United States Department of Agriculture
Agricultural Marketing Service | National Organic Program
Document Cover Sheet
https://www.ams.usda.gov/rules-regulations/organic/national-list/petitioned

Document Type:

☐ **National List Petition or Petition Update**

A petition is a request to amend the USDA National Organic Program's National List of Allowed and Prohibited Substances (National List).

Any person may submit a petition to have a substance evaluated by the National Organic Standards Board (7 CFR 205.607(a)).

Guidelines for submitting a petition are available in the NOP Handbook as NOP 3011, National List Petition Guidelines.

Petitions are posted for the public on the NOP website for Petitioned Substances.

☒ **Technical Report**

A technical report is developed in response to a petition to amend the National List. Reports are also developed to assist in the review of substances that are already on the National List.

Technical reports are completed by third-party contractors and are available to the public on the NOP website for Petitioned Substances.

Contractor names and dates completed are available in the report.

Pullulan

Handling/Processing

Identification of Petitioned Substance

Chemical Names:
5-[[3,4-dihydroxy-6-(hydroxymethyl)-5-[[3,4,5-trihydroxy-6-(methoxymethyl)oxan-2-yl] methoxymethyl]oxan-2-yl]methoxymethyl]-6-(hydroxymethyl)oxane-2,3,4-triol (IUPAC)

Other Name:
Pullulan [National Formulary]
Polymaltotriose

Trade Name:
Pullulan

CAS Number:
9057-02-7

EC/EINECS Number:
232-945-1

Other Codes:
PubChem CID: 92024139
EPA Chem. Sub. Inventory Nos.: 1224323-71-0, 152743-43-6; 58252-16-7; 58391-35-8
INS No. 1204 [E1204]

Summary of Petitioned Use

The petitioned use of pullulan is as an allowed non-synthetic ingredient in tablets and hard and soft capsules for dietary supplements labeled "made with organic (specified ingredients or food group(s)" through its addition to the National List at § 205.605(a).

Characterization of Petitioned Substance

Composition of the Substance:
Pullulan is a linear carbohydrate biopolymer consisting of repeating units of maltotriose joined by α-D-(1→6) linkages, creating a long stair-step-type structure. Maltotriose is a trisaccharide of three glucosyl moieties; the molecular structure of maltotriose is shown in Figure 1. The mean molecular weight (MW) of pullulan can range from 8 kilodaltons (kDa) to more than 2,000 kDa depending upon the conditions under which the source organism is grown. The petition cites two pullulan materials with mean molecular weights of 100 and 200 kDa.

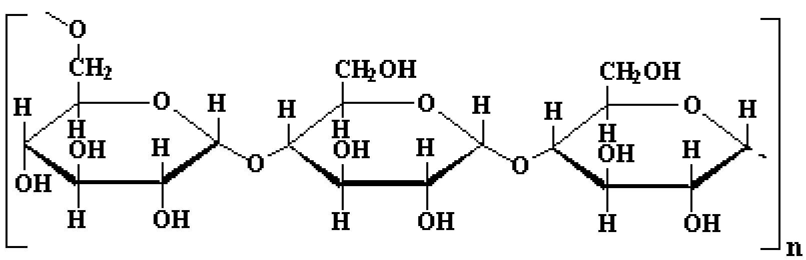

Figure 1. Chemical structure of maltotriose, the subcomponent of pullulan (JECFA 2011).

Source or Origin of the Substance:
Pullulan is a natural extracellular polysaccharide excreted by the black yeast-like fungus *Aureobasidium pullulans* and by several other non-toxigenic strains of fungi during fermentation of a carbohydrate-containing substrate.

A. pullulans is a ubiquitous saprophyte mold generally considered an environmental contaminant. It is most common in temperate zones, with numerous recordings from the British Isles and the United States, but is also found in Canada, Alaska, Antarctica, Europe, and Russia. The genus is found in forest soil, freshwater, on aerial portions and leaf surfaces of plants, as well as on seeds (e.g., wheat), cereals (e.g., barley, oats), and some nuts (e.g., pecans). *A. pullulans* is found throughout all ecological niches, including forest soils, fresh water and seawater, and plant and animal tissues (Shingel 2004; Wolf et al. 2003). It is also found as a spoilage agent on fruits (e.g., pears, grapes, tomatoes) or in fruit drinks. It has been associated with the deterioration of pears and oranges in storage or in transit (Institut National de Santé Publique du Québec 2016).

