Safety Assessment of HydroFibre (bacterial cellulose suspension)

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Novel Food Classification: *Article 1.2 (d)*

Introduction

An application for the authorisation of a bacterial cellulose aqueous suspension (HydroFibre) was submitted to the Food Safety Authority of Ireland (FSAI) by Satisfibre S.A. of Portugal in accordance with *Article 4* of the novel food Regulation (EC) No 258/97. The application was accepted by the FSAI on December 19th of 2016.

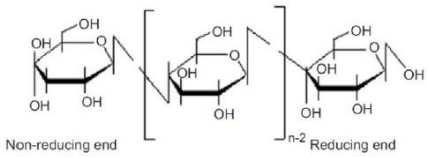
HydroFibre is produced by fermentation with *Komagataeibacter sucrofermentans* (formerly known as *Gluconacetobacter xylinus*), a gram negative aerobic bacterium that belongs to the acetic acid bacteria family. The novel ingredient has the same chemical composition as that of vegetable cellulose and morphologically is made up of bundles of ribbon-shaped pure cellulose microfibrils. The bacterial polysaccharide used in the manufacture of HydroFibre is free of lignin, pectin, hemicellulose and other biogenic compounds. The applicant intends to incorporate the novel ingredient as a source of dietary fibre to a final level of 2-20% in a variety of foods such as, composite food, meat and meat products, milk and dairy products, fruit and vegetable juices, ice-creams and bakery wares.

In Asia, bacterial cellulose has traditionally been produced from the fermentation of coconut waste-water by cellulose-producing acetic acid bacteria and used in low-calorie foods. However, bacterial cellulose has not been consumed to a significant degree in the EU prior to 1997 and so requires authorisation as a novel food. The novel ingredient falls under *Article 1.2(d)* of the novel food Regulation (EC) No 258/97: "*foods and food ingredients consisting of or isolated from micro-organisms, fungi or algae".* In order to assess wholesomeness, the application dossier was prepared pursuant to Class 2 of Commission Recommendation 97/618/EC; *"Complex NF from non-GM source",* and subclass (2); *"the source of the NF has no history of food use in the Community".*

I. Specification of the novel food

The novel ingredient is a bacterial cellulose (exopolysaccharide) produced by fermentation with *Komagataeibacter sucrofermentans*, a gram negative strict aerobe. It is chemically

identical to plant cellulose (CAS number 9004-34-6), though in the form of a hydrated paste consisting of 5% (m/v) bacterial cellulose and 95% water. The cellulose molecule $(C_6H_{10}O_5)n$ is composed of repeating D-anhydroglucose units joined by β (1-4) linkages, each glycoside containing three hydroxyl groups at the 2, 3 and 6 positions. Similar to plant cellulose, bacterial cellulose is not branched nor does it have any substituent groups.



Chemical Structure of Bacterial Cellulose

The degree of polymerization of bacterial cellulose typically falls within the range 800-11000, corresponding to a molecular mass of 130-1782 kDa.

Characteristic	**Specification**
Physical	White moist cake/paste with no taste or odour
Moisture	92 – 96%
Protein (F=6.25)	<0.15%
Nitrogen	<0.02%
Crude fibre	4 – 6%
Ash	<1.0%
PH	6.5 – 7.5

The novel ingredient is in the form of a highly hydrated random assembly of ribbon shaped fibrils. It is insoluble in water, ethanol, ether and dilute mineral acids while being slightly soluble in sodium hydroxide solution, similar to microcrystalline cellulose from vegetable sources. The melting point of the novel ingredient is in the range of 325-350°C while its stability is established at 6 months when stored at 4°C.

II. Effect of the production process applied to the novel food

The production process for the novel ingredient is currently operating at pilot scale under standardised conditions and the applicant contends that the specifications and purity criteria presented will be representative of the industrial scale process. Fermentation is carried out with *Komagataeibacter sucrofermentans* (ATCC 7001781, lot number 3556952 - from the American Type Culture Collection) grown on food-grade nutrients. The biosynthetic pathway

producing bacterial cellulose is presented by the applicant. The recovered cellulose is washed, ground to a pulp and concentrated to 5% (m/v) by centrifugation, before autoclaving and packaging in vacuum sealed plastic bags. The applicant intends to implement ISO 22000:2005 which includes a HACCP plan, though certification to this effect is not yet available for full scale production.

