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## Safety of xylo-oligosaccharides (XOS) as a novel food pursuant to Regulation (EU) 2015/2283

EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA),  
Dominique Turck, Jean-Louis Bresson, Barbara Burlingame, Tara Dean,  
Susan Fairweather-Tait, Marina Heinonen, Karen Ildico Hirsch-Ernst, Inge Mangelsdorf,  
Harry J McArdle, Androniki Naska, Monika Neuhäuser-Berthold, Grażyna Nowicka,  
Kristina Pentieva, Yolanda Sanz, Alfonso Siani, Anders Sjödin, Martin Stern, Daniel Tomé,  
Marco Vinceti, Peter Willatts, Karl-Heinz Engel, Rosangela Marchelli, Annette Pötting,  
Morten Poulsen, Josef Rudolf Schlatter, Emanuela Turla and Henk van Loveren

### Abstract

Following a request from the European Commission, the EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) was asked to deliver a scientific opinion on a mixture of xylo-oligosaccharides (XOS) as a novel food (NF) pursuant to Regulation (EU) 2015/2283. The NF is obtained from corncobs (*Zea mays* subsp. *mays*) via enzyme-catalysed hydrolysis and subsequent purification. The main components of the NF, the oligosaccharides, are resistant to human digestive enzymes and are fermented by colonic bacteria. The intention is to add the NF to a variety of foods such as bakery and dairy products, fruit jelly, chocolates and soy-drinks. The information provided on composition, specifications, production process and stability of the NF, does not raise safety concerns. There were effects observed in the animal studies with the NF or with other XOS which were considered by the Panel to be expected from the intake of non-digestible carbohydrates. The Panel notes that the acute and transient gastrointestinal observed in human intervention studies with the NF or with other XOS have also been associated with the consumption of other non-digestible carbohydrates. The Panel concludes that the NF, a mixture of XOS, is safe under the proposed uses and use levels. The target population is the general population.

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**Keywords:** xylo-oligosaccharides, XOS, novel food, safety

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**Correspondence:** [nda@efsa.europa.eu](mailto:nda@efsa.europa.eu)

**Panel members:** Jean-Louis Bresson, Barbara Burlingame, Tara Dean, Susan Fairweather-Tait, Marina Heinonen, Karen Ildico Hirsch-Ernst, Inge Mangelsdorf, Harry J McArdle, Androniki Naska, Monika Neuhäuser-Berthold, Grażyna Nowicka, Kristina Pentieva, Yolanda Sanz, Alfonso Siani, Anders Sjödin, Martin Stern, Daniel Tomé, Dominique Turck, Henk van Loveren, Marco Vinceti and Peter Willatts.

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## Summary

Following a request from the European Commission, the EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) was asked to deliver a scientific opinion on a mixture of xylo-oligosaccharides (XOS) as a novel food (NF) pursuant to Regulation (EU) 2015/2283. The assessment, which follows the methodology set out in the EFSA Guidance on the preparation and presentation of an application for authorisation of a novel food Regulation (EU) 2015/2283 and in the Commission Implementing Regulation (EU) 2017/2469, is based on the data supplied in the original application, the initial assessment by the competent authority of Hungary, the concerns and objections of a scientific nature raised by the other Member States and the responses of the applicant.

The NF is a mixture of XOS which are obtained from corncobs (*Zea mays* subsp. *mays*) via enzyme-catalysed hydrolysis and subsequent purification. The main components of the NF, the oligosaccharides, are resistant to human digestive enzymes and are fermented by colonic bacteria. The intention is to add the NF to a variety of foods such as bakery and dairy products, fruit jelly, chocolates and soy-drinks.

The information provided on composition, specifications, production process and stability of the NF, does not raise safety concerns.

The Panel considers that there are no concerns with respect to genotoxicity of the NF.

There were effects observed in the animal studies with the NF or with other XOS which were considered by the Panel to be expected from the intake of non-digestible carbohydrates.

Human intervention studies, which were carried out with the NF or with other XOS, indicated the occurrence of acute and transient gastrointestinal effects at the beginning of the consumption of XOS at doses of 10–12 g/day. The Panel notes that these effects have also been associated with the consumption of other non-digestible carbohydrates. Therefore, under the proposed condition of use the Panel considers that the available human data do not raise safety concerns in relation to the NF.

