews on spirulina. A comprehensive spirulina safety database was also provided by the petitioner (see petition,

¹² Nebebech Belai to Molly Harry, February 1, 2017, Review of CAP 6C0306 – Amend 21 CFR 73.530 to provide for the expanded safe use of spirulina extract as a color additive to seasonally color the shells of hard-boiled eggs

¹³ Hyoung Lee to Molly Harry, February 1, 2017, CAP 6C0306: Exponent, Inc., on behalf of McCormick & Company, Inc. Petition to amend 21 CFR 73.530 for the safe use of spirulina extract to seasonally color the shells of hard-boiled eggs. Submission of 8/10/16

¹⁴ Jin-Young Park to Molly Harry, February 2, 2017, Color Additive Petition (CAP) 6C0306: *Spirulina* Extract (Water Extract from *Arthrospira Platensis*) to Seasonally Color the Shells of Hard-Boiled Eggs – Toxicology Review

¹⁵ Jin-Young Park to Molly Harry, April 13, 2017, Addendum to Feb 2, 2017 Color Additive Petition (CAP) 6C0306 Toxicology Review: *Spirulina* Extract (Water Extract from *Arthrospira Platensis*) to Seasonally Color the Shells of Hard-Boiled Eggs

¹⁶ Jin-Young Park to Molly Harry, April 24, 2017, Addendum to Feb 2, 2017 Color Additive Petition (CAP) 6C0306 Toxicology Review: *Spirulina* Extract (Water Extract from *Arthrospira Platensis*) to Seasonally Color the Shells of Hard-Boiled Eggs

¹⁷ The complete citation list of publications identified by the petitioner's updated literature search (July 1, 2016 to November 8, 2019) is provided in Appendices III and IV.

Appendix II) that included five newly identified studies¹⁸ as well as relevant historical toxicology and clinical studies.

DFI Toxicology reviewed the newly published safety studies identified by the petitioner's updated literature search and confirmed that all studies referenced by the petitioner in the comprehensive database (see petition, Appendix II) had been previously evaluated in the Office of Food Additive Safety toxicology memoranda.¹⁹ Additionally, DFI Toxicology conducted an independent literature search and review of spirulina and phycocyanins.¹⁹ No safety concerns relating to spirulina or phycocyanin were identified in the recently published studies and no new safety data were found that would change the previously determined ADI of phycocyanins.¹⁹

5. DFI Toxicology's Safety Evaluation of a Potential Toxic Constituent

β -N-methylamino-L-alanine (BMAA) is a non-proteinogenic amino acid that has been implicated in multiple publications in the development of neurodegenerative diseases. BMAA may be produced by a wide variety of cyanobacteria species with levels dependent on species, strain, and environmental conditions.²⁰ DFI Toxicology identified several publications which reported the detection of BMAA in products containing spirulina and spirulina powder.^{21,22} In the original submission, the petitioner did not address the presence or absence of BMAA in either the *A. platensis* biomass or the color additive.

In an amendment dated July 31, 2020, the petitioner provided analytical data demonstrating that the levels of BMAA and its isomers, β -amino-N-methyl-alanine (BAMA), diaminobutyric acid (2,4-DAB), and N-2-aminoethylglycine (AEG), were all below the limit of detection in five lots of the spirulina biomass and the commercially produced spirulina extract. The petitioner also provided a published study from Downing et al., 2011²³ which demonstrated that BMAA forms when there is a nitrogen deficiency in cyanobacteria during cultivation. The petitioner states that phycocyanins, the main coloring components of spirulina extract, act as nitrogen storage compounds and that if there was a nitrogen deficiency during the cultivation of the *A. platensis* biomass, the phycocyanin content would decrease to such a level as to be unusable for the manufacture of the coloring extract; thus, BMAA formation would not be expected to occur.