III. History of the organism used for the novel food

Acetic acid bacteria are used in the production of certain food and drinks including vinegar, kombucha tea and cocoa. The novel ingredient is produced by fermentation with *Komagataeibacter sucrofermentans* which belongs to a genus that produces relatively high levels of acetic acid and cellulose. It is a rod-shaped gram-negative bacterium without flagellae. The non-pathogenic obligatory aerobe is found ubiquitously in natural sources such as flowers, vegetables, nuts, sugar cane, fruits, fruit products and, in particular, rotten fruits The bacterial strain (ATCC 700178) used is well characterised with classification details publically available and has been assigned the lowest biosafety level of 1 (CDC, NIH).

IV. Anticipated intake/extent of use of the novel food

HydroFibre is intended as a dietary fibre (5% bacterial cellulose and 95% water) in a range of foodstuffs such as composite food, meat and meat products, milk and dairy products, fruit and vegetable juices, ice-creams and bakery wares. The final products will contain concentration ranges of 2-20%, corresponding to a bacterial cellulose concentration of 0.1-1% in food. Recommended or maximum daily doses or intakes are not indicated and food supplements are not a target for inclusion of the novel ingredient.

The bacterial cellulose from HydroFibre shares the same chemical identity as that of microcrystalline cellulose obtained from vegetable sources therefore, background exposure was calculated for microcrystalline cellulose (MCC) and powdered cellulose. MCC is a food additive which is present in foods such as power bars, dressings, fillings and icings. Regulation (EC) 1333/2008 states that the maximum permitted level of cellulose in food is *quantum satis*. As there is some overlap between the proposed food categories for HydroFibre and MCC, intake assessments for MCC include only those food categories not intended for incorporation of HydroFibre and so could represent a potential underestimate.

Using the EFSA FAIM model, the applicant has provided data on the mean and P95 daily intake values of cellulose from HydroFibre and MCC for each food category and population

groups including toddlers, children, adolescents, adults and the elderly. Assuming maximal (0.5 – 1.0%) presence in foods, the highest mean intake of cellulose from HydroFibre was found to be 287 mg/kg bw/day and the high exposure was found to be 422 mg/kg bw/day.

Food group	FAIM category FCS level 2	Concentration levels of bacterial cellulose from HydroFibre (%)
Composite Food		
Meat burger	8.1	0.5-1.0
Meat-based meals	8.1; 8.2	0.5-1.0
Vegetable based meals	4.2	0.1-0.5
Ready-to-eat soups	12.5	0.1-0.5
Meat and Meat Products		
Sausages	8.2	0.5-1.0
Meat imitates	12.9	0.1-1.0
Textured soy protein	12.9	0.1-1.0
Milk and Dairy Products		
Fermented milk products	1.4; 1.23	0.1-0.5
Yoghurt	1.4; 1.23	0.1-0.5
Cheese (unripened and ripened)	1.7; 1.7.1; 1.7.2	0.1-0.5
Whipped cream	1.6	0.1-0.5
Juices and Drinks		
Fruit juice	14.1.2.1	0.1-0.5
Fruit and vegetable smoothies	14.1.2.2	0.1-0.5
Dairy fruit and vegetable shakes	14.1.4	0.1-0.5
Flavoured drinks	14.1.4	0.1-0.5
Flavoured drinks with sweeteners	14.1.4.2	0.1-0.5
Snacks, Desserts and Other Foods		
Ices and desserts	3; 16	0.1-0.5; 0.5-1.0
Ice cream	3	0.1-0.5
Sauces and Dressings		
Salad dressing	12.6	0.1-1.0
Mayonnaise	12.6	0.1-1.0
Fine Bakery Wares	7.2	0.1-1.0

Based on more ‘normal use levels’ (0.1 – 0.5%), the highest mean value of cellulose from HydroFibre found was 72 mg/kg bw/day and the high exposure was found to be 124 mg/kg

bw/day. It should be noted that these intake values were for toddlers and children, whereas for adolescents and adults the intake values are approximately a factor of 2 to 4 lower.

