Vireo Advisors, LLC

Jo Anne Shatkin, Ph.D.
President

4 June 2020

Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Pkwy
College Park, MD
20740-3835

ATTN: Richard Bonnette
Re: GRAS Notice for Fibrillated Cellulose

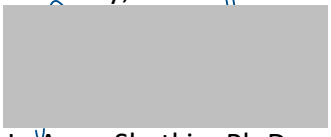
Dear Dr. Bonnette,

In accordance with 21 CFR §170 Subpart E consisting of §170.203 through §170.285, the Alliance for Food Safety Acceptance of Fibrillated and Crystalline Celluloses (Alliance), on behalf of Borregaard AS, Evergreen Packaging, LLC, Fiberlean Technologies Limited, Sappi Papier Holding GmbH, Sappi North America Inc., Sappi Southern Africa Limited, Stora Enso Oyj, and Weidmann Fiber Technology by Weidmann Electrical Technology AG, hereby notifies the United States (U.S.) Food and Drug Administration (FDA) that fibrillated cellulose is not subject to the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act based on scientific procedures and our conclusion that the notified substance is Generally Recognized as Safe (GRAS) under the conditions of its intended use as described in this Notice.

Vireo Advisors, LLC, as the agent to the Alliance (notifier) of this GRAS Notice, certifies that all data and information presented in this notice represents a complete, representative, and balanced submission, and has considered all unfavorable as well as favorable pertinent information known to Vireo Advisors, LLC, to evaluate the safety and GRAS status of fibrillated cellulose to be used in food. In addition to the determination of safety, an Expert Panel of qualified persons was assembled to assess all relevant information. This GRAS Notice is submitted online via FDA's Electronic Submissions Gateway (ESG) containing Form 3667, the fibrillated cellulose GRAS Notice, and attachments further referenced.

Thank you for the review of this GRAS Submission. If additional clarification or information is needed, please feel free to contact me via telephone or email.

Sincerely,



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GRAS NOTICE FOR FIBRILLATED CELLULOSE

Prepared for: Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD
20740-3835

Prepared by: Vireo Advisors, LLC
111 Perkins St., # 223
Boston, MA
02130

On behalf of: **The Alliance for Food Safety Acceptance of Fibrillated and Crystalline Celluloses:**
Borregaard AS
Evergreen Packaging, LLC
Fiberlean Technologies Limited
Sappi North America Inc.
Sappi Papier Holding GmbH
Sappi Southern Africa Limited
Stora Enso Oyj
Weidmann Fiber Technology by Weidmann Electrical Technology AG

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PART 1. SIGNED STATEMENTS AND CERTIFICATION

1.1. GRAS Notification to FDA for fibrillated cellulose

In accordance with 21 CFR §170 Subpart E consisting of §170.203 through §170.285, Vireo Advisors, LLC, on behalf of the Alliance for Food Safety Acceptance of Fibrillated and Crystalline Celluloses (Alliance), including Borregaard AS, Evergreen Packaging, LLC, Fiberlean Technologies Limited, Sappi Papier Holding GmbH, Sappi North America Inc., Sappi Southern Africa Limited, Stora Enso Oyj, and Weidmann Fiber Technology by Weidmann Electrical Technology AG, hereby notifies the United States (U.S.) Food and Drug Administration (FDA) that fibrillated cellulose is not subject to the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act based on scientific procedures and our conclusion that the notified substance is Generally Recognized as Safe (GRAS) under the conditions of its intended use as described in this Notice. Vireo Advisors, LLC as the agent for the Alliance, certifies that all data and information presented in this notice represents a complete, representative, and balanced submission, and has considered all unfavorable as well as favorable pertinent information known to Vireo Advisors, LLC, to evaluate the safety and GRAS status of fibrillated cellulose to be used in food.

Signed,

4 June 2020

Jo Anne Shatkin, Ph.D. Date
President
Vireo Advisors, LLC on behalf of:

The Alliance for Food Safety Acceptance of Fibrillated and Crystalline Celluloses
Borregaard AS
Evergreen Packaging, LLC
Fiberlean Technologies Limited
Sappi North America Inc.
Sappi Papier Holding GmbH
Sappi Southern Africa Limited
Stora Enso Oyj
Weidmann Fiber Technology by Weidmann Electrical Technology AG

1.2 Name and address of organizations

Notifier: The Alliance for Food Safety Acceptance of Fibrillated and Crystalline Celluloses
Care of:
Vireo Advisors, LLC
111 Perkins St, # 223
Boston, Massachusetts 02130
United States of America

Manufacturers:

Borregaard AS
Hjalmar Wesselsvei 6, 1721
Sarpsborg, Norway

Evergreen Packaging, LLC
5350 Poplar Avenue #600
Memphis, Tennessee 38119
United States of America

Fiberlean Technologies Limited
Par Moor Centre
Par Moor Road, Par, Cornwall
PL24 2SQ, United Kingdom

Sappi North America Inc.
255 State Street
Boston, Massachusetts, 02109
United States of America

Sappi Papier Holding GmbH
21 Brucker Strasse
Gratkorn, 8101
Austria

Sappi Southern Africa Limited
108 Oxford Road Rosebank
Johannesburg, 2196
South Africa

Stora Enso Oyj
Kanavaranta 1
00101 Helsinki
Finland

Weidmann Fiber Technology by Weidmann Electrical Technology AG
Neue Jonastrasse 60
8640 Rapperswil-Jona
Switzerland

1.3 Name of notified substance
Fibrillated cellulose (synonyms: cellulose; microfibrillated cellulose; microfibrils)

1.4 Conditions of intended use in foods
Fibrillated cellulose is proposed for use as a component in food additives and edible and protective food coatings. It is intended to be used in baked goods and baking mixes, alcoholic beverages, non-alcoholic beverages, cheeses, confections and frostings, fats and oils, fresh fruits and fruit juices, frozen dairy desserts and mixes, gelatins, puddings and fillings, gravies and sauces, milk and milk products, processed fruits and fruit juices, and as a food coating. Because fibrillated cellulose products will effectively substitute for traditional microcrystalline

or cellulose substances as a food additive, we anticipate no substantial increases in global consumption. The conditions of intended use and levels are summarized in Table 1-1 below.

Table 1-1 Summary of conditions of use of fibrillated cellulose in foods

Intended food use	Proposed Use Levels
Baked goods and baking mixes	
Batters and breading	0.5-3%
Cake (fat-reduced)	5%
Fillings (Bakery products)	0.8-2.0%
Puffed snacks	2-5%
Beverages, alcoholic	
Alcoholic formulations (e.g. piña colada mix)	0.3-0.5%
Beverages, non- alcoholic	
Soy milk beverages	0.28-0.4%
High fiber drinks	0.5-1.0%
Coffee beverage	0.4-0.6%
Nutritional beverage	0.4-0.8%
Cheeses	
Fresh cheese	0.10%
Low-fat processed cheese	1.00%
Cheese sauce and dip	0.65-1.25%
Confections and frostings	
Confectionary	0.5-2.8%
Icings	0.2-1%
Mixes for power bars	3-5%
Mixes for candy bars	3-5%
Fats and oils	
Salad Dressings	1-3%
Fresh fruits and fruit juices	
Fruit juice	0.5-2.0%
Protective fruit coating	0.05-100%
Fresh vegetables	
Protective vegetable coating	0.05-100%
Frozen dairy desserts and mixes	
Ice cream, frozen desserts	0.1-1.0%
Frozen whipped toppings; vegetable fat	0.3-0.6%
Gelatins, puddings and fillings	
Puddings, Mousse	1.4-4.8%
Gravies and sauces	
Cooking cream sauces	0.25-1.0%
Tomato sauce	0.3-1.3%
Milk, Milk products	
Fat-free milk, Chocolate milk	0.25-0.7%
Low-fat sour cream	0.35-0.5%
Whipped toppings; dairy	0.3-0.6%
Processed fruits and fruit juices	
Fruit drink	0.5-2.0%

1.5 Statutory Basis of GRAS conclusion

The statutory basis of GRAS Conclusion is through scientific procedures in accordance with 21 CFR §170.30(a) and (b). The GRAS determination is based on information generally available to the public, as discussed herein, as well as through consensus among a panel of experts who are qualified by scientific training and experience to evaluate the safety of fibrillated celluloses in food.

1.6 Availability of Data and Information

A complete copy of the data and information used as the basis for this GRAS conclusion will be provided to the FDA upon request, in either electronic format that is accessible for FDA evaluation, or on paper, and is available for reviewing and copying during customary business hours at:

Vireo Advisors, LLC
111 Perkins St, # 223
Boston, Massachusetts 02130

1.7 Freedom of Information Act, 5 U.S.C. 552

All data and information presented in Parts 2 through 7 of this notice do not contain any trade secret, commercial, or financial information that is privileged or confidential, and therefore all data and information presented herein are not exempt from the Freedom of Information Act, 5 U.S.C. 552.

1.8 Statement for this GRAS Notice Submission

To the best of our knowledge, Vireo Advisors, LLC certifies that this GRAS Notice is a complete, representative and balanced submission.

PART 2. IDENTITY, METHOD OF MANUFACTURE, SPECIFICATIONS, AND TECHNICAL EFFECT

2.1 Identity

2.1.1. Overview of Cellulose and its Derivatives

Cellulose is the most abundant natural biopolymer on earth. It is widely distributed in plants, where, combined with lignin and hemicelluloses, it plays an essential role in maintaining structure and providing support to cell walls. Cellulose is also found in invertebrates, algae, bacteria and fungi, and can be produced by some bacteria (Habibi *et al*., 2010). Cellulose is a linear homopolymer of β-1,4-linked anhydro-D-glucose units. The base unit of cellulose, termed cellobiose, consists of two molecules of glucose rotated 180° along the axis of the polymer (Figure 2-1).

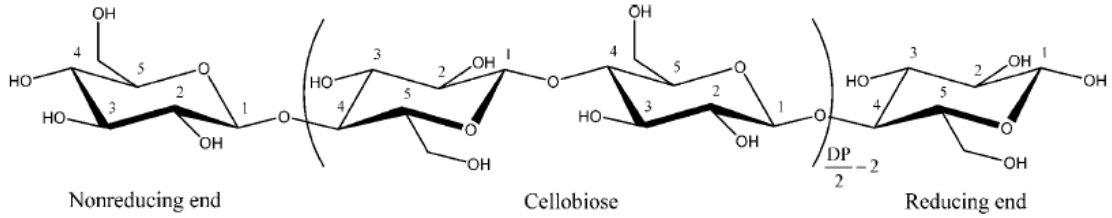

Figure 2-2. The molecular structure of cellulose (Habibi et al., 2010).

In nature, cellulose does not occur as isolated individual molecules; rather, cellulose chains are assembled into a hierarchy of cellulose fibers. During biosynthesis, cellulose chains aggregate into larger units known as elementary fibrils (crystallite strands of cellulose) maintained by hydrogen bonds. The elementary fibrils have an average diameter of 10 nm and an average length of 1000 nm. Elementary fibrils are subsequently bundled and assembled to form macrofibrils, commonly known as cellulose fibers, the major structural unit of plant cell walls (Wustenberg, 2015) (Figure 2-2).

Cellulose can be obtained from a variety of sources, though lignocellulosic materials (*e.g.,* wood) are the most common (Wustenberg, 2015). Wood pulp is created through a pulping process that consists of two steps: mechanical processing to wood chips, and chemical processing to remove lignin and free the cellulose fibers. This purified cellulose pulp serves as the base material to create a variety of different morphological forms and functional cellulose derivatives. Each derivative has numerous commercial applications, and several related cellulosic materials are already used in food and Generally Recognized as Safe (GRAS), including bacterial cellulose (also called “fermentation-derived cellulose” or “microbial cellulose”) and microcrystalline cellulose (MCC). While not the subject of this Notice, the rich database of uses, history and safety demonstration for bacterial cellulose, microcrystalline cellulose and conventional cellulose is referenced as supporting information toward the safety of fibrillated cellulose.

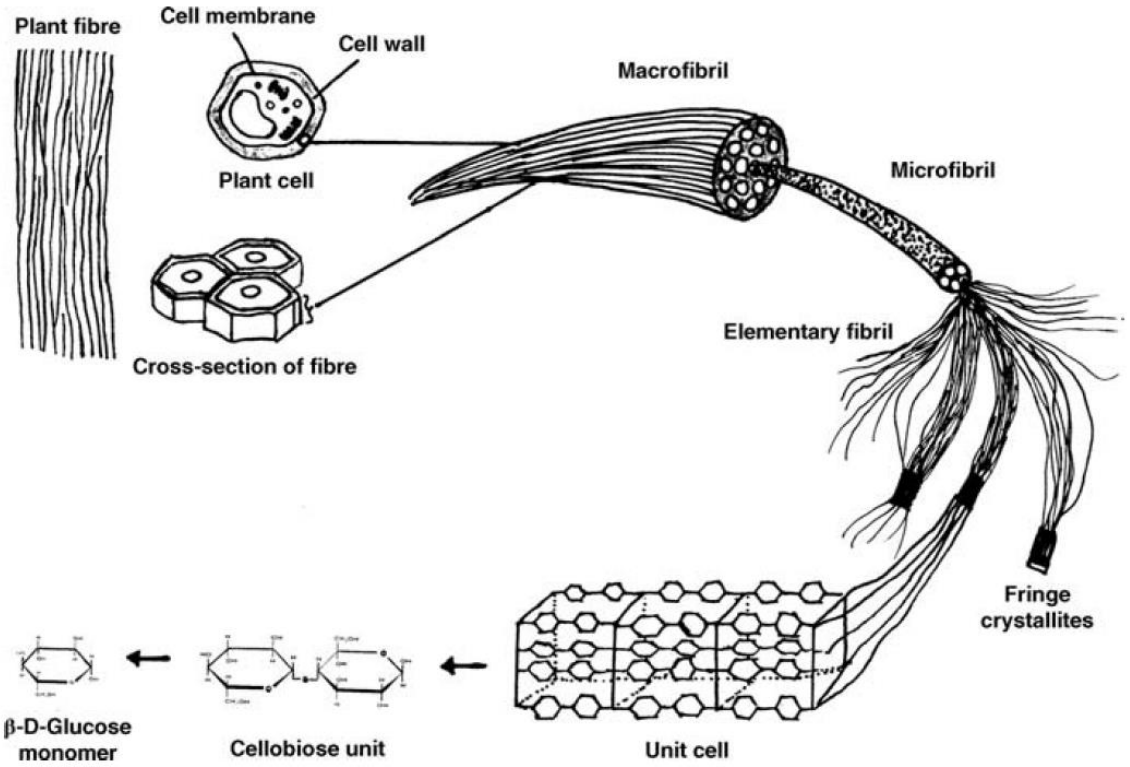

Figure 3-2. Fibrils are formed from the hierarchical assembly of cellulose (Wustenberg, 2015).

2.1.2. Fibrillated Cellulose

The six notified materials of this GRAS dossier are each a form of fibrillated cellulose, produced by six separate manufacturers, identified in Table 2-1. These manufacturers use similar approaches for producing fibrillated cellulose, by a mechanical process which involves freeing cellulose fibrils from bleached or unbleached wood pulp (either from hardwood or softwood). There are slight differences in production as described in Section 2.2 Method of Manufacture. Physical and chemical characterization of the six fibrillated celluloses (labeled C20-C25) side-by-side with two related materials that were used in a 90-day subchronic dietary study (OECD TG 407, 408). The forms of cellulose tested in the 90-day dietary study were a non-commercial form of fibrillated cellulose from the University of Maine Process Development Centre (Ref FC) as well as a conventional cellulose (Ref CC) material, Solka Floc®, a powdered form of cellulose that is GRAS and has been used as a food and feed ingredient for over 85 years. Solka Floc is representative of conventional forms of cellulose; it is a food grade cellulose that serves as a functional ingredient in a wide range of commercial food and feed products, used for fiber enrichment and various other technical effects, for binding, anti-caking, improved flowability, dimensional stability and volume enhancement, texturizing, filtration, etc.

The physical and chemical characterization of the six Notified fibrillated celluloses and two Reference celluloses (Ref FC and Ref CC) demonstrates the similarity of these materials, which share the same fundamental molecular structure (Figure 2-1) and exhibit similar general morphology, size, size distribution and surface charge. These similarities allow for grouping and

read-across (the ability to use endpoint information for one substance to predict the end-point for another substance) among the six Notified forms and Ref FC, as well as read-across to the extensive safety literature for conventional celluloses (Ref CC). The physical, chemical and biological characterization in support of grouping and read-across is discussed in Section 6.2.3, including *in vitro* simulated gastrointestinal and lysosomal digestion of these materials, followed by an assessment of physical, chemical and toxicological properties in an intestinal tri-culture model. The physical and chemical characterization of neat materials is reported here. These results are available in the publication by Pradhan et al. 2020 (Attachment 2).

Table 2-1. Manufacturers producing fibrillated cellulose that are the subject of this GRAS Notice.

Manufacturer	**Trade Name**
Borregaard AS	Exilva
Evergreen Packaging LLC	Evergreen Fibrillated Cellulose
Fiberlean Technologies Limited	FiberLean® MFC
Sappi North America Inc. Sappi Papier Holding GmbH Sappi Southern Africa Limited	Valida
Stora Enso Oyj	Integrant
Weidmann Fiber Technology by Weidmann Electrical Technology AG	Celova®

2.1.2.1 Description

The six Notified forms of fibrillated cellulose are white, odorless solids that are insoluble in water. They are typically sold as gels or wet crumbles, ranging from 2-30% (wt.) fibrillated cellulose, but can also be dried solids up to 100%. Some forms of fibrillated cellulose also contain either

Fibrillated cellulose is insoluble, forms tangled fibrous networks, and has high molecular weight, typically ranging from 32,400 – 243,000 g/mol, calculated based on degree of polymerization values around 200-1500, and molar mass of a glucose unit of 162 g/mol (Henriksson et al. 2007, Asrofi et al. 2017).

2.1.2.2

Fibrillated celluloses are derived from cellulose, a linear homopolymer of β-1,4-linked anhydro-D-glucose units, and share the same molecular structure (Figure 2-1), molecular formula [$(C_6H_{10}O_5)_n$], and Chemical Abstract Service (CAS) Registry Numbers: Cellulose (CAS RN 9004-34-6) and cellulose pulp (CAS RN 65996-61-4). Synonyms include cellulose, microfibrillated cellulose, or microfibrils; or by their commercial names (Table 2-1).

Inductively coupled plasma-mass spectrometry analysis (ICP-MS) was used to measure the concentration of total metals [magnesium (Mg), vanadium (V), nickel (Ni), cobalt (Co), copper

(Cu), zinc (Zn), arsenic (As), cadmium (Cd), and lead (Pb)] in the six Notified materials and reference fibrillated cellulose (Ref FC). Figure 2-3 and Table 2-2 demonstrate the low levels of metal impurities, in the low parts per billion (ppb) range. Ref FC had total metal impurities of 0.73 ppb; C20-C25 values ranged from 0.3-3.0 ppb.

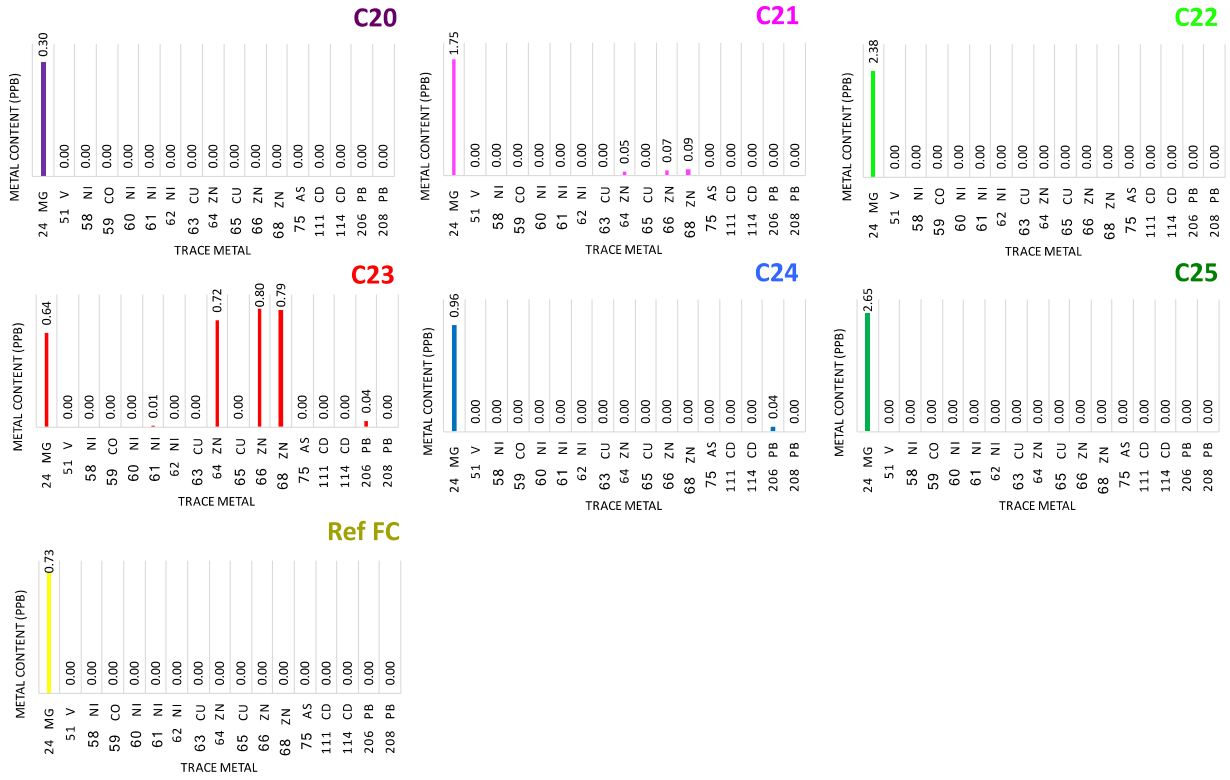

Figure 2-3. Trace metal concentrations of fibrillated celluloses C20-C25 and Ref FC as determined by ICP-MS.

2.1.2.3 Morphology

When dried, fibrillated celluloses are odorless, white films or powders. Representative phase microscopy images are shown in Figure 2-4.

