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Safety of cellobiose as a novel food pursuant to regulation (EU) 2015/2283

EFSA Panel on Nutrition, Novel Foods and Food Allergens (NDA), Dominique Turck, Torsten Bohn, Jacqueline Castenmiller, Stefaan De Henauw, Karen Ildico Hirsch-Ernst, Alexandre Maciuk, Inge Mangelsdorf, Harry J McArdle, Androniki Naska, Carmen Pelaez, Kristina Pentieva, Alfonso Siani, Frank Thies, Sophia Tsabouri, Marco Vinceti, Francesco Cubadda, Thomas Frenzel, Marina Heinonen, Miguel Prieto Maradona, Rosangela Marchelli, Monika Neuhäuser-Berthold, Morten Poulsen, Josef Rudolf Schlatter, Henk van Loveren, Océane Albert and Helle Katrine Knutsen

Abstract

Following a request from the European Commission, the EFSA Panel on Nutrition, Novel Foods and Food Allergens (NDA) was asked to deliver an opinion on cellobiose as a novel food (NF) pursuant to Regulation (EU) 2015/2283. The NF consists of two glucose monomers linked by a β-(1–4) glucosidic bond. The applicant intends to add the NF to a variety of foods, and to food supplements aimed at the general population 3 years and older. The information provided on the manufacturing process, composition and specifications of the NF is sufficient and does not raise safety concerns. The applicant provided a subchronic toxicological study which did not raise safety concerns. The applicant provided a human dose-escalation study from which the Panel concludes that the consumption of 20 g per day of cellobiose (equivalent to 290 mg/kg body weight (bw) per day in a 70-kg adult) does not raise concern regarding gastrointestinal tolerability. The maximum anticipated daily intake of cellobiose from the proposed uses is below 290 mg/kg bw per day in the target population. Considering the nature, source, compositional characterisation, and production process of the NF, as well as the toxicological data provided, the Panel considers that the NF does not raise safety concerns under the proposed conditions of use.

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Keywords: novel foods, cellobiose, food supplement

Requestor: European Commission

Question number: EFSA-Q-2020-00465

Correspondence: nif@efsa.europa.eu

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Panel members: Dominique Turck, Torsten Bohn, Jacqueline Castenmiller, Stefaan De Henauw, Karen Ildico Hirsch-Ernst, Helle Katrine Knutsen, Alexandre Maciuk, Inge Mangelsdorf, Harry J McArdle, Androniki Naska, Carmen Pelaez, Kristina Pentieva, Alfonso Siani, Frank Thies, Sophia Tsabouri and Marco Vinceti.

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1. Introduction

1.1. Background and Terms of Reference as provided by the requestor

On 28 May 2020, the company SAVANNA Ingredients GmbH submitted a request to the European Commission in accordance with Article 10 of Regulation (EU) 2015/2283 to authorise placing on the Union market of cellobiose as a novel food.

The application requests to authorise use of cellobiose in a number of foods.

The applicant has also requested data protection in accordance with Article 26 of Regulation (EU) 2015/2283.

In accordance with Article 10(3) of Regulation (EU) 2015/2283, the European Commission asks the European Food Safety Authority to provide a scientific opinion on cellobiose as a novel food.

In addition, the European Food Safety Authority is requested to include in its scientific opinion a statement as to if, and if so to what extent, the proprietary data for which the applicant is requesting data protection was used in elaborating the opinion, in line with the requirements of Article 26(2)(c) of Regulation (EU) 2015/2283.

2. Data and methodologies

2.1. Data

The safety assessment of this NF is based on data supplied in the application and information submitted by the applicant following EFSA requests for supplementary information. During the assessment, the Panel identified additional literature data that were not included in the application.

Administrative and scientific requirements for NF applications referred to in Article 10 of Regulation (EU) 2015/2283 are listed in the Commission Implementing Regulation (EU) 2017/2469[1].

A common and structured format on the presentation of NF applications is described in the EFSA guidance on the preparation and presentation of a NF application (EFSA NDA Panel, 2016). As indicated in this guidance, it is the duty of the applicant to provide all of the available (proprietary, confidential and published) scientific data (including both data in favour and not in favour) that are pertinent to the safety of the NF.

This NF application includes a request for protection of proprietary data in accordance with Article 26 of Regulation (EU) 2015/2283. The data requested by the applicant to be protected comprise information about the identity, production process and composition of the NF, as well as the genotoxicity, subchronic toxicity and human tolerance studies carried out with the NF.

2.2. Methodologies

The assessment follows the methodology set out in the EFSA guidance on NF applications (EFSA NDA Panel et al., 2016) and the principles described in the relevant existing guidance documents from the EFSA Scientific Committee. The legal provisions for the assessment are laid down in Article 11 of Regulation (EU) 2015/2283 and in Article 7 of the Commission Implementing Regulation (EU) 2017/2469.

Additional information that was not included in the application was retrieved by literature search, following a search strategy and standard operating procedure as described by the University of Chemistry and Technology (UCT) Prague (Dibusz and Vejvodova, 2020).

This assessment concerns only the risks that might be associated with the consumption of the NF under the proposed conditions of use and is not an assessment of the efficacy of the NF with regard to any claimed benefit.

3. Assessment

3.1. Introduction

The NF which is the subject of the application is cellobiose, a disaccharide consisting of two glucose units linked by a β-(1–4) glucosidic bond, produced by a two-step enzymatic conversion from sucrose

[1] Commission Implementing Regulation (EU) 2017/2469 of 20 December 2017 laying down administrative and scientific requirements for applications referred to in Article 10 of Regulation (EU) 2015/2283 of the European Parliament and of the Council on novel foods. OJ L 351, 30.12.2017, pp. 64–71.

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and glucose. With reference to Article 3 of Regulation (EU) 2015/2283[2], cellobiose falls under the category 2(a)(i): '*a food with a new or intentionally modified molecular structure, where that structure was not used as, or in, a food within the Union before 15 May 1997*'. The NF is proposed to be used as ingredient in several food categories to replace sucrose or lactose or function as a sweetener. The target population is the healthy general population.

3.2. Identity of the NF

Cellobiose is a disaccharide consisting of two glucose monomers linked by a β-(1–4) glucosidic bond. The chemical identity of the NF was verified by ^{1}H and ^{13}C NMR spectroscopy. The signal from the proton linked to the carbon involved in the glucosidic bond appears as a doublet with a coupling constant of 8.0 Hz, which is consistent with a β-glucosidic bond. The structure was confirmed by two-dimensional NMR experiments: heteronuclear multiple bond correlation (HMBC) and correlation spectroscopy (COSY) and by mass spectrometry. The identification parameters are reported in Table 1.

Table 1: Identity of the NF

Common name	**Cellobiose**
Other names	D-(+)-cellobiose, D-cellobiose
CAS number	528–50-7
Chemical (IUPAC) name	4-O-β-D-glucopyranosyl-β-D-glucopyranose
Molecular formula	$C_{12}H_{22}O_{11}$
Molecular weight	342.3 g/mol
Melting point	239°C (decomposition)

The molecular structure of cellobiose is represented in Figure 1.

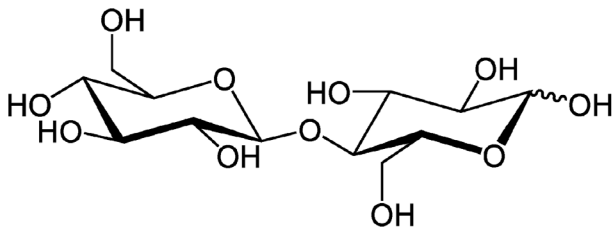

Figure 1: Molecular structure of cellobiose

3.3. Production process

According to the information provided, cellobiose is produced in line with good manufacturing practice (GMP) and Hazard Analysis Critical Control Points (HACCP) principles.

The production of cellobiose is catalysed by a two-step enzymatic reaction converting sucrose to cellobiose (Figure 2). The general principle of the process was described by Brucher and Häßler (Brucher and Häßler, 2019).

Briefly, sucrose phosphorylase converts sucrose into glucose-1-phosphate (G1P) and fructose. Cellobiose phosphorylase then couples one molecule of glucose to one molecule of G1P via a β-(1–4) glucosidic bond. After conversion, cellobiose is separated from the enzymes by ultrafiltration, followed by further purification using electrodialysis. The liquor is concentrated by evaporation and the final product is obtained from the concentrated liquor by crystallisation (*c.f.* manufacturing flow scheme; Figure 3). The Panel notes that the applicant demonstrated the absence of DNA from the genetically modified microorganisms producing sucrose phosphorylase and cellobiose phosphorylase in both enzyme preparations used as manufacturing aids.