The 95th percentile anticipated daily intake of the NF among the EU surveys ranges between 0.6 and 2.6 g/day for infants, 1.2 and 4.2 g/day for toddlers, 1.5 and 5.9 g/day for children, 1.7 and 7.4 for adolescents and 4.0 and 7.7 for adults. The Panel notes that the anticipated daily intake of the NF is based on the assumption that a person would consume all proposed food products containing the maximum added amount of the NF.

In order to assess the anticipated daily intake of the NF, the Panel considers the current dietary reference value (DRV) and intake data (in the case of infants) of dietary fibre, as a proxy for non-digestible carbohydrates. The DRVs are 10 g/day for toddlers, 14–16 g/day for children, 19–21 g/day for adolescents and 25 g/day for adults. The Panel notes that the highest 95th percentile anticipated daily intake of the NF is below the DRV for dietary fibre in these population groups. Regarding infants, no DRV for dietary fibre has been determined for this population group. The Panel notes that the highest 95th percentile anticipated daily intake of the NF for infants is similar or below the average intake of dietary fibre for this population group.

The Panel considers that the consumption of the NF in the intended foods and at the intended use levels, in addition to the background dietary exposure of fibre, does not raise safety concerns.

The Panel concludes that the NF, a mixture of XOS, is safe under the proposed uses and use levels. The target population is the general population.

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## 1. Introduction

### 1.1. Background and Terms of Reference as provided by the European Commission

On 4 May 2016, the company Longlive Europe Food Division Ltd. submitted a request under Article 4 of the Novel Food Regulation (EC) No 258/97<sup>1</sup> to place on the market xylo-oligosaccharides (XOS) as a novel food (NF).

On 18 July 2016, the competent authority of Hungary forwarded to the Commission its initial assessment report, which came to the conclusion that XOS meets the criteria for acceptance of a NF defined in Article 3(1) of Regulation (EC) No 258/97.

On 20 July 2016, the Commission forwarded the initial assessment report to the other Member States (MS). Several MS raised objections or submitted comments.

The concerns of a scientific nature raised by the MS can be summarised as follows:

- Complete specifications for the three forms of the NF are needed.
- More information is needed on the stability of the NF, in particular when added to the intended food categories.
- Questions were raised on the anticipated daily intake of the NF presented by the applicant as it should take into account all proposed food categories in which the NF is intended to be added, considering each population group in the target population and mean and high intake.
- Clarifications were sought on toxicological studies: whether they were performed according to GLP standards and were in line with OECD guidances; whether the test materials corresponded to the NF.

On 6 September 2017 and in accordance with Article 29(1)(a) of Regulation (EU) No 178/2002<sup>2</sup>, the Commission asked EFSA to provide a scientific opinion by carrying out the additional assessment for XOS as a NF in the context of Regulation (EU) No 258/97.

According to Article 35 (1) of Regulation (EU) 2015/2283<sup>3</sup>, any request for placing a NF on the market within the Union submitted to a Member State in accordance with Article 4 of Regulation (EU) No 258/97, and for which the final decision has not been taken before 1 January 2018, shall be treated as an application under Regulation (EU) 2015/2283. This is the case for this application.

In accordance with Article 10 (3) of Regulation (EU) 2015/2283, EFSA shall give its opinion as to whether the update of the Union List referred to in Article 10 (1) is liable to have an effect on human health.

## 2. Data and methodologies

### 2.1. Data

The safety assessment of this NF is based on data supplied in the original application, the initial assessment by the competent authority of Hungary, the concerns and objections of a scientific nature raised by the other MS along with the responses from the applicant and information submitted by the applicant following EFSA requests for supplementary information.

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<sup>4</sup>.

<sup>1</sup> Regulation (EC) No 258/97 of the European Parliament and of the Council of 27 January 1997 concerning novel foods and novel food ingredients. OJ L 43, 14.2.1997, p. 1–6.

<sup>2</sup> Regulation (EU) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. OJ L 31, 1.2.2002, p. 1–24.

<sup>3</sup> Regulation (EU) 2015/2283 of the European Parliament and of the Council 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 (2013/0435 (COD)). OJ L 327, 11.12.2015, p. 1–22.

<sup>4</sup> 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.

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.<sup>5</sup> 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 to support the safety of the proposed NF.

The application did not include a request for the protection of proprietary data (Article 26 of Regulation (EU) 2015/2283).

## 2.2. Methodologies

The assessment follows the methodology set out in the EFSA guidance on NF applications 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.

This assessment concerns only risks that might be associated with consumption of the NF under the proposed conditions of use (i.e. added to a variety of foods) and is not an assessment of the efficacy of the NF with regard to any claimed benefit.