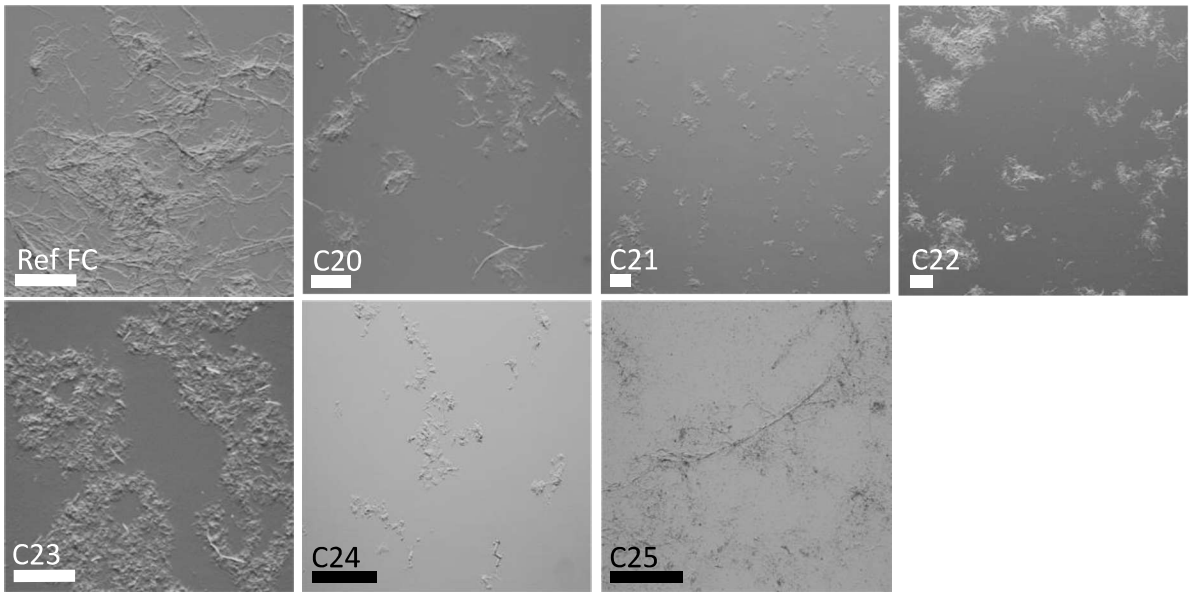

Figure 2-4. Representative phase microscopy images of the 6 Notified forms of fibrillated cellulose (C20-C25) alongside reference fibrillated cellulose (Ref FC). The scale bars in the images are 100 µm.

Electron microscopy images show the fibrillar morphology of fibrillated celluloses (Figure 2-5, A-G), consisting of an entangled network of fibers and fibrils of varying widths. Note that one material (C25) is made by grinding with a GRAS mineral agent, calcium carbonate, observable in micrograph G. In comparison, conventional cellulose (Figure 2-5, H) has a lower aspect ratio than fibrillated cellulose and does not form an entangled network of fibers. Solka Floc®, the conventional form of cellulose used for comparison in the Notifier's studies, has an amorphous morphology that is tens of microns in length and width (Figure 2-5, H).

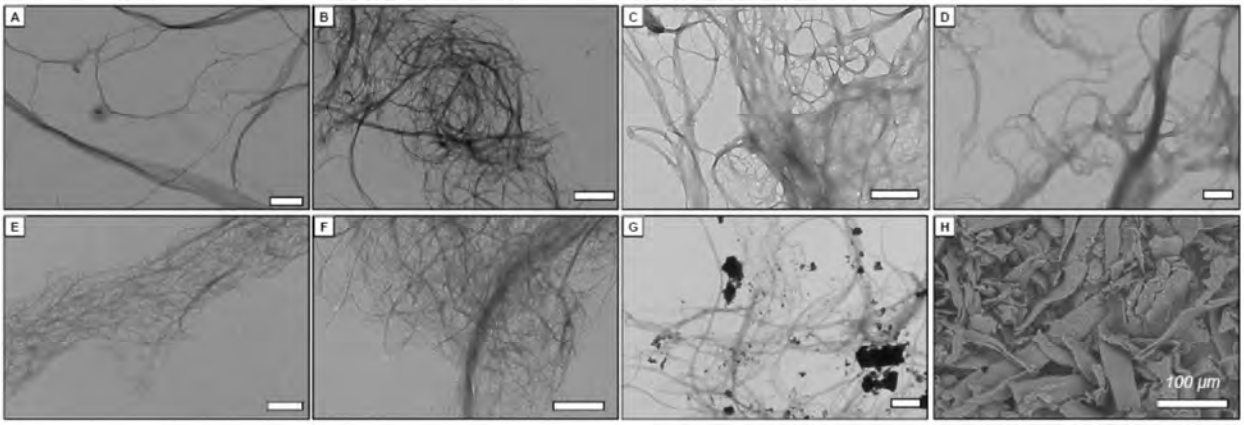

Figure 2-5. Morphology of fibrillated cellulose materials imaged by transmission electron microscopy (TEM): (A) Ref FC, (B) C20, (C) C21, (D) C22, (E) C23, (F) C24, and (G) C25. The scale bar in the images represent 600 nm, unless otherwise noted. (H) Shows a representative scanning electron micrograph (SEM) of Ref CC.

Dynamic light scattering (DLS) was used to measure the hydrodynamic diameter (HDD) and dispersity indices (DI) of the fibrillated celluloses. Each of the 11 measurements for each sample

was completed in triplicate. Ref FC has an average HDD of 1.89 µm, similar to Notice substances C20-C25, which range from 0.65-2.47 µm. In contrast, the HDD of conventional cellulose is an order of magnitude larger, with average values of 26.66 µm (Figure 2-6A; Table 2-2). Note that the HDD is a relative, not absolute, measure of size because it calculates size assuming a spherical shape in aqueous media. All eight of the studied cellulose materials have broad size distributions, characterized by DI >0.4. The DI for Ref FC and Ref CC were 0.55 and 0.65, respectively, while C20-C25 ranged from 0.53-0.92 (Figure 2-6B; Table 2-2).

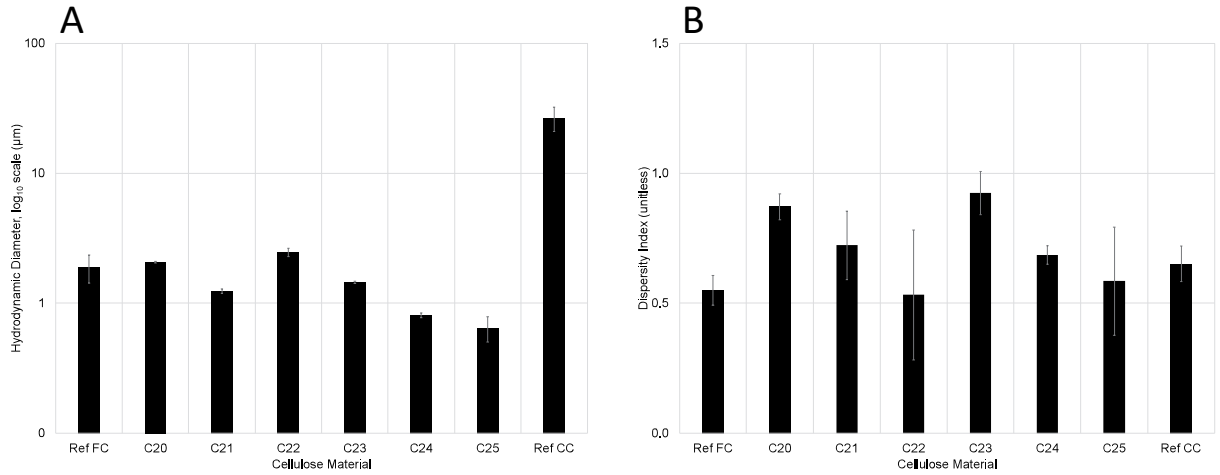

Figure 2-6. Hydrodynamic diameter and dispersity indexes of the 6 Notified forms of fibrillated cellulose (C20-C25) alongside reference fibrillated cellulose (Ref FC) and conventional cellulose (Ref CC).

2.1.2.4. Surface charge

Measurement of surface charge (zeta potential) determined all fibrillated celluloses were negatively charged. Ref FC had an average zeta potential in 3 measurements of -33.87 mV, similar to C20-C25 which ranged from -46.40 to -5.20 mV. Ref CC has an average zeta potential of -2.14 mV (Figure 2-7; Table 2-2).

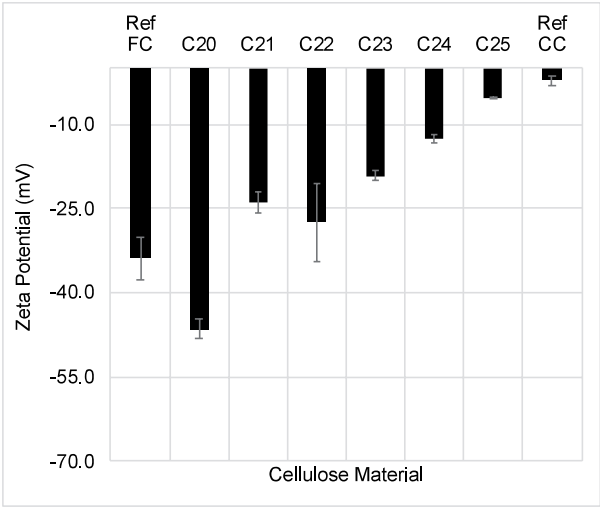

Figure 2-7. Zeta potential of the 6 Notified forms of fibrillated cellulose (C20-C25) alongside reference fibrillated cellulose (Ref FC) and conventional cellulose (Ref CC).

2.1.2.5. Evidence for grouping and read-across

The physical and chemical characterization of fibrillated celluloses (Ref FC; C20-C25) demonstrate that these materials are similar physically and chemically; composed of the same molecular structure and exhibiting similar morphology, size, size distribution, surface charge and low levels of impurities, summarized in Table 2-2. Conventional cellulose (Ref CC) exhibits a different morphology with particles an order of magnitude larger than fibrillated forms.

Table 2-2. Summary physical and chemical characteristics of fibrillated and conventional cellulose.

Metric	Ref FC (Value)	C20-C25 (Range)	Ref CC (Value)
pH	7.2	5.4-7.9	-
Total Metal Impurities (ICP-MS)	0.73 ppb	0.3-3.0 ppb	-
HDD (DLS)	1.89 µm	0.65-2.47 µm	26.66 µm
DI (DLS)	0.55	0.53-0.92	0.65
Zeta Potential	-33.87 mV	-46.40 to -5.20 mV	-2.14 mV

2.2. Method of Manufacture

2.2.1 Manufacturing Process

Production of fibrillated cellulose generally follows the processes described in U.S. Patent No. 8,546,558. Bleached or unbleached wood pulp is optionally first treated by mechanical refining or fibrillation, which may be followed by one or more of the following steps:

1) Addition of a cellobiohydrolase enzyme 2-60 ECU/g cellulose;
2) Use of a standard paper filler, calcium carbonate or kaolin, as grinding media at ~65°C;
3) Addition of endoglucanase enzyme 50-300 ECU/g cellulose or xylanase <100 ECU/g cellulose, followed by adjustment of pH with NaOH, with temperature adjustment';
4) Addition of carboxymethyl cellulose;
5) Washing of pulp to sodium form, and dilution of slurry with reverse osmosis water before pulp is refined through recirculation over the refiner and washed to remove residuals;
6) In one variation, pulp is heated to >120°C for >1 hour;
7) In another, the temperature specification is >65°C for up to 23 hours;
8) Pulp may be optionally processed by high shear methods such as high pressure homogenization or refining;
9) Fibrillated cellulose may be autoclaved;
10) Fibrillated cellulose is processed into sterile containers;
11) Concentration of fibrillated cellulose is increased through mechanical and/or thermal processes.

Production processes are as described in patents: US 8546558B2, US 8231764B2, WO 2015180844A1, US 8778134B2, WO 2018185227A1, WO 2015092146

2.2.2. Raw Materials and Processing Aids

All raw materials, processing aids, and purification equipment used to manufacture fibrillated cellulose are food grade ingredients, have GRAS status, or have been self-affirmed as safe for use in food for their respective uses. All enzymes have GRAS status and come from a strain with a long history of use that is commonly used for production of food enzymes, *Trichoderma longibrachiatum* (formerly *T. reesei*).

2.2.2.1 Regulatory status of enzymes

The enzymes cellobiohydrolase (2-60 ECU/g cellulose), endoglucanase (50-300 ECU/g cellulose), and xylanase (<100 ECU/g cellulose) used as processing aids in select manufacturing processes of fibrillated cellulose are GRAS, listed under 21 CFR §184.1250 - Cellulase enzyme preparation derived from *T. longibrachiatum*, and GRAS Notice (GRN) 675, 628, and 567 for xylanases from *T. reesei*. According to 21 CFR §184.1250, cellulase preparations are authorized for the breakdown of cellulose, and can be used in food with no limitation other than being produced according to current good manufacturing practice (cGMP) while meeting general and additional requirements for enzyme preparations (Food Chemicals Codex 1996). The Joint FAO/WHO Expert Committee on Food Additives (JECFA) evaluation of cellulase from *T. longibrachiatum* also established an acceptable daily intake (ADI) of 'not specified', used for food substances of very low toxicity (JECFA 1992a, 1992b).

Cellulase refers to any of several enzymes that can catalyse the degradation of cellulose. A 'cellulase enzyme preparation' derived from *T. longibrachiatum,* referred to in 21 CFR §184.1250, includes both cellobiohydrolase and endoglucanase cellulases, along with other cellulase types. Figure 2-8 (from Seiboth *et al.* 2011) outlines the different cellulase types (*e.g.* cellobiohydrolase, endoglucanase, B-glucosidase, etc.) that can be obtained from cellulase enzyme preparations derived from *T. longibrachiatum* and includes both cellulases used in select manufacturing processes of fibrillated cellulose. The FDA has noted, in response to GRN 584 for cellulase enzyme preparation (from a different species, *Penicillium funiculosum*), that "… the cellulase enzyme preparation is a mixture of a number of cellulose-degrading enzymes including three endoglucanases, two cellobiohydrolases and a beta-glucosidase, that all catalyze the hydrolysis of cellulose" (Footnote 1, in FDA 2015). JECFA specifies that cellulase enzyme preparation from *T. longibrachiatum* consists of enzyme activities from endo-1,4-β-glucanase (synonyms cellulase and endoglucanase) and exo-cellobiohydrolase (JECFA 1992a, 1992b).

2.2.2.2 Production strain and enzyme safety

The host strain*, T. longibrachiatum*, is well established to be safe for use in food and has a long history of use in industrial-scale enzyme production (Nevalainen *et al.* 1994; Sewalt *et al.* 2016), and is the subject of a number of existing GRAS notices (*e.g.* GRN 863, 817, 756, 628, 230, *etc.*).

The enzymes referred to in the production of fibrillated cellulose conform with GRAS requirements of cellulase preparations. They are affirmed to be produced from a safe strain with a long history of use in food (*T. longibrachiatum*), are prepared according to cGMP, and meet the general and additional requirements for enzyme preparation in the Food Chemicals Codex (1996), and as such are safe for use in the manufacture of fibrillated cellulose.

GH family	CAZy nomenclature	Previous designation	Cellulase type	Size in amino acids	Position of CBM	Stereo-selectivity
1	CEL1A	BGL2	β-glucosidase	466	-	Retaining
1	CEL1B		β-glucosidase+	484	-	Retaining
3	CEL3A	BGL1	β-glucosidase	744	-	Retaining
3	CEL3B		β-glucosidase+	874	-	Retaining
3	CEL3C		β-glucosidase+	833	-	Retaining
3	CEL3D		β-glucosidase+	700	-	Retaining
3	CEL3E		β-glucosidase+	765	-	Retaining
5	CEL5A	EG2	endoglucanase	397	N	Retaining
5	CEL5B		endoglucanase+	438	GPI anchor	Retaining
6	CEL6A	CBH2	cellobiohydrolase	447	N	Inverting
7	CEL7A	CBH1	cellobiohydrolase	497	C	Retaining
7	CEL7B	EG1	endoglucanase	436	C	Retaining
12	CEL12A	EG3	endoglucanase	218	-	Retaining
45	CEL45A	EG5	endoglucanase	270	C	Inverting
61	CEL61A	EG4	endoglucanase	344	C	Not known
61	CEL61B		Endoglucanase (?)#	249	-	Not known
74	CEL74A	EG6	endoglucanase/ xyloglucanase*	818	C	Inverting

Table 1. The cellulose degrading enzyme system of *T. reesei*

Figure 2-8. The cellulose degrading enzyme system of *T. reesei* (from Seiboth et al. 2011).

2.3. Product Specification and Batch Analysis

2.3.1 Product Specification

Food grade specifications for fibrillated cellulose have been established based on the Impurities and Specific Test specifications outlined for cellulose (CAS 9004-34-6) by the Food Chemicals Codex, Eleventh Edition (FCC 11) (Food Chemicals Codex 2018). Additional microbial analysis included total aerobic microbial counts, total yeast and mold counts, and testing for Salmonella. All analytical measurements to confirm fibrillated cellulose meets these specifications followed the methods outlined in the FCC 11, with modifications for pH measurement due to the rheological characteristics of fibrillated cellulose. Solutions were diluted further to 1.8% to allow for pH measurement. Certificate of Analysis (CoA) results for Ref FC are shown in Table 2-3. All Notified forms of fibrillated cellulose (C20-C25) conform to these specifications.

Table 2-3. Certificate of Analysis for Ref FC

Chemical Test	Test Result	FCC 11 Specification
Ash (total), %	< 0.01	≤ 0.3

Chloride, %	< 0.01	≤ 0.05
Heavy Metals, ppm as Pb	< 0.01	≤ 3.0
pH (1.8% suspension)	7.13	*5.0 - 7.5
Sulfur, %	< 0.01	≤ 0.01
Water Soluble Substance, %	< 0.01	≤ 1.5

Microbiological test (25g sample)	**Test Result**	**Specification**
Standard Plate Count, cfu/g	< 30.0	≤ 1,000
Yeast and Mold, cfu/g	0.00	≤ 100
Salmonella	Negative	Negative

* pH values may deviate from FCC 11 to a lower pH value of 4 and a higher pH value of 10

2.3.2 Batch Analysis

All Notifiers of fibrillated cellulose (C20-C25) have optimized manufacturing processes to ensure they consistently meet the above established product specifications.

2.4 Stability of Product

Fibrillated cellulose is a chemically inert and non-reactive substance. Exposure of fibrillated cellulose to chemical conditions representing intracellular or gastrointestinal digestion demonstrated no significant change in material physical or chemical characteristics (see Section 6.2.3). Fibrillated cellulose is stable when stored in a tightly sealed container in a cool, dry, well-ventilated area and protected from high heat, freezing, or degrading enzymes. Stability analysis of fibrillated cellulose under both accelerated and real-time storage conditions is ongoing.

2.5 Technical Effect

Similar to forms of cellulose currently used in food, the physical and chemical properties of fibrillated cellulose provide several technical effects useful for a variety of applications in the food additive industries. The technical effects of fibrillated cellulose as a food additive include use as a: (i) rheology modifier, (ii) stabilizer, (iii) low calorie substitute, (iv) fiber supplement, (v) component to improve food quality and (vi) processing aid.

Fibrillation of cellulose increases strength, optical and thixotropic properties, creating a high quality inert fiber source and zero-calorie bulking agent. Fibrillated cellulose may also replace current carbohydrate additives commonly used as rheology modifiers and stabilizers in a wide variety of food products. The rheological properties of fibrillated cellulose enhance its potential use as a viscosity modifier, improving the texture, consistency and mouthfeel in several food applications, including use as a low-calorie fat substitute to mimic full-fat texture. Fibrillated cellulose can impart stability to foods, acting as an emulsifier and adding thermal stability to products that undergo freeze-thaw cycles. Fibrillated celluloses may also be used for edible and protective food coatings for weather protection, increased shelf-life and the preservation of

anthocyanin-containing fruits such as cherries and blueberries, where the coatings allow the fruit to be preserved while retaining the nutritional benefits and color (Jung *et al*., 2018). **Table 2-4** includes a list of some of the proposed technical effects of fibrillated cellulose for use in the food industry (Wustenberg, 2015). This petition proposes GRAS designation for fibrillated cellulose as a multipurpose food additive for broad use according to the technical effects and proposed use-levels in **Table 2-5.**

Table 2-4. Technical effects of celluloses

Name	CAS No./E No.	Technical Effects Used in the Food Industry
Fibrillated cellulose; Cellulose; Cellulose pulp; Microcrystalline cellulose	9004-34-6 (cellulose); 65996-61-4 (cellulose pulp); 9004-34-6 E 460 (i) (microcrystalline cellulose) E460 (ii) (Powdered cellulose)	1. Food Additives (i) Rheology Modifier -Viscosity modifier -Thixotropic -Gelling agent (ii) Stabilizer -Carrier -Emulsifier -Stabilizing agent -Thermal stability (high temperature processing; freeze thaw cycles) -Anti-caking agent (iii) Low Calorie Substitute -Non-caloric bulking agent -Fat replacement (iv) Fiber Supplement -Source of dietary fiber (v) Improved Food Qualities -Control ice crystal growth -Reduced fat absorption during frying -Humectant -Control ice crystal growth -Improved mouthfeel to mimic full fat texture -Adds body and creaminess -Improved texture -Improved flavor retention -Opacifier -Protection -Barrier properties (vi) Processing aid -Filtration aid in beverage processing -Prevents boil-out -Aids in extrusion -Tableting aid 2. Food Coating -Protective coating to prevent spoilage

Adapted from (Wustenberg, 2015).