[2] Regulation (EU) 2015/2283 of the European Parliament and of the Council of 25 November 2015 on novel foods, amending Regulation (EU) No 1169/2011 of the European Parliament and of the Council and repealing Regulation (EC) No 258/97 of the European Parliament and of the Council and Commission Regulation (EC) No 1852/2001.

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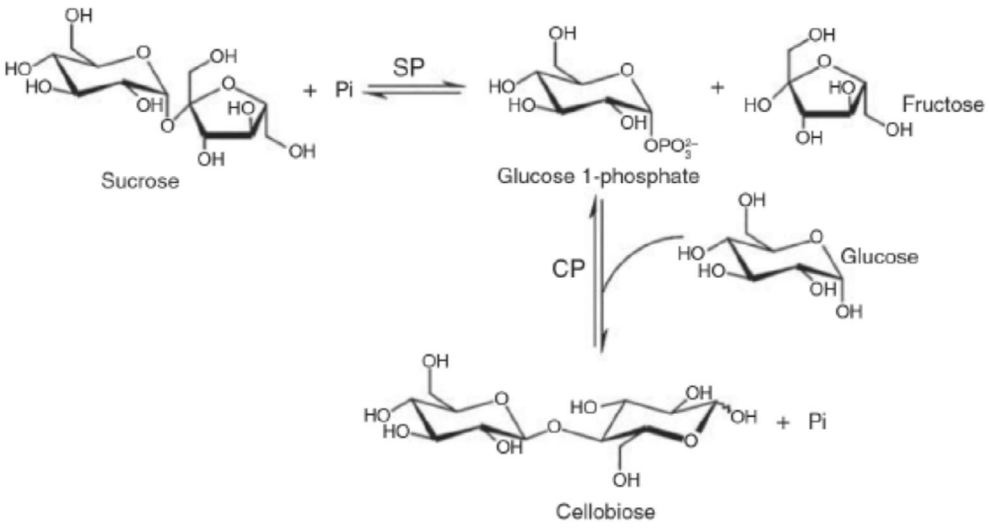

Figure 2: Enzymatic conversion of sucrose into glucose-1 phosphate (G1P) and fructose; synthesis of cellobiose from G1P and glucose. SP: sucrose phosphorylase; CP: cellobiose phosphorylase

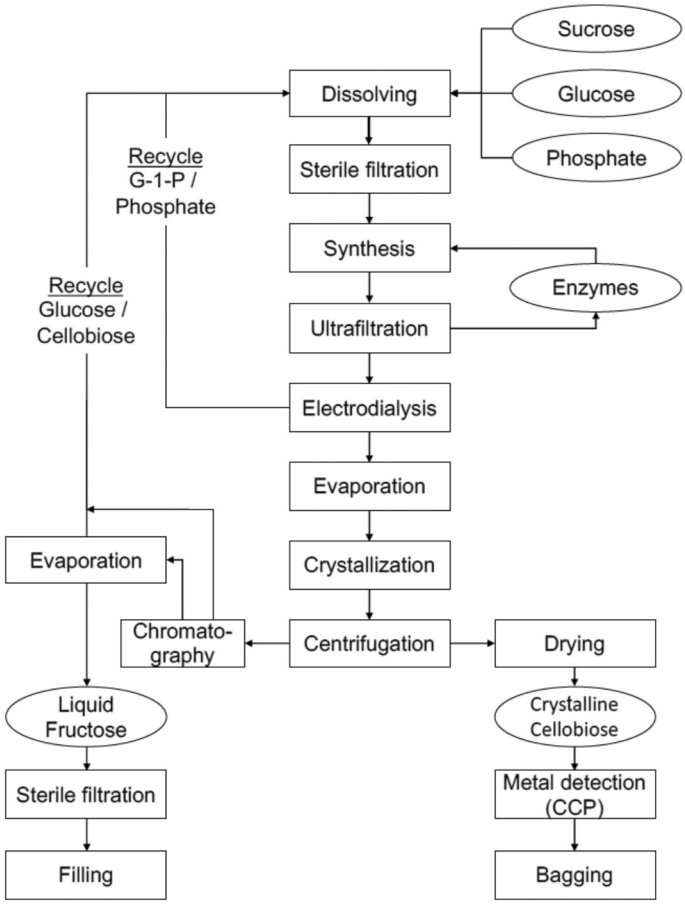

Figure 3: Manufacturing flow scheme for cellobiose. G-1-P: glucose-1-phosphate; CCP (critical control point): sifter with metal detection

The Panel notes that the production process described by the applicant is that of a pilot plant. The Panel does not expect the scaling up of the production process to generate safety issues.

The Panel considers that the production process is sufficiently described and does not raise safety concerns.

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3.4. Compositional data

In order to confirm that the manufacturing process is reproducible and adequate to produce on a commercial scale a product with certain characteristics, the applicant provided analytical information for five independent batches of the NF (Table 2).

Table 2: Batch to batch analysis of the NF

Parameter (unit)	Batch number					Method of analysis
	#1	#2	#3	#4	#5	
Identity						
NMR spectra	Complies	Complies	Complies	Complies	Complies	^{1}H-NMR
Physico-chemical properties						
Refraction index (°Brix)	1.348	1.348	1.349	1.349	1.348	Refractometry Ph. Eur. 2.2.6: 2008–01
Optical rotation $[\alpha]_D$ (c 10, water)	+35.1	+34.8	+33.9	+34.4	+35.1	Polarimetry Ph. Eur.: 2017–01
Rel. density (kg/L)	1.0389	1.0393	1.0403	1.0415	1.0391	Densitometry Ph. Eur. 2.2.5: 2008–01
pH	5.4	4.2	3.7	3.7	4.3	Potentiometry Ph. Eur. 2.2.3: 2016–07
Melting point (°C)	237.4	236.6	234.6	235.1	237.5	Open capillary method Ph. Eur. 2.2.15, mod.
Dry matter (g/100 g)	≥ 99	≥ 99	≥ 99	≥ 99	≥ 99	Gravimetry § 64 LFGB L 39.00-2 (EG): 1981–04
Water activity	0.495	0.491	0.463	0.436	0.487	Equilibrium relative humidity (ERH) Rotronic for AwTherm (2015–11)
Chemical analysis						
Cellobiose (g/100 g)	99.4	99.5	99.7	99.3	99.8	HPLC-RI § 64 LFGB L 40.00-7/ KIN CH 013
Fructose (g/100 g)	0.19	0.18	0.28	0.54	0.21	Enzymatic method § 64 LFGB L 26.11.03–8: 1983–05
Glucose (g/100 g)	0.13	0.11	0.24	0.26	0.36	Enzymatic method § 64 LFGB L 26.11.03–8: 1983–05
Sucrose (g/100 g)	< 0.10	< 0.10	< 0.10	0.12	0.21	Enzymatic method § 64 LFGB L 26.11.03–8: 1983–05
Maltose (g/100 g)	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	Enzymatic method § 64 LFGB L 26.11.03–8: 1983–05
Ash (g/100 g)	< 0.10	< 0.10	< 0.10	< 0.10	< 0.10	Gravimetry § 64 LFGB L 17.00–3: 1982–05
Ash, HCl insoluble (g/100 g)	< 0.10	< 0.10	< 0.10	< 0.10	< 0.10	Gravimetry § 64 LFGB L 53.00–4: 1996–02
Phosphate (mg/kg d.m.)	55	24	25	20	31	Spectrometric method DIN EN ISO 6878 (D11): 2004–09
Protein (g/100 g)	0.0031	0.0025	0.0026	0.0029	0.0031	Bradford (Roth NanoQuant®)

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Parameter (unit)	Batch number					Method of analysis
	#1	#2	#3	#4	#5	
Microbial analysis						
Total aerobic count (CFU/g)	< 10	< 10	< 10	160	< 10	Plate counting method DIN EN ISO 4833-1: 2013–12
TYMC (CFU/g)	< 10	< 10	< 10	< 10	< 10	Plate counting method § 64 LFGB L 01.00–37: 1991–12
Enterobacteriaceae (cfu/g)	< 10	< 10	< 10	< 10	< 10	Plate counting method DIN EN ISO 21528-2: 2017–09
Coliforms (CFU/g)	< 10	< 10	< 10	< 10	< 10	Plate counting method ISO 4832: 2006–02
Staphylococcus aureus, coag. Pos. (CFU/g)	< 10	< 10	< 10	< 10	< 10	Plate counting method DIN EN ISO 6888-1: 2003–12
Salmonella (/25 g)	n.d.	n.d.	n.d.	n.d.	n.d.	Qualitative method § 64 LFGB L 00.00–20: 2018–03
E. coli (/10 g)	n.d.	n.d.	n.d.	n.d.	n.d.	Qualitative method ISO 7251: 2005–02
Heavy metals						
Lead (mg/kg)	< 0.020	< 0.020	< 0.020	< 0.020	< 0.020	ICP-MS: 2010–04 DIN EN 15763
Cadmium (mg/kg)	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	ICP-MS: 2010–04 DIN EN 15763
Mercury (mg/kg)	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	ICP-MS: 2010–04 DIN EN 15763
Arsenic (mg/kg)	< 0.040	< 0.040	< 0.040	< 0.040	< 0.040	ICP-MS: 2010–04 DIN EN 15763

NMR: nuclear magnetic resonance; Ph. Eur.: European Pharmacopoeia; LFGB: Lebensmittel und Futtermittelgesetzbuch, German Food and Feed Act; DIN: Deutsches Institut für Normung; ISO: International Organization for Standardization; cfu: colony forming unit; n.d.: not detected; TYMC: total yeast and mould count; ICP-MS: inductively coupled plasma mass spectrometry.