## 3. Assessment

### 3.1. Introduction

The NF is a mixture of XOS which are obtained from corncobs (*Zea mays* subsp. *mays*) via enzyme-catalysed hydrolysis and subsequent purification. The intention is to add the NF in a variety of foods such as bakery and dairy products, fruit jelly, chocolates and soy-drinks.

### 3.2. Identity of the NF

The NF is a mixture of XOS, which are oligosaccharides constituted of chains of D-xylose molecules linked via  $\beta(1-4)$  bonds with a degree of polymerisation (DP) ranging from 2 to 7. The NF is obtained from corncobs (*Z. mays* subsp. *mays*) via hydrolysis using a xylanase followed by a purification process.

The NF is available in syrup (XOS 70L) or powder (XOS 70P and XOS 95P) form, and is predominantly composed of the disaccharide xylobiose, the trisaccharide xylotriose and the tetrasaccharide xylotetraose. Small amounts of higher oligosaccharides (i.e. xylopentaose, xylohexaose and xyloheptaose) are also present. The XOS content in the NF is at least 70% in the XOS 70L and XOS 70P forms, and 95% in the XOS 95P form.

### 3.3. Production process

The NF is obtained from corncobs of *Z. mays* subsp. *mays* (genus *Zea*; family Poaceae), which do not include kernels, via hydrolysis by a xylanase (EC 3.2.1.8) followed by a purification process.

Corn cob powder is soaked in water; after addition of acetic acid, the mixture is subjected to heating under high temperature and pressure conditions to break down the hemicelluloses. After cooling to the temperature optimum of the enzyme, xylanase is added to hydrolyse the shortened xylan chains into oligosaccharides. After separation from the remaining solid material, the oligosaccharide-containing liquid is purified by treatment with activated carbon powder and by means of two consecutive cation and anion exchange chromatographic steps. For the liquid XOS product, the purified solution is concentrated by evaporation. For XOS products in powder form, the liquid is subjected to filtration using a nano-filter membrane and subsequently concentrated and dried via spray drying. For XOS 70P, maltodextrin is added to adjust the intended concentration of XOS in the final product.

According to the applicant, the corncobs used as raw material for the production of the NF are grown and produced in Northwest Plain of Shandong (China). The corn plants are not genetically

<sup>5</sup> EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition and Allergies), 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, Pötting 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(11):4594, 24 pp. <https://doi.org/10.2903/j.efsa.2016.4594>

modified; negative results from respective PCR tests on corncobs and on a batch of XOS 70L were provided. Corncobs were also tested for the presence of aflatoxins, heavy metals and residual levels of pesticides, which were reported to be below the limits of quantification.

According to a certificate provided, the enzyme manufacturer obtained *Trichoderma reesei* CICC 13052 from the China Center of Industrial Culture Collection (CICC) for the production of the xylanase.

*T. reesei* has a long history of use in the production of cellulolytic enzymes. The safety of *T. reesei* has been reviewed by Nevalainen et al. (1994) and Blumenthal (2004). *T. reesei* is considered to be non-pathogenic. However, the Panel noted that *Trichoderma reesei* was not eligible for Qualified presumption of safety (QPS) status considering its capacity to produce peptaibols, antimicrobial peptides, and additional compounds with unknown biological activity (EFSA BIOHAZ Panel, 2013).

Upon request by EFSA, the applicant provided additional information on the specification of the xylanase. The enzyme complied with the JECFA requirements for food enzymes (JECFA, 2006) with regard to lead and microbiological criteria (i.e. *Salmonella* species, total coliforms). However, for *Escherichia coli*, the limit is 'Not more than 3 MPN (Most Probable Number)/g', whereas JECFA requires that *Escherichia coli* should be 'absent in 25 g sample'. The enzyme did not show antibacterial activity. The fact that the enzyme does not show antibacterial activity indicates that peptaibols are not present in the food enzyme in amounts that would raise safety concern.

The applicant also provided results on several mycotoxins in the xylanase (i.e. ochratoxin A, aflatoxins, fumonisins, deoxynivalenol, zearalenone, T-2 toxin and HT-2 toxin) which were below the limits of detection.

Upon an EFSA request for information on the absence of the enzyme in the NF, the applicant referred to the low protein concentration (< 0.2 g/100 g), as indicated in the specifications of the NF in Table 3. In addition, xylanase activity in the NF was reported to be below the limit of detection (10 U/g) of the applied assay. These results are to be expected taking into account the employed purification steps of the NF, i.e. active carbon treatment, ion exchange chromatography, concentration and drying.