A. pullulans requires high levels of available water to grow; it is commonly found growing indoors on surfaces that are continually damp as well as in liquid waste materials (Institut National de Santé Publique du Québec 2016).

The preferred saccharide substrate in the growth medium for *A. pullulans* is known as 'starch syrup' (i.e., partially hydrolyzed food starch), although other saccharides such as glucose, maltose, malt oligosaccharides, sucrose, fructose (Ozaki, Nomura and Miyake 1996), and tapioca (Capsugel 2018) are also acceptable sources. Less refined materials also can be used as substrates and include beet molasses (Lazaridou et al. 2002), corn steep liquor (Sharma, Prasad and Choudhury 2013; West and Strohfus 1999), Jerusalem artichoke tubers (Xia et al. 2017), date extract (Kato and Shiosaka 1975), dairy whey (Roukas 1999), and agricultural wastes such as Asian palm kernel (Sugumaran, Gowthami, et al. 2013), cassava bagasse (Sugumaran, Jothi and Ponnusami 2014), and jack fruit seed (Sugumaran, Sindhu, et al. 2013).

Properties of the Substance:

Pullulan is a white to off-white odorless powder highly soluble in water and practically insoluble in ethanol and other organic solvents. A 10 percent aqueous solution has a pH of 5.0–7.0. Pullulan films are thin, clear, readily dissolved, highly oxygen-impermeable, fat-resistant, odorless, colorless, and biodegradable (Leathers 2003; Farris et al. 2014).

Specific Uses of the Substance:

According to the FDA Center for Drug Evaluation and Research (CDER), pullulan is a "product used for tablet coating, as an excipient to aid tableting processes, in the production of edible films, and as an alternative to gelatin in capsule production" (FDA 2014). The unique film-forming property of pullulan enables the production of clear capsules and coatings for dietary supplements (Farris et al. 2014). Capsules made with pullulan provide ease of formulation while still maintaining a disintegration/dissolution profile equal to that of gelatin. A capsule shell made with pullulan can also help eliminate "spotting" concerns that occur when vitamin C is encapsulated in gelatin. Pullulan creates a more effective oxygen barrier than other available plant-based products, and, similar to gelatin capsules, pullulan capsules are highly machinable on all capsule filling machines (Capsugel 2012).

The self-affirmed Generally Recognized as Safe (GRAS) notification letter sent to FDA in 2002 specified general uses of pullulan in foods as a multiple-use direct additive and enumerated nine specific physical and technical effects described at 21 CFR § 170.3(o): (8) "Emulsifiers and emulsifier salts," (14) "Formulation aides," (16) "Humectants," (20) "Nutrient supplements," (24) "Processing aids," (28) "Stabilizers and thickeners," (29) "Surface-active agents," (31) "Synergists," and (32) "Texturizers."

In addition to the petitioned use of pullulan as an ingredient in tablets and capsules for dietary supplements, edible pullulan films are used to extend the shelf life of various foods. These films prevent moisture loss and reduce surface exposure to oxygen and spoilage bacteria in intact berries (Krasniewska et al. 2017; Trevino-Garza et al. 2015; Diab et al. 2001), Brussels sprouts (Krasniewska et al. 2016), baby carrots (Gniewosz et al. 2013), nuts (Gounga et al. 2008), fresh eggs (Ozaki, Nomura and Miyake 1996), intact apples (Chlebowska-Śmigiel, Gniewosz and Świńczak 2007), and cut fruits such as apple slices (Wu and Chen 2013).

Approved Legal Uses of the Substance:
FDA issued an Agency Response Letter GRAS Notice No. GRN 000099 on August 1, 2002, indicating that FDA had no objections to the self-affirmed GRAS status for pullulan (Rulis 2002). The self-affirmed Generally Recognized as Safe (GRAS) notification letter sent to FDA in 2002 specified general uses of pullulan in foods as a multiple-use direct additive and enumerated nine specific physical and technical effects described at 21 CFR § 170.3(o): (8) "Emulsifiers and emulsifier salts," (14) "Formulation aides," (16) "Humectants," (20) "Nutrient supplements," (24) "Processing aids," (28) "Stabilizers and thickeners," (29) "Surface-active agents," (31) "Synergists," and (32) "Texturizers." EPA classifies pullulan as a 2016 Chemical Data Reporting (CDR) Full Exempt substance; these substances are fully exempt from reporting under 2016 CDR as long as they are not also found in certain Toxic Substances Control Act (TSCA) actions (EPA Substance Registry Services (SRS)).