Table 2-5. Technical effects and proposed use levels of fibrillated cellulose in food

Intended food use	Technical Effects						Proposed Use Levels
	Rheology Modifier	Stabilizer	Low Calorie Substitute	Fiber Supplement	Improved Food Qualities	Processing Aid	
Baked goods and baking mixes							
Batters and breading					x	x	0.5-3%
Cake (fat-reduced)			x				5%
Fillings (Bakery products)					x	x	0.8-2.0%
Puffed snacks					x	x	2-5%
Beverages, alcoholic							
Alcoholic formulations (e.g. piña colada mix)		x			x	x	0.3-0.5%
Beverages, non-alcoholic							
Soy milk beverages		x			x	x	0.28-0.4%
High fiber drinks		x		x	x		0.5-1.0%
Coffee beverage		x			x	x	0.4-0.6%
Nutritional beverage		x		x	x	x	0.4-0.8%
Cheeses							
Fresh cheese	x		x		x		0.10%
Low-fat processed cheese	x		x		x		1.00%
Cheese sauce and dip	x	x			x	x	0.65-1.25%
Confections and frostings							
Confectionary			x		x		0.5-2.8%
Icings	x	x			x		0.2-1%
Mixes for power bars		x			x		3-5%
Mixes for candy bars		x			x		3-5%
Fats and oils							
Salad Dressings	x	x	x		x		1-3%
Fresh fruits and fruits juices							
Fruit juice	x						0.5-2.0%
Protective fruit coating					x		0.05-100%
Fresh vegetables							
Protective vegetable coating					x		0.05-100%
Frozen dairy desserts and mixes							
Ice cream, frozen desserts			x		x		0.1-1.0%
Frozen whipped toppings; vegetable fat		x					0.3-0.6%
Gelatins, puddings and fillings							
Puddings, Mousse		x	x		x		1.4-4.8%
Gravies and sauces							
Cooking cream sauces	x	x	x		x	x	0.25-1.0%
Tomato sauce	x	x			x	x	0.3-1.3%
Milk, Milk products							
Fat-free milk, Chocolate milk		x			x	x	0.25-0.7%
Low-fat sour cream		x	x		x		0.35-0.5%
Whipped toppings; dairy		x					0.3-0.6%
Processed fruits and fruit juices							
Fruit drink	x						0.5-2.0%

PART 3. DIETARY EXPOSURE

3.1 Intended Uses

Fibrillated cellulose is proposed for use in baked goods and baking mixes, alcohol beverages, non-alcoholic beverages, cheeses, confections and frostings, fats and oils, fresh fruits and fruit juices, frozen dairy desserts and mixes, gelatins, protective produce coatings, puddings and fillings, gravies and sauces, milk and milk products, and in processed fruits and fruit juices (**Table 2-5)**. Use of fibrillated celluloses in these categories ranges from 0.1-5% as a food additive, and 0.05-100% in protective produce coating. **Table 2-5** includes the range of proposed use-levels, listed as the weight/weight percentage (w/w %) of fibrillated cellulose. Because fibrillated cellulose products will effectively substitute for traditional microcrystalline or cellulose substances as a food additive, we anticipate no major increases in global consumption. Estimates of microcrystalline cellulose intake indicate that heavy consumer intake of MCC (90th percentile) ranges from 5.4 to 10.2 g/person per day (CanTox Inc. 1993 in JECFA 1998).

3.2 Calculation of Estimated Daily Intake of Fibrillated Cellulose (EDI)

An estimated concentration in the daily diet is made using the estimated daily intake (EDI), calculated as milligrams (mg) fibrillated cellulose per person per day, according to FDA's *Guidance for Industry: Estimating Dietary Intake of Substance in Food* (FDA, 2006).

For each category of food additive, the EDI of fibrillated cellulose (gram/person/day fibrillated cellulose) is calculated by multiplying the maximum proposed use level (%) by the reported food intake (g/person/day) of food (**Table 3-1**). Since fibrillated cellulose is proposed as a multiple-use additive, intake is calculated using food consumption data for the total sample, rather than for the eaters-only population, because of the high probability that the entire population would consume some foods containing fibrillated cellulose (FDA, 2006). The choice to use total sample is reasonable, given the likelihood that the number of eaters of at least one of the specified foods would be close to 100%.

EDIs were calculated using daily food intake data (g/day) from previously submitted GRAS petitions that were FDA reviewed and received "no further questions", as well as surveys performed by the Center for Disease Control and Prevention's (CDC) National Health and Nutrition Examination Surveys [NHANES 1999-2006] (CDC, 2011-2012), and the US Department of Agriculture's 1994-1996 Continuing Survey of Food Intakes by Individuals (CSFII, 1994-1996). Because use in produce coatings is an additional application for fibrillated cellulose not estimated for conventional forms of cellulose, a separate calculation is performed in Section 3.3.2 to include in the total EDI. Any contribution from uses in food packaging is not expected to measurably contribute to the overall EDI, and would be lower than the conservative estimate made here for edible produce coatings, also insignificant. Fibrillated cellulose is insoluble, forms tangled fibrous networks, and has high molecular weight, inhibiting diffusion from packaging.

The intake calculation provides "worst case estimates" as a result of several conservative (*i.e.* overestimating) assumptions. The EDI calculations assume that all food products within a food category would include fibrillated cellulose at the maximum proposed use level. Furthermore, for some specific uses (*e.g.,* fillings for bakery products), no consumption statistics were located, and instead, intake values for the *whole food category* (*i.e.*, Baked Goods and Baking Mixes) are used, which will grossly overestimate intake levels. In addition, it is established that the length of dietary surveys affects the estimated consumption of individual users. The surveys used are short-term surveys, such as the typical 2-day dietary surveys, which tend to overestimate the consumption of food products that are consumed relatively infrequently (Lambe et al. 2000).

For two categories, high fiber drinks and nutritional beverages, no survey data were located on daily intake, and these are not expected to be high-intake categories; however, a rough calculation based on some market estimates of consumption of functional drinks in the U.S. demonstrates that this category will contribute relatively small amounts of fibrillated cellulose to the EDI. Here, the overestimates likely compensate for the missing data.

Table 3-1. Estimated daily intake of fibrillated celluloses (2+ years, mean intake, total-sample basis).

Food Additives	Proposed Use Levels	Food intake (g/person/day)	Fibrillated cellulose EDI (g/person/day)	Reference
Baked goods and baking mixes	0.5-5%	28[1]	1.4	Smiciklas-Wright, 2002 1994-1996 CSFII
Batters and breadings	0.5-3%			
Cake (fat-reduced)	5%			
Fillings (Bakery products)	0.8-2.0%			
Puffed snacks	2-5%			
Beverages, alcoholic				
Alcoholic formulations (e.g. piña colada mix)	0.3-0.5%	103	0.5	GRAS notice 470 1994-1996 CSFII
Beverages, non-alcoholic				
Soy milk beverages	0.28-0.4%	9.6	0.04	GRAS notice 609
High fiber drinks	0.5-1.0%	0.225	0.00225	Calculation in section 3.3.1
Coffee beverage	0.4-0.6%	121.1[2]	0.7	GRAS notice 607 1965 MRCA
Nutritional beverage	0.4-0.8%	0.225	0.002	Calculation in section 3.3.1
Cheeses	0.1-1.25%	21	0.3	Smiciklas-Wright, 2002 1994-1996 CSFII
Fresh cheese	0.10%			
Low-fat processed cheese	1.00%			
Cheese sauce and dip	0.65-1.25%			
Confections and frostings				
Confectionary	0.5-2.8%	7	0.2	GRAS notice 470

[1] Includes quickbreads and muffins, doughnuts and sweet rolls, cakes and pie.
[2] For all instant coffees and instant teas.

Food Additives	Proposed Use Levels	Food intake (g/person/day)	Fibrillated cellulose EDI (g/person/day)	Reference
Icings	0.2-1%	Partially covered elsewhere[3]		1994-1996 CSFII
Mixes for power bars	3-5%	10	0.5	GRAS notice 613 1994-1996 CSFII
Mixes for candy bars	3-5%	3.7	0.2	GRAS notice 640 2011-2012 NHANES
Fats and oils				
Salad Dressings	1-3%	5	0.2	Smiciklas-Wright, 2002 1994-1996 CSFII
Fresh fruits and fruits juices				
Fruit juice	0.5-2%	61[4]	1.2	Smiciklas-Wright, 2002 1994-1996 CSFII
Protective fruit coating	0.05-100%		0.30	Calculation in Section 3.3.2.
Fresh vegetables				
Protective vegetable coating	0.05-100%		0.26	Calculation in Section 3.3.2.
Frozen dairy desserts and mixes	0.1-1.0%	27	0.3	GRAS notice 470 1994-1996 CSFII
Ice cream, frozen desserts	0.1-1.0%			
Frozen whipped toppings; vegetable fat	0.3-0.6%			
Gelatins, puddings and fillings	1.4-4.8%	20.4	1.0	GRAS notice 607 1965 MRCA
Puddings, Mousse	1.4-4.8%			
Gravies and sauces	0.25-1.3%	11.4	0.1	GRAS notice 640 2011-2012 NHANES
Cooking cream sauces	0.25-1.0%			
Tomato sauce	0.3-1.3%			
Milk products	0.25-0.7%	271[5]	1.9	Smiciklas-Wright, 2002 1994-1996 CSFII
Fat-free milk, Chocolate milk	0.25-0.7%			
Low-fat sour cream	0.35-0.5%			
Whipped toppings; dairy	0.3-0.6%			
Processed fruits and fruit juices				
Fruit drink	0.5-2%	87	1.7	Smiciklas-Wright, 2002 1994-1996 CSFII
TOTAL			**10.8**	

The EDI for food additives is 10.8 g/person/day. At an average American adult body weight (bw) of 82 kg (CDC 2012), the equivalent intake is 132 mg/kg bw/day.

[3] Icing on and in cakes is captured in the 'Baked Goods and Baking Mixes' category
[4] Includes orange juice (42 g/day), apple juice (17 g/day), and lemon juice (2 g/day)
[5] Includes all cows' milk reported separately or as an ingredient in another food including all milk in ice creams, pudding, yogurt, creams and processed foods except cheese and margarine

In comparison, the mean microcrystalline cellulose (MCC) intake in the U.S., as reported in the safety evaluation of MCC by the Joint FAO/WHO Expert Committee of Food Additives (JECFA 1998), ranged from 2.7 g/person per day (children 2 years of age) to 5.1 g/person per day (young adult males). For heavy consumer intake of MCC (90th percentile) the intake values are an estimated 5.4 to 10.2 g/person per day for the same age groups (CanTox Inc. 1993 in JECFA 1998). These calculated EDI values are similar to previously reported intake rates for cellulose, although are likely to be lower for the reasons already discussed.

The notifier's OECD 408 repeated dose 90-day oral toxicity study in rats demonstrated no observable adverse effects (NOAELs) at the highest dietary concentration of 2194.2 mg/kg/day (males) and 2666.6 mg/kg/day (females) fibrillated cellulose, 17-20 times the EDI. Publicly available peer-reviewed data (in Section 6) demonstrate there are no adverse effects of cellulose fiber consumption, at levels as high as 5000 mg/kg oral consumption, or when fed diets consisting of up to 30% cellulose.

3.3 Supporting calculations

3.3.1 Calculations – High fiber drinks and nutritional beverages

Functional foods are a poorly defined category that can include fortified juices, probiotics, energy drinks and others. An estimate was made here for functional beverages not already included in existing food additive categories. A market report from 2017 reports global consumption of functional drinks to be 7,718 million liters, with the U.S. accounted for 36.7% of the overall global market value (Market Research 2018), equaling 2,832 million liters per annum. With an adult population (>18 years old) of ~287 million people in 2017[6], where an estimated 12% of the overall population consume functional beverages[7], an estimated 34,440,000 people consume functional drinks per year. In the U.S. daily consumption of functional beverages is estimated as 2,832,000,000 L/34,440,000 people = 82 L/person/year, or 225 mL/person/day. At a maximum intended use level of 1% fibrillated cellulose in these drinks, the daily intake would be 2.25 mg/day. While the basis for this estimate is from market studies, which are typically of low reliability. However, the resulting consumption rate is likely to be conservative, given coverage of portions of this application in other categories. For example, the consuming population is based on consumption rates of tea and coffee, which is likely larger than the proportion of functional beverage consumers in excluded categories.

3.3.2 Calculations – Protective produce coatings

Fibrillated celluloses are intended to be used as part of an edible coating to protect fresh fruit and vegetables (Zhao 2013, Patent WO 2014153210 A1). For the purposes of dietary intake estimate, it was assumed that that there would be 100% penetration of the product into the market (*i.e.*, all produce consumed would be coated in fibrillated cellulose) at the highest concentration (100 wt/v%) applied at a maximum rate of 10 grams per square meter (gsm). This

[6] United States Census Bureau. QuickFacts. https://www.census.gov/quickfacts/fact/table/US/PST045218
[7] Ozen et al. 2012

assumption produces the highest (most conservative) estimate of potential fibrillated cellulose consumption from this use, creating an estimate expected to be far higher than what will realistically occur. Both edible peel and inedible peel applications are proposed. The intake estimates assume that consumers will eat the skins of 'exposed' produce (*i.e.*, produce with edible peels), and will therefore consume 100% of the fibrillated cellulose coating on the produce. In the case of fruits and vegetables with removable peels (*e.g.*, oranges and squash), it is assumed that peels will not be consumed and that the fibrillated cellulose will not migrate through the skin into the fruit. These types of produce are therefore excluded from the intake calculation. For edible peel 'exposed' produce, the calculation is highly conservative, as it assumes that 100% of the original coating stays on the produce and does not get rubbed off or washed off.

Fibrillated cellulose concentration is in the range of 0.05 wt/v% to 100 wt/v% in the coating formulation. *The maximum* (100 wt/v%) concentration of fibrillated cellulose at 10 gsm is expected to be approximately **1000 µg/cm^2** on the surface of a whole item of produce.

To calculate the EDI of fibrillated cellulose from the protective coating on produce, first an estimate for fruit with edible peels is calculated based on data on average consumer intake, and then, based on this calculation, an intake value for vegetables is calculated. The rationale is that fruit generally has a more regular shape (*i.e.* a sphere) than vegetables, given the calculation takes application rate and surface area into account.

Average daily intake of exposed fruit (g/day)

The average daily intake (ADI) of exposed fruits (edible peel) for different age groups is from the Continuing Food Survey on Intakes by Individuals [CFSII] 1994-1996 data (**Table 3-2**), as found in Chapter 9, Table 9-18 EPA Exposure Factors Handbook 2011 Edition. This value is presented in g/kg body weight/day. To calculate a daily intake of exposed fruit in g/day, the ADI values were multiplied by the average body weight (bw) in each age group (data from the Center for Disease Control and Prevention's (CDC) National Health and Nutrition Examination Survey [NHANES 1999-2006], in Chapter 8, Table 8-3 EPA Exposure Factors Handbook 2011 Edition).

Table 3-2. Average daily intake of exposed fruit by age group.

	Age Group							
	<1 year	**1-2**	**3-5**	**6-11**	**12-19**	**20-39**	**40-69**	**70+**
Average daily intake of exposed fruits, per capita (g/kg bw/day)	10	10.9	5.6	2.2	0.87	0.58	0.69	0.97
Average weight of an individual (kg bw)	6.8	11.4	18.6	31.8	64.2	79.6	83.2	72.5
Average daily intake of exposed fruit (g/day)	68.3	124.3	104.2	70	55.9	46.2	57.4	70.3

Dietary intake of fibrillated cellulose per gram fruit

Table 3-3 shows the calculation of the average surface areas of fruit. The fruit dimensions used for calculations were based on commonly consumed varieties of fruit in the United States (Agricultural Marketing Resource Center, 2017). Apple data were based on Golden Delicious and Red Delicious apples (Tabatabaeefar 2005). Grape data were based on flame seedless grapes (*Vitis vinifera*) (Dimovska et al. 2014). Cherry data were based on sweet cherries (*Prunus avium*) (Zeman et al. 2011). Fruits were assumed to be spherical, therefore *surface area* was calculated using the equation $4\pi r^2$ (where the radius [r], is half the diameter).

At the maximal application rate of 1000 µg/cm^2, the amount of fibrillated cellulose per gram fruit is 930 µg/g for apples, 2330 µg/g for grapes and 2420 µg/g for cherries (**Table 3-3**).

Table 3-3. Estimated amount of fibrillated cellulose per gram of fruit - 100 w/v% formulation.

	Apples	**Grapes**	**Cherries**
Diameter (cm)	7.0	1.25	2.42
Surface area (cm^2)	153.9	4.9	18.4
Weight (g)	165.0	2.1	7.6
Surface area/weight (cm^2/g)	0.9	2.3	2.4
Application rate (µg/cm^2)	1000	1000	1000
Fibrillated cellulose per gram fruit (µg/g)	**930**	**2330**	**2420**
Average fibrillated cellulose (µg/g)	**1893** (all fruit)		
Maximum fibrillated cellulose (µg/g)	**2420** (cherries)		

Estimated Daily Intake (EDI) of fibrillated cellulose in fruit coatings

To calculate the exposed fruit coating EDI of fibrillated cellulose (µg/day) = Average daily intake of fruit (g/day) * Amount of fibrillated cellulose per gram fruit (µg/g)

No data were readily available to elucidate the types and proportions of 'exposed fruits' that comprise the average daily intake. Therefore, to calculate a range of daily fibrillated cellulose intake, an average value and a maximum value were calculated. For the average value, an equal mixture of apple, grape, and cherry consumption was assumed; for the maximum value, it was assumed that 100% of the consumption of fruit with fibrillated cellulose coating was of a single fruit, cherries, as they had the highest surface-to-weight ratio.

Table 3-4 Average and maximum EDI for each age group - maximal 100 w/v% formulation

Age group	Average daily intake of exposed fruit (g/day)	Fibrillated cellulose per gram fruit (µg/g)		Estimated daily intake of fibrillated cellulose (mg/day)	
		Average (all fruit)	*Only Cherries*	*Average*	*Maximum*
<1 years	68.3	1893	2420	129.3	165.3
1-2	124.3	1893	2420	235.3	300.8
3-5	104.2	1893	2420	197.3	252.2
6-11	70.0	1893	2420	132.5	169.4
12-19	55.9	1893	2420	105.8	135.3
20-39	46.2	1893	2420	87.5	111.8
40-69	57.4	1893	2420	108.7	138.9
70+	70.3	1893	2420	133.1	170.1

According to this calculation, the 1-2 year-old age group would have the highest intake of fruit per day (EPA 2011), consuming an average of 235.5 mg/day or a maximum of 300.8 mg/day (**Table 3-4**) of fibrillated cellulose from a coating. Therefore, the maximum amount of **0.301 g/day is considered the potential dietary intake from fruit coating**, which is added to the total EDI in Table 3-1.

Estimated Daily Intake (EDI) of fibrillated cellulose in vegetable coatings
Consumer intake is estimated at an average of 1.33 g/kg/day of exposed vegetables (CFSII 1994-1996), in comparison to 1.53 g/kg day of exposed fruits, for a ratio of 1:1.15. Considering that intake of fibrillated cellulose on fruit is calculated to be 300.8 mg/day, then the intake of fibrillated cellulose from vegetables is estimated to be 300.8 mg/day / 1.15 = 261.6 mg/day, or **0.262 g/day**, which is added to the total EDI in Table 3-2.

PART 4. SELF-LIMITING LEVELS OF USE

The use of fibrillated cellulose in wet formulations is largely limited by its properties as a rheology modifier, and therefore will be controlled through the product formulation, and is intended to be used only to the level that imparts the desired characteristics.

PART 5. EXPERIENCE BASED ON COMMON USE IN FOOD BEFORE 1958

Not applicable.

PART 6. GRAS NARRATIVE

This section provides data and information that form the basis for the conclusion that fibrillated cellulose is Generally Recognized as Safe (GRAS) based on scientific procedures. The recognition of safety is based on the 90-day subchronic dietary feeding study performed by the notifier, supported by the read-across studies, the general conclusions of safety of cellulose and its derivatives by experts, genotoxicity assays, as well as publicly available scientific data demonstrating the safety of fibrillated cellulose and similar materials, the long-term use of conventional cellulose materials in the food industry and the regulatory acceptance of cellulose and its derivatives globally. This narrative reviews all available data and information relevant and available for fibrillar cellulose; we are not aware of any data that is inconsistent with our conclusion of GRAS status.

6.1 History of Use and Regulatory Status of Cellulose

6.1.1 History of Use

Cellulose is the most abundant organic polymer and is consumed regularly in the diet from plant-based foods. Conventional cellulose and its derivatives have been safely used as food additives globally for decades. Cellulose occurs naturally in plants and other types of biomass, with a long history of use in the food industry in applications as a food additive (**Table 6-1**) (Wustenberg, 2015). Cellulose is used extensively in the animal feed and pet food industries, serving as a crude fiber or a feed supplement. Several forms of cellulose (*i.e.*, powdered cellulose, regenerated cellulose and microcrystalline cellulose [MCC]) are also approved organic ingredients (U.S. Department of Agriculture [USDA], 2001]).

Cellulose derived from bacteria is used to make 'nata de coco,' a commercially available product widely eaten in Asian countries; this use is GRAS in the United States. Bacterial cellulose is accepted as a food additive in Japan. Recently, production has increased for export to Europe, United States and the Middle East (Dourado *et al*., 2016).

A 1973 U.S. Food and Drug Administration (FDA) Select Committee on GRAS Substances (SCOGS) review outlined the common uses of conventional forms of cellulose and its derivatives in various products (Table 0-1), showing the percentages of cellulose and several cellulose derivatives added to foods. Cellulose is used in a wide range of food types, including beverages, candy, fats and oils, grain products and baked goods. In most uses, cellulose comprises 0.25% to 4.83% of the product, but it can comprise up to 29% of baked goods and baking mixes. Fibrillated cellulose will displace some conventional cellulose uses, but not all (*e.g*. fibrillated cellulose is not intended for use in meat products). It was calculated that 438,526 kg of cellulose was used in foods in 1970, equaling 5.7 mg/person/day (SCOGS, 1973). There are indications that the mean intake of dietary MCC in the USA is increasing; in 1993, mean intake was estimated to range from 2.7 g/person/day (children 2 years of age) to 5.1 g/person/day (young adult males). For heavy consumers, intake of MCC (90^{th} percentile) was 5.4 to 10.2 g/person/day for the same age groups (CanTox Inc., 1993, referenced in the Joint FAO/WHO Committee on Food Additives report [JECFA 1998]).

Table 0-1. Percentages of cellulose and several cellulose derivatives added to foods (Adapted from SCOGS 1973).