Information was provided on the accreditation of the laboratories that conducted the analyses presented in the application.

The Panel considers that the information provided on the composition is sufficient to characterise the NF.

3.4.1. Stability

The applicant performed stability tests with five independently produced batches of the NF. The tests were carried out in normal conditions at 25°C and 60% relative humidity (RH) for 24 months and in accelerated conditions at 40°C and at 75% RH for 6 months. The batches were analysed for microbial, chemical and sensory stability of cellobiose.

Under normal storage conditions, no degradation of cellobiose was observed over 24 months, and water activity ranged between 0.34 and 0.56. Under accelerated conditions over 6 months, concentrations of cellobiose remained ≥ 99%, and glucose concentration remained < 1%. The water activity reached a maximum value of 0.66 in the accelerated test conditions but remained low enough to inhibit microbial growth over the whole time of investigation.

Stability of cellobiose was also tested in 5% water solution at acidic pH (2.5 and 3) over 15 months and in real foods (9–10% in soft drinks, jams and jellies). Only minor variations in cellobiose and glucose content were observed, indicating a high stability of the NF.

The Panel considers that the data provided sufficient information with respect to the stability of the NF for up to 24 months.

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3.5. Specifications

The specifications of the NF are indicated in Table 3.

Table 3: Specifications of the NF

Description: Cellobiose is a disaccharide with two glucose monomers linked by a β-(1–4) glucosidic bond	
Source:	
Parameter	**Specification**
Appearance	White powder
General characteristics	
Cellobiose DM (%)	≥ 99
Moisture (%)	< 1
Other identified sugars (%)	≤ 1
Optical rotation $[\alpha]_D$ (c 10, water)	+33–36
Ash (g/100 g)	< 0.1
Protein content (g/100 g)	< 0.01
Heavy metals	
Arsenic	< 0.1 mg/kg
Microbiological	
Total aerobic count (cfu/g)	≤ 1,000
Yeast and moulds (cfu/g)	≤ 100
Salmonella (in 25 g)	n.d.
Coliforms (cfu/g)	≤ 10
E. coli (in 10 g)	n.d.

CFU: colony forming units; n.d.: not detected.

The Panel considers that the information provided on the specifications of the NF is sufficient and does not raise safety concerns.

3.6. History of use of the NF and/or of its source

3.6.1. History of use of the source

Sucrose and glucose are naturally occurring sugars abundantly present in vegetables, fruits and other sugar-rich foods such as honey or sugarcane. In isolated form, both have a long history of safe use as food ingredients.

3.6.2. History of use of the NF

Trace amounts of cellobiose have been found in honey and developing maize grains. Gentinetta et al. (1979) detected free cellobiose at levels of up to 0.05 mg/g in maize embryos and 0.06–0.13 mg/g in the endosperm. In honey, concentrations are in the same order (0.06–0.28 g/100 g honey (Sanz et al., 2004; de la Fuente et al., 2006)).

Pure cellobiose has not been used as food ingredient before.

3.7. Proposed uses and use levels and anticipated intake

3.7.1. Target population

The target population proposed by the applicant is the general population.

For use in food supplements, the target population proposed by the applicant is the general population excluding infants and young children below 3 years of age.

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3.7.2. Proposed uses and use levels

The NF is proposed to be used as an ingredient in several food products. These food products as defined using the FoodEx2[3] hierarchy, and their maximum use levels, are reported in Table 4.

The applicant also intends to market the NF for use in food supplements, at a maximum dose of 3 g per day, in the general population excluding infants and young children.

Table 4: Food categories and maximum use levels intended by the applicant

FoodEx2 level	FoodEx2 code	Food category	Max use level (g NF/100 g)
2	A022L	Animal meat dried	2
2	A024B	Canned-tinned meat	2
3	A022R	Raw cured (or seasoned) meat	2
3	A023G	Cooked cured (or seasoned) meat	2
3	A024G	Fresh raw sausages	2
3	A026K	Meat based spreadable-textured specialties	2
3	A026M	Liver based spreadable-textured specialties	2
3	A0EYP	Preserved or partly preserved sausages	2
3	A16GK	Savoury sauce dry preparation	40
3	A0F7V	Table-top sweeteners in powder form	60
3	A0F7X	Table-top sweeteners in tablets	60

3.7.3. Anticipated intake of the NF

EFSA performed an intake assessment of the anticipated daily intake of the NF based on the applicant's proposed uses and maximum proposed use levels (Table 4) using the EFSA Dietary Exposure (DietEx) Tool,[4] which is based on individual data from the EFSA Comprehensive European Food Consumption Database (EFSA, 2011[5]). The lowest and highest mean and 95th percentile anticipated daily intake of the NF (on a mg/kg body weight (bw) basis), among the EU dietary surveys, are presented in Table 5.

The estimated daily intake of the NF for each population group from each EU dietary survey is available in the Excel file annexed to this scientific opinion (under supporting information).

Table 5: Intake estimate resulting from the use of the NF as an ingredient in the intended food categories at the maximum proposed use levels per age class

Population group	Age (years)	Mean intake (mg/kg bw per day)		P95th intake (mg/kg bw per day)	
		Lowest[(a)]	Highest[(a)]	Lowest[(b)]	Highest[(b)]
Infants	< 1	0	21	0	121
Young children[(c)]	1 to < 3	4.1	49	21	137
Other children	3 to < 10	3.2	45	16	127
Adolescents	10 to < 18	6.5	26	21	79
Adults[(d)]	≥ 18	4.2	21	17	65

(a): Intakes are assessed for all EU dietary surveys available in the food comprehensive database on 29/08/2022. The lowest and the highest averages observed among all EU surveys are reported in these columns.
(b): Intakes are assessed for all EU dietary surveys available in the food comprehensive database on 29/08/2022. The lowest and the highest P95th observed among all EU surveys are reported in these columns (P95th based on less than 60 individuals are not considered).
(c): Referred as 'toddlers' in the EFSA food consumption comprehensive database (EFSA, 2011).
(d): Includes elderly, very elderly, pregnant and lactating women.

[3] FoodEx2 is an EFSA standardised food classification and description system https://www.efsa.europa.eu/en/data/data-standardisation
[4] https://www.efsa.europa.eu/it/science/tools-and-resources/dietex
[5] https://www.efsa.europa.eu/en/data-report/foodconsumption-data#the-efsa-comprehensive-european-food-consumption-database

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Table 6 presents the proposed maximum daily intake of the NF used as food supplement according to the applicant's proposed uses (3 g/day), expressed on a mg/kg bw per day basis.

Table 6: Use of the NF as food supplement and resulting intake expressed as mg/kg bw per day

Population group	Age (years)	Body weight[(a)] (kg)	Use level (mg/day)	Intake (mg/kg bw per day)[(b)]
Other children	3 to < 10	23.1	3,000	130
Young adolescents	10 to < 14	43.4	3,000	69
Old adolescents	14 to < 18	61.3	3,000	49
Adults	≥ 18	70	3,000	43

(a): Default and average body weights for each population group are available in EFSA Scientific committee, 2012.
(b): Intake in 'mg/kg bw per day' are calculated by considering the proposed maximum use levels in 'mg/day' and body weights defined in EFSA Scientific committee (2012).

Table 7 presents the total intake resulting from the uses of the NF both as ingredient and as food supplement.