Action of the Substance:
The primary actions of pullulan are to serve as coatings on tablets, preservative films that prolong the shelf life of certain foods, and as an alternative to gelatin capsules for certain oral supplements. The regular occurrence of α-(l-6) linkages in pullulan interrupts what would otherwise be a linear amylose chain. This unique linkage pattern is believed to be responsible for the structural flexibility and solubility of pullulan, resulting in distinct film- and fiber-forming characteristics not exhibited by other polysaccharides. These characteristics permit pullulan to be formed into edible films and capsules, including capsules used for certain dietary supplements. Pullulan's high water solubility makes it a useful ingredient in tablet coatings (Izutsu et al. 1987). Its oxygen barrier properties are ideal for final tablet coating to protect oxygen-sensitive vitamins and other active ingredients and to keep the tablets from darkening over time.

Combinations of the Substance:
Pullulan films generally contain a plasticizer to improve physio-chemical properties such as tensile strength and stretch ability (Pan et al. 2014; Vuddanda et al. 2017); glycerol (glycerin) is the most effective plasticizer, which is on the National List at 7 CFR 205.605(b). Both pullulan and glycerol are highly soluble in water, a key requirement for an encapsulation material used for oral dietary supplements.

In some applications, synthetic substances may be combined with pullulan to achieve specific properties. For example, Kim (2018) patented a modified starch composed of modified waxy corn starch and modified waxy potato starch to create a soft capsule.

Status

Historic Use:
Hayashibara Co., Ltd. initiated commercial production of pullulan in 1976 in Japan (Tsujisaka and Mitsuhashi 1993) for use as a polysaccharide thickener and edible film matrix, commercializing pullulan film production in 1982 (Leathers 2003). In March 2002, Hayashibara submitted the original notification of the GRAS status of pullulan to the FDA. The FDA issued the Agency Response Letter GRAS Notice No. GRN 000099 on August 1, 2002 indicating that the FDA had no objections to the self-affirmed GRAS status for pullulan (Rulis 2002).

In February 2004, the Capsugel Division of Pfizer Inc. submitted a petition to the National Organic Standards Board (NOSB) to add pullulan to § 205.605 of the National List as a substance for use in foods "made with organic (specified ingredient or food group(s)". The NOSB subsequently put the petition on hold, and no final recommendation was ever made. Information as to why the original petition was put on hold is not publicly available.

From 2004 until December 2016, non-organic pullulan was commonly classified by accredited certifiers as agricultural, enabling its use as an ingredient in products labeled "made with organic (specified ingredient or food group(s))." USDA organic regulations allow nonorganic agricultural ingredients that do not appear on the National List at § 205.606 only in "made with organic (specified ingredient or food group(s))."

Because of this interpretation, the organic supplement industry developed around the use of nonorganic pullulan capsules (ACA 2017). In contrast, *nonagricultural* ingredients that do not appear on the National List at § 205.605 are not allowed in organic products, including products labeled as "made with organic (specified ingredient or food group(s))."

In December 2016, the National Organic Program (NOP) released the guidance document "NOP Guidance 5033 Classification of Materials,"[1] which describes the procedure to be used to classify materials as synthetic or nonsynthetic, and as agricultural or nonagricultural, under the USDA organic regulations. This guidance includes a decision tree[2] for classifying agricultural and nonagricultural materials for organic livestock production or handling. In response to this guidance, some certifiers reclassified pullulan as a nonagricultural ingredient, which would disallow pullulan as an ingredient in products labeled "made with organic (specified ingredient or food group(s))." Because of the dissent in the organic community regarding pullulan's classification, the Accredited Certifiers Association (ACA) created a working group to address this topic. The working group strongly agreed with the nonagricultural classification of pullulan, as noted in their October 2017 report entitled "Best Practices for Classification and Evaluation of Pullulan" (Accredited Certifiers Association Inc. 2017). Subsequently, the ACA agreed to suspend phase-out efforts for existing formulations containing pullulan contingent upon the submission of a new petition to the NOSB and a decision by NOSB about whether pullulan should be added to the National List (Accredited Certifiers Association Inc. 2017).

The current National List of Allowed and Prohibited Substances does not include pullulan at § 205.605, "Nonagricultural (nonorganic) substances allowed as ingredients in or on processed products labeled as "organic" or "made with organic (specified ingredients or food group(s))." In January 2018, the Organic Trade Association filed a petition on behalf of its National List Innovation Working Group to add pullulan to the National List at § 205.605(a) as an allowed non-agricultural, non-synthetic ingredient used in tablets and capsules for dietary supplements labeled "made with organic (specified ingredients or food group(s))." This petition aims to enable the continued production and availability of certified "made with organic" encapsulated dietary supplements and to support the commercial development of certified organic pullulan.