Food Category	Identified as Use for Fibrillated Cellulose	Cellulose, pure and regenerated, including MCC		Methyl cellulose		Carboxy-methyl cellulose		Sodium Carboxy-methyl cellulose		Ethyl cellulose	
		Usual	Max.	Usual	Max.	Usual	Max.	Usual	Max.	Usual	Max.
Baking goods, baking mixes	x	4.83	29.00	0.15	0.36	0.12	0.30	0.21	0.31		
Other grain products, pastas				0.75	1.00	0.01	0.01	0.07	0.08		
Fats and oils	x	3.00	4.50	0.07	0.12			0.60	0.78		
Milk, milk products	x	0.25	0.39			0.02	0.04	0.05	0.36		
Cheese	x	1.00	1.00			0.40	0.40	0.08	0.08		
Frozen dairy desserts, mixes	x	0.40	0.85	0.41	0.46	0.01	0.04	0.09	0.17		
Processed fruits, juices and drinks	x					0.08	0.08	0.06	0.08		
Fruit ices, water ices								0.07	0.12		
Meat products		0.94	1.41	0.04	0.07	0.06	0.13	0.01	0.01		
Poultry products		2.00	3.00	0.01	0.02	0.36		0.20	0.35		
Eggs, egg products								0.06	0.06		
Fish products		2.50	3.00								
Processed vegetables, juices	x	<0.01		0.20	0.66	0.07	0.49	0.01	0.03		
Candy, soft	x			<0.01	<0.01			0.10	0.10		
Sugar, confections	x	0.40	0.68			0.25	0.25	0.14	0.16		
Jams, jellies and sweet spreads								0.50	0.50		
Sweet sauces, toppings, syrups		0.90	1.03	<0.01	<0.01	0.07		0.51	1.00		
Gelatins, puddings, fillings	x	0.75	1.00			0.20	1.00	0.06	0.06		
Soup, soup mixes		0.35	0.75					0.01	0.02		
Beverages Type 1 (nonalcoholic)	x			<0.01	0.01	0.01	0.02	0.07	0.09		
Nuts, nut products		0.54	0.54	1.00	1.00						
Gravies, sauces	x	0.75	1.00	<0.01	<0.01	0.22	0.46	0.52	0.53		
Dairy product analogs	x	0.52	1.25	0.15	0.20			0.23	0.47		
Hard candy										0.01	0.01
Chewing gum				2.20	2.20					0.02	0.04

6.1.2 Non-food uses

Cellulose fibers are currently used in many non-food uses, including several of the applications in Table 6.2. A renewable, bio-based material, conventional forms of cellulose have been used for packaging, construction and transportation materials, electrical insulation, water and air

filtration, among others. Fibrillated celluloses possess additional desirable properties as non-food use materials. They are demonstrated to impart high strength to composites; are lightweight; highly absorbent; modify viscosity; improve barrier properties for sound, grease, oil, and oxygen; and can be made to be optically transparent (Shatkin *et al.,* 2014; Siró, 2010; Lavoine, 2012). These properties offer several advantages for commercial applications outside of the food industry, including the automotive, coatings, construction, paper, aerospace, pharmaceutical, personal hygiene and electronics industries. Table 0-2 summarizes some potential high-volume, low-volume, novel and emerging applications of fibrillated celluloses (Shatkin *et al.*, 2014).

Table 0-2. USDA-identified proposed applications of fibrillated celluloses.

High-Volume Applications	Low-Volume Applications	Novel and Emerging Applications
Cement	Wallboard facing	Sensors
Automotive body	Insulation	Reinforcement fiber
Packaging coatings	Aerospace structure	Water filtration
Paper coatings	Aerospace interior	Air filtration
Paper filler	Aerogels for oil and gas industry	Viscosity modifiers
Packaging filler	Paint – architectural	Purification
Replacement – plastic packaging	Paint – special purpose	Cosmetics
Plastic film replacement	Paint – OEM applications	Excipients
Hygiene and absorbent products		Organic LED
Textiles for clothing		Flexible electronics
		Photovoltaics
		Recyclable electronics
		3D printing
		Photonic films

Modified from (Shatkin et al.*, 2014).*

Cellulose has a long history of safe use in the pharmaceutical industry. Powdered cellulose and MCC are used as bulking agents, adsorbents, suspending agents and capsule diluents, and MCC can also be used as a thickening agent (Marques-Marinho, 2013). A number of forms of cellulose are listed as approved inactive ingredients for drug products. This includes approval for use of MCC and powdered cellulose in oral tablets and capsules (FDA, 2016).

Bacterial celluloses are widely used in biomedical applications, ranging from topical wound dressings to durable scaffolds for tissue engineering (Park, 2009). Biomedical products using bacterial cellulose were introduced in the early 1980s, and there is currently a wide range of available products (Park, 2009).

Cellulose and its derivatives are widely used in cosmetics and toiletries as thickeners, suspending agents, film formers, stabilizers, emulsifiers, emollients, binders and water-retention agents. The majority of uses are in hair products, eye and facial makeup and skin care preparations. The concentration of use can range up to 88%; however, celluloses are most frequently used in concentrations of >0.1-1% (Cosmetics Ingredient Review [CIR] Expert Panel, 2009).

An independent expert panel convened by the Cosmetic Ingredient Review (CIR) completed a in-depth review and analysis of relevant safety studies and concluded that cellulose, calcium carboxymethyl cellulose (Ca-CMC), carboxymethyl cellulose acetate butyrate (CMCAB), carboxymethyl hydroxyethyl cellulose (CMHEC), cellulose acetate (CA), cellulose acetate butyrate (CAB), cellulose acetate propionate carboxylate (CAPC), cellulose gum (CG), cellulose acetate propionate (CAP), cellulose succinate (CS), cetyl hydroxyethyl cellulose (CHEC), ethyl cellulose (EC), hydrolyzed cellulose gum (HCG), hydroxybutyl methyl cellulose (HBMC), hydroxyethyl cellulose (HEC), hydroxyethyl ethyl cellulose (HEEC), hydroxypropyl cellulose (HPC), hydroxypropyl methyl cellulose (HPMC), hydroxypropyl methyl cellulose acetate/succinate (HPMCA/S), methyl cellulose (MC), ethyl methyl cellulose (EMC), hydroxyethyl methyl cellulose (HEMC), microcrystalline cellulose (MCC), potassium cellulose succinate (K-CS) and sodium cellulose sulfate (Na-CS) are safe as cosmetic ingredients in the practices of use and concentrations given in their safety assessments.

6.1.3 Regulatory Status of Cellulose Materials in the United States

Cellulose, powdered cellulose, and microcrystalline cellulose were affirmed GRAS, though do not appear in the Code of Federal Regulations (CFR) as they are considered to belong in the 'prior sanctioned category' (used in food prior to January 1, 1958) (FDA 2018). A re-evaluation of their safety status by the FDA Select Committee of GRAS Substances (SCOGS) confirmed safety and GRAS status (SCOGS 1973). Sodium carboxymethyl cellulose (Na-CMC, 21 CFR 182.1745) and MC (21 CFR 182.1480) are listed in 21 CFR 18 'Substances Generally Recognized as Safe' as multiple purpose additives. EC (182.90) is listed in 21 CFR 182 as GRAS for 'Substances Migrating to Food from Paper and Paperboard Products.' Other important cellulose derivatives (HPC (172.870), HPMC (172.874), EMC (172.872) and EC (172.868)) are approved under 21 CFR 172 'Food Additives Permitted for Direct Addition to Food for Human Consumption' as multipurpose additives. In addition, there are approvals for other cellulose derivatives that define their use in specific food products such as 'Artificially Sweetened Fruit Jellies' (21 CFR 150.141) or for specific uses such as 'Adhesives and Coatings for Food Use' (21 CFR 175.300). Currently, carboxymethyl cellulose (CMC) and MC are also approved as food additives by the USDA for use as an 'Extender or Stabilizer in Meat and Vegetable Patties' (9 CFR 318.7) and for use as an 'Extender and Stabilizer for Poultry Products' (9 CFR 381.147).

Bacterial cellulose was demonstrated GRAS as a food ingredient through the independent conclusion process under 21 CFR 182.1 using scientific procedures in accordance with 201 (s) (21 USC Section 321 (s)) of the Federal Food, Drug and Cosmetic Act. A GRAS affirmation petition was filed on the basis of the GRAS determination of bacterial cellulose on December 11, 1991, which was accepted by the FDA in 1992. Amendment of the GRAS affirmation petition to GRAS notification was requested under the Interim Policy provision of the FDA's April 17, 1997, GRAS notification proposal (Sec. 21 CFR 170.36 (g) 2) (Park 2009).

Producers of fibrillated cellulose have achieved authorization for its use as a food contact substance. Imerys Minerals, Inc., Omya AB, Fiberlean Technologies Ltd. and Billerud Korsnäs AB, multi-national packaging producers, were granted Food Contact Notifications (FCNs) 1582,

1864, 1887, and 2022 by the FDA for the use of microfibrillated cellulose (MFC) pulp as an additive in the manufacture of paper and board and as banana peel coating. These FCNs authorize MFC for use at levels not to exceed 5.0 % by weight of the dry fibers in the finished paper and paperboard in contact with all types of food, and is also permitted for uses in contact with infant formula, and as a component of coatings applied to banana peels, up to 13% by dry weight in the final dry coating.

6.1.4. Regulatory Status of Cellulose Materials in Europe

Cellulose (E460) and several cellulose derivatives such as MC (E461), EC (E462), low-substituted HPC (E463a), HPMC (E464), EMC (E465) and Na-CMC (E466), cross-linked Na-CMC (E468), and enzymatically hydrolysed CMC (E469) are permitted to be used in foods in Europe, all allowed *quantum satis*, where 'no maximum numerical level is specified and substances shall be used in accordance with good manufacturing practice (GMP), at a level not higher than is necessary to achieve the intended purpose and provided the consumer is not misled' (Regulation (EC) No 1333/2008). An evaluation of ten forms of dietary celluloses concluded that short term exposure to celluloses up to 10% did not indicate any adverse effects, and chronic exposure NOAEL values ranging up to 9000 mg/kg bw/day (EFSA ANS Panel 2018). Further, the panel concluded that there was no need for a numerical ADI and that there would be no safety concern at the reported uses and use levels for the unmodified and modified celluloses (EFSA ANS Panel 2018).

In addition to use as food additives, cellulose and several of its derivatives are used extensively as additives for food contact materials. A safety evaluation of cellulose and HPC conducted by the European Food Safety Authority (EFSA) Panel on Food Contact Materials, Enzymes, Flavorings and Processing Aids (CEF Panel) in 2013 concluded that cellulose and HPC are safe to use in packaging that is in direct contact with food (EFSA, 2013).

6.1.5. Regulatory Status of Cellulose Materials in Canada

In Canada, cellulose and several of its derivatives are authorized for food use. HPC and EMC are allowed in any unstandardized food as long as GMP is followed. HPMC is allowed in salad dressings and milks and unstandardized foods, as long as GMP is followed. MC is allowed in beers, dressings, and unstandardized foods, as long as GMP is followed. Cross-linked CMC can be used in table-top sweetener tablets. Powdered cellulose is listed for a large range of applications including as a bulking agent in batter, edible ices, fillings, tablets, icings, seasonings, baked goods and dry mixes from 0.5-50% of the total product. MCC may be used for a number of functions, such as a bodying and texturizing agent in ice milk and ice cream mix; sherbet; whipped vegetable oil topping; unstandardized frozen desserts, dips, and spreads; breath freshener products and sausage casings from 0.5-9% total product. MCC may also be used as a filler in 'reduced in energy' food products, for tablet disintegration at a maximum of 2.2%, in table-top sweetener tablets containing aspartame and as a stabilizing and thickening agent for whipping cream, up to 2%. Na-CMC can be used in sausage casings as a coating to enable peeling (up to 0.25% of the casing) and can be used to inhibit crystal formation in wine and canned mandarin oranges.

6.2 Safety

6.2.1. Safety Evaluation by Authoritative Bodies

Several expert panels have reviewed the safety of cellulose and its derivatives and offered conclusions and recommendations concerning their use as food additives, in food paper and packaging and in cosmetics. Expert panels have all reached similar conclusions: current applications of cellulose and its derivatives are not hazardous and are recognized as safe.

Select Committee of GRAS Substances. From 1958 to 1969, the FDA granted GRAS status to many forms of cellulose for a variety of applications. The FDA, through a SCOGS, conducted an evaluation of cellulose and its derivatives in 1973 (NTIS PB No. 221-28, 1972; SCOGS Report No. 25, 1973). After a comprehensive review by the SCOGs and after public comment, the committee concluded that:

> *"There is no evidence in the available information […] that demonstrates, or suggests reasonable grounds to suspect a hazard to the public when it is used at levels that are now current and in the manner now practiced."*

Cellulose and its derivatives were incorporated into the CFR by rulemaking and resulted in GRAS designations for several cellulose derivatives.

Joint FAO/WHO Committee on Food Additives. The Food and Agriculture Organization of the United Nations (FAO) and World Health Organization (WHO) published comprehensive reviews of available toxicology literature on celluloses and associated derivatives. The Joint FAO/WHO Committee on Food Additives (JECFA) is an international expert committee that has been meeting since 1956 to evaluate food additives. MCC was evaluated at the 15th, 17th and 19th meetings of the committee, and again at the 49th meeting (JECFA, 1998). At the 19th meeting an acceptable daily intake (ADI) of "not specified" was allocated. The JECFA Committee states that an ADI without an explicit indication of the upper limit of intake ("ADI not specified") is assigned to substances of very low toxicity, and that on the basis of the available data (toxicological, biochemical and other), the total daily intake of the substance, arising from its use or uses at the levels necessary to achieve the desired effect and from its acceptable background in food, does not, in the opinion of the Committee, represent a hazard to health (JECFA, 1975). The committee concluded in the 1998 report that there was no evidence of toxicity from the ingestion of MCC based on toxicological data from humans and animals when used in foods according to GMP (JECFA, 1998).

Nordic Council of Ministers. Supporting the conclusions of JECFA, the Nordic Council of Ministers reviewed the status of food additives presently permitted in the EU, including cellulose, MCC, MC, HPC, HPMC, EMC, CMC and Na-CMC. The conclusion from the report was that re-evaluation of the use of these materials as food additives was not necessary given their demonstrated history of safety (TemaNord, 2002). These conclusions were largely based on evaluations by the Scientific Committee on Food (SCF) and JECFA reflecting the notion that for

current uses and levels of intake, these materials do not represent a hazard to health (TemaNord, 2002).

Cosmetic Ingredient Review. An expert panel for Cosmetic Ingredient Review published their conclusions on the safety assessment of cellulose and 25 of its derivatives. After thorough review of the potential acute, subchronic and chronic animal and human toxicity from all exposure routes (dermal, mucosal, and ocular irritation, phototoxicity, reproductive and development toxicity, genotoxicity, carcinogenicity), as well as other related effects (*e.g.*, laxative, dental caries), the panel concluded that all of the substances evaluated "are safe as cosmetic ingredients in the practices of use and concentrations given in this safety assessment" (CIR Expert Panel, 2009).

6.2.2. Toxicity Tests performed by notifier on fibrillated cellulose

In addition to the extensive peer-reviewed literature supporting the safety of similar forms of cellulose, three additional tests have been performed by the notifier to support the conclusion of safety for GRAS status: an OECD Test Guideline (TG) 487 *in vitro* mammalian cell micronucleus test, a 7- and 14-day range finding oral toxicity study in rats, and an OECD TG 408 Repeated dose 90-day oral toxicity study in rats (OECD 1998).

Genotoxicity. An Organization for Economic Cooperation and Development (OECD) 487 study (in vitro micronucleus test in L5178Y $TK^{+/-}$ mouse lymphoma cells) was performed at CiToxLAB France, using 2500 µg/mL of fibrillated cellulose, in compliance with the OECD Principles of Good Laboratory Practice (GLP) (ENV/MC/CHEM (98) 17). One of the notifier's fibrillated cellulose was tested in two independent experiments, with and without a metabolic activation system, the S9 mix, prepared from a liver microsomal fraction (S9 fraction) of rats induced with Aroclor 1254, as follows:

	First experiment	Second experiment
Without S9 mix	3 h treatment + 24 h recovery	24 h treatment + 20 h recovery
With S9 mix	3 h treatment + 24 h recovery	3 h treatment + 24 h recovery

Each treatment was coupled to an assessment of cytotoxicity, as determined by population doubling. Micronuclei were counted after exposure to three dose levels; 625, 1250 and 2500 µg/mL for the 3-hour treatments with or without S9 mix, and 312.5, 625 and 1250 µg/mL for the 24-hour treatment without S9 mix. Exposure to fibrillated cellulose did not induce any cytotoxicity, nor induce any chromosome damage, or damage to the cell division apparatus in TK+/- mouse lymphoma cells, in the absence or in the presence of a rat metabolizing system.

Sub-acute toxicity. Two sub-acute toxicity studies were performed using a non-commercial form of fibrillated cellulose (Ref FC) and conventional cellulose, Solka Floc (Ref CC). These tests served as range-finding studies for the subchronic 90-day study, and to evaluate palatability and general toxicity following 7 or 14 days of dietary administration. The study was based on principles outlined in OECD TG 407, and performed at Product Safety Labs in a GLP compliant facility. Five male and five female rats fed 0.6%, 0.9%, or 1.2% fibrillated cellulose in the diet

daily showed no signs of gross toxicity, behavioral changes, or clinical changes after 14-days. The Ref FC or Ref CC was mixed into a fiber-free diet and dried into pellets. Piloerection was consistently observed in most of the control and the test substance rats from days 5-14; however, this was not observed again in the subsequent 7-day pilot study nor the full 90-day subchronic dietary study and therefore not considered to be related to an effect of the test substance. The 14-day study determined that standard methods typically used to measure fiber in a dietary matrix were not sensitive enough to validate the amount of fibrillated cellulose added to the diet.

A second pilot study was performed exposing rats to 5% fibrillated cellulose in the diet for 7-days using the same parameters as the initial 14-day study. There were no biologically significant changes attributed to the dietary administration of fibrillated cellulose under the conditions of the study and based on the toxicological endpoints evaluated. Green feces were observed in every rat in the 5% fibrillated male and female groups – this was attributed to the green dye used in the feed (a standard technique to distinguish the different feeds). The dye was not employed in the 90-day study, and no additional observations of green feces were noted. The mean dietary intake of fibrillated cellulose was calculated to be 4961.8 mg/kg/day for male rats and 4131.1 mg/kg/day for the female rats, and was concluded to be palatable.

Subchronic toxicity. The notifier performed an OECD TG 408 (OECD 1998[8]) study to assess the 90-day dietary toxicity of fibrillated cellulose (Ref FC), as compared to a widely used conventional cellulose (Ref CC) Solka Floc®, a powdered form of cellulose that is GRAS and has been used as a food ingredient for over 85 years. The fibrillated cellulose was obtained from the University of Maine Process Development Center. The study was performed in a commercial lab under GLP. Sprague Dawley rats were fed 2%, 3%, or 4% fibrillated cellulose for 90 consecutive days; parallel Ref CC groups were used as controls. A maximum dose of 4% was estimated to equal an exposure level of 2000-2500 mg/kg. This was selected following recommendations in OECD documents as the upper limit of doses to be used in rodent studies (*e.g.* OECD 425), in line with many other studies on fibrillated and conventional forms of cellulose, and as a concentration approximately 17-20 times higher than the conservatively calculated EDI. Survival, clinical observations, body weight (BW), food consumption, ophthalmologic evaluations, hematology, serum chemistry, urinalysis, and a post-mortem anatomic pathology were monitored and performed. A complete histopathological analysis performed on the high dose control and fibrillated cellulose groups found no lesions or histopathological abnormalities indicating irritation or tissue damage[9]. No adverse observations

[8] Note that a newer version of OECD TG 408 was released on 25 June 2018 that was updated to add endocrine-sensitive endpoints. However, the Notifier's study was initiated in April 2018 and therefore follows the prior version of the Test Guidelines. There are no indications from available studies or from the Notifier's study that exposure to dietary fibrillated cellulose would result in effects on the endocrine system, and therefore is not deemed to warrant measurement (*e.g.* EFSA ANS Panel 2018, Behall 1984).

[9] The following tissues and organs were examined: adipose tissue, aorta, bone marrow, bone (femur, sternum), brain, cervix, epididymis, esophagus, eye, adrenal gland, Harderian gland, mammary gland, parathyroid gland, pituitary gland, prostate gland, salivary glands (parotid, sublingual, submandibular), seminal vesicle gland, thyroid gland, heart, kidney, large intestine (cecum, colon, rectum), larynx, liver, lungs, lymph node (mandibular, mesenteric), nasal turbinate, nose, optic nerve, ovary, oviduct, pancreas, peripheral sciatic nerve, Peyer's patch, pharynx, skin, skeletal muscle, small intestine (duodenum, ileum, jejunum), snout, spinal cord (cervical, lumbar, midthoracic), spleen, stomach, testes, thymus, trachea, urinary bladder.

were found in relation to the administration of fibrillated cellulose. Under the conditions of this study and based on the toxicological endpoints evaluated, the no-observed-adverse-effect level for fibrillated cellulose is 2194.2 mg/kg/day (males) and 2666.6 mg/kg/day (females), corresponding to the highest dose tested (4%) for male and female Sprague Dawley rats. These results demonstrate that fibrillated cellulose behaves similarly to conventional cellulose and raises no safety concerns when used as a food ingredient at these concentrations. These results have been peer-reviewed and published in a journal, Toxicology Reports (Ong et al., 2020) (Attachment 1).

The results of the notifier's study on fibrillated cellulose are in agreement with other cellulose studies (Table 6-3), where feeding or gavaging of diets up to 21% fibrillated cellulose, and 30% conventional cellulose do not significantly affect biological, biochemical, or histological parameters in rats and other mammals.

6.2.3. Toxicity Tests performed by notifier on fibrillated cellulose for read-across

Physical, chemical and toxicological characterization of eight cellulose materials (Ref FC, Ref CC, C20-C25) was conducted to demonstrate the physical, chemical and biological similarity of these materials and provide substantive evidence to support their grouping and read-across between the notified materials and the materials tested in the acute and subchronic dietary studies.

The eight materials are derived from cellulose and share the same molecular structure, described in Section 2.1. The six Notified fibrillated cellulose materials (C20-C25) were compared side-by-side with the two cellulose materials that were evaluated in the subchronic dietary studies (OECD TG 407, 408): a non-commercial form of fibrillated cellulose (Ref FC) obtained from the University of Maine Process Development Center, as well as a conventional cellulose (Ref CC) material, Solka Floc® currently used in food (see Section 6.2.2).

Experimental overview and aims. Cellulose materials 'as produced' and following simulated *in vitro* gastrointestinal and lysosomal digestion were characterized to allow comparison of their physical and chemical properties. The pristine and digested cellulose materials were also assessed for a number of toxicological endpoints including cytotoxicity, barrier integrity, oxidative stress, and inflammation in a gastrointestinal (GI) tri-culture model.