Table 7: Total intake resulting from the uses of the NF as an ingredient and as a food supplement

Population group	Age (years)	Body weight[(a)] (kg)	Highest[(b)] P95th intake from the NF used as an ingredient (mg/kg bw per day)	Intake from the NF used as a food supplement (mg/kg bw per day)[(c)]	Total intake[(d)] (mg/kg bw per day)
Infants	< 1	5	121	N/A	121
Young children	1 to < 3	12	137	N/A	137
Other children	3 to < 10	23.1	127	130	257
Adolescents	10 to < 18	61.3	79	69	148
Adults	≥ 18	70	65	43	108

N/A: not appropriate.
(a): Default and average body weights are defined in EFSA Scientific committee, 2012.
(b): Intakes are assessed for all EU dietary surveys available in the food comprehensive database. The highest P95th observed among all surveys is reported in this column (P95th calculated based on less than 60 individuals are not considered).
(c): Intake in 'mg/kg bw per day' is calculated by considering the use levels in 'mg/d' and default body weights defined in EFSA, 2012.
(d): Total intake is the sum of the intake from NF ingredient use (maximum 95th percentile) and from the NF used as a food supplement, for each population group.

3.8. Absorption, distribution, metabolism and excretion (ADME)

3.8.1. Absorption

The applicant provided data from the literature on the gastrointestinal fate of cellobiose.

Cellobiose has been used in medical settings for several decades in the cellobiose/mannitol (Ce/Ma) test, a non-invasive technique for the investigation of intestinal permeability. Briefly, 5 g cellobiose and 2 g mannitol are taken orally after a 6-h fast in a hypertonic solution consisting of 20 g lactose and 20 g sucrose in 100 mL water. After 5 h, a urinary sample is collected and analysed for both probe molecules. Results are expressed as a ratio of the percentage recovery of cellobiose over that of mannitol. Studies conducted in healthy volunteers, summarised in Appendix A, consistently report average recovery levels ranging from 0.32 to 0.54% cellobiose (Cobden et al., 1978; Cobden et al., 1980; Hamilton et al., 1982; Strobel et al., 1984; Generoso et al., 2003).

These studies also consistently report higher recovery levels of cellobiose in patients suffering from various gastrointestinal disorders (Appendix A), such as villous atrophy (Cobden et al., 1978; Cobden et al., 1980; Hamilton et al., 1982), coeliac disease (Cobden et al., 1978; Cobden et al., 1980; Hamilton et al., 1982; Strobel et al., 1984), irritable bowel syndrome and Crohn's disease (Strobel et al., 1984).

Additionally, Cobden et al. showed that 92 ± 11.6% cellobiose was recovered in the urine of five healthy volunteers within 10 h of intravenous injection with 50 mg cellobiose, indicating no major systemic metabolism of cellobiose in humans (Cobden et al., 1985).

Based on the high excretion in the faeces, the Panel concludes that cellobiose is not absorbed as such in significant quantities by the human intestine.

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3.8.2. Metabolism

Studies investigating the metabolism of cellobiose are summarised in Appendix B.

3.8.2.1. *In vitro* models

The applicant sponsored experiments to investigate the digestibility of the NF. Both experiments were carried out with appropriate controls.

In a first experiment, the resistance of cellobiose to gastric acidity and digestive enzymes was investigated (Unpublished report, 2013). For that purpose, cellobiose at a concentration of 1.5 g/L was serially incubated (i) at pH 2 with 0.2% pepsin at 40°C for 45 min to simulate gastric conditions, (ii) at pH 6.8 with 0.2% porcine pancreatin at 40°C for 2 h to simulate intestinal conditions. The quantification of sugars did not reveal the presence of reducing sugars or glucose other than cellobiose (found at a concentration of 1.4 g/L) in incubated samples. These data suggest that cellobiose is not hydrolysed under the test conditions and can resist acidic conditions and endogenous digestive enzymes.

A second experiment investigated the resistance of cellobiose to brush border cell-derived enzymes and intestinal absorption. For that purpose, differentiated human colorectal carcinoma (Caco-2) cells grown on a permeable microporous membrane were used as an intestinal epithelium model. Sugar concentrations in the apical and basolateral sides of the cells were measured after 2 h of incubation at 37°C with 15 μg/mL cellobiose. Quantification revealed that 97% cellobiose was recovered in the apical compartment, while none was found in the basolateral compartment, demonstrating that cellobiose is not hydrolysed and transported through the brush-border cells.

3.8.2.2. Rodent models

Ileorectostomised rats fed a diet containing 6% cellobiose in drinking water for 7 days displayed a 36.2% cellobiose faecal recovery rate, suggesting that about 65% is digested in the small intestine (Morita et al., 2008). The same recovery rate was found when neomycin was added to the diet, indicating that the uptake was not due to bacterial activity in the small intestine. This indicates that cellobiose is metabolised in the rat small intestine, likely by β-glucosidases.

The same authors investigated the digestibility of cellobiose (*i.e.*, glucose release) in the mucosa of the small intestine of adult and suckling rats *in vitro* and compared it with lactose digestibility. Cellobiase activity was about half that of lactase, and about threefold higher in suckling rats than in adult rats. As the same pattern was observed for lactase, it was concluded that lactase may be responsible for cleaving cellobiose.

3.8.2.3. Human studies

In humans, several *in vitro* studies from 1962 to 1987, report on cellobiase activity of lactase ranging from 11 to 19% of lactase activity (Dahlqvist, 1962; Gray and Santiago, 1969; Skovbjerg et al., 1981; Lau, 1987).

In a study investigating the reliability of the Ce/Ma test (as described in Section 3.8.1), Strobel et al. reported no difference in the cellobiose recovery rate between five lactase-deficient and fifteen healthy volunteers, suggesting that human intestinal lactase does not influence cellobiose absorption or metabolism (Strobel et al., 1984). The Panel, however, notes that two of the five lactase-deficient subjects reported loose bowel motions 6–8 h after ingestion of the solution, suggesting that lactase may play a role in cellobiose metabolism.

In a study conducted in Japan, Nakamura et al. reported that a single ingestion of 25 g cellobiose in ten healthy women did not produce any increase in blood sugar or insulin secretion up to 3 h after the intake (Nakamura et al., 2004). The prevalence of lactase insufficiency in the age-related primary form is higher in African and Asian populations than in European populations due to genetic polymorphism. In the absence of information about the lactase insufficiency status of the subjects, it is unclear whether the results from this study can be transferred to a European population, for which a higher level of intestinal lactase may lead to cellobiose digestion to glucose.

The Panel considers that the available evidence is insufficient to conclude on whether, or to what extent, cellobiose may be hydrolysed in the small intestine.

3.8.3. Fermentation

The fermentability of cellobiose by the human microbiota is substantiated by several studies (Nakamura et al., 2004; Gill et al., 2006; Andersen et al., 2012; Cantarel et al., 2012; van Zanten

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et al., 2012; Andersen et al., 2013; van Zanten et al., 2014; van Zanten et al., 2015; Ilhan et al., 2017; Magnusdottir et al., 2017). The Panel considers that cellobiose fermentation in the gut is not of concern.

3.9. Nutritional information

It remains unclear whether and to which extent cellobiose may be hydrolysed in the small intestine, to what extent fermentation by human microbiota takes place, and the extent to which short-chain fatty acids are produced and enter the circulation for energy supply to the host.

However, considering the source and nature of the NF, the Panel considers that the NF is not nutritionally disadvantageous.

3.10. Toxicological information

The applicant provided five toxicological studies on the NF, which were conducted in compliance with OECD (Organisation for Economic Co-operation and Development) principles of good laboratory practice (GLP) (OECD, 1998) and in accordance with the test guidelines (TG) No 471 (OECD, 1997), 487 (OECD, 2014) and 408 (OECD, 1998). These studies, which were claimed proprietary by the applicant, are listed in Table 8.

Table 8: List of toxicological studies with the NF

Reference	Type of study	Test system	Dose
Unpublished report (2017d); Messinger et al. (2020)	Bacterial reverse mutation test (GLP, OECD TG 471)	*S. Typhimurium* TA98, TA100, TA1535 and TA1537, *E. coli* WP2 uvrA [pKM101]	Up to 5,000 μg/plate (with and without metabolic activation)
Unpublished report (2017c); Messinger et al. (2020)	*In vitro* mammalian cell micronucleus test (GLP, OECD TG 487)	Cultured human peripheral lymphocytes	Up to 2,000 μg/mL (with and without metabolic activation)
Unpublished report (2017a); Winkler et al. (2020)	28-day dose-range finding study (GLP)	Sprague Dawley rats	0, 5, 10, 15% in drinking water
Unpublished report (2017b); Winkler et al. (2020)	90-day repeated dose oral toxicity study with a 28-day recovery period (GLP, OECD TG 408)	Sprague Dawley rats	0, 2.5, 5, 10% in drinking water
Unpublished report (2018); More et al. (2019)	Dose-escalation study (WMA, CPMP/ICH/135/95, ICH E6)[(a)]	Healthy subjects	Single dose of 10, 15, 20 or 25 g, or two doses of 15 or 20 g/day for 14 days

(a): Study performed in compliance with the principles of the World Medical Association (Declaration of Helsinki) as well as the EU recommendations for Good Clinical Practice (CMPH/ICH/135/95), ICH E6.