Organic Foods Production Act, USDA Final Rule:

Pullulan is not listed in OFPA or the current rule (7 CFR, Part 205).

International

Canada, Canadian General Standards Board – CAN/CGSB-32.311-2015 Amended March 2018, Organic Production Systems Permitted Substances List

http://www.inspection.gc.ca/food/organic-products/standards/eng/1300368619837/1300368673172

Pullulan is not included in the Canadian General Standards Board—CAN/CGSB-32.311-2015, Organic Production Systems Permitted Substances List.

CODEX Alimentarius Commission – Guidelines for the Production, Processing, Labelling and Marketing of Organically Produced Foods (GL 32-1999)

http://www.fao.org/docrep/005/Y2772E/Y2772E00.HTM

Pullulan does not appear in the CODEX Alimentarius Commission—Guidelines for the Production, Processing, Labelling and Marketing of Organically Produced Foods (GL 32-1999).

European Economic Community (EEC) Council Regulation – EC No. 834/2007 and 889/2008

http://eur-lex.europa.eu/legal-content/EN/ALL/?uri=CELEX:32008R0889

Pullulan does not appear in the European Economic Community (EEC) Council Regulation—EC No. 834/2007 and 889/2008.

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[1] *NOP 5033 Classification of Materials 12/2/2016*, https://www.ams.usda.gov/sites/default/files/media/NOP-5033.pdf

[2] *NOP 5033-2 Decision Tree for Classification of Agricultural and Nonagricultural Materials for Organic Livestock Production or Handling 12/2/2016*, https://www.ams.usda.gov/sites/default/files/media/NOP-Ag-NonAg-DecisionTree.pdf

Japan Agricultural Standard (JAS) for Organic Production
http://www.maff.go.jp/e/jas/specific/criteria_o.html
The Japan Agricultural Standard (JAS) for Organic Production does not address the use of pullulan. Pullulan is not listed in Table 1 "Additives" of the JAS for Organic Processed Foods Notification No. 1606, partially revised March 27, 2017.

IFOAM – Organics International
http://www.ifoam.bio/en/ifoam-norms
Pullulan is not included in the IFOAM Norms.

Evaluation Questions for Substances to be used in Organic Handling

__Evaluation Question #1:__ **Describe the most prevalent processes used to manufacture or formulate the petitioned substance. Further, describe any chemical change that may occur during manufacture or formulation of the petitioned substance when this substance is extracted from naturally occurring plant, animal, or mineral sources (7 U.S.C. § 6502 (21)).**

All pullulan is created by microbial fermentation. The microorganism is usually the black, yeast-like fungus *A. pullulans*, although other species from this genus of black fungus—such as *A. fermentans* (Ozaki, Nomura and Miyake 1996) and *A. melanogenum* (Jiang et al. 2018)—have also been shown to produce pullulan. Nitrogen is provided in the growth medium in the form of inorganic nitrogen sources such as ammonium salts and nitrates and biological sources such as a glutamate, peptone, yeast extract, and corn steep liquor. Essential nutrient minerals are provided as phosphates, magnesium salts, and the sulfates of iron, manganese, and zinc.

The petitioned pullulan is produced using the following steps.

1. Fermentation of saccharide substrate by a microorganism creates pullulan.
2. Microfiltration separates microorganism cells and cellular debris from the aqueous medium containing water-soluble pullulan.
3. Heat-sterilization inactivates the heat-labile enzyme pullulanase, a co-product of the fermentation which causes the degradation of pullulan. This step also ensures the microbiological safety of the pullulan solution.
4. Deionization using insoluble ion exchange resins removes electrolytes and other nutrients, such as minerals, from the pullulan solution, thereby purifying it.
5. Intermediate concentration (water evaporation) increases the pullulan concentration in the solution.
6. Decolorization with activated carbon binds the black pigment melanin produced by the microorganism during the fermentation.
7. Filtration removes the activated carbon and adsorbed melanin.
8. Drying removes the water and yields a solid material.