The applicant provides similar estimates of intake of cellulose from MCC. Assuming maximal presence (0.5 – 1.0%), the highest mean intake of cellulose from MCC found was 198 mg/kg bw/day and the high exposure was found to be 487 mg/kg bw/day. For 'use levels' (0.1 – 0.5%), the highest mean value of cellulose from MCC was 112 mg/kg bw/day and the high exposure was found to be 285 mg/kg bw/day. Similarly, intakes were highest in toddlers and children and considerably lower in adolescents and adults.

The applicant calculates a conservative combined maximal intake of cellulose from both sources (i.e. from HydroFibre and MCC) of 902 mg/kg bw/day for the high intake exposure calculations and 397 mg/kg bw/day for mean intake exposure calculations. The conservative nature of this estimate calculated for children is noted, combined intakes for adolescents and adults are about a factor of 2 to 4 lower.

The initial NOAEL proposed by the applicant is 5,331mg/kg bw/day for males and 5,230 mg/kg bw/day for females, based on a sub-acute toxicity study using 5% fermented cellulose which is considerably higher than that calculated in the exposure assessment. Subsequently, the applicant states that an ADI of 7,000 mg/kg bw/day is more suitable based on a rat sub-chronic study and considering that cellulose is not digested or absorbed. The target population of the novel ingredient is listed as adults, including the elderly. However, it is not clear whether there are any sub-groups such as pregnant and lactating women who should not consume the novel ingredient.

V. Information from previous human exposure to the novel food or its source

Bacterial cellulose is currently produced and marketed on a large scale and has a long history of use in Asian countries under the trade name 'nata de coco' and was exported to Europe, USA, and the Middle East between 2006 and 2013. It can be produced from a variety of *Acetobacter* and *Komagataeibacter* strains and has GRAS status the in the USA since 1992. The applicant has no knowledge of any health related issues having been associated with the consumption of the novel ingredient.

VI. Nutritional information on the novel food

The novel ingredient is composed of 5% fibre and 95% water, with relatively low amounts of fat (<0.2%), carbohydrates (<0.5%) and protein (<0.15%). It is indigestible and passes

essentially unchanged through gastrointestinal tract following ingestion. Bacterial cellulose and plant cellulose are chemically identical and as such would be considered nutritionally equivalent. Information on the impact of bacterial cellulose on lipid metabolism in animals has been provided, with some inconsistent reductions in triglyceride and cholesterol concentrations reported.

VII. Microbiological information on the novel food

The novel ingredient is autoclaved (sterilised) prior to packaging and therefore microbiological contamination is not a significant concern. Microbiological analysis of the novel ingredient demonstrates the absence of 'Total microorganisms' (total microorganisms include bacteria, yeasts and molds).

VIII. Toxicological information on the novel food

The results of toxicity tests on bacterial cellulose formulations were provided by the applicant, although none were carried out specifically on the cellulose isolated from *Komagataeibacter sucrofermentans*. There is no indication that any of these studies were conducted to GLP or followed recognised test guidelines such as those issued by the OECD.

Acute Toxicity

The acute toxicity of a 10% suspension of a commercial product, Cellulon^{TM} fibre (dried bacterial cellulose: sucrose at a ratio of 1:1), was investigated in Sprague-Dawley rats at a dose corresponding to 1,000 mg bacterial cellulose/kg bw via oral intubation, with no adverse treatment-related effects recorded.

Sub-acute Toxicity

F344 rats fed diets containing 0, 1.25, 2.5, or 5.0% 'fermented cellulose' (composed of 60% bacterial cellulose, 20% carboxymethyl cellulose and 20% sucrose) for 28 days did not display any treatment-related clinical signs of toxicity. Statistically significant differences in some blood biochemistry parameters were sporadic, sex-specific and not corroborated by other treatment-related effects and therefore not considered to be biologically significant. Increases in relative cecum (filled) and cecum (empty) weights in mid and high-dose males and females, relative salivary gland weights in both sexes at the high dose, and relative kidney and adrenal weights in high dose females were not corroborated by histopathological changes (there were no histopathological findings of note). The increase in cecum weight was considered to be a physiological adaptation related to the ingestion of large amounts of

modified starch, fibrous ingredients, or other carbohydrates which are poorly absorbed and have a high osmotic potential. The No Adverse Observed Effect Level (NOAEL) was set at the highest dose which was calculated to be 5,331 mg/kg bw/day in males and 5,230 mg/kg/day in females.