3.10.1. Genotoxicity

The applicant investigated the mutagenicity of the NF with a bacterial reverse mutation test according to OECD TG 471 (Unpublished report, 2017d; Messinger et al., 2020). The NF was tested using four *Salmonella Typhimurium* strains (TA98, TA100, TA1535, and TA1537) and one *Escherichia coli* strain (WP2 uvrA [pkM101]) in two independent experiments, both carried out with and without metabolic activation with a microsomal preparation derived from Aroclor 1254-induced rat liver.

The cytotoxicity of the NF was investigated in a preliminary plate incorporation test with the Salmonella Typhimurium strain TA100. No signs of cytotoxicity were noted after exposure to ten different concentrations of cellobiose ranging from 0.316 to 5,000 μg/plate. In the main mutagenicity test, no increase in revertant colony numbers as compared with control counts was observed for the NF tested at six concentrations ranging from 31.6 to 5,000 μg/plate, with all strains tested, with or without metabolic activation.

The applicant further investigated the genotoxicity of the NF with an *in vitro* mammalian cell micronucleus test according to OECD TG 487 (Unpublished report, 2017c; Messinger et al., 2020).

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Cellobiose samples were tested using human peripheral lymphocytes, both with and without metabolic activation by a rat liver post-mitochondrial fraction (S9 mix) from Aroclor 1254 induced animals.

The cytotoxicity of the NF was investigated in a preliminary experiment with and without metabolic activation at concentrations ranging from 3.14 to 2000 μg/mL. No cytotoxic effects were observed up to 2000 μg/mL. The main genotoxicity experiment showed no increase in the frequency of micronucleated cells after exposure to concentrations of cellobiose ranging from 125 to 2000 μg/mL in the presence or absence of metabolic activation, suggesting the absence of chromosomal damage following exposure to the NF.

Taking into account the test results provided and considering the nature, source and production process of the NF, the Panel considers that there are no concerns regarding genotoxicity.

3.10.2. Subacute toxicity

The applicant carried out a 28-day dose-range finding study by repeated oral administration of the NF (Unpublished report, 2017a; Winkler et al., 2020). Briefly, four groups of three male and three female Crl:CD(SD) (Sprague Dawley) rats were exposed to 0, 5, 10 and 15% cellobiose via the drinking water. The calculated mean cellobiose intake was 3.95 (♂) - 5.13 (♀), 6.95 (♂) - 6.85 (♀), and 8.10 (♂) – 8.40 (♀) g/kg bw per day in the 5, 10 and 15% exposure groups, respectively.

Notable observations included:

- A reduction in body weight in male rats in the 10% (by approximately 10–12% until day 28, not significant) and 15% groups (up to 26.6% below the control group, solely statistically significant at test day 8 but sustained until day 28).
- A decrease in body weight in female rats from the 15% group (6.9–12% below the control until day 28), including significant values at test day 8 and 15.
- A significant decrease in food intake in male rats from the 10% group during the first (23.3% below the control) and third test week (14.1% below control), and in male rats from the 15% group (38.7, 29.0 and 15.5% below control on week 1, 2 and 3, respectively).
- A dose-dependent decrease in water consumption in rats of up to 21.1% in males and 27.2% in females in the 10% group; in the 15% groups, this decrease reached 51.1% in males and 41.4% in females.
- A significant decrease in absolute heart weight in males exposed to 5 and 10% cellobiose (−18.3 and −30.8%, respectively).
- Non-dose-dependent statistically significant modifications in haematological parameters.

After consideration of these data, it was suggested to have 10% cellobiose in drinking water as the highest dose for the 90-day study (*c.f.* Section 3.10.3).

Further to a literature search, the applicant also provided a study investigating the effects of feeding diets containing cellobiose on the dry weights of cleaned gastrointestinal organs in the rat (Moinuddin and Lee, 1958). Briefly, weanling male SD rats were fed a diet supplemented with 15% cellobiose (as compared to a basal cornstarch diet) ad libitum for 4 weeks. The body weight gains of cellobiose-fed rats after 4 weeks were significantly lower than the basal diet control (−16%). Severe or moderate diarrhoea occurred almost every day in each of the cellobiose-fed rats during the first 2 weeks. After that, the frequency and severity of diarrhoea tapered off gradually. At the time of killing the rats, diarrhoea had disappeared in four of the six rats and was only slight in the other two rats. The weight of the small intestine relative to body weight (+22%) and both the absolute (+18%) and relative (+41%) weights of the caecum and colon plus rectum were significantly greater in cellobiose-treated rats.

3.10.3. Subchronic toxicity

The applicant investigated the toxicity of cellobiose in a 90-day repeated dose oral toxicity study according to OECD TG 408 (OECD, 1998) (Unpublished report, 2017b; Winkler et al., 2020). Briefly, four groups of 10 male and 10 female SD rats were exposed to either 0 (tap water vehicle), 2.5, 5 or 10% cellobiose in drinking water ad libitum for 90 days. Additionally, five male and five female rats were exposed to either 0 or 10% cellobiose in drinking water for 90 days, followed by a 28-day recovery period. The calculated mean cellobiose intake was 1.93 (♂) - 2.41 (♀), 3.82 (♂) - 4.95(♀) and 6.85 (♂) - 8.04 (♀) g/kg bw per day in the 2.5, 5 and 10% exposure groups, respectively.

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Notable observations included:

- In male rats, a dose-related decrease in body weight starting with 5% cellobiose (up to 6% below the control as of test day 15), statistically significant in the 10% cellobiose group (5–7% below the control group as of test day 8). The body weight of the male animals previously treated with 10% cellobiose for 90 days was still marginally reduced during the recovery period. The body weight gain revealed a tendency towards normalisation over the course of the treatment.
- A dose-related decrease of food consumption: (i) in female rats from the low-dose group (5–7% below the control group); (ii) in both male (5–10%) and female (8–13%) rats from the middle-dose group; (iii) in both male (9–17%) and female (10–22%) rats from the high-dose group.
- Occasional, statistically significant reductions in daily drinking water consumption of the male rats from the 5% group on few test days as of test week 2 (up to 19% below the control animals)
- Statistically significant decreases in daily drinking water consumption, up to 34% and 33% in males and females, respectively, in the high-dose group compared to the control group. The weekly average drinking water consumption was decreased in a statistically significant manner (up to 16% or 26% below the control group) in male and female rats from that same group.
- The values for food and drinking water consumption of the previously high dosed animals increased again to or slightly above the values consumed by the control group during the recovery period.

Following a request from EFSA, the applicant provided an analysis of food efficiency and caloric intakes. The food efficiency ratio did not show any statistically significant difference between groups. The total caloric intake (feed and drinking water) over the exposure period of all treated female and of the male rats from the middle- and high-dose groups was reduced by up to 7.6% in a dose-related way in comparison to the control group. The caloric intake via drinking water (up to 8.4% of the overall caloric intake, based on an energy content of 2 kcal/g as proposed by the applicant, *i.e.*, 8.368 kJ/g cellobiose) in the groups exposed to the test item partially compensated the lower caloric intake via food. The mean differences in the total caloric intake in comparison to the control group indicated a tendency towards normalisation during the exposure. This indicates that the effect observed is non-adverse and thus provides an explanation for the reduced food intake.

The Panel considers that the NF did not cause adverse effects up to the highest dose tested (*i.e.*, 6.85 g/kg bw per day in male and 8.04 g/kg bw per day in female rats). The Panel notes that the decision to apply the treatment via drinking water, which according to the applicant facilitated a homogeneous distribution of the test item compared to a feed-test item mixture, inherently limited the dose testing range.

3.10.4. Human data

The applicant carried out a literature search on human trials using cellobiose as test substance which yielded three studies (summarised in Table 10).

In van Zanten et al. (2014), 18 healthy subjects were enrolled in a double-blinded, randomised and placebo-controlled cross-over study, where they received 5 g cellobiose together with 10^9 CFU *Lactobacillus acidophilus* daily for 3 weeks (van Zanten et al., 2014). The majority of volunteers reported overall well-being as 'neither good nor bad', 'good' or 'very good' in both the placebo and symbiotic treatment groups, and self-reported gastrointestinal symptoms did not differ between groups.

As described in the ADME section, Nakamura et al. also investigated the effects of a single intake of 25 g cellobiose in 10 healthy women. No abdominal symptoms or side effects were reported (Nakamura et al., 2004).