No organic solvents are used in the process described above, but another successful commercial process employs an alcohol (e.g., isopropyl alcohol) for solvent precipitation of pullulan as an alternate to Steps 4, 5, and 7 in the manufacturing process above (Kato and Nomura 1977). Because pullulan is insoluble in alcohol, adding an alcohol precipitates the pullulan (Thorne, Pollock and Armentrout 2002), facilitating its isolation and purification. This process does not modify the extracted pullulan, and no solvent residues persist in the finished pullulan with either of these methods.

Once pullulan is created/produced in the fermentation process (Step 1), it does not undergo any further chemical change during either of the manufacturing processes described above. If chemically changed, the substance would no longer be considered pullulan per the JECFA monograph (JECFA 2011) or Food Chemical Codex (U.S. Pharmacopeia 2010).

Evaluation Question #2: Discuss whether the petitioned substance is formulated or manufactured by a chemical process or is created by naturally occurring biological processes (7 U.S.C. § 6502 (21)). Discuss whether the petitioned substance is derived from an agricultural source.

Pullulan is created by aerobic fermentation of saccharides (sugar) from plant matter, a naturally occurring biological process. Pullulan is a carbohydrate polymer. Each of the useful saccharide sources cited in the literature as suitable substrates—starch syrup, tapioca, glucose, fructose, corn steep liquor, Jerusalem artichoke tubers, date extract, dairy whey and agricultural waste products—is derived from an agricultural source, but it is the microorganism that creates the carbohydrate configuration that identifies the substance pullulan. When the described microorganism ferments any of these saccharide substrates, the product is always pullulan.

The question of whether pullulan is derived from an agricultural source, and whether it should be considered an agricultural ingredient for the purposes of organic certification, is the subject of interest and the reason for its petitioned addition to the National List. As described under *Historic Use*, pullulan was previously considered agricultural, as it did not clearly fit the definition of "nonagricultural substance" at 7 CFR 205.2, which reads:

> "Nonagricultural substance. A substance that is not a product of agriculture, such as a mineral or a bacterial culture, that is used as an ingredient in an agricultural product. For the purposes of this part, a nonagricultural ingredient also includes any substance, such as gums, citric acid, or pectin, that is extracted from, isolated from, or a fraction of an agricultural product so that the identity of the agricultural product is unrecognizable in the extract, isolate, or fraction."

Pullulan is neither a mineral nor a bacterial culture. It is not an extract, an isolate, or a fraction of an agricultural product. Not meeting these criteria in the definition of "nonagricultural substance" led certifiers to classify it as agricultural. However, the identity of the agricultural product used as the substrate in the fermentation is completely unrecognizable in the substance pullulan. Thus, the analysis of pullulan as an agricultural or non-agricultural substance according to this definition is not conclusive. When the NOP published the clarifying Decision Tree for Classification of Agricultural and Nonagricultural Materials for Organic Livestock Production or Handling (NOP 5033-2), the ACA Working Group used this decision tree to determine whether pullulan should be classified as agricultural or nonagricultural (Accredited Certifiers Association Inc. 2017). The specifics of their analysis are not available to the public but appear to be based on the logic noted in response to Question 3 in the decision tree, which asks if the substance is a crop or livestock product or if it is derived from crops or livestock.

If one considers that pullulan is derived from the microorganism that produces it, rather than from the agricultural substrates used to cultivate the microorganism, the conclusion is that pullulan is nonagricultural. Historic NOSB decisions on similar carbohydrate polymer substances (gums) currently on the National List are consistent with classification of pullulan as a nonagricultural substance. Seven gums are currently allowed as nonorganic ingredients and processing aids under the National Organic Program (NOP) regulations. These gums are identified in four listings on the National List of Allowed and Prohibited Substances.

- At § 205.605(a) as an allowed nonsynthetic substance, "Gellan gum" is listed with the annotation, "high acyl form only."
- At § 205.605(b) as an allowed synthetic substance, "Xanthan gum" is listed without any additional annotation.
- At § 205.606 as allowed agricultural substances, "Gums" are listed with the annotation, "water extracted only (Arabic; Guar; Locust bean; and Carob bean)."
- Also at § 205.606 as an allowed agricultural substance, "Tragacanth gum" is listed without any additional annotation.

The four gums listed at § 205.606 are very different than the two gums listed at § 205.605. Gum Arabic, guar gum, locust bean gum, and carob bean gum, being derived from plants in the family Leguminosae (alternatively called Fabaceae), are classified as agricultural at § 205.606. Gum arabic and tragacanth gum are both exudates of leguminous plants (Nexight Group 2018), and guar gum and locust bean gum are storage polysaccharides obtained from the endosperms of leguminous seeds simply extracted from the raw agricultural commodity. In contrast, gellan gum and xanthan gum—classified as nonagricultural at § 205.606—are derived from microorganisms, as is pullulan.