Sub-chronic Toxicity

The sub-chronic toxicity of bacterial cellulose was investigated in Sprague-Dawley rats fed diets containing 0, 5, and 10% Cellulon^{TM} fibre or microcrystalline cellulose for 13 weeks. There were no treatment-related deaths or treatment-related clinical signs of toxicity. Body weight was unaffected. Food consumption was generally increased in treated animals (both Cellulon^{TM} fibre and microcrystalline cellulose) and this was attributed to the relatively high test article concentrations in the feed. Statistically significant changes in haematology and clinical chemistry parameters were not considered to be biologically significant. There were no notable gross pathologic or histopathological findings at necropsy and organ weights were unaffected. The outcomes were interpreted as proof that Cellulon^{TM} fibre and microcrystalline cellulose are toxicologically equivalent and that sub-chronic exposure to Cellulon^{TM} fibre did not adversely affect these animals. The NOAEL was 7,000 mg/kg/day for male rats and 8,500 mg/kg/day for female rats.

Genotoxicity

Four *in vitro* and one *in vivo* genotoxicity studies carried out on bacterial cellulose (Cellulon^{TM}) did not identify any genotoxic or mutagenic effects under any of the conditions tested.

Chronic Toxicity and Carcinogenicity

A brief description was provided of the findings of a 72-week oral chronic toxicity study in rats administered either 30% ordinary cellulose or dry microcrystalline cellulose or microcrystalline cellulose gel. There were no clinical signs of toxicity or effects on food consumption, mortality or haematology. The body, liver and kidney weights of male rats given microcrystalline cellulose gel were higher than those of the controls. The significance of this was not discussed. Gross and histo-pathology showed some dystrophic calcification of renal tubules in females on microcrystalline cellulose but all other organs appeared unremarkable. The incidence of neoplasia did not differ between treatment and control groups.

Human Safety Data

The applicant has not identified any human studies relating to HydroFibre in the literature.

Allergenicity

The applicant has not addressed allergenicity issues.

Conclusions

Authorisation is being sought to add HydroFibre as a novel source of dietary fibre to a variety of foods. The applicant contends that the novel ingredient is safe based on the historical human consumption of bacterial cellulose in Asian countries and its physical and chemical similarity to microcrystalline cellulose (MCC) from plant sources. Most of the toxicological data provided does not relate specifically to the novel ingredient, but instead to a related ingredient which is acceptable considering the physical and chemical similarities. It is also not clear whether any of the studies cited were carried out to GLP or OECD standards.

Unusually, production of the novel ingredient appears to have been carried out only to pilot scale so far and without inclusion of a HACCP plan. However, scale up is not expected to impact on the safety of the product and it will be carried out to ISO 22000 standards and in line with HACCP principles. While products containing the novel ingredient will carry a recommendation that they are not for use by children, it is not clear if there are any similar restrictions for other sub-populations such as pregnant or lactating women.

The product will be used as a source of dietary fibre for adults aimed at increasing satiety. In 1975 and again in 1998, JECFA concluded that an ADI for microcrystalline cellulose was "not deemed necessary". Furthermore, JECFA has allocated a group ADI "not specified" to seven modified cellulose derivatives. The Scientific Committee on Food of the European Commission allocated an ADI 'not specified' to a number of authorised food additives including E460 and E466 on the basis that they do not present any hazard to the health of consumers. On this basis, the use of intra- and inter-species uncertainty factors is deemed unnecessary and an ADI of 7 grams/kg bw/day is acceptable. The maximum total intake of cellulose fibre as a result of the proposed use of bacterial cellulose (HydroFibre) is calculated to be 902 mg/kg bw/day for the high intake exposure calculations and 397 mg/kg bw/day for the mean intake exposure calculations. Therefore, the intake of bacterial cellulose from HydroFibre is well below the ADI. This gives a margin of 16 for the maximum exposure estimate to HydroFibre intake in toddlers of 422 mg/kg bw/day and a margin of 8 for

combined exposure to HydroFibre and E460 in children under maximum exposure conditions (902 mg/kg bw/day).

Recommendation

The FSAI has not identified any health concerns associated with the consumption of HydroFibre at the dosage recommended by the applicant. As long as the scaled up production process for the novel ingredient can achieve the product specifications, the FSAI is of the opinion that HydroFibre meets the criteria for novel food set out in *Article 3.1.* of the novel food Regulation (EC) No 258/97.