¹⁸ Along with Bigagli et al., 2017; Hernández-Lepe et al., 2018; and Hernández-Lepe et al., 2019, Yadav et al., 2016 and Gavric et al., 2018 were included by the petitioner in Appendix II. Yadav et al., 2016 and Gavric et al., 2018 were identified by DFI Toxicology, but not specifically by the petitioner in Volume I of the petition, as being relevant to the safety of spirulina extract

¹⁹ Danica DeGroot to Stephanie Hice, October 8, 2022, Literature Review Memorandum. CAP 0C0316: Hogan Lovells US LLP on behalf of GNT USA, Inc: Petition for the use of spirulina (*Arthrospira platensis*) extract as a color additive in various foods. Submissions dated January 28, 2020 and July 31, 2020

²⁰ Cox et al. Diverse taxa of cyanobacteria produce β -N-methylamino-L-alanine, a neurotoxic amino acid [published correction appears in *Proc Natl Acad Sci U S A*. 2005 Jul 5;102(27):9734]. *Proc Natl Acad Sci U S A*. 2005;102(14):5074-8. doi:10.1073/pnas.0501526102

²¹ Baker et al. Assessing environmental exposure to β -N-methylamino-L-alanine (BMAA) in complex sample matrices: A comparison of the three most popular LC-MS/MS methods. *Neurotox Res*. 2018;33(1):43-54. doi:10.1007/s12640-017-9764-3

²² Glover et al. Determination of β -N-methylamino-L-alanine, N-(2-aminoethyl)glycine, and 2,4-diaminobutyric acid in food products containing cyanobacteria by ultra-performance liquid chromatography and tandem mass spectrometry: Single-laboratory validation. *J AOAC Int*. 2015;98(6):1559-1565. doi:10.5740/jaoacint.15-084

²³ Downing et al. Nitrogen starvation of cyanobacteria results in the production of β -N-methylamino-L-alanine. *Toxicon*. 2011;58(2):187-194. doi:10.1016/j.toxicon.2011.05.017

DFI Toxicology Comments: We note that the study by Downing et al., 2011, did not utilize *A. platensis* and it is unclear if the mechanisms of BMAA formation presented in the study are generalizable to *A. platensis*. Therefore, the critical data to support the safe use of spirulina extract is the analytical data demonstrating no detectable levels in the spirulina biomass and color additive.²⁴

5. Conclusions

DFI Toxicology has evaluated the relevant safety studies submitted in the petition and in its associated amendments. Additionally, DFI Toxicology has performed its own literature search and reviewed relevant publications relating to the safety, including allergenicity, of spirulina and its major coloring components, the phycocyanins. Based on the literature review, no new information or data were identified that would change the ADI of 1.0 to 1.8 g/p/d for phycocyanins nor indicate a safety concern at levels below this ADI. DFI Toxicology notes that based on information provided by the petitioner, DFI Chemistry does not expect that the estimated dietary exposure to the phycocyanins from the new proposed food uses will increase the current upper bound cumulative dietary exposure of 1.14 g/p/d to the phycocyanins.

We have no safety concerns for the proposed uses of spirulina extract as a color additive in alcoholic beverages (with less than 20% alcohol-by-volume content), non-alcoholic beverages, condiments and sauces, dips, dairy product alternatives (identified as non-dairy yogurt alternatives, non-dairy frozen desserts, and non-dairy puddings), salad dressings, and seasoning mixes (unheated) when consumed up to the calculated CEDI for the phycocyanins. DFI Toxicology recommends that FDA continue to monitor updated safety and exposure data for spirulina and the phycocyanins as they become available.

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²⁴ Nebebech Belai to Stephanie Hice, September 30, 2022, CAP 0C0316: Hogan Lovells US LLP on behalf of GNT USA, Inc.: Spirulina extract for use as a color additive in alcoholic beverages (with less than 20% alcohol-by-volume content), non-alcoholic beverages, condiments and sauces, dips, dairy product alternatives, salad dressings, and seasoning mixes (unheated). Submissions dated January 28, 2020 and July 31, 2020

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