As noted above, a petition was submitted to the National Organic Standards Board in February 2004 to add pullulan to § 205.605 of the National List. The NOSB took no action on this petition, and no final recommendation for this substance was ever made.

Evaluation Question #3: If the substance is a synthetic substance, provide a list of nonsynthetic or natural source(s) of the petitioned substance (7 CFR 205.600(b)(1)).

Pullulan is a non-synthetic substance produced by fermentation of an agricultural input. See *Evaluation Question #1* for more information on pullulan manufacturing processes. All of the processes discussed in this report result in the production of a non-synthetic substance. No sources were found that indicate the existence of a chemically synthesized form.

Evaluation Question #4: Specify whether the petitioned substance is categorized as generally recognized as safe (GRAS) when used according to FDA's good manufacturing practices (7 CFR 205.600(b)(5)). If not categorized as GRAS, describe the regulatory status.

The FDA issued an Agency Response Letter GRAS Notice No. GRN 000099 on August 1, 2002, indicating that the FDA had no objections to the self-affirmed GRAS status for pullulan (Rulis 2002).

Evaluation Question #5: Describe whether the primary technical function or purpose of the petitioned substance is a preservative. If so, provide a detailed description of its mechanism as a preservative (7 CFR 205.600(b)(4)).

The petitioned use of pullulan is as a component of dietary supplements, comprising the vegetable-sourced capsule or an inert ingredient in a dietary supplement formulation. The Food Protection Committee of the National Academy of Sciences/National Research Council established the classification of GRAS Substances by Technical Effect in 1972, and defined "preservatives" as "including antimicrobial agents, fungistats, mold and rope inhibitors, etc." FDA adopted this definition at 21 CFR 170(3)(o). Pullulan is not a preservative because it has none of these properties.

While pullulan is not considered a preservative by FDA, pullulan films can help to preserve food quality by limiting oxygen access and moisture loss. A pullulan film applied to fruits such as berries (Krasniewska et al. 2017; Trevino-Garza et al. 2015) extends storage life by excluding oxygen and reducing moisture loss. A pullulan film on cut fruit surfaces (Wu and Chen 2013) and red meats (Morsy et al. 2014; Gennadios and Sumner 1999) can also extend shelf-life. Pullulan films containing spice oils (e.g., oregano, rosemary, caraway) improved the keeping quality of meat (Morsy et al. 2014), Brussel sprouts (Krasniewska et al. 2016) and baby carrots (Gniewosz et al. 2013), respectively. However, the high cost of pullulan limits these applications (Gennadios and Sumner 1999).

Evaluation Question #6: Describe whether the petitioned substance will be used primarily to recreate or improve flavors, colors, textures, or nutritive values lost in processing (except when required by law) and how the substance recreates or improves any of these food/feed characteristics (7 CFR 205.600(b)(4)).

Pullulan is a high molecular weight glucose polymer, roughly similar to corn starch or oat glucan, but much more soluble. It cannot restore or recreate flavors, colors, textures, or nutritive values lost in processing, but it has been shown to reduce the rate of quality deterioration when used as a protective film.

Evaluation Question #7: Describe any effect or potential effect on the nutritional quality of the food or feed when the petitioned substance is used (7 CFR 205.600(b)(3)).

The petitioned use of pullulan is as an ingredient in tablets and capsules for dietary supplements, which involve only milligram (mg) amounts per serving. Very large vegetarian capsules (greater than one inch in length with capacities of 1.37 mL) contain just 163 mg of pullulan, while medium-sized vegetarian capsules (three-quarters of an inch in length with capacities of 0.48 mL) contain just 63 mg of pullulan (Capsuline 2018). The pullulan used for capsules has a molecular weight of 100 or 200 kDa.

Nutritional studies involving pullulan have focused on much greater levels of intake—10 to 50 g per dose—to determine its use either as a glucose polysaccharide that might provide soluble fiber if it is slowly or poorly digested, or as a source of food energy if it is rapidly digested. The results of these feeding trials in various species including humans indicate that the extent of pullulan digestion can depend upon its molecular weight (Bauer et al. 2003; Cervantes-Pahm et al. 2013; de Godoy et al. 2013; Knapp et al. 2010; Knapp et al. 2008; Peters et al. 2011; Spears, Karr-Lilienthal and Fahey 2005; Spears et al. 2005). Very low molecular weight pullulan is generally more digestible than higher molecular weight pullulan.