The testing is designed to allow read-across for the eight related cellulose materials as part of a food safety demonstration for fibrillated cellulose. The testing strategy follows guidance released by the European Food Safety Authority (EFSA 2018) that represents a currently accepted approach for screening level testing of food ingredients intended to limit the need for excessive animal testing to demonstrate the safety of chemically similar materials. The testing strategy simulates realistic gastrointestinal exposure conditions *in vitro*, and demonstrates the physical, chemical, and biological similarity of the six Notified forms of fibrillated cellulose (C20-C25) to the fibrillated cellulose (Ref FC) tested in acute and subchronic oral toxicity testing *in vivo* (OECD TG 407 and 408). By providing evidence to support the grouping of these seven forms of fibrillated cellulose, results for Ref FC from the 90-day dietary toxicity study can be read-across to the six Notified materials, demonstrating the safe use of the six forms of fibrillated cellulose up to 4% in food, without additional, excessive animal testing of each form.

Similarly, the side-by-side comparison of the Notified forms to conventional cellulose fiber allows read across to the extensive safety data dossiers for currently used forms of GRAS cellulose. The read-across *in vitro* work is currently under peer review at (Pradhan et al. 2020) (Attachment 2), and 90-day study results have been peer-reviewed and published in a journal, *Toxicology Reports* (Ong et al. 2020) (Attachment 1).

Physical and chemical characterization. The Notified and OECD TG studied cellulose materials were characterized for several physical and chemical endpoints including: morphology, hydrodynamic size, fiber width, size distribution, surface charge and impurities. All materials are similarly derived from cellulose and share the same fundamental molecular structure described in Section 2.1. Inductively coupled plasma-mass spectrometry (ICP-MS) analysis found low levels of metal impurities (low ppb range) in the six Notified materials and Ref FC, shown in Section 2.1.2, Figure 2-3 and Table 2-2.

Electron microscopy images in Section 2.1.2 and Figure 2-4 show the fibrillar morphology of fibrillated celluloses, consisting of an entangled network of fibers and fibrils of varying widths. Note that one material (C25) is made by grinding with a GRAS mineral agent, calcium carbonate, observable in micrograph G. In comparison, conventional cellulose (Figure 2-4, H) has a lower aspect ratio than fibrillated cellulose and does not form an entangled network of fibers. Solka Floc®, the conventional form of cellulose, has an amorphous morphology that is tens of microns in length and width.

Dynamic light scattering (DLS) measured the hydrodynamic diameter (HDD) and dispersity indices (DI) of the fibrillated celluloses, demonstrating similar size and size distributions in aqueous media, described in Section 2.1.2.3.

Measurements of surface charge (zeta potential) determined all fibrillated celluloses were negatively charged. Ref FC has an average zeta potential (n=3) of -33.87mV, similar to C20-C25 which ranged from -46.40 to -5.20 mV. Ref CC has an average zeta potential of -2.14 mV (Table 2-2).

Physical, chemical and biological characterization of the eight materials before and after simulated *in vitro* gastrointestinal and lysosomal digestion followed guidance released by EFSA. *In vitro* gastrointestinal digestion exposed cellulose materials *ex vivo* to chemical conditions, enzymes and salts representative of those in the mouth, stomach and intestinal compartments, simulating physiological digestion in the gastrointestinal tract, following an internationally agreed upon simulated digestion model for food (Minekus et al. 2014).

Briefly, the materials were exposed to simulated oral phase fluid that included alpha-amylase and salts for a total of 2 minutes at 37 °C. Simulated gastric fluid included the enzyme pepsin, phospholipids, and salts, with pH adjusted to 3.0 with hydrochloric acid. The fibrillated and conventional celluloses were incubated in gastric fluid for 2 hours at 37 °C. Subsequently, materials were exposed to simulated intestinal fluid that contained pancreatin (a complex mixture of digestive enzymes isolated from porcine), bile, and salts, with pH adjusted to 7.0 with sodium hydroxide. Fibrillated celluloses were incubated in the simulated intestinal fluid at 37 °C for one of four timepoints: 15, 30 minutes, 1, 4 hours (Pradhan et al. 2020).

In vitro lysosomal digestion exposed cellulose materials to conditions within lysosomes, simulating intracellular digestion conditions. Artificial lysosomal fluid (ALF) is a complex mixture of sodium chloride, sodium hydroxide, citric acid, calcium chloride, disodium phosphate, sodium sulfate, magnesium chloride, glycerol, trisodium citrate, sodium tartrate, sodium L-lactate, sodium pyruvate, and formaldehyde. ALF was pH-adjusted to 4.5 and cellulose materials were incubated at 37 °C for one of four timepoints: 30 minutes, 2, 24 or 72 hours (Pradhan et al., 2020).

Fibrillated celluloses (C20-C25) remained physically and chemically similar post-digestion, retaining fibrillar morphologies, with no significant changes in HDD, DI, or zeta potential compared to pristine forms for both simulated gastrointestinal and lysosomal digestion. Similarly, Ref CC showed no significant change in morphology, HDD, DI, or zeta potential following simulated gastrointestinal and lysosomal digestion. Cellulose, an insoluble fiber, does not undergo digestion along the human gastrointestinal tract due to a lack of degrading enzymes and results in excretion in the feces (Chassaing et al. 2017, Holscher 2017). Results for post-digestion characterization demonstrate that as expected, fibrillated cellulose also does not undergo degradation under simulated digestion conditions. These results are summarized here and presented in Pradhan *et al.*, 2020.

Biological characterization. Cellulose materials were evaluated pre- and post-simulated gastrointestinal digestion for a variety of toxicological endpoints including cytotoxicity, barrier integrity, oxidative stress and inflammation, using an *in vitro* tri-culture model of the intestinal epithelium. The tri-culture includes HT29-MTX cells, an intestinal epithelial cell line that resembles goblet cells capable of secreting mucus; Caco-2 cells, an intestinal epithelial cell line that resembles enterocytes; and Raji-B cells, a lymphocyte cell line which can induce M-cell phenotypes in Caco-2 cells when co-cultured. The tri-culture model is an advanced *in vitro* system to reproduce the morphology and physiology of the human gastrointestinal tract. It was employed to assess and compare the biological activity of all eight cellulose materials, pre- and post-digestion. The tri-culture model was exposed to 2% cellulose solutions (pristine or digested) in cell culture media (final cellulose exposure 0.4% wt).

All toxicological assays were optimized and validated; the experimental design included negative control treatments (untreated), vehicle control treatments (either deionized water or simulated gastrointestinal fluid), and positive control treatments. For cytotoxic endpoints, Rotenone was the positive control, a pesticide known to induce cytotoxicity through induction of oxidative stress (Heinz et al., 2017). For inflammation, lipopolysaccharides (LPS) served as a positive control. LPS are found in the outer membrane of gram-negative bacteria and are known to induce pro-inflammatory mediator production and release (Bisig et al., 2019). Hydrogen peroxide (H_2O_2), a known inducer of oxidative stress, was used as a positive control treatment in flow cytometry experiments examining this endpoint.

Cytotoxicity following material exposure (1-48 hours) was assessed with the MTS assay, a colorimetric assay that measures changes in cellular metabolism to indicate toxicity in individual cell type and triculture assays. Cytotoxicity following material exposure (1-4 hours)

was also assessed using a combination of the fluorescent dye SYTOX Red and flow cytometry. Integrity of the *in vitro* intestinal epithelium was assessed using transepithelial electrical resistance (TEER) up to 8 days post-exposure. Potential for induction of oxidative stress following material exposure (15 minutes – 4 hours) was assessed with the fluorescent dye CellROX Green, measured by flow cytometry. Finally, potential for a pro-inflammatory response following material exposure (1-48 hours) was assessed using the marker interleukin (IL)-6, measured by enzyme-linked immunosorbent assay (ELISA).

No adverse effects were observed in the seven forms of fibrillated cellulose (Ref FC, C20-C25) or with conventional cellulose (Ref CC), in both the pristine form and following simulated gastrointestinal digestion, in comparison to vehicle controls, when cells of the in vitro intestinal model were exposed to 2% cellulose solutions (final cellulose exposure 0.4% wt). Results demonstrate no significant increase in cytotoxicity (up to 48 hours post-exposure), no decrease in the integrity of the intestinal epithelium (up to 8 days post-exposure), no induction of oxidative stress (up to 4 hours post exposure), and no significant increase in the pro-inflammatory marker IL-6 (up to 48 hours post exposure) in the intestinal tri-culture following material exposure (pristine and digested forms).

The eight cellulose materials were also characterized following simulated lysosomal digestion to assess the potential for intracellular digestion of these materials, because this pathway is considered in the EFSA guidance. However, there is no evidence to date to suggest that any insoluble cellulose fiber, including fibrillated cellulose, can be taken up intracellularly or cross the gastrointestinal tract, or interact in any way with lysosomes. Human digestive enzymes are not capable of metabolizing cellulose. The large molecular size means fibrillated forms of cellulose will not cross the intestinal epithelium or travel through intercellular pathways. Studies examining the absorption, distribution, metabolism and excretion (ADME) of cellulose find it to be excreted in the feces, as described in Section 6.2.4.1.

The *in vitro* toxicological assessment of fibrillated cellulose by the notifier demonstrates a lack of cytotoxicity, barrier impairment, oxidative stress response, and inflammation, evidence that these materials are do not interact with cells in the human gastrointestinal tract.

Evidence for grouping and read-across. The physical and chemical characterization of fibrillated cellulose (Ref FC; C20-C25) demonstrates these materials are similar physically and chemically, being composed of the same fundamental molecular structure and exhibiting the same general morphology, size, size distribution and surface charge. These similarities remained following simulated gastrointestinal and lysosomal digestion. Along with the *in vitro* toxicological assessment, the study demonstrates the tested materials also have similar biological behavior. Exposure to each of the fibrillated forms of cellulose (Ref FC; C20-C25), in either the pristine or digested form at 0.4% by weight, showed no adverse effects following exposure in the intestinal tri-culture model.

The *in vitro* studies demonstrate the similar biological behavior of the Notified fibrillar celluloses to the forms in the subchronic dietary study. The results demonstrate the physical,

chemical, and biological similarities of these materials, and provide substantive evidence to support their grouping. Based on this grouping, results from the *in vivo* subchronic oral toxicity testing of Ref FC, which found no adverse effects in rats over 90 days up to 4% in the diet can be read-across to the six forms of fibrillated cellulose (C20-C25) which are the subject of this Notice.

Conventional cellulose (Ref CC) exhibits a different morphology and is an order of magnitude larger than fibrillated forms; however, side-by-side mammalian and *in vitro* toxicological testing by the notifier demonstrates the fibrillar materials behave similarly in the gastrointestinal tract. No adverse effects were observed following dietary exposure to either fibrillated or conventional cellulose in rats up to 90-days post exposure, or during *in vitro* testing up to 2 days post exposure. The similar biological behavior in the notifier's testing warrants the consideration of the extensive historical data sets demonstrating the safe use of conventional cellulose in food (which is GRAS) as supporting evidence toward the conclusion that fibrillated forms of cellulose are similarly GRAS.

6.2.4. Published toxicology studies

Conventional cellulose and its derivatives have been employed as food additives for decades. Collected evidence has established their safety; expert reviews of these data have similarly concluded these materials are safe. Several studies have reported health benefits associated with consumption of cellulose, citing its use as a caloric substitute and as a source of dietary fiber.

The publicly available studies examining the safety of fibrillated cellulose and related forms are summarized to support the determination that fibrillated cellulose is GRAS. The most relevant studies are summarized in Table 0-**3,** with more detailed description of results in the text. As described in 6.1.8 in the read-across demonstration, fibrillated celluloses have molecular, physical, chemical, and biological characteristics similar to conventional celluloses. In the sections that follow, available information for fibrillated cellulose, conventional cellulose, and bacterial cellulose are discussed. As supporting data, some relevant studies on microcrystalline celluloses are also reported. There is a large body of literature available on the safety of derivatives of celluloses which are not reported here, because these functionalized forms are different chemically than fibrillated celluloses.

6.2.4.1. Human Safety

There is no evidence to date to suggest that fibrillated cellulose in food is harmful to humans.

Several studies have examined the effects of human oral consumption of cellulose. Cellulose is already safely and legally used in foods globally, with no known records of toxicity in the diet. Scientific studies demonstrate that incorporation of cellulose into the diet results in no adverse effects. Studies have examined gastrointestinal function, nutrient absorption, mineral balance and metabolic function following oral administration of cellulose. Frey *et al.* (1928) found increased bowel movements as the only noted effect on gastrointestinal function following

incorporation of cellulose into the diets of children. In a study examining nutrient absorption, Kasper *et al.* (1979) observed an increase in vitamin A absorption in meals that incorporated MCC. Mineral balance has also been examined following incorporation of cellulose into the diet. Behall *et al.* (1987) found no change in calcium, magnesium, manganese, iron, copper or zinc when cellulose was added to the diet, while Godara *et al.* (1981) found a slight reduction in calcium, phosphorous and iron levels with cellulose addition. Examinations of metabolic function have reported few effects from oral administrations of cellulose. Behall *et al.* (1984) found a diet supplemented with cellulose fibers did not affect serum cholesterol, triglyceride or free fatty acid levels, although an increase in LDL cholesterol was observed. A similar study by Hillman *et al.* (1985) found no effect from cellulose in the diet on total serum cholesterol, triglycerides or HDL cholesterol. Two studies cited in the 1973 cellulose SCOGS document administered MCC to adults and found no pathological changes in the blood or urine samples. In one study, humans were fed 30 g of MCC as dry flour or gel for 5 weeks; no significant changes in the gastrointestinal tract were observed during the administration period (Tusing, 1964 in JECFA, 1998). The other study did not cite the dose but involved administering MCC for over two weeks to eight humans (Asahi Chemical Company, 1966 in SCOGS, 1973). These results suggest that cellulose is well tolerated in the diets of both men and women with few negative effects.

6.2.4.2. Absorption, Distribution, Metabolism, Excretion

There is no evidence to date to suggest that fibrillated cellulose in food will be absorbed, distributed, metabolized or excreted any differently than conventional cellulose fiber. Cellulose is a major constituent of dietary fiber (along with lignin and hemicellulose), which cannot be broken down by human digestive enzymes. The large molecular size of undigested cellulose and physico-chemical characteristics (*e.g*., lack of lipophilicity) prevent its absorption across the digestive epithelium. Therefore, ingested cellulose is excreted from the body undigested.

In the notifier's OECD TG 408 90-day subchronic study, there were no observations of irritation of the gastrointestinal tract, indicating that there was little interaction nor absorption across the GI tract (Section 6.2.2). The physical and chemical properties of fibrillated cellulose did not change after simulated gastrointestinal and lysosomal digestion, further indicating that it remains an insoluble fiber, and that there is little digestion or breakdown of cellulose, and therefore not likely metabolized, similar to conventional celluloses. Finally, the integrity of an *in vitro* intestinal epithelium was not affected after exposure to fibrillated cellulose, as demonstrated by the notifier using transepithelial electrical resistance (TEER) (Section 6.2.3).

Fibrillated celluloses. Fibrillated celluloses share the same molecular structure as conventional celluloses; which are insoluble fibers that are unlikely to be bioavailable. Humans lack digestive enzymes that can break down insoluble fibers, and therefore, similar to conventional cellulose, fibrillated cellulose would not be metabolized in the mammalian gut, and therefore would be excreted in the feces.

Other celluloses. Studies examining oral administration of conventional crystalline cellulose have documented little or no absorption and metabolism of these materials, indicating the majority of ingested cellulose is excreted in the feces. For example, a study conducted in rats

found no evidence of cellulose degradation or digestion when subjects were fed a diet containing 10 or 20% 14C-labelled MCC. Examination of fecal radioactivity found recovery rates ranging from 96-104% with no radioactivity noted in the urine (JECFA, 1998). Kotkoskie *et al.* (1996) noted a lack of tissue accumulation of MCC particles and also no qualitative evidence of intestinal uptake of MCC in rats that were orally gavaged with 500, 2500, and 5000 mg/kg/day for 90 consecutive days. Absorption, distribution, metabolism, and excretion (ADME) studies in humans found complete recovery of administered MCC-radioactivity (98.9±3%) from the feces in 2 days with no noted radioactivity in the urine or expired CO_2 (JECFA, 1998), indicating no metabolism of the MCC nor absorption or distribution to other parts of the body.

Collectively, the studies in the literature on conventional cellulose and the notifier's studies on both conventional and fibrillar cellulose demonstrate excretion as the only pathway following ingestion, no absorption, distribution or metabolism was found.

Table 0-3 Summary of published studies examining the safety of cellulose, fibrillated celluloses, and crystalline celluloses.

	Fibrillated cellulose	Other celluloses
Acute and Sub-acute Toxicity Studies	(1) Mice orally gavaged with a one-time dose of 300 mg/kg body fibrillated cellulose. No mortality nor acute adverse effects were observed, such as dyspnea, vomiting, diarrhea, and constipation (Shimotoyodome *et al*. 2011). (2) No deaths nor other clinical signs of toxicity were noted in rats treated by gavage or intraperitoneal injection of 2000 mg/kg bw bacterial cellulose (Pinto *et al.* 2016).	(1) Observations of BW, appearance, behavior, or gross pathology found no difference between treated and untreated rats in oral exposures to alpha-cellulose; LD_{50} > 3160 mg/kg (Pallotta 1959 in JECFA 1998). (2) Oral gavage of 2000 mg/kg cellulose resulted in no mortality nor toxicologic lesions (Schmitt et al. 1991). (3) Observations of BW, appearance, behavior, or gross pathology found no difference between treated and untreated animals in oral exposures to 85% MCC; LD_{50} > 5000 mg/kg (Freeman 1991a in JECFA 1998).
Subchronic and Chronic Toxicity Studies	(1) Mice were fed diets containing 7%, 14%, or 21% cellulose fibrils. After 30 days, the researchers conducted biological, biochemical, and histological tests and found no evidence of toxicity (Andrade et al. 2014). (2) Rats gavaged twice weekly for five weeks with 1% w/w suspensions of fibrillated cellulose in both water and cream did not undergo any significant changes in weight, organ function or blood chemistry in comparison to rats fed control diets (DeLoid *et al.* 2019) (3) A mixture of 60% fermentation-derived fibrillated cellulose produced from *A. aceti*/20% Na-CMC/20% sucrose was administered to rats for 28 days at dietary levels ranging from 0-5%. No adverse effects were observed; necropsy showed slight increase in cecum weights. No-observed-adverse-effects level (NOAEL) is 5% in the diet (5,331 mg/kg bw per day) (Hagiwara *et al.* 2010).	(1) 100 rats were fed diets of 30% dry, gel, or fibrous forms of cellulose for 72 weeks with no effects on appearance, behavior, food consumption, or survival (Paynter 1963 in SCOGS 1973) (2) No mortality, diarrhea or abnormal feces were observed in African Green Monkeys fed a 9.71% diet of cellulose for 3.5 years (Paulini et al., 1987); (3) In a 52-day study, rats were fed diets supplemented with 5% or 10% cellulose, and no mortality was observed (Yoshioka, Shimomura, and Suzuki, 1994) (4) No mortality was observed in rats fed a 21-day diet consisting of 5-20% MCC (Sundaravalli *et al.* 1971). (5) Diet containing 5, 10 or 20% MCC over 4 week period did not report any toxicity in rats (Hove and King, 1979) (6) RTECS lists the lowest published 'toxic' dose of cellulose to be 420 g/kg exposure for 4 weeks with continuous feeding (RTECS #FJ5691460) (7) Daily oral gavage of MCC for 90 days in rats reported no adverse effects. NOEL > 5000 mg/kg/day (Kotkoskie *et al* 1996). (8) No evidence of toxicity when rats were fed diets containing 0%, 5%, or 10% Cellulon fiber or MCC for approximately 13 weeks (Schmitt et al., 1991). (9) MCC added as dietary food supplement (7.5%) for 17 weeks; no toxic effect in rats (Paturi *et al.* 2010)

		(10) 30 g/day of MCC fed to humans in a free-choice diet for 5 weeks resulted in no gastrointestinal changes (Tusing *et al.* 1964, in JECFA, 1998).
Genotoxicity Studies	(1) Highest tolerated dose >2000 mg/L in bacterial Ames test (OECD 471); no mutagenicity for fibrillated cellulose (Pitkänen *et al.* 2010). (2) Neither gavage nor intraperitoneal injection of 2000 mg/kg bw bacterial cellulose in mice caused any significant effects, as measured by the *in vivo* bone marrow micronucleus assay (Pinto *et al.* 2016).	(1) Eight individual genotoxicity studies reported in JECFA (1998) did not find any adverse effects of MCC, even at 5000 mg/kg bw. (2) Exposure to Cellulon in the Ames assay (2500 µg/plate), an assay for chromosomal aberrations in Chinese hamster ovary (CHO) cells (8000 µg/mL), an assay for induction of unscheduled DNA synthesis in rat primary hepatocytes (5010 µg/mL), and the CHO/HGPRT assay (5.0 mg/mL), no genotoxic effects were observed (Schmitt et al., 1991).
Carcinogenicity Studies	No data	(1) In studies examining chronic ingestion of cellulose (*i.e.*, diets containing 1-5%) in animal feed over the lifespan of mice and rats, there were no reports of increased spontaneous disease or neoplasia (McCollister *et al.*, 1973; Maurer *et al.*, 1990; Anastasia *et al.*, 1990; Medinsky *et al*., 1982 in JECFA, 1998). (2) High oral exposures (*i.e.*, 30% of the diet for 72 weeks) to cellulose did not result in any changes in tumor incidence (Hazleton Labs, 1963 in JECFA, 1998). (3) High cellulose diets (4.5 or 9% Avicel, MCC) reduced the incidence of induced colonic neoplasia and number of colonic tumors compared to calorically equivalent cellulose free diet (Freeman *et al.* 1978, 1980).
Teratogenicity/ Reproductive Toxicity Studies	(1) Inclusion of 3% cellulose (Arbocel FD 00, cellulose fiber) in the diet improved the rate of egg production and fertility in hens (Mohiti-Asli *et al.* 2012).	(1) No teratogenic effects in pregnant rats fed 25 or 50 g/kg bw MCC (Avicel RCN-15, mix of 85% MCC and 15% guar gum) during gestation; NOEL > 50 g/kg (Freeman 1992b in JECFA 1998). (2) No effects on fetuses, change of sex ratio in pups, or eye defects when pregnant rats fed 25 or 50 g/kg bw MCC (Avicel CL-611, mix of 85% MCC and 15% SCM) (Freeman 1994b in JECFA 1998) (3) Rats fed diet containing 30% MCC over 4 generations noted no teratogenic effects; caloric deprivation linked to decline in fertility, number and survivability of pups (Hazleton Labs 1964 in JECFA 1998).