The applicant also provided a dose escalation study with the NF (Unpublished report, 2018; More et al., 2019). The experimental design was two-phased: (i) a single-ascending dose (SAD) phase during which 24 volunteers received a single daily dosing of 10, 15, 20 or 25 g cellobiose (six subjects/dose) and (ii) a multiple-ascending dose (MAD) phase with two 15 or 20 g cellobiose dosing a day (12 subjects/dose) for 14 days. Subjects presented no changes in body weight and BMI and no clinically relevant changes in vital signs (blood pressure and pulse rate). In the SAD phase, there were no significant differences in bowel movement frequency and stool consistency between groups, and there were no statistically significant differences in the total gastrointestinal symptom rating scale (GSRS) score.

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In the MAD phase, however, stool consistency was significantly softer and GSRS scores were significantly higher after the consumption of 20 g of cellobiose twice a day compared to 15 g twice a day.

No serious adverse events (AEs) were reported. Reported AEs mainly affected the gastrointestinal system (*e.g.*, flatulence, borborygmus and diarrhoea). Data on AEs are summarised below (Table 9):

Table 9: Adverse events as reported in More et al. (2019)

	SAD phase				MAD phase	
	10 g	**15 g**	**20 g**	**25 g**	**2 × 15 g**	**2 × 20 g**
Number of subjects reporting AEs	0/6	3/6	1/6	3/6	5/12	8/12
Number of AEs	0	5	4	7	7	21
Intensity of AEs						
Light	N/A	5	4	1	6	9
Moderate	N/A	0	0	5	1	12
Severe	N/A	0	0	1	0	0
Causality to the intake of the NF						
Unlikely	N/A	5	0	0	6	6
Possible	N/A	0	4	1	1	2
Probable	N/A	0	0	6	0	13

SAD: single-ascending dose; MAD: multiple-ascending dose; AEs: adverse events; N/A: not appropriate.

In the SAD phase, global tolerability to single daily doses of 10, 15 and 20 g of the NF was evaluated by all study subjects as 'very good' or 'good'. In the MAD phase, global tolerability to two daily doses of 15 g of the NF was evaluated as only 'moderate' by 8.3% of the subjects.

Based on the study results, reporting mild gastrointestinal symptoms in one of six subjects after consumption of 20 g per day of the NF, the Panel considers that the consumption of 20 g per day of cellobiose (equivalent to 290 mg/kg bw per day in a 70-kg adult[6]) does not raise concern regarding gastrointestinal tolerability. The Panel notes that intakes higher than 20 g per day of the NF may increase the risk of adverse gastrointestinal effects in humans.

Table 10: Summary of human study protocols

Reference	Study Design	Study Population	Study duration	Doses; route of administration	Safety-related parameters investigated
van Zanten et al. (2014)	Double-blind, randomised, placebo-controlled cross-over study	18 healthy subjects (10♀, 8♂); 20–30 years	3 weeks 3-week washout 3-week cross experiment	5 g cellobiose + 10^9 CFU lyophilised L. acidophilus; oral ingestion Placebo: maltodextrin Matsutani Chemical Industry Co, Japan; 97% cellobiose	– Faecal samples – Overall health and well-being – Defecation frequency and consistency – Gastrointestinal symptoms

[6] EFSA Scientific Committee; Guidance on selected default values to be used by the EFSA Scientific Committee, Scientific Panels and Units in the absence of actual measured data. EFSA Journal 2012;10(3):2579, 32 pp. https://doi.org/10.2903/j.efsa.2012.2579

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Reference	Study Design	Study Population	Study duration	Doses; route of administration	Safety-related parameters investigated
Nakamura et al. (2004)	Tolerance and breath hydrogen excretion test	10 healthy subjects (♀); 20.5 ± 2.1 years	Single occasion	25 g cellobiose; oral ingestion Control: glucose Matsutani Chemical Industry Co, Japan; 97% cellobiose	– Tolerance test – Breath hydrogen tests – Blood collection – Recording of adverse events
Unpublished report (2018); More et al. (2019)	Single-arm, monocentric, dose-escalation, nutritional study	48 healthy Caucasian subjects (30♀, 18♂); 40.5 ± 12.4 years	Single ascending dose (24 subjects; 6 subjects/dose)	Single cellobiose dosing of 10, 15, 20 or 25 g/day dissolved in hot herbal infusion	– Stool consistency – Gastrointestinal well-being (Gastrointestinal Symptom Rating Scale) – Recordings of adverse events
			14 days, multiple ascending doses (24 subjects; 12 subjects/dose)	Cellobiose dosing of 15 or 20 g dissolved in hot herbal infusion twice daily (≥8 h in between) Savanna Ingredients GmbH	

3.11. Allergenicity

The Panel considers that, owing to the low amount of protein present, the NF is unlikely to trigger allergic reactions in the target population under the proposed conditions of use.

4. Discussion

The NF which is the subject of the application is cellobiose, a disaccharide consisting of two glucose units linked by a β-(1–4) glucosidic bond, produced by a two-step enzymatic conversion from sucrose and glucose. The applicant intends to market the NF as an ingredient in a number of food products, and as food supplement in individuals aged 3 years and above at levels of 3 g per day.

The highest 95th percentile anticipated daily intake of the NF when used as a food ingredient was calculated for young children at 137 mg NF/kg bw per day. Consumption of the NF as food supplement leads to a highest intake estimate of 130 mg NF/kg bw per day in children aged 3–10 years.

The genotoxicity and subchronic toxicity studies carried out with the NF did not raise safety concerns. In the repeated dose 90-day oral toxicity study in rodents, the NF did not cause adverse effects up to the highest dose tested (*i.e.*, 6,850 mg/kg bw per day in males and 8,000 mg/kg bw per day in females). However, the Panel notes that the dose testing range was limited by the mode of application of the treatment (*i.e.*, drinking water).

The applicant also provided a human dose-escalation study. Based on the study results, the Panel considers that the consumption of 20 g per day of cellobiose (equivalent to 290 mg/kg bw per day in a 70-kg adult) does not raise concern regarding gastrointestinal tolerability. The Panel notes that intakes higher than 20 g per day of the NF may increase the risk of adverse gastrointestinal effects in humans.

The Panel notes that the intake estimates resulting from the use of the NF as food ingredient and as food supplement, both individually or in combination, are below 290 mg/kg bw per day.

Considering the source, compositional characterisation, production process and nature of the NF, as well as the toxicological data provided on the NF, the Panel considers that the NF does not raise safety concerns under the proposed conditions of use.

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5. Conclusions

The Panel concludes that the NF, cellobiose, is safe under the proposed conditions of use.

5.1. Protection of proprietary data in accordance with Article 26 of Regulation (EU) 2015/2283

The Panel could not have reached the conclusion on the safety of the NF under the proposed conditions of use without the data claimed as proprietary by the applicant (information about the identity, production process, compositional data, and genotoxicity, subchronic toxicity and human studies).

6. Steps taken by EFSA

1) On 23/09/2020, EFSA received a letter from the European Commission with the request for a scientific opinion on the safety of Cellobiose [Ref. Ares(2020)4973916].
2) On 23/09/2020, a valid application on Cellobiose, which was submitted by name of the company, was made available to EFSA by the European Commission through the Commission e-submission portal (NF 2020/1805) and the scientific evaluation procedure was initiated.
3) On 18/01/2021, EFSA requested the applicant to provide additional information to accompany the application and the scientific evaluation was suspended.
4) On 5 July 2021, additional information was provided by the applicant through the Commission e-submission portal and the scientific evaluation was restarted.
5) On 18/02/2022, EFSA requested the applicant to provide additional information to accompany the application and the scientific evaluation was suspended.
6) On 13/04/2022, additional information was provided by the applicant through the Commission e-submission portal and the scientific evaluation was restarted.
7) During its meeting on 28/09/2022, the NDA Panel, having evaluated the data, adopted a scientific opinion on the safety of Cellobiose as a NF pursuant to Regulation (EU) 2015/2283.

References

Andersen JM, Barrangou R, Hachem MA, Lahtinen SJ, Goh YJ, Svensson B and Klaenhammer TR, 2012. Transcriptional analysis of prebiotic uptake and catabolism by Lactobacillus acidophilus NCFM. PLoS One, 7, e44409. https://doi.org/10.1371/journal.pone.0044409

Andersen JM, Barrangou R, Abou Hachem M, Lahtinen SJ, Goh YJ, Svensson B and Klaenhammer TR, 2013. Transcriptional analysis of oligosaccharide utilization by Bifidobacterium lactis Bl-04. BMC Genomics, 14, 312. https://doi.org/10.1186/1471-2164-14-312

Brucher B and Häßler T, 2019. Enzymatic process for the synthesis of cellobiose. Industrial Enzyme Applications, Wiley-VCH, Weinheim, Germany. pp. 167–178.