Knapp et al. (Knapp et al. 2010) compared the glucose and insulin levels in dogs weighing 25 kilograms (kg) that were given water solutions consisting of 25 grams (g) of a pullulan sample with molecular weights of 100 kDa, 250 kDa, or 500 kDa, and found no significant differences in the dogs' physiological responses to the pullulan samples over a three-hour period.

Peters et al. (Peters et al. 2011) fed drinks providing 15 g of test carbohydrates (maltodextrin and pullulan) to 35 healthy human adults. They found that over a five-hour period, maltodextrin was rapidly digestible, pullulan with a MW of 22.6 kDa was slowly but completely digestible, and pullulan with a MW of 200 kDa was indigestible.

Providing 50 grams of pullulan with a MW of 100 kDa to non-diabetic, healthy subjects in the form of a sterilized nutritional beverage yielded a slow rate of digestion that makes a food containing pullulan useful in the dietary management of diabetics (Wolf 2005). Twenty-eight non-diabetic, healthy U.S. adults consumed 50 g of either pullulan or maltodextrin (the control substance) in a randomized, double-blinded, cross-over study in which subjects participated in two separate three-hour meal tolerance tests. The incremental peak blood glucose concentration was reduced by 54 percent when subjects consumed pullulan compared to the control group (4.24 ± 0.35 vs. 1.97 ± 0.10 mmol/L) ($P < 0.001$). At 180 minutes, the blood glucose concentration was higher when subjects consumed pullulan, supporting the hypothesis that pullulan is digested slowly ($P < 0.05$). The positive incremental area under the curve was reduced by 50 percent when subjects consumed pullulan compared with the control ($P < 0.001$). With the pullulan beverage, flatulence and breath hydrogen over eight hours were higher, reflecting slower and less complete digestion of pullulan (Wolf et al. 2003). Pullulan can be considered a "resistant starch" that acts as a source of dietary fiber.

Stewart et al. (Stewart et al. 2010) found that flatulence was greatest among subjects consuming a pullulan sample with a MW of 486 kDa compared to resistant starch, soluble fiber dextrin, soluble corn fiber, and the control maltodextrin. There was no significant difference in the number of recorded stools per day between the pullulan and control diets ($P > 0.05$).

Evaluation Question #8: List any reported residues of heavy metals or other contaminants in excess of FDA tolerances that are present or have been reported in the petitioned substance (7 CFR 205.600(b)(5)).

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) established a limit for lead in pullulan of not more than 1 mg/kg (i.e., < 1 ppm) (JECFA 2011). In the U.S., the Food Chemicals Codex (FCC) monograph—accepted by FDA—limits lead in pullulan to less than 0.1 mg/kg (i.e., <0.1 ppm) (U. S. Pharmacopeia 2010).

<u>Evaluation Question #9:</u> Discuss and summarize findings on whether the manufacture and use of the petitioned substance may be harmful to the environment or biodiversity (7 U.S.C. § 6517 (c) (1) (A) (i) and 7 U.S.C. § 6517 (c) (2) (A) (i)).

Pullulan is completely biodegradable (Farris et al. 2014). It may be digested directly to glucose by the consumer, fermented by the intestinal flora, or broken down by microflora digesting human waste in a sewage treatment plant. In all cases, the carbon, oxygen, and hydrogen that constitute pullulan are converted to carbon dioxide, water, and sometimes hydrogen gas (produced in the colon). In the small and large intestines of monogastric animals such as humans, glucose resulting from digested pullulan is absorbed in the small intestine and short-chain fatty acids resulting from fermented pullulan are absorbed in the colon.

Each byproduct of the production of pullulan is either biodegradable (the carbohydrate and nitrogen in the cell debris from the microorganism), recyclable (the ion exchange resin), biologically available (the mineral elements), or soil-compatible (activated charcoal). Thus, no harm to the environment or biodiversity is expected from the manufacture or use of pullulan as petitioned.

<u>Evaluation Question #10:</u> Describe and summarize any reported effects upon human health from use of the petitioned substance (7 U.S.C. § 6517(c)(1)(A)(i), 7 U.S.C. § 6517(c)(2)(A)(i)) and 7 U.S.C. § 6518(m)(4)).