6.2.4.3. Acute and sub-acute Toxicity

The available evidence indicates that fibrillated cellulose in food is not acutely toxic.

Fibrillated celluloses. In a study determining the ability of fibrillated celluloses to reduce post-prandial blood response after ingestion of dietary carbohydrates, mice were orally gavaged with a single dose of 300 mg/kg BW (Shimotoyodome *et al*., 2011). No mortality or acute adverse effects, such as dyspnea, vomiting, diarrhea and constipation, were observed. Fibrillated cellulose did not cause changes in blood concentrations of glucose, plasma insulin, glucose-dependent insulinotropic polypeptide or triglycerides (Shimotoyodome *et al*., 2011). No deaths or other clinical signs of toxicity were noted in rats treated by gavage or intraperitoneal injection of 2000 mg/kg bw bacterial cellulose (Pinto *et al.*, 2016), and no gross pathological abnormality was observed in major organs at necropsy.

Other celluloses. Studies examining the acute toxicity of cellulose have evaluated many routes of exposure including oral, intraperitoneal and dermal exposure. These studies describe effects of a single cellulose exposure or multiple cellulose exposures within a short time period (<24 hours). Single oral doses, administered by stomach tube, of a refined form of α-cellulose (Cellan 300) up to 3160 mg/kg rats produced no acute deaths, and no pathological effects in rats observed for 7 days following administration. Therefore, the acute oral LD_{50} was determined to be >3160 mg/kg (Palotta, 1959, in JECFA, 1998). In similar acute toxicity testing, rats were orally gavaged with a 2000 mg/kg dose of Cellulon fibers (bacterial cellulose) and then observed for toxic or pharmacotoxic effects: no deaths occurred, and no toxicologic lesions were observed during necropsy; some clinical effects (gasping respiration and hunched posture) were observed, but these were attributed to the dosing regimen (Schmitt *et al*., 1991).

Acute studies of oral exposure to crystalline celluloses have not demonstrated toxicity. Mice were fed 5000 mg/kg bw Avicel RCN-15 (85% MCC with 15% guar gum) mixed with parmesan cheese, and after a 14-day period, no gross lesions or acute effects were observed (LD_{50} > 5000 mg/kg) (Freeman 1991a in JECFA 1998).

6.2.4.4. Subchronic and Chronic Oral Toxicity

There is no evidence to date to suggest that fibrillated cellulose in food is toxic over sub-acute, subchronic, or chronic periods of time or after repeated daily intake.

Fibrillated Cellulose. A review of studies examining the subchronic (< 90 days), and chronic (>90 days) oral toxicity of cellulose revealed no indication of toxicity, even when cellulose contributed a relatively high percentage of the diet. Andrade *et al*. (2014) fed mice diets containing 7%, 14%, or 21% fibrillar cellulose (derived from peach palm residue). After 30 days, the researchers conducted biological, biochemical, and histological tests and found no evidence of toxicity. Rats gavaged twice weekly for five weeks with 1% w/w suspensions of fibrillar cellulose in both water and cream did not undergo any significant changes in weight, organ function or blood chemistry in comparison to rats fed control diets (DeLoid, 2019). A study examining the sub-acute oral toxicity of a mixture of 60% bacterial cellulose from *Acetobacter*

aceti, 20% Na-CMC, and 20% sucrose reported no effects. The researchers examined the effects of a 28-day exposure to this mixture in rats at dietary levels ranging from 0-5% (corresponding to 5331 mg/kg bw per day in males; 5230 mg/kg bw per day in females). No adverse clinical effects were noted, and no change in mortality, BW, food or water consumption were observed. Results from urinalysis, ophthalmology, hematology, blood chemistry and histopathology were all similar to controls with the exception of a slight increase in cecum weight in exposed animals, resulting in a no observable effects level (NOEL) of 5% in the diet for fermentation-derived fibrillated cellulose mixture (Hagiwara *et al.*, 2010).

Other celluloses. Other studies investigating the effects of added cellulose to the diet also indicate a lack of toxicity. In one study, groups of 100 rats were fed diets of 30% dry, gel, or fibrous forms of cellulose for 72 weeks with no effects on appearance, behavior, food consumption, or survival (Paynter 1963, in SCOGS, 1973). African Green Monkeys fed a diet of 9.71% cellulose for 3.5 years were healthy (Paulini *et al*., 1987); no mortality, diarrhea or abnormal feces were observed, and absorption of iron, zinc, and copper were within normal range. Some histopathological changes were observed in the intestine, and there were some necrotic cells observed in the lamina propria, but these did not cause any obvious deleterious effects. In a 52-day study, rats were fed diets supplemented with 5% or 10% cellulose, and no mortality was observed (Yoshioka, Shimomura, and Suzuki, 1994). A 21-day diet consisting of 5-20% MCC fed to rats resulted in no adverse toxic effects. In fact, inclusion of cellulose in the diet effectively reduced dietary caloric content, and the authors noted a decrease in body fat; cellulose was also observed to lower levels of plasma and liver cholesterol (Sundaravalli *et al.*, 1971). In a similar study, Hove and King (1979) examined how a casein diet with 2.5, 5, 10 or 20% MCC impacted BW gain and food consumption over a 4-week period in rats. No toxicity was observed at the exposure doses used, and the authors concluded MCC was an inert diet diluent (Hove and King, 1979). RTECS lists the lowest published 'toxic' dose of cellulose to be 420 g/kg exposure for 4 weeks with continuous feeding; this dose resulted in weight loss or decreased weight gain in rats (RTECS #FJ5691460). A study in which rats were orally gavaged with MCC at 500, 2500, and 5000 mg/kg/day for 90 consecutive days did not result in any adverse outcomes (Kotkoskie *et al*., 1996). The researchers did not find any effects on BW gain, food consumption, ophthalmoscopic examinations, clinical chemistry measurements, hematology, absolute or relative organ weight, and no histopathological lesions or inflammation were observed. Another study examining oral toxicity of Cellulon (bacterial cellulose) in rats found no evidence of toxicity when rats were fed diets containing 0%, 5%, or 10% Cellulon fiber or MCC for approximately 13 weeks (Schmitt *et al*., 1991). The authors found no effect on survival or bodyweight, no pathologic findings at necropsy and no dose-dependent effects on hematology or blood chemistry results. Indirect evidence, in which cellulose is added as a dietary food supplement to animal feed (4-50%), also reveals no major effects in rats (Juskiewicz *et al*., 2004; Mallett *et al*., 1983; Paturi *et al.*, 2010) or sheep (Oltjen *et al*., 1962). Finally, in a study on mice examining subcutaneous implants of bacterial cellulose secreted by *Gluconacetobacter xylinus*, researchers found that implants elicited a mild inflammatory response but no foreign body reaction; no differences in BW between animals with implants and animals without were observed (Pertile *et al*., 2011).

6.2.4.5. Genotoxicity

There is no evidence to suggest that fibrillated cellulose is genotoxic.

Fibrillated cellulose. Pitkänen *et al.* (2010) evaluated the genotoxicity of fibrillated cellulose in the bacterial reverse mutation assay (Ames test; OECD TG 471) and found no genotoxicity at the highest dose examined (2000 mg/L). Neither gavage nor intraperitoneal injection of 2000 mg/kg bw bacterial cellulose in mice caused any significant genotoxic effects, as measured by the *in vivo* bone marrow micronucleus assay (Pinto *et al*., 2016).

Other celluloses. Eight studies in the 1998 JECFA report investigated the genotoxic potential of commercially available forms of MCC (Avicel RCN-15, Avicel AC-815, Avicel CL-611, Avicel RCN-15). The studies included the bacterial reverse mutation assay, the mouse lymphoma forward mutation assay, and the mammalian cell micronucleus test. All genotoxicity assays were negative after cellulose exposures even at the highest doses examined (*i.e*., 5000 mg kg^{-1} bw for mammalian cell micronucleus test) (Batt, 1992; Lawlor, 1996; Cifone, 1992; Cifone, 1994; McKeon, 1992; Murli, 1992; Murli, 1994a; Murli, 1994b in JECFA, 1998). In another study on a commercially available bacterial cellulose (Cellulon), four genotoxicity assays, the Ames assay (2500 µg/plate), an assay for chromosomal aberrations in Chinese hamster ovary (CHO) cells (8000 µg/mL), an assay for induction of unscheduled DNA synthesis in rat primary hepatocytes (5010 µg/mL), and the CHO/HGPRT assay (5.0 mg/mL) found no genotoxic effects; results held across a range of doses tested (Schmitt *et al*., 1991).

6.2.4.6. Carcinogenicity

Other celluloses. Several repeat dose ingestion studies demonstrate a lack of carcinogenic activity for cellulose materials (Anderson *et al.*, 1992). In studies examining chronic ingestion of cellulose (*i.e.*, diets containing 1-5%) in animal feed over the lifespan of mice and rats, there were no reports of increased spontaneous disease or neoplasia (McCollister *et al.*, 1973; Maurer *et al.*, 1990; Anastasia *et al.*, 1990; Medinsky *et al*., 1982 in JECFA, 1998). Even high oral exposures (*i.e.*, 30% of the diet for 72 weeks) to cellulose did not result in any changes in tumor incidence (Hazleton Labs, 1963 in JECFA, 1998). In studies examining the incidence of tumors in the colon, mammary gland and bladder, chronic ingestion of cellulose (*i.e.*, diets containing 4.5-46%) did not display any tumor promoting activities despite high oral doses (Nigro *et al*., 1979; Freeman, 1982; Kritchevsky *et al.*, 1984; Anderson, 1989). Further evidence comes from studies examining the effect of purified cellulose on induced rat colonic neoplasia. Here, high cellulose diets (4.5 or 9%) reduced the incidence of colonic neoplasia and number of colonic tumors after parenteral administration of 1,2-dimethylhydrazine dihydrochloride compared to a nutritional and calorically equivalent cellulose-free diet (Freeman *et al.*, 1978, 1980). Together, these studies demonstrate there is no evidence of carcinogenicity for cellulose.

6.2.4.7. Teratogenicity/Reproduction Toxicity

Fibrillated cellulose. Most studies of the reproductive toxicity and teratogenicity of cellulose have reported beneficial effects, rather than adverse effects. In studies examining cellulose in the diet, the inclusion of cellulose may improve fertility. For example, Mohiti-Asli *et al.* (2012) found that the inclusion of 3% cellulose (Arbocel FD 00, a cellulose fiber) in the diet improved

the rate of egg production and fertility in hens. Ogonowshi *et al.* (2018) also found that there was a stimulatory effect on the reproduction cycles of *Daphnia magna* at intermediate concentration of fibrillated cellulose (2.06 mg/L) in a filtered tank that mimicked an environmentally relevant 'worst case scenario' for aquatic exposure. While negative effects on reproduction were observed during chronic exposure to very high fibrillated cellulose concentration (> 20 mg/L) this was most likely related to the caloric restriction driven by the low algae:cellulose ratios in the tank (Ogonowshi, 2018).

Other celluloses. Reports examining the embryonic toxicity of crystalline cellulose materials have found no evidence of any effects on fetuses. For example, several studies fed pregnant rats MCC at a concentration of 25 or 50 g/kg bw during gestation. In one study rats were exposed to Avicel RCN-15 (a mix of 85% MCC and 15% guar gum) for 10 days consecutively during gestation at either 2.1 or 4.5 g/kg bw per day). There were no teratogenic effects, demonstrated by lack of effects on the number and distribution of implantation sites, early and late resorptions or live and dead fetuses; furthermore, no effects on the corpora lutea and no external, visceral and skeletal fetal effects were observed (Freeman, 1992b in JECFA, 1998). In a similar study, rats were administered Avicel CL-611 (a mix of 85% MCC and 15% SCM) equal to 2.2 and 4.6 g kg^{-1} bw per day from day 6 to 16 of gestation. No adverse effects were seen in the number and distribution of implantation sites, early and late resorptions or live and dead fetuses; the corpora lutea was not affected, no external, visceral, skeletal or ocular fetal effects were noted, and there was no evidence of a change in pup sex ratio (Freeman, 1994b in JECFA, 1998). In one of the few studies noting an adverse effect of cellulose in the diet, four generations of rats were fed diets of 30% MCC flour or gel. While no teratological deformities were observed, the authors noted declines in fertility and in the number and survivability of live pups. However, these effects were attributed to caloric deprivation (30% of the diet was MCC, a non-nutritious material that contributes no calories) rather than to a direct toxic effect of MCC (Hazleton Labs, 1964 in JECFA, 1998). It should be noted that a continual diet of 30% MCC is far higher than would be expected in any realistic diet (equivalent to 300 000 mg/kg).

6.2.4.8. Other relevant studies

Other simulated digestion and *in vitro* studies provide supporting data that digested fibrillated cellulose does not result in adverse reactions. DeLoid et al. (2019) employed a gastrointestinal tract simulator to digest fibrillated cellulose (0.75% and 1.5% w/w), then exposed these digested fibrillated cellulose to a triculture model of the intestinal epithelium. After exposure, there were no significant effects on cytotoxicity, reactive oxygen species, or monolayer integrity. Addition of 1% fibrillated cellulose to high fat food appeared to reduce fat digestion and absorption in the small intestine, supported by results demonstrating a temporary decrease in serum triglycerides in a supporting *in vivo* gavage study (DeLoid et al. 2018). Another study demonstrated that 0.5% addition of fibrillated cellulose could bind glucose, delay glucose diffusion, delay amylolysis and inhibit starch *in vitro* digestion (Liu et al. 2018). It is known that different types of fiber influence parameters such as lipid uptake, cholesterol levels, glycemic response, insulin sensitivity, etc. (Isken et al. 2010, Oda et al. 1993, Wolever et al. 1990). In other simulated digestion studies, derivatives of cellulose such as methyl cellulose have been demonstrated to also reduce lipid digestion (Espinal-Ruiz 2014).

Publicly available information on the potential effects of fibrillated cellulose ingestion on the microbiome were reviewed to assess any potential effects. Cellulose, an insoluble dietary fiber, moves through the gut quickly, reducing the amount of time available for colonic bacterial fermentation of non-digested foodstuff. Cellulose is resistant to degradation in the human gut due to a lack of enzymes specialized in breakdown of celluloses (Chassaing et al. 2017, Holscher 2017). Since fibrillated cellulose has the same molecular structure as currently authorized celluloses, it will be similarly resistant to breakdown in the gut. In addition, the notifier's simulated digestion studies demonstrate that fibrillated cellulose is resistant to breakdown under digestive conditions (see Section 6.2.3).

Khare et al. (2020) gavaged rats with 1% fibrillated cellulose biweekly for five weeks. Fibrillated cellulose did not have substantial effects on the fecal metabolome, but did alter microbial diversity in the gut and diminish some species that produce short chain fatty acids. Some effects were seen on epithelial cell junction gene expression and increased production of cytokines. However, due to the lack of associated pathological effects, the authors concluded that these perturbations likely represent minor effects. It is well established the dietary fibers (and other dietary components) will alter microbiota in the gut by affecting bacterial fermentation, colony size, and species composition (*e.g.* Sawicki *et al.* 2017, Holscher 2017, O'Grady *et al*. 2018, Patnode *et al.* 2019). Consuming a diet of a variety of different dietary fibers (*e.g.* cellulose, pectins, gums, fructans, etc.) is considered more supportive of a varied gastrointestinal microbial community compared to a refined diet (Holscher 2017). There are no indications in the literature that replacing conventional cellulose with fibrillated cellulose at levels similar to those in use now will result in any adverse effects related to the microbiome.

6.3. Basis for Conclusion that Fibrillated Cellulose is Generally Recognized as Safe

In summary, the intended uses of fibrillated cellulose have been determined safe through scientific procedures, thereby satisfying the technical element of the GRAS determination. Fibrillated cellulose is molecularly and chemically identical to celluloses already authorized and used in food for many decades. The notifier's *in vitro*, genotoxicity, and subchronic 90-day studies demonstrate that exposure to fibrillated cellulose at 17-20 times the predicted EDI does not result in any adverse effects, resulting in similar biological outcomes as conventional celluloses. The publicly available scientific data on fibrillated cellulose, conventional cellulose, and related cellulose forms support these conclusions, demonstrating that short and long-term consumption of celluloses do not result in any significant adverse effects in mammals.

The basis for conclusion that fibrillated cellulose is GRAS is based on the following:

1. Cellulose is the most abundant natural biopolymer on earth, providing structure and strength to cell walls of plants and provides natural fiber in the human diet. Fibrillated cellulose is produced by mechanically freeing cellulose fibrils from wood or plants and has the same molecular and chemical structure as celluloses that already authorized for use.

The CAS for cellulose (9004-34-6) or cellulose pulp (65996-61-4) is used for fibrillated cellulose.

2. Fibrillated cellulose is proposed for use as a partial replacement for current available celluloses in diverse types of foods. A highly conservative calculation of estimated daily intake is 10.8 g/day, which is comparable to previous calculations performed for microcrystalline cellulose, where heavy consumer intake of MCC (90th percentile) ranges from 5.4 to 10.2 g/person per day (CanTox Inc. 1993 in JECFA 1998). All calculations were based on the most conservative assumptions, where fibrillated cellulose has 100% market penetration and is used at the highest predicted concentrations across an entire food category, resulting in overestimates in consumption and food uses that will include fibrillated cellulose.
3. There is a long history of safe use of diverse forms of cellulose and its various derivatives as food additives, in food contact materials, as part of animal feed and as part of pharmaceuticals and cosmetics. The U.S. Food and Drug Administration Select Committee on GRAS Substances (SCOGS) Committee concluded that there is no evidence that cellulose demonstrates a hazard to the public when used in the manner now practiced, and granted GRAS status to many forms of cellulose for a variety of applications.
4. Cellulose and its various derivatives, including structurally similar fiber-like bacterial celluloses, are authorized for many food-use purposes globally, including in the United States, the European Union and Canada. Cellulose, and at least seven different cellulose derivatives, along with bacterial cellulose, have GRAS status in the U.S. Cellulose and several cellulose derivatives are authorized to be used *quantum satis* in the E.U. In Canada, cellulose and several derivatives are permitted in foods, in some cases up to 50% of the total product.
5. The Joint FAO/WHO Committee on Food Additives (JECFA) international expert committee assigned an acceptable daily intake of celluloses as “not specified,” which is assigned to substances of very low toxicity (JECFA, 1975).
6. The notifier performed an OECD 408 repeated dose 90-day oral toxicity study in rats fed diets of up to 4% fibrillated cellulose. There were no adverse effects, including effects on survival, clinical observations, body weight, food consumption, ophthalmologic evaluations, hematology, serum chemistry, urinalysis, pathology, and histopathology. The no-observed-adverse-effect level (NOAEL) for fibrillated cellulose was 2194.2 mg/kg/day (males) and 2666.6 mg/kg/day (females), corresponding to the highest dose tested of 4% in the diet. Concentrations used in other subchronic or chronic studies ranged up to 14% fibrillated cellulose and 30% conventional celluloses in rodent and mammalian studies. The actual consumption of fibrillated cellulose is likely to be much lower than the calculated EDI value of 132 mg/kg bw/day, because of conservative assumptions in the calculations. Regardless, the EDI is significantly lower than the measured NOAELs, with a margin of exposure (MoE) is more than 17-20 times the intake levels at maximum use.
7. Fibrillated cellulose is demonstrated to behave similarly to conventional cellulose in the OECD TG 408 study, including lack of GI tract irritation, indicative of lack of interaction and uptake in the digestive tract. Fibrillated celluloses demonstrate a similar lack of adverse biological effects in the Notifier’ read-across *in vitro* toxicological studies demonstrating no effect on the integrity of the *in vitro* intestinal epithelium.

8. The read-across testing demonstrates the similar physical, chemical and biological aspects of the six Notified forms of fibrillated cellulose, as well as the fibrillated and conventional cellulose studied in the OECD TG 408 study. These materials have identical molecular structures and a similar lack of adverse biological effects in *in vitro* toxicological studies, providing substantive evidence to support their grouping. Results from subchronic oral toxicity testing in rats demonstrating a lack of adverse effect from fibrillated cellulose up to 4% of the diet can be read-across to all forms of fibrillated cellulose that are the subject of this Notice.
9. Publicly available, published animal toxicity studies for celluloses and different forms of celluloses exhibit sufficient support that there are no indications that acute, subchronic or chronic consumption results in any toxic effects, even at doses as high as 30% diet.
10. Three studies demonstrate a lack of genotoxicity, including an OECD 487 *in vitro* micronucleus test obtained by the notifier demonstrating no toxicity at an exposure to 2500 mg/L fibrillated cellulose, and two publicly available studies, an OECD 471 Ames test (Pitkänen et al. 2010) and an *in vivo* bone marrow micronucleus assay (Pinto et al. 2010).
11. There is no evidence from published studies that there will be any other negative effects, including carcinogenic, teratogenic or other effects, from exposure to cellulose or fibrillated celluloses.
12. The fibrillated cellulose is manufactured using a process that qualifies it for 'food grade,' under current Good Manufacturing Practices.
13. Based on independent, critical evaluation of all the available information presented in this dossier, the Expert Review Panel concludes that the intended uses of fibrillated cellulose, meeting food-grade specifications, for use in the applications stated in this Notice, is safe and qualifies for GRAS status. The Expert Review Panel Statement can be found in Attachment 3.

6.4. Supplemental information on safety by non-oral exposure

6.4.1.Eye Irritation Studies

Several studies examining the potential for cellulosic materials to be skin or eye irritants have concluded it to be only minimally or non-irritating, the least harmful categorization of irritant.

Fibrillated cellulose. No data on fibrillated celluloses are available for eye irritation studies.

Other cellulosics. Instillation of cellulose (Avicel RNC-15 or AC-815) into the eyes of New Zealand White Rabbits was reported to be minimally irritating in a series of unpublished reports (Freeman 1991c, 1996a; JECFA, 1998).

6.4.2. Dermal Irritation Studies

Fibrillated cellulose. Napavichayanun *et al.* (2016) found that bacterial cellulose wound dressings (impregnated with an antibacterial and a silk water-soluble protein) were a safe and efficient wound dressing material. A clinical trial patch tests was performed on the normal skin of human volunteers, and was found to not irritate the skin of any volunteers (as characterized by normal levels of erythema and melanin, and the absence of edema, papule, vesicle, and bullae).

Other cellulosics. Examination of 4 hours of occlusive dermal contact (where skin is covered directly or indirectly by impermeable films or substances) in New Zealand White rabbits found cellulose (Avicel RNC-15 or AC-815) to be non-irritating to the skin (Freeman 1991d, 1996b in JECFA 1998).