Cantarel BL, Lombard V and Henrissat B, 2012. Complex carbohydrate utilization by the healthy human microbiome. PLoS One, 7, e28742. https://doi.org/10.1371/journal.pone.0028742

Cobden I, Dickinson RJ, Rothwell J and Axon AT, 1978. Intestinal permeability assessed by excretion ratios of two molecules: results in coeliac disease. Br Med J, 2, 1060. https://doi.org/10.1136/bmj.2.6144.1060

Cobden I, Rothwell J and Axon AT, 1980. Intestinal permeability and screening tests for coeliac disease. Gut, 21, 512–518. https://doi.org/10.1136/gut.21.6.512

Cobden I, Hamilton I, Rothwell J and Axon AT, 1985. Cellobiose/mannitol test: physiological properties of probe molecules and influence of extraneous factors. Clin Chim Acta, 148, 53–62. https://doi.org/10.1016/0009-8981(85)90300-6

Dahlqvist A, 1962. Specificity of the human intestinal disaccharidases and implications for hereditary disaccharide intolerance. J Clin Invest, 41, 463–470. https://doi.org/10.1172/JCI104499

Dibusz K and Vejvodova P, 2020. Systematic literature search to assist EFSA in the preparatory work for the safety assessment of Novel Food applications and Traditional Food notifications. EFSA Supporting Publications, 17, 1774E. https://doi.org/10.2903/sp.efsa.2019.EN-1774

EFSA Scientific Committee, 2012. Guidance on selected default values to be used by the EFSA Scientific Committee, Scientific Panels and Units in the absence of actual measured data. EFSA Journal 2012;10(3):2579, 32 pp. https://doi.org/10.2903/j.efsa.2012.2579

EFSA NDA Panel (EFSA Panel on Nutrition, Novel Foods and Food Allergens), Turck D, Bresson J-L, Burlingame B, Dean T, Fairweather-Tait S, Heinonen M, Hirsch-Ernst KI, Mangelsdorf I, McArdle H, Naska A, Neuhäuser-Berthold M, Nowicka G, Pentieva K, Sanz Y, Siani A, Sjödin A, Stern M, Tomé D, Vinceti M, Willatts P, Engel K-H, Marchelli R, Pöting A, Poulsen M, Salminen S, Schlatter J, Arcella D, Gelbmann W, de Sesmaisons-Lecarré A, Verhagen H and van Loveren H, 2016. Guidance on the preparation and presentation of an application for authorisation of a novel food in the context of Regulation (EU) 2015/2283. EFSA Journal 2016;14:e04594, 24 pp. https://doi.org/10.2903/j.efsa.2016.4594

18314732, 2022, 11, Downloaded from https://efsa.onlinelibrary.wiley.com/doi/10.2903/j.efsa.2022.7596 by University Of Aberdeen, Wiley Online Library on [19/04/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

de la Fuente E, Sanz ML, Martinez-Castro I and Sanz J, 2006. Development of a robust method for the quantitative determination of disaccharides in honey by gas chromatography. J Chromatogr A, 1135, 212–218. https://doi.org/10.1016/j.chroma.2006.09.035

Generoso M, De Rosa M, De Rosa R, De Magistris L, Secondulfo M, Fiandra R, Carratu R and Carteni M, 2003. Cellobiose and lactulose coupled with mannitol and determined using ion-exchange chromatography with pulsed amperometric detection, are reliable probes for investigation of intestinal permeability. J Chromatogr B Analyt Technol Biomed Life Sci, 783, 349–357. https://doi.org/10.1016/s1570-0232(02)00766-3

Gentinetta E, Zambello M and Salamini F, 1979. Free sugar in developing maize grain. Annali dell' Istituto Sperimentale per la Cerealicoltura, Roma (Italy). Cereal Chemistry, v. 56(2) p. 81–83. ISSN : 0374-535X

Gill SR, Pop M, Deboy RT, Eckburg PB, Turnbaugh PJ, Samuel BS, Gordon JI, Relman DA, Fraser-Liggett CM and Nelson KE, 2006. Metagenomic analysis of the human distal gut microbiome. Science, 312, 1355–1359. https://doi.org/10.1126/science.1124234

Gray GM and Santiago NA, 1969. Intestinal beta-galactosidases. I. Separation and characterization of three enzymes in normal human intestine. J Clin Invest, 48, 716–728. https://doi.org/10.1172/JCI106029

Hamilton I, Cobden I, Rothwell J and Axon AT, 1982. Intestinal permeability in coeliac disease: the response to gluten withdrawal and single-dose gluten challenge. Gut, 23, 202–210. https://doi.org/10.1136/gut.23.3.202

Ilhan ZE, Marcus AK, Kang DW, Rittmann BE and Krajmalnik-Brown R, 2017. pH-mediated microbial and metabolic interactions in fecal enrichment cultures. mSphere, 2, 17 pp. https://doi.org/10.1128/mSphere.00047-17

Lau HK, 1987. Physicochemical characterization of human intestinal lactase. Biochem J, 241, 567–572. https://doi.org/10.1042/bj2410567

Magnusdottir S, Heinken A, Kutt L, Ravcheev DA, Bauer E, Noronha A, Greenhalgh K, Jager C, Baginska J, Wilmes P, Fleming RM and Thiele I, 2017. Generation of genome-scale metabolic reconstructions for 773 members of the human gut microbiota. Nat Biotechnol, 35, 81–89. https://doi.org/10.1038/nbt.3703

Messinger H, Winkler A and Bar A, 2020. Genotoxic potential of cellobiose. Regul Toxicol Pharmacol, 111, 104554. https://doi.org/10.1016/j.yrtph.2019.104554

Moinuddin JF and Lee HW, 1958. Effects of feeding diets containing sucrose, cellobiose or glucose on the dry weights of cleaned gastrointestinal organs in the rat. Am J Physiol, 192, 417–420. https://doi.org/10.1152/ajplegacy.1958.192.2.417

More MI, Postrach E, Bothe G, Heinritz S and Uebelhack R, 2019. A dose-escalation study demonstrates the safety and tolerability of cellobiose in healthy subjects. Nutrients, 12, 64 pp. https://doi.org/10.3390/nu12010064

Morita T, Ozawa M, Ito H, Kimio S and Kiriyama S, 2008. Cellobiose is extensively digested in the small intestine by beta-galactosidase in rats. Nutrition, 24, 1199–1204. https://doi.org/10.1016/j.nut.2008.06.029

Nakamura S, Oku T and Ichinose M, 2004. Bioavailability of cellobiose by tolerance test and breath hydrogen excretion in humans. Nutrition, 20, 979–983. https://doi.org/10.1016/j.nut.2004.08.005

OECD, 1997. Test No. 471: Bacterial Reverse Mutation Test, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris. https://doi.org/10.1787/9789264078536-en

OECD, 1998. OECD Principles on Good Laboratory Practice, OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring, No. 1, OECD Publishing, Paris.

OECD, 2014. Test No. 487: In Vitro Mammalian Cell Micronucleus Test, OECD Publishing, Paris. https://doi.org/10.1787/9789264224438-en

Sanz ML, Sanz J and Martinez-Castro I, 2004. Gas chromatographic-mass spectrometric method for the qualitative and quantitative determination of disaccharides and trisaccharides in honey. J Chromatogr A, 1059, 143–148. https://doi.org/10.1016/j.chroma.2004.09.095

Skovbjerg H, Sjostrom H and Noren O, 1981. Purification and characterisation of amphiphilic lactase/phlorizin hydrolase from human small intestine. Eur J Biochem, 114, 653–661. https://doi.org/10.1111/j.1432-1033.1981.tb05193.x

Strobel S, Brydon WG and Ferguson A, 1984. Cellobiose/mannitol sugar permeability test complements biopsy histopathology in clinical investigation of the jejunum. Gut, 25, 1241–1246. https://doi.org/10.1136/gut.25.11.1241

Unpublished report, 2013. Evaluation of prebiotic properties of cellobiose. Adisseo, Centre of Expertise and Research in Nutrition. Unpublished document.

Unpublished report, 2017a. 28-day dose-range-finding study of cellobiose by repeated oral administration via the drinking water in rats, Laboratory of Pharmacology and Toxicology GmbH. Study no. 32941. Unpublished document.

Unpublished report, 2017b. 90-day repeated dose toxicity study of cellobiose by oral administration via the drinking water in rats, Laboratory of Pharmacology and Toxicology GmbH. Study no. 32942. Unpublished document.

Unpublished report, 2017c. In vitro assessment of cellobiose in the micronucleus test in cultured human peripheral lymphocytes according to OECD guideline 487, Laboratory of Pharmacology and Toxicology GmbH. Study no. 32944. Unpublished document.