Pullulan has extremely low toxicity, with a median lethal dose (LD_{50}) of greater than 24,000 mg/kg in rats and greater than 14,000 mg/kg in mice (JECFA 2011). Feeding a diet with 10 percent pullulan for 62 weeks had no adverse effect in male and female rats (Kimoto, Shibuya and Shiobara 1997). Human clinical studies included feeding 12 grams daily for 14 days (Stewart et al. 2010) and one-time consumption of a beverage containing 50 grams (Wolf et al. 2003).

As discussed under *Evaluation Question #7*, significant amounts (i.e., 10–50 grams) of high molecular weight (~200 kDa) pullulan can produce flatus as undigested carbohydrate enters the colon and is metabolized by intestinal bacteria. Pullulan of lower molecular weight is slowly but completely digestible, making it useful in dietary products for diabetics and others for whom slower glucose availability and lower insulin levels are desirable (Wolf 2005). Pullulan of high molecular weight acts as dietary fiber (e.g., causing flatus) (Stewart et al. 2010; Wolf et al. 2003).

<u>Evaluation Question #11:</u> Describe any alternative practices that would make the use of the petitioned substance unnecessary (7 U.S.C. § 6518(m)(6)).

Pullulan is a plant-derived polysaccharide with a unique linear molecular structure that allows it to be easily formed into film for use in capsules. Historically, the materials used to make capsules for dietary supplements and medications have been based on gelatin, an animal product extracted from the bones and skin of swine and cattle through boiling. Gelatin derived from swine has the disadvantage of religious proscription: it can never be considered Kosher or Halal. Gelatin derived from cattle may be derived from animals susceptible to bovine spongiform encephalopathy, or mad cow disease. Pullulan serves as a vegetarian alternative in these applications.

Another alternative would be to omit a coating altogether. However, coatings make tablets easier to swallow, prevent oxygen penetration which leads to color change and degradation of active components (Izutsu et al. 1987), and help control tablet disintegration and dissolution rates (Patel et al. 2012). Thus, the alternative practice of omitting a coating is a less viable option.

<u>Evaluation Question #12:</u> Describe all natural (non-synthetic) substances or products which may be used in place of a petitioned substance (7 U.S.C. § 6517(c)(1)(A)(ii)). Provide a list of allowed substances that may be used in place of the petitioned substance (7 U.S.C. § 6518(m)(6)).

As noted under *Evaluation Question #11*, from a technical perspective, gelatin capsules can be used in place of pullulan capsules. Gelatin is permitted as a nonorganically produced agricultural product at 7 CFR

205.606(j). However, regardless of the animal species that is the source of gelatin, such capsules would not satisfy the consumer need for vegetarian capsules.

A variety of edible, film-forming ingredients have been tested in the past; several lipid (e.g., waxes, long-chain fatty acids, acetylated glycerides), polysaccharide (e.g., starch and its derivatives, cellulose ethers, alginate, carrageenan, pectin, pullulan, gellan gum), and protein (e.g., collagen, gelatin, whey protein, casein, wheat gluten, corn zein, soy protein, egg albumen) biopolymers have been investigated as edible film-forming ingredients (Gennadios 1999). Protein substances were also tested as coatings but are more likely to trigger allergic reactions. Because of its oxygen-barrier properties, pullulan is the polymer of choice for this application despite its high cost.

<u>Evaluation Information #13:</u> Provide a list of organic agricultural products that could be alternatives for the petitioned substance (7 CFR 205.600(b)(1)).

The petitioner (Organic Trade Association 2018) communicated (petition page 14 of 15) that organic pullulan-based capsules are not commercially available in North America currently. According to the Organic Integrity Database, certified organic pullulan capsules are available from Shanxi JC Biology Technology Co Ltd in China. The corporate headquarters of their North America subsidiary, Bright Pharma Caps, are located in Hood River, Oregon. Bright-Poly Pullulan Capsules are offered in Certified Organic version now, but are not currently available for distribution in the United States and Canada (Bright Pharma Caps Inc. 2016).

Because pullulan is manufactured by fermentation of agricultural substances such as starch syrup by naturally occurring microorganisms, and because the production process described in *Evaluation Question #1* does not include the use of volatile organic solvents to precipitate or purify pullulan, this pullulan production process appears to be organic-compatible. According to the petition, organic pullulan is under development and should be available in North America in the future. Until then, no other organic option for capsules is commercially available.

Report Authorship

The following individuals were involved in research, data collection, writing, editing, and/or final approval of this report:

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All individuals are in compliance with Federal Acquisition Regulations (FAR) Subpart 3.11—Preventing Personal Conflicts of Interest for Contractor Employees Performing Acquisition Functions.

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