6.4.3. Dermal Sensitization Studies

Other cellulosics. Although there is a paucity of data, completed studies suggest that cellulose exposure does not result in dermal sensitization. One of the few studies available determined that topical exposure of MCC (Avicel RCN-15 and Avicel AC-815) to Hartley guinea-pigs was non-sensitizing (JECFA, 1998).

PART 7. LIST OF SUPPORTING DATA AND INFORMATION

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GRAS Notice 607. Documentation Supporting the Evaluation of Glucosylated Steviol Glycosides (GSG) as Generally Recognized as Safe (GRAS) for Use as a Flavoring Agent**.**

GRAS Notice 609. Rice Protein Concentrate.

GRAS Notice 613. Self-determined Generally Recognized As Safe Assessment of AppleActive DAPP™/Leahy DAPP™.

GRAS Notice 628. Endo-1,4-beta-xylanase from *Trichoderma reesei.*

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7.2 Acronyms

ADI	Acceptable Daily Intake
ADME	Absorption, Distribution, Metabolism, and Excretion
ALF	Artificial Lung Fluid
BW	Body Weight
CA	Cellulose Acetate
CAB	Cellulose Acetate Butyrate
Ca-CMC	Calcium Carboxymethyl Cellulose
CAP	Cellulose Acetate Propionate
CAPC	Cellulose Acetate Propionate Carboxylate
CAS	Chemical Abstract Number
CC	Conventional Cellulose
CFR	Code of Federal Regulations
CG	Cellulose Gum
CHEC	Cetyl Hydroxyethyl Cellulose
CHO/HGPRT	Chinese Hamster Ovary/ hypoxanthine-guanine phosphoribosyl-transferase
CIR	Cosmetic Ingredient Review
CMC	Carboxymethyl Cellulose
CMCAB	Carboxymethyl Cellulose Acetate Butyrate
CMHEC	Carboxymethyl Hydroxyethyl Cellulose
CoA	Certificate of Analysis
CS	Cellulose Succinate
DI	Dispersity Indices
DLS	Dynamic Light Scattering
EC	Ethyl Cellulose
EDI	Estimated daily intake
EFSA	European Food Safety Authority
ELISA	Enzyme-Linked Immunosorbent Assay
EMC	Ethyl Methyl Cellulose
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
FC	Fibrillated Cellulose
FCC	Food Chemicals Codex
FCN	Food Contact Notification
FDA	Food and Drug Administration
GI	Gastrointestinal
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice
GRAS	Generally Recognized as Safe
GRN	GRAS Notice
HBMC	Hydroxybutyl Methyl Cellulose
HDD	Hydrodynamic Diameter
HEMC	Hydroxyethyl Methyl Cellulose

HEC	Hydroxyethyl Cellulose
HEEC	Hydroxyethyl Ethyl Cellulose
HPC	Hydroxypropyl Cellulose
HPMC	Hydroxypropyl Methyl Cellulose
HPMCA/S	Hydroxypropyl Methyl Cellulose Acetate/Succinate
ICP-MS	Inductively Coupled Plasma-Mass Spectrometry Analysis
IL-6	Interleukin 6
JECFA	Joint FAO/WHO Expert Committee on Food Additives
K-CS	Potassium Cellulose Succinate
LD_{50}	Lethal Dose at 50% of tests system mortality
LPS	Lipopolysaccharides
MC	Methyl Cellulose
MCC	Microcrystalline Cellulose
MDDC	Monocyte Derived Dendritic Cells
MEC	Methylethylcellulose
MFC	Microfibrillated Cellulose
MoE	Margin of Exposure
Na-CMC	Sodium Carboxymethyl Cellulose
Na-CS	Sodium Cellulose Sulfate
NIOSH	National Institute for Occupational Safety and Health
No.	Number
NOAEL	No Observable Adverse Effects Level
NOEL	No Observable Effects Level
OECD	Organization for Economic Cooperation and Development
REF CC	Reference Conventional Cellulose
REF FC	Reference Fibrillated Cellulose
RTECS	Registry of Toxic Effects of Chemical Substances
SCF	Scientific Committee on Food
SCOGS	Select Committee on GRAS Substances
SEM	Scanning Electron Microscopy
SNAc	Significant New Activity Notice
TEER	Transepithelial Electrical Resistance
TG	Test Guideline
USDA	U.S. Department of Agriculture
WHO	World Health Organization

Expert Panel Opinion Regarding the Generally Recognized as Safe (GRAS) Status of Fibrillated Cellulose

Background

Vireo Advisors, LLC (Vireo), as the agent for The Alliance for Food Safety Acceptance of Fibrilllate and Crystalline Celluloses (the Notifier) of the GRAS Notice for Fibrillated Cellulose, commissioned an independent panel of experts (GRAS Expert Panel) including: Mitchell Cheeseman, Ph.D., Steptoe and Johnson, LLP and Richard C. Pleus, Ph.D., Intertox, Inc., qualified by their scientific training and national and international experience, to determine whether: (1) there is sufficient information available to support the safety of Fibrillated Cellulose (FC) in food additives; and (2) there is basis to conclude that this technical evidence of safety is generally known and accepted by qualified experts.

To assist the Panel in its review, Vireo provided a comprehensive package (GRAS Notice for FC and attachments) with detailed information about the intended uses and use levels, manufacturing, specifications, and analytical data, along with a summary of data supporting the safety of FC. The Panel, independently and collectively, critically examined the comprehensive package and supporting literature, and evaluated other information deemed appropriate or necessary.

Following their independent and collaborative critical evaluation of the data and information, the Panel convened *via* teleconference on December 5, 2019. The Panel reviewed their findings and following discussion, unanimously concluded that the intended uses and concentrations proposed for food-grade FC are Generally Recognized as Safe (GRAS) based on scientific procedures.

Expert Panel Review

In nature, cellulose does not occur as isolated individual molecules; rather, cellulose chains are assembled into a hierarchy of cellulose fibers. The six notified materials of the GRAS dossier are each a form of FC, produced by six separate manufacturers that use similar approaches for producing FC.

The use of FCs in various applications include as a food additive (0.1-5%), and as protective produce coatings (0.05-100%). Because FC products will effectively substitute for traditional microcrystalline or cellulose substances as a food additive, no major increase in global consumption of cellulose is anticipated. The maximum estimated daily intake (EDI) was calculated to be 133 mg/kg bw/day. Calculation of the EDI is accomplished by multiplying the maximum proposed use level (%) by reported food intake (g/person/day), for each food group. This approach is based on *FDA's Guidance for Industry: Estimating Dietary Intake of Substance in Food* (FDA 2006) using daily food intake data (g/day) from Food Consumption Surveys and data from previously submitted GRAS petitions that were FDA reviewed and received 'no further questions' responses. The calculated EDI is expected to be an overestimate, as the Notifier used conservative estimates for whole food groups rather than specific types of food, used the maximum estimates of daily consumption, and assumed 100% market penetration of FC in all proposed food categories.

The Notifier conducted a 90-day subchronic dietary feeding study to form the basis for the conclusion that FC is GRAS based on scientific procedures. The subchronic 90-day study was deemed to have been responsibly designed, performed at a reputable lab, and conducted according to Good Laboratory Practices (GLP). The study followed the OECD Test Guideline 408 Repeated Dose 90-Day Oral Toxicity Study in Rodents test guideline (OECD 1998) as well as US FDA Toxicological Principles for the Safety Assessment of Food Ingredients, Redbook 2000, IV.C. 4. a. (FDA 2007). The study used a preferred and commonly used species, CRL Sprague Dawley IGS Rats, with at least 10 males and 10 females in each of the dose groups, and paired conventional cellulose control groups for comparison. The dietary route of administration represents the route of human exposure, and doses were selected following recommendations in OECD documents for the upper limit of doses to be used in rodent studies (*e.g.,* OECD 425), in line with many other studies on fibrillated and conventional forms of cellulose, and at a dose rate approximately 16-20 times higher than the conservatively calculated EDI. 4% was chosen as the upper dose level to target approximate dietary intakes up to 2500 mg/kg/day based upon a food consumption rate of 25 g for a 400 g rat. Solka Floc was used as a conventional cellulose control because it is already authorized use in food. Dietary fiber such as conventional cellulose can have a non-adverse but significant effect on some physiological and morphological parameters. Therefore, control groups with paired fiber dose levels were used to ensure that any differences observed between groups could be ascribed to either a difference in fiber levels, or to FC. The stability, homogeneity, and concentrations of the FCs and conventional cellulose were all appropriately verified, and storage conditions were adequate.

All OECD TG 408 recommended endpoints (OECD 1998) were measured, including daily clinical observations for viability and cage-side observations including visual and behavioral observations, ophthalmological observations at the beginning and end of the study, weekly body weight and body weight gains, including food consumption, food efficiency, and dietary intake of FC and conventional cellulose. There were no mortalities or negative changes in any of these parameters attributable to the administration of FC. At the termination of the study, gross necropsy was performed and eleven different organs (adrenals, brain, epididymides, heart, kidneys, liver, ovaries with oviducts, spleen, testes, thymus, and uterus) were weighed to determine absolute and organ to body/brain weight ratios, and 53 other organs were collected and preserved for histopathological examination. No significant differences were noted that indicate adverse gross pathology. Histopathological examination performed on the preserved organs and tissues of the FC and control cellulose high dose groups found no toxicologically significant findings. At terminal sacrifice, all recommended clinical pathology parameters, including hematology, coagulation, clinical chemistry, and urinalysis were measured, and no statistically significant changes were observed at any of the dose levels. It was established that the study design and initiation pre-dated a newly adopted OECD TG 408 (25 June 2018), which was updated to add endocrine-sensitive endpoints. The Notifier's pilot study was initiated in April 2018. However, there are no indications from any publicly available studies that exposure to dietary celluloses results in any negative endocrine effects that would warrant measurement (*e.g.* EFSA ANS Panel 2018, Behall 1984).

The Notifier also performed a series of *in vitro* experiments to provide a basis for read-across to conventional cellulose, to demonstrate similarity amongst the notified forms, and to further assess any potential effects on the gastrointestinal tract. Conventional cellulose, the non-commercial form of FC tested in the 90-day study, and the six notified FC materials were subjected to *in vitro* gastrointestinal and lysosomal digestion, then were characterized to allow comparison of their physical and chemical properties. The pristine and digested cellulose materials were also assessed for a number of toxicological endpoints including cytotoxicity, barrier integrity, oxidative stress, and inflammation in a gastrointestinal (GI) tri-culture model. FCs and conventional cellulose remained physically and chemically similar post-digestion, retaining fibrillar morphologies, with no significant changes in hydrodynamic diameter (HDD), dispersity index (DI), or zeta potential compared to pristine forms for both simulated gastrointestinal and lysosomal digestion. Results demonstrate no significant increase in cytotoxicity (up to 48 hours post-exposure), no decrease in the integrity of the intestinal epithelium (up to 8 days post-exposure), no induction of oxidative stress (up to 4 hours post exposure), and no significant increase in the pro-inflammatory marker IL-6 (up to 48 hours post exposure) in the intestinal tri-culture following material exposure (pristine and digested forms). The *in vitro* studies demonstrate the similar biological behavior of the notified FCs to the forms in the subchronic 90-day dietary study, and provide substantive evidence to support their grouping. Based on this grouping, results from the *in vivo* subchronic 90-day oral toxicity testing, which found no adverse effects in rats over 90 days exposed to up to 4% in the diet, can be read-across to the six forms of FC which are the subject of this Notice. Further, the *in vitro* studies provide supporting evidence that FC will not adversely affect the gastrointestinal tract or the integrity of the intestinal barrier.

These studies adequately demonstrated the safety of the FC materials and did not raise questions about the safety of FC in food at the levels tested (2-4%), which is equivalent to a no observed adverse effects level (NOAEL) of >2194.2 mg/kg/day (males) and >2666.6 mg/kg/day (females), corresponding to the highest tested dose (4%). The maximum dose tested is approximately 16-20 times the calculated EDI, providing an adequate margin of exposure to support a conclusion of safety of dietary FC. Published studies were referenced that provide further support of safety. Subchronic or chronic feeding of a 1-10% diet of conventional or fibrillated celluloses does not result in any adverse effects in mammals (Andrade et al. 2014, De Loid et al. 2019, EFSA ANS 2018, Hagiwara 2010, Kotkoskie et al. 1996, Paturi et al. 2010, Schmitt et al. 1991, Yoshioka et al 1994). One referenced study has shown that consumption of a 30% dry, gel, or fibrous form of cellulose for 72 weeks does not have any effects on rat appearance, behavior, food consumption, or survival (Paynter 1963).

The GRAS Expert Panel concurred with the Notifier's determination that existing toxicity data for FC and related forms can be used to support the safety of FC. The Panel notes the following elements discussed in Vireo's GRAS dossier as evidence of the general safety of FC:

- There are no indications, as supported by the OECD TG 408 study and *in vitro* studies, to suggest that FC will be absorbed, distributed, metabolized, or excreted differently than conventional celluloses.

- The results of the OECD TG 487 genotoxicity study, as well as the broader literature, support a conclusion of 'no genotoxic effects'.

- The evidence and rationale presented for reading-across safety data from conventional cellulose to fibrillated forms of cellulose is valid, given the demonstrated similar chemical and molecular structure, as well as similar lack of biological activity (90-day oral toxicity, simulated digestion, cytotoxicity, oxidative stress and inflammation) among these materials.

- There is a vast database of publicly available literature reviewed in the GRAS Notice that demonstrates that FC and related forms are safe. The literature studies demonstrate there are no adverse effects of cellulose fiber consumption, at levels as high as 5000 mg/kg oral consumption, or when fed diets consisting of up to 30% cellulose. The summary of the safety literature (including acute, subchronic, chronic, genotoxicity, carcinogenicity, and teratogenicity/reproductive toxicity studies) in the GRAS Notice supports the conclusion that FC is safe at the proposed concentrations for the intended use.

- The TG 408 study performed by the Notifier established a no observed adverse effects level (NOAEL) of >2194.2 mg/kg/day (males) and >2666.6 mg/kg/day (females). The maximum dose tested is approximately 16-20 times the calculated EDI, providing an adequate margin of exposure to support a conclusion of safety of dietary FC.

Expert Panel Opinion Statement

We, the members of the GRAS Expert Panel, have independently and collectively, critically evaluated all the relevant information summarized in the *GRAS NOTICE FOR FIBRILLATED CELLULOSE* or otherwise publicly available. All the appropriate resources have been objectively summarized and cited and it is our opinion as qualified experts that there is reasonable certainty that no harm will result from the use of FC as a food or packaging-additive at levels resulting in consumer exposures within the EDI of 133 mg/kg bw/day.

We further conclude that the such uses would be considered Generally Recognized as Safe (GRAS) based on scientific procedures, and that other qualified experts would agree.

Signed	Signed
Mitchell Cheeseman, Ph.D. Managing Director Steptoe and Johnson, LLP 1330 Connecticut Avenue, NW Washington, DC 20036 mcheeseman@steptoe.com	Richard C. Pleus, Ph.D., M.S. Founder, CEO, Chief Toxicologist Intertox, Inc. 600 Stewart St #1101 Seattle, WA 98101 rcpleus@intertox.com
Date:	Date:

Nine pages have been removed in accordance with copyright laws. The removed reference is:

Ong, K., Ede, J., Pomeroy-Carter, C., Sayes, C., Mulenos, M., Shatkin, J. 2020. "A 90-day dietary study with fibrillated cellulose in Sprague-Dawley rats." *Toxicology Reports 7*.

Twelve pages have been removed in accordance with copyright laws. The removed reference is:

Pradhan, S., Mulenos, M., Steele, L., Gibb, M., Ede, J., Ong, K., Shatkin, J., Sayes, C. 2020. "Physical, chemical, and toxicological characterization of fibrillated forms of cellulose using an in vitro gastrointestinal digestion and co-culture model." *Toxicology Research*.

Form Approved: OMB No. 0910-0342; Expiration Date: 07/31/2022
(See last page for OMB Statement)

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

GENERALLY RECOGNIZED AS SAFE *(GRAS)* NOTICE (Subpart E of Part 170)

FDA USE ONLY

GRN NUMBER	DATE OF RECEIPT
ESTIMATED DAILY INTAKE	INTENDED USE FOR INTERNET
NAME FOR INTERNET	
KEYWORDS	

Transmit completed form and attachments electronically via the Electronic Submission Gateway *(see Instructions)*; OR Transmit completed form and attachments in paper format or on physical media to: Office of Food Additive Safety *(HFS-200)*, Center for Food Safety and Applied Nutrition, Food and Drug Administration,5001 Campus Drive, College Park, MD 20740-3835.

SECTION A – INTRODUCTORY INFORMATION ABOUT THE SUBMISSION

1. Type of Submission *(Check one)*

☒ New ☐ Amendment to GRN No. _____ ☐ Supplement to GRN No. _____

2. ☒ All electronic files included in this submission have been checked and found to be virus free. *(Check box to verify)*

3 Most recent presubmission meeting *(if any)* with FDA on the subject substance *(yyyy/mm/dd):* 2019-07-29

4 For Amendments or Supplements: Is your amendment or supplement submitted in response to a communication from FDA? *(Check one)* ☐ Yes ☐ No If yes, enter the date of communication *(yyyy/mm/dd):* _____

SECTION B – INFORMATION ABOUT THE NOTIFIER

1a. Notifier

Name of Contact Person	Position or Title
Jo Anne Shatkin	Agent

Organization *(if applicable)*
The Alliance for Food Safety Acceptance of Fibrillated and Crystalline Celluloses

Mailing Address *(number and street)*
111 Perkins Street Apartment 223

City	State or Province	Zip Code/Postal Code	Country
Boston	Massachusetts	02130	United States of America

Telephone Number	Fax Number	E-Mail Address
508-612-8807		JAShatkin@vireoadvisors.com

1b. Agent or Attorney *(if applicable)*

Name of Contact Person	Position or Title
Jo Anne Shatkin	President

Organization *(if applicable)*
Vireo Advisors, LLC

Mailing Address *(number and street)*
111 Perkins Street Apartment 223

City	State or Province	Zip Code/Postal Code	Country
Boston	Massachusetts	02130	United States of America

Telephone Number	Fax Number	E-Mail Address
508-612-8807		JAShatkin@vireoadvisors.com

SECTION C – GENERAL ADMINISTRATIVE INFORMATION

1. Name of notified substance, using an appropriately descriptive term

Fibrillated Cellulose

2. Submission Format: *(Check appropriate box(es))*

☒ Electronic Submission Gateway

☐ Electronic files on physical media

☐ Paper

If applicable give number and type of physical media

_____

3. For paper submissions only:

Number of volumes _____

Total number of pages _____

4. Does this submission incorporate any information in CFSAN's files? *(Check one)*

☒ Yes *(Proceed to Item 5)* ☐ No *(Proceed to Item 6)*

5. The submission incorporates information from a previous submission to FDA as indicated below *(Check all that apply)*

☐ a) GRAS Notice No. GRN _____

☐ b) GRAS Affirmation Petition No. GRP _____

☐ c) Food Additive Petition No. FAP _____

☐ d) Food Master File No. FMF _____

☒ e) Other or Additional *(describe or enter information as above)* FCN 1582, 1864, 1887, 2022; SCOGS Report 25 (1973)

6. Statutory basis for conclusions of GRAS status *(Check one)*

☒ Scientific procedures *(21 CFR 170.30(a) and (b))* ☐ Experience based on common use in food *(21 CFR 170.30(a) and (c))*

7. Does the submission (including information that you are incorporating) contain information that you view as trade secret or as confidential commercial or financial information? (see 21 CFR 170.225(c)(8))

☐ Yes *(Proceed to Item 8*

☒ No *(Proceed to Section D)*

8. Have you designated information in your submission that you view as trade secret or as confidential commercial or financial information *(Check all that apply)*

☐ Yes, information is designated at the place where it occurs in the submission

☐ No

9. Have you attached a redacted copy of some or all of the submission? *(Check one)*

☐ Yes, a redacted copy of the complete submission

☐ Yes, a redacted copy of part(s) of the submission

☐ No

SECTION D – INTENDED USE

1. Describe the intended conditions of use of the notified substance, including the foods in which the substance will be used, the levels of use in such foods, and the purposes for which the substance will be used, including, when appropriate, a description of a subpopulation expected to consume the notified substance.

Fibrillated cellulose is proposed for use as a food additive and component of edible and protective food coatings. It is intended to be used in baked goods and baking mixes, alcoholic beverages, non-alcoholic beverages, cheeses, confections and frostings, fats and oils, fresh fruits and fruit juices, frozen dairy desserts and mixes, gelatins, puddings and fillings, gravies and sauces, milk and milk products, and processed fruits and fruit juices up to 5.0% and as a food coating up to 100%. Because fibrillated cellulose products will effectively substitute for traditional microcrystalline or cellulose substances as a food additive, we anticipate no substantial increases in global consumption. Specific use levels can be found in Table 1-1 of the GRAS Notice.

2. Does the intended use of the notified substance include any use in product(s) subject to regulation by the Food Safety and Inspection Service (FSIS) of the U.S. Department of Agriculture?

(Check one)

☐ Yes ☒ No

3. If your submission contains trade secrets, do you authorize FDA to provide this information to the Food Safety and Inspection Service of the U.S. Department of Agriculture?

(Check one)

☐ Yes ☐ No , you ask us to exclude trade secrets from the information FDA will send to FSIS.

SECTION E – PARTS 2 -7 OF YOUR GRAS NOTICE

(check list to help ensure your submission is complete – PART 1 is addressed in other sections of this form)

☒ PART 2 of a GRAS notice: Identity, method of manufacture, specifications, and physical or technical effect (170.230).

☒ PART 3 of a GRAS notice: Dietary exposure (170.235).

☒ PART 4 of a GRAS notice: Self-limiting levels of use (170.240).

☒ PART 5 of a GRAS notice: Experience based on common use in foods before 1958 (170.245).

☒ PART 6 of a GRAS notice: Narrative (170.250).

☒ PART 7 of a GRAS notice: List of supporting data and information in your GRAS notice (170.255)

Other Information

Did you include any other information that you want FDA to consider in evaluating your GRAS notice?

☒ Yes ☐ No

Did you include this other information in the list of attachments?