Unpublished report, 2017d. Mutagenicity study of cellobiose in the Salmonella typhimurium and Escherichia coli reverse mutation assay (in vitro) according to Regulation (EC) No. 440/2008 method B.13/14 and OECD Guideline 471, Laboratory of Pharmacology and Toxicology GmbH. Study no. 32943. Unpublished document.

Unpublished report, 2018. Dose-escalation study to evaluate safety and tolerability of cellobiose in healthy subjects, analyze & realize GmbH. Study no. PLA/012617. Unpublished document.

18314732, 2022, 11, Downloaded from https://efsa.onlinelibrary.wiley.com/doi/10.2903/j.efsa.2022.7596 by University Of Aberdeen, Wiley Online Library on [19/04/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Winkler A, Messinger H and Bar A, 2020. Subchronic (91-day) oral toxicity study of cellobiose in rats. Regul Toxicol Pharmacol, 110, 104518. https://doi.org/10.1016/j.yrtph.2019.104518

van Zanten GC, Knudsen A, Roytio H, Forssten S, Lawther M, Blennow A, Lahtinen SJ, Jakobsen M, Svensson B and Jespersen L, 2012. The effect of selected synbiotics on microbial composition and short-chain fatty acid production in a model system of the human colon. PLoS One, 7, e47212. https://doi.org/10.1371/journal.pone.0047212

van Zanten GC, Krych L, Roytio H, Forssten S, Lahtinen SJ, Abu Al-Soud W, Sorensen S, Svensson B, Jespersen L and Jakobsen M, 2014. Synbiotic Lactobacillus acidophilus NCFM and cellobiose does not affect human gut bacterial diversity but increases abundance of lactobacilli, bifidobacteria and branched-chain fatty acids: a randomized, double-blinded cross-over trial. FEMS Microbiol Ecol, 90, 225–236. https://doi.org/10.1111/1574-6941.12397

van Zanten GC, Sparding N, Majumder A, Lahtinen SJ, Svensson B and Jacobsen S, 2015. The differential proteome of the probiotic lactobacillus acidophilus NCFM grown on the potential prebiotic cellobiose shows upregulation of two beta -glycoside hydrolases. Biomed Res Int, 2015, 347216. https://doi.org/10.1155/2015/347216

Abbreviations

13C-NMR	carbon-13 nuclear magnetic resonance
1H-NMR	proton nuclear magnetic resonance
ADME	Absorption, distribution, metabolism and excretion
AEs	Adverse events
BMI	Body Mass Index
bw	Body weight
CAS	Chemical Abstracts Services
CCP	Critical control point
CD	Coeliac disease
Ce	Cellobiose
CFU	Colony Forming Units
COSY	Correlation spectroscopy
CP	Cellobiose phosphorylase
DIN	Deutsches Institut für Normung
DNA	Deoxyribonucleic acid
FAIM	Food Additive Intake Model
G1P	Glucose-1-phosphate
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice
GSRS	Gastrointestinal symptom rating scale
HACCP	Hazard Analysis Critical Control Points
HMBC	Heteronuclear multiple bond correlation
IBS	Irritable bowel syndrome
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
ISO	International Organization for Standardization
IUPAC	International Union of Pure and Applied Chemistry
LGFB	Lebensmittel und Fiuttermittelgesetsbuch, German Food and Feed Act
Ma	Mannitol
MAD	Multiple-ascending dose
n.d.	Not detected
NDA	EFSA Panel on Nutrition, Novel Foods and Food Allergens
NF	Novel food
NMR	Nuclear magnetic resonance
OECD	Organisation for Economic Co-operation and Development
Ph. Eur.	European Pharmacopoeia
RH	Relative humidity
SAD	Single-ascending dose
SD	Sprague Dawley
SP	Sucrose phosphorylase
TG	Test guideline
TYMC	Total yeast and mould count
UCT	University of Chemistry and Technology (Prague)

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Appendix A – Summary of studies on cellobiose excretion in the urine

Reference	Species	Doses	Design	Urinary recovery[(a)] (%)
Cobden et al. (1978)	Human	5 g cellobiose + 2 g mannitol in 100 mL (+ 20 g sucrose and 20 g lactose)	16 patients with normal jejunal histological appearances (control group)	0.02–0.62
			10 untreated adults with villous atrophy	0.34–2.76
			11 treated patients with CD	Data not given
Cobden et al. (1980)	Human	5 g cellobiose + 2 g mannitol in 100 mL (+ 20 g sucrose and 20 g lactose)	55 patients with normal jejunal biopsy but various intestinal disorders (control group)	0.32 ± 0.20
			24 untreated adults with villous atrophy (incl. 13 confirmed CD)	0.97 ± 0.57
Cobden et al. (1985)	Human	5 g cellobiose + 2 g mannitol in 100 mL (+ 20 g sucrose and 20 g lactose)	55 control patients with normal jejunal biopsy	*cf.* Cobden et al., 1978, 1980
			Serial urinary recoveries in 3 healthy volunteers	≥ 11 h: 65
			37 patients with untreated CD	*cf.* Cobden et al., 1978, 1980
			6 patients with chronic renal failure	0.09–0.44
			5 patients with hepatic dysfunction	0.10–0.55
		IV injection of 50 mg cellobiose + 500 mg mannitol	5 normal volunteers	52 ± 14.3 92 ± 11.6
Hamilton et al. (1982)	Human	5 g cellobiose + 2 g mannitol in 100 mL (+ 20 g sucrose and 20 g lactose)	55 patients with normal jejunal biopsy and no evidence of significant GI pathology (control group)	0.32 ± 0.20
			21 untreated adults with villous atrophy	Before gluten-free diet: 0.96 ± 0.61 After gluten-free diet (≥ 3 months): 0.29 ± 0.24
			10 patients with CD treated with gluten withdrawal	0.57 ± 0.50
			3 patients unresponsive to gluten-free diet	0.66 ± 0.22
			6 patients with CD on gluten challenge	0.30 ± 0.28
Strobel et al. (1984)	Human	5 g cellobiose + 2 g mannitol in 100 mL (+ 20 g sucrose and 20 g lactose) in 150 mL water	15 healthy volunteers (21–42 years)	0.54 ± 0.16
			11 patients with IBS	0.51 ± 0.25
			4 patients with eczema	0.82 ± 0.26
			27 patients with CD	1.27 ± 0.9
			7 patients with Crohn's disease	0.86 ± 0.33
			5 patients with folate deficiency	0.79 ± 0.41
Generoso et al. (2003)	Human	5 g cellobiose + 2 g mannitol	25 healthy volunteers (12♀, 13 ♂; 28.6 ± 10.3 years)	0.335 ± 0.25

CD: Coeliac disease; IBS: Irritable bowel syndrome.
(a): After 5 h unless indicated otherwise.

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Appendix B – Summary of studies on cellobiose metabolisation

Reference	Species	Design	Doses	Measurement
Morita et al. (2008)	Rats	Ileorectostomised rats	6% cellobiose or fructo-oligosaccharide ± 0.1% neomycin in drinking water for 7 days	Faecal recovery: 36.2%
Dahlqvist (1962)	Human (*in vitro*)	Homogenates of human intestinal mucosa, obtained from piece of small intestine cut out during surgical operation Jejunal sample: ♀, 40 years Ileal sample: ♀, 26 years	N/A	Cellobiase activity: – Distal jejunum: 19% of lactase activity – Distal ileum: practically no activity
Gray and Santiago (1969)	Human (*in vitro*)	Assessment of enzyme activities in homogenates of human intestinal mucosa, obtained from piece of small intestine (10 samples)	7 mM/L cellobiose	Cellobiase activity: Ninefold lower than lactase activity in isolated lactase enzymes from human intestinal mucosa, maximum activity in jejunum, less in duodenum and ileum
Skovbjerg et al. (1981)	Human (*in vitro*)	Purified human intestinal lactase	28 mM cellobiose at pH 6.0 in 50 mM sodium maleate	Cellobiase activity: 14% of lactase activity
Lau (1987)	Human (*in vitro*)	Characterisation of human lactase isolated from solubilised small-intestinal brush-border membranes (infants of Chinese extraction, ≤ 2 years)	28 mM cellobiose	Cellobiase activity of lactase: 17.6% of lactase activity
Strobel et al. (1984)	Human	15 healthy volunteers; 21–42 years 5 lactase deficient subjects	5 g cellobiose + 2 g mannitol in 100 mL (+ 20 g sucrose and 20 g lactose) in 150 mL water	Urinary recovery after 5 h: – Controls: 0.54 ± 0.16% – Lactase deficient: 0.47 ± 0–24% (0.17–0.79 g)
Nakamura et al. (2004)	Human	10 healthy subjects (♀); 20.5 ± 2.1 years	Single oral ingestion of 25 g cellobiose	0–180 min after ingestion: no increase in blood sugar or insulin secretion

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