☒ Yes ☐ No

SECTION F – SIGNATURE AND CERTIFICATION STATEMENTS

1. The undersigned is informing FDA that The Alliance for Food Safety Acceptance of Fibrillated and Crystalline Celluloses
(name of notifier)

has concluded that the intended use*(s)* of Fibrillated Cellulose
(name of notified substance)

described on this form, as discussed in the attached notice, is (are) not subject to the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act based on your conclusion that the substance is generally recognized as safe recognized as safe under the conditions of its intended use in accordance with § 170.30.

2. The Alliance for Food Safety Acceptance of Fibrillated
(name of notifier)
agrees to make the data and information that are the basis for the conclusion of GRAS status available to FDA if FDA asks to see them; agrees to allow FDA to review and copy these data and information during customary business hours at the following location if FDA asks to do so; agrees to send these data and information to FDA if FDA asks to do so.

111 Perkins St. Apt. 223 Boston, MA 02130
(address of notifier or other location)

The notifying party certifies that this GRAS notice is a complete, representative, and balanced submission that includes unfavorable, as well as favorable information, pertinent to the evaluation of the safety and GRAS status of the use of the substance.The notifying party certifies that the information provided herein is accurate and complete to the best or his/her knowledge. Any knowing and willful misinterpretation is subject to criminal penalty pursuant to 18 U.S.C. 1001.

3. Signature of Responsible Official, Agent, or Attorney	**Printed Name and Title**	**Date (mm/dd/yyyy)**
	Jo Anne Shatkin, President	06/04/2020

SECTION G – LIST OF ATTACHMENTS

List your attached files or documents containing your submission, forms, amendments or supplements, and other pertinent information. Clearly identify the attachment with appropriate descriptive file names (or titles for paper documents), preferably as suggested in the guidance associated with this form. Number your attachments consecutively. When submitting paper documents, enter the inclusive page numbers of each portion of the document below.

Attachment Number	Attachment Name	Folder Location (select from menu) (Page Number(s) for paper Copy Only)
	Form3667.pdf	Administrative
	1.CoverLetter_FibrillatedCellulose_2020-06-04.pdf	Administrative
	2.Form3667_FibrillatedCellulose_2020-06-04.pdf	Administrative
	3.Form3667FullListofNotifiers_FibrillatedCellulose_2020-06-04.pdf	Administrative
	4.GRASNotice_FibrillatedCellulose_2020-06-04.pdf	GRAS Notice
	5. Attachment1Dietary90DayStudy_FibrillatedCellulose_2020-01-20.pdf	GRAS Notice
	6. Attachment2ReadAcrossInVitroStudies_FibrillatedCellulose_2020-05-20.pdf	GRAS Notice
	7. Attachment3ExpertPanelStatement_FibrillatedCellulose_2020-06-04.pdf	GRAS Notice

From: jashatkin@vireoadvisors.com
To: DiFranco, Stephen
Cc: Honigfort, Mical; Carlson, Susan
Subject: [EXTERNAL] GRN 954 Follow up
Date: Tuesday, May 25, 2021 12:45:15 PM
Attachments: image007.png

Dear Dr. DiFranco,

Thank you for sharing this detailed follow-up. As mentioned previously, we would like to request that we schedule a meeting with FDA personnel to discuss these points, as we plan to address them in detail.

However, due to timing concerns, I am writing to formally request that FDA Office of Food Additive Safety cease to evaluate GRN 954 *GRAS Notice for Fibrillated Celluloses*, submitted by Vireo Advisors on behalf of the Alliance for Food Safety Acceptance of Fibrillated and Crystalline Celluloses (AFSAC) in June 2020.

I look forward to receiving electronic and written confirmation of FDA receipt of this request.

Kind regards,

Jo Anne Shatkin, Ph.D.
President
Vireo Advisors, LLC
Boston, MA USA
@josthoughts

From: DiFranco, Stephen <Stephen.DiFranco@fda.hhs.gov>
Sent: Wednesday, May 12, 2021 5:03 PM
To: Jo Anne Shatkin <jashatkin@vireoadvisors.com>
Cc: Honigfort, Mical <Mical.Honigfort@fda.hhs.gov>; Carlson, Susan <Susan.Carlson@fda.hhs.gov>
Subject: GRN 954 Follow up

Dear Dr. Shatkin,

We appreciate you and your team joining us on March 24, 2021 to discuss our issues with GRN 954 for fibrillated celluloses. I wanted to follow up with some points we discussed during our teleconference and provide additional detail.

Firstly, the FDA staff in attendance were:

Stephen DiFranco, OFAS-DFI (Division of Food Ingredients), CSO
Shayla West-Barnette, OFAS-DFI, Regulatory Team Lead
Jeremy Mihalov, OFAS-DFI, Chemistry
Jannavi Srinivasan, OFAS-DFI, Chemistry Team Lead
Diana Doell, OFAS-DFI, Acting Chemistry Branch Chief
Troy Hubbard, OFAS-DFI, Toxicology
Supratim Choudhuri, OFAS-DFI, Toxicology Team Lead

As was discussed in the meeting, we have several issues with the content of the notice and would like to give Vireo Advisors, LLC. (Vireo) the opportunity to request that we cease to evaluate this notice. As currently written, we would likely not issue a "No Questions" letter in response to Vireo's conclusion that fibrillated celluloses is/are GRAS for the stated uses.

Overall, we identified 3 major deficiencies in the current notice: lack of an adequate physiochemical characterization of the notified substance (six fibrillated celluloses) and a robust comparison among them and the reference fibrillated cellulose substance; a tenuous toxicological read-across between these substances coupled with data gaps; and issues with the notified substance being considered as GRAS within the greater scientific community. Each of these points are discussed in detail below.

We do not feel these issues can be remedied through clarifications via an amendment to the current notice. If Vireo requests that we cease to evaluate their notice for the use of fibrillated celluloses in food, you may resubmit your conclusions as either a new GRAS notice or as a food additive petition that addresses the issues discussed herein.

As we discussed, fibrillated celluloses are materials engineered to exhibit properties or phenomena, including physical, chemical, or biological properties that are attributed to its dimensions. Per GRN 000954, fibrillated celluloses contain material that has at least one external dimension or surface structure in the nanoscale range (~1 nm—100 nm). The "engineered" nature of substances are not limited to manufacture or selection of a particular morphology through chemical means; and may include mechanical, enzymatic, or other processing steps that intentionally favor a particular morphology.

Moreover, as outlined in our 2014 guidance document, 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, the notified materials exhibit properties different from a constituently equivalent bulk ingredient already used in food, namely bulk cellulose. As such, FDA would consider fibrillated cellulose as a novel ingredient which warrants a new review, as the safety data on bulk cellulose may be incomplete or not applicable to establishing safety for fibrillated cellulose.

1. The notified substance (six fibrillated celluloses) is composed of fibril agglomerations containing ordered crystallite and amorphous fibril regions. However, dietary exposure to individual fibrils, which could be considered nanomaterials, may occur with the intended use of this ingredient. This is not addressed in the notice nor is a safety narrative discussing the possible dimension-dependent properties or phenomena, or corresponding functional effects that may be of concern.

2. The notice states that six distinct fibrillated cellulose products, produced by six different manufacturers using different manufacturing processes, are GRAS. Differences in manufacturing for the six products are not adequately described. Although a variety of optional manufacturing steps are listed, their intended effects on the identity and composition of the final products is not discussed. The raw material, the pre-treatment, mechanical treatment, and any post-treatment are not adequately described for each of the six products. Variations in starting materials and individual manufacturing processes could result in fibrillated celluloses with different physiochemical properties.
3. The notice discusses numerous physiochemical measures to demonstrate the similarity among reference cellulose materials and fibrillated celluloses. However, the data presented indicate that there are also differences among fibrillated celluloses, such as the surface charge (zeta potential), which may not support read across/grouping. The zeta potential is one physiochemical parameter correlated to the stability in colloidal dispersions via electrostatic repulsions. Known zeta potentials are reported with a polarity and a magnitude ranging from ±0-5 mV (typically indicative of instability and rapid coagulation) to ±40-60+ mV (indicative of high electronic stability). The zeta potentials reported for the six fibrillated celluloses span nearly the entire range (-5.20 mV to -46.40 mV) with Ref CC and Ref FC reported as -2.14 mV and -33.87 mV, respectively. The notice does not discuss how these differences in zeta potential are insignificant to the behavior or safety of these fibrils. The notice also does not discuss how varying zeta potentials support the physicochemical similarity of the six fibrillated celluloses to the reference cellulose material. Additionally, the surface charge of conventional cellulose is significantly different from fibrillated celluloses. The variable surface charges suggest differences in surface area and surface chemistry among fibrillated celluloses and conventional cellulose. Surface charge, in addition to other parameters, can contribute to observed properties and phenomena of nanomaterials, not observed in constituently similar materials with more conventional dimensions/aspect ratios.
    a. In the Pradhan et al. 2020 manuscript, there are statistically significant differences in zeta potentials of C20, C21, C23, C24, C25, and Ref FC, relative to the reference conventional cellulose. These statistically significant differences are neither discussed in the manuscript nor in the notice. Given that surface charge is a known physiochemical parameter of nanomaterials which can impact biological function, these differences need to be adequately discussed to support read-across of fibrillated and conventional celluloses.
    b. In the manuscript Ong et al., (2020), the reference conventional cellulose (Solka Floc™) has an average zeta potential of -24 mV. However, on p. 13 of the notice, Vireo states that the reference conventional cellulose has a zeta potential of -2 mV. This discrepancy in the reported zeta potential for the reference cellulose (Solka Floc™) is not discussed.
    c. Increased mucus penetration has been observed by nanomaterials with diameters less than or equal to 50 nm and a neutral surface charge. The notice does not address this potential safety issue.
    d. Negatively charged cellulose nanocrystal (-46.4 mV) induces increased cytotoxic responses in human embryonic kidney (HEK 293) and insect (Sf9) cells. Given the measured anionic surface charge of some of the six fibrillated celluloses, this should also be addressed in the notice.
4. There are physiochemical measurements often relevant to nanomaterials, which are omitted. For example, in Lavoine et al. (2012), the authors state that key properties of fibrillated celluloses are linked to its high surface area (which is at least ten times greater than conventional cellulose) and its extensive hydrogen bonding ability. Such properties would likely be pertinent to the technical effects of fibrillated celluloses and the intended uses in food.
5. Hydrodynamic diameter of conventional cellulose is an order of magnitude higher than the notified fibrillated celluloses. Moreover, there is variability in the hydrodynamic diameter of

the six fibrillated celluloses. The notice does not discuss how these differences are inconsequential or affect the validity of the read-across approach used to address safety.

6. The notice describes the length and width parameters for conventional cellulose (Solka Floc) but does not provide similar measurements for the six assayed fibrillated celluloses.
7. The notifier states that the specifications provided for fibrillated celluloses meet those established by the Food Chemicals Codex (FCC) for powdered cellulose, with the exception of pH due to the rheological characteristics.
    a. FCC specifications (12th edition) for powdered cellulose include identification and assay procedures, and a microscopy test. We note that these specifications are not discussed in the notice and similar criteria are not included for fibrillated celluloses.
    b. FCC specifications (12th edition) include a limit for loss on drying that is not a specification for fibrillated celluloses. The notifier describes forms of fibrillated cellulose to include gels or wet crumbles that are 2-30% fibrillated celluloses, in addition to a dried form that is up to 100%. The notice should discuss the water content of fibrillated celluloses and clarify that the specifications are on a dry matter basis.
    c. The notice should identify the methodologies used as part of the specifications provided and confirm that the methods are validated for the intended use. If referencing published methods, please provide a complete citation.
    d. The notice should also address what are the acceptance criteria used to identify fibrillated celluloses or differentiate fibrillated celluloses from conventional cellulose. In addition, please indicate if there are specifications or classification criteria for grading or differentiating forms of fibrillated celluloses.
    e. The manufacture of fibrillated celluloses may involve treatment with one or more enzymes. For example, we note that the FCC specifications for enzyme-modified sodium carboxymethyl cellulose (CMC) includes a viscosity test to distinguish enzymatically hydrolyzed CMC from non-hydrolyzed CMC. It also includes a determination of the percentage of enzymatically hydrolyzed CMC as well as criteria for residual enzyme activity. The notice should discuss limits for enzyme activity and criteria for evaluation of fibrillated cellulose produced by enzyme modification.
    f. The notice should discuss limits for optional components that may be added to the fibrillated celluloses (i.e., kaolin, carboxymethyl cellulose, calcium carbonate) and provide citation to applicable regulation or GRAS notice for these substances under the conditions of the intended use.
    g. Given the range of zeta-potentials for the six fibrillated celluloses, the notice should discuss whether the test indicated in the notice is informative as to the identification of the fibrillated celluloses as compared to other celluloses, and whether a specification for zeta-potential is appropriate for identification purposes.
8. The notice should include a discussion of additional properties relevant to the characterization of fibrillated celluloses and to further support their grouping and read-across to conventional cellulose. Such parameters could include:
    a. Microfibril diameter and length distributions (particle size distributions)
    b. Particle/agglomeration size
    c. Degree of polymerization
    d. Degree of crystallinity
    e. Surface chemistry, such as carboxylate and aldehyde content
    f. Specific surface area
9. Substances with similar surface characteristics to that of the six fibrillated celluloses have been shown to exhibit interactions with their environment different from their constituent bulk material. A discussion of any impacts on digestion or interactions with food matrices that might be anticipated to impact the physiochemical properties of the ingested fibrillated cellulose (e.g. formation of a protein corona) should be included.

The notice describes widespread use of conventional forms of cellulose in foods and published studies using conventional (non-nanoscale) cellulose materials in support of the safety determination of the six fibrillated celluloses. Generally, a case for read-across should be based on the limited differences in physicochemical properties between the ingredient of interest and conventional materials. The similarities and differences between materials should be presented and discussed. Additionally, the effect of these differences on dietary exposure, toxicokinetics, and

hazard should be discussed and may be substantiated with additional physicochemical, in vitro and/or in vivo data as needed, as was recommended in previous pre-submission meetings with FDA.

10. The notice does not adequately explain how the physiochemical differences between fibrillated celluloses and conventional cellulose are relevant to the safety of the notified substance.
11. Additionally, the notice does not describe information/studies utilizing cellulose nanocrystals as the test material. Explanation as to why such data is or is not relevant to the safety of fibrillated celluloses should be included.
12. Numerous forms of conventional celluloses used in food are discussed in the safety narrative in support of the safety of fibrillated celluloses. However, the notice only describes the physiochemical similarity of fibrillated celluloses to a conventional cellulose, specifically, a microcrystalline form (Solka Floc™). If this approach is used, addressing the similarities and differences of the six fibrillated celluloses relative to these other forms of conventional celluloses in order to qualify the use of these studies/data in support of the safety.

The notice claims that due to the chemical nature of fibrillated celluloses it is not anticipated to undergo differential metabolism relative to conventional celluloses used in food. However, due to the nanoscale dimensions, it is unclear if such materials would exhibit similar absorption, distribution and excretion properties relative to conventional celluloses. The presented in vitro digestion assays are inadequate to characterize the comprehensive ADME profile of fibrillated cellulose. Shatkin et. al. (2017) states:

> *"Although the available data do not suggest significant or novel hazards (relative to conventional cellulose), there are fundamental gaps in knowledge that preclude a conclusion of CN (cellulosic nanomaterials) safety for certain markets and product classes. For instance, toxicokinetic data of CN have yet to be published."*

In previous meetings with FDA, it was stated that a 14-day toxicokinetic study (mass balance after ingestion) was ongoing. This is also mentioned in the introduction of the cited Pradhan et al. study (2020). When available, these data should be discussed, as it is critical to the safety determination and for establishing read-across to other cellulose materials.

Although conventional cellulose is considered safe for use in food, the novel physicochemical properties and nanoscale dimensions of fibrillated celluloses may confer different biological effects. The notice fails to discuss biological activities in support of establishing the safety of the six fibrillated celluloses. Furthermore, some biological activities which may raise a safety concern may not be addressed by traditional animal toxicity studies.

13. In previous meetings, the FDA expressed concern regarding chronic exposure to fibrillated celluloses and possible unintended effects on the ultrastructural morphology of the intestinal tract. The safety narrative does not discuss the potential changes to the intestinal mucosa from consumption of the notified substance.
    a. Mucoadhesion describes the behavior in which a substance absorbs on the mucosal layer, which may influence gastrointestinal transit time. The notice does not address the mucoadhesive properties of fibrillated celluloses relative to conventional celluloses.
14. Nanoscale celluloses are reported to have increased solubility, water holding capacity, swelling capacity and fermentability relative to conventional celluloses. How these effects impact fecal bulking or alimentary transit time is not discussed in the notice.
15. The potential long-term effects of ingesting fibrillated cellulose on the gut microbiota is not addressed. Specifically, increased production of short chain fatty acids (SCFAs) has been reported following incubation of human fecal samples with milled cellulose. How the increased fermentability of fibrillated cellulose is anticipated to impact the overall nutrition and intestinal health of consumers is not discussed.
16. Certain fiber materials may delay absorption of lipids and lipid soluble vitamins and minerals related to binding/entrapment of these materials within the fiber matrix. Given the nanoscale dimensions and increased surface area of the fibrillated cellulose matrix, altered absorption of dietary lipids and lipid-soluble vitamins may be a safety concern. This issue was not addressed in the notice.
17. A previous publication indicates the potential for nanofibrillated cellulose to promote

increased secretion of inflammatory cytokines TNF-α and IL-1β in treated THP-1 macrophages. This effect was lessened when surface charges were introduced following functionalization of nanofibrils. These conflicting reports in the literature regarding the potential for nano-cellulosic substances to induce inflammatory signaling pathways are not discussed in the notice.

18. In inflammatory bowel disease (IBD) patients, consuming insoluble fibers can aggravate symptoms, causing increased bloating, diarrhea, gas and pain. Consumption of fibrillated celluloses may possibly influence the severity of such symptoms differently than conventional cellulose. The argument that use of the six fibrillated celluloses would be substitutional for the presently consumed cellulose in the diet would expose this subpopulation. As such, the notice should discuss how the six fibrillated celluloses do not pose an increased safety risk.
19. Uses are stated as being substitutional for conventional celluloses in the diet, with similar use levels. However, the subject material is engineered to have specific technical effects. While some of these technical effects may be similar to conventional cellulose, they may be of increased potency. For example, the water holding capacity of fibrillated cellulose is stated to be at least 10 times that of conventional cellulose. Based on this notion, it is unclear if use levels substitutional for conventional cellulose on a mass or percentage basis would present a safety concern due to the enhanced technical effect.

The estimate of dietary exposure provided in the notice is based on the intended uses in certain food categories. We note that the estimates of food consumption are drawn from a variety of sources, including previous GRAS notices and published reports that are based on food consumption surveys such as the Continuing Survey of Food Intakes by Individuals (1994-1996), National Health and Nutrition Examination Survey (NHANES, 2011-2012), and the Market Research Corporation of America (1965). For consistency, we would recommend using the same food consumption survey as a data source to the extent possible. Similarly, we note that current food consumption data should be used. For example, NHANES, What We Eat in America is an on-going food consumption survey and the data is publicly available and released every 2 years; the current data available is for survey year 2017-2018.

a. We note an error in Section 3.3.1 of the notice. The calculated dietary exposure to fibrillated celluloses from the intended use in high-fiber drinks and nutritional beverages at a maximum level of 1% is reported to be 2.25 mg/person/day. However, the estimated daily consumption of functional beverages is reported as 225 mL/person/day, which would correspond to approximately 225 g/person/day, rather than mg/person/day. Therefore, a use level of 1% would equate to 2.25 g/person/day.
b. The notifier states that consumption data for high-fiber drinks and nutritional beverages were not available and provides an estimate based on published market research. We note that beginning with NHANES 2009-10, consumption data is available for various nutritional beverages including nutritional drinks, shakes, and powders.
c. In addition to dietary exposure estimates, the notifier states that the use of fibrillated celluloses would be substitutional for microcrystalline (conventional) cellulose or other cellulose ingredients used in food, and therefore, does not expect dietary exposure to change. The notifier reports the estimates of dietary exposure to microcrystalline cellulose for the high percentile consumer to be 5.4 to 10.2 g/person/day with citation to CanTox Inc., 1993 in the Joint FAO/WHO Expert Committee on Food Additives (JECFA) 1998. The notice should discuss the intended uses and food consumption data that were considered in this report and how these compare to the intended uses described in your notice.

Given the current state of science on intentionally, morphologically engineered materials exhibiting novel properties as compared to the bulk, we recognize that there are numerous questions to address, and that it may be difficult to demonstrate consensus within the relevant scientific communities. Due to the lack of previous use of fibrillated celluloses in foods and incomplete understanding of the properties or phenomena of nanoscale materials, there is currently no clear scientific consensus regarding the overall safety of these types of materials. As such, it currently may not be feasible to establish that the safety of these materials is “generally recognized”.

Per the GRAS final rule (81 FR 54960), general recognition requires that information is both publicly available and that this generally available data and information supports a conclusion that the substance is generally recognized, among qualified experts, to be safe under the conditions of its intended use.

Concerning the appropriate regulatory pathway for the use of fibrillated celluloses in food (i.e., a GRAS notice versus a food additive petition), FDA has maintained that Vireo is welcome to choose either, provided they have sufficient data and information to fulfill the requirements of the respective program. However, in the 2014 guidance document referenced above, concerning the assessment of significant changes to manufacturing processes, including use of nanotechnology, FDA stated:

> *At this time, we are not aware of any food substances intentionally engineered on the nanometer scale for which there are generally available safety data sufficient to serve as the foundation for a determination that the use of a food substance is GRAS.*

Further, in the 2016 GRAS final rule (81 FR 54960), FDA discusses a report by the Government Accounting Office (GAO) and how we are addressing the recommendations made, including the guidance that FDA issued. This final rule reiterates that:

> *….at present, for nanotechnology applications in food substances, there are questions related to the technical evidence of safety as well as the general recognition of that safety, that are likely to be sufficient to warrant formal premarket review and approval by FDA, rather than to satisfy criteria for GRAS status.*

As such, FDA would need compelling evidence that the proposed use of this engineered material is both reasonably certain to cause no harm and meets the general recognition standard of the program.

Please review the above comments, and respond within 2 weeks, whether you wish for us to cease to evaluate the notice or if we should move forward with our response letter. As stated in our meeting, should you request that we cease to evaluate the current notice, we would encourage you to meet with us prior to resubmitting a new GRN or food additive petition.

I hope you find the above useful. If you have any additional questions or concerns, please don't hesitate to contact me at stephen.difranco@fda.hhs.gov or by phone at 240.402.2710.

Best,

Steve

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