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

### 3.4. Compositional data

The applicant provided compositional data on 56 batches of XOS 70L, 73 batches of XOS 70P and 127 batches of XOS 95P produced between 2012 and 2015 (Table 1).

In addition, data on heavy metal contents and microbiological data were provided for three batches of XOS 70L, XOS 70P and XOS 95P, respectively (Table 2).

The analyses have been performed at a laboratory accredited according to ISO/IEO 17025:2005 General Requirements for the Competence of Testing and Calibration Laboratories (CNAS-CL01 Accreditation Criteria for the Competence of Testing and Calibration Laboratories) in China.

The Panel considers that the information provided on the composition and the batch-to-batch variability of the NF is sufficient and does not raise safety concerns.



**Table 1:** Compositional data of batches of XOS-95P, XOS-70P and XOS-70L produced between 2012 and 2015

Parameter	XOS 95P (56 batches)			XOS 70P (73 batches)			XOS 70L (127 batches)		
	Mean	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.
Moisture (%)	2.63	2.58	2.73	2.59	2.23	2.67	72.3	70.3	73.1
Protein (g/100 g)	0.10	0.071	0.2	0.17	0.12	0.18	0.17	0.14	0.18
Ash (%)	0.05	0.03	0.06	0.06	0.04	0.09	0.12	0.10	0.15
pH	3.89	3.5	4.1	4.13	3.96	4.15	4.22	4.00	4.31
Total carbohydrate content (g/100 g)	98.0	97.67	98.33	96.90	94.27	97.90	75.7	74.75	75.91
XOS content (dry basis) (g/100 g)	95.07	95.01	95.39	73.62	71.5	88.2	76.54	71.5	88.2
Other carbohydrates (g/100 g) <sup>(a)</sup>	2.87	2.50	2.94	7.24	1.5	8.2	23.13	17.31	25.54
Monosaccharides total (g/100 g)	2.23	1.35	2.72	4.49	3.23	5.72	21.1	15.61	23.14
Glucose (g/100 g)	0.95	0.82	1.45	2.38	0.76	3.72	3.19	2.01	3.75
Arabinose (g/100 g)	0.69	0.13	0.89	0.25	0.17	0.58	6.45	4.6	6.59
Xylose (g/100 g)	0.59	0.40	0.98	0.86	0.60	0.98	11.46	9.0	12.8
Disaccharides total (g/100 g)	33.42	33.13	35.86	28.71	24.2	30.9	31.71	30.5	34.0
Xylobiose XOS DP2 (g/100 g)	30.65	30.63	32.96	25.96	21.7	28.0	29.68	28.8	31.6
Cellobiose (g/100 g)	2.77	2.5	2.9	2.75	2.5	2.9	2.03	1.7	2.4
Oligosaccharides total (g/100 g)	65.08	41	77	44.65	41.61	51.1	46.87	43.41	53.16
Xylotriose (XOS DP3) (g/100 g)	31.6	26.7	34.0	21.1	18.1	20.9	21.98	19.9	22.8
Xylotetraose (XOS DP4) (g/100 g)	12.7	7.5	36.9	11.8	10.6	12.3	12.28	12.2	13.2
Xylopentaose (XOS DP5) (g/100 g)	17.5	8.0	19.71	6.31	4.98	7.19	6.46	6.13	7.14
Xylohexaose (XOS DP6) (g/100 g)	4.7	4.0	5.03	3.81	3.26	3.96	2.65	2.38	4.22
XOS DP ≥ 7 (g/100 g)	6.4	5.8	6.6	4.70	4.67	7.75	3.50	2.8	5.8
Maltodextrin (g/100 g) <sup>(b)</sup>	0	0	0	21	20	25	0	0	0

XOS: xylo-oligosaccharides; DP: degree of polymerisation.

(a): Other carbohydrates include monosaccharides (glucose, xylose and arabinose) and cellobiose.

(b): Maltodextrin content is calculated according to the amount added in the process.



**Table 2:** Heavy metal contents and microbiological data for batches of XOS 95P, XOS 70P and XOS 70L

Parameter	XOS 95P			XOS 70P			XOS 70L			Method
	Batch 28.12.2012	Batch 25.4.2013	Batch 1.9.2013	Batch 7.6.2012	Batch 10.9.2013	Batch 28.11.2013	Batch 8.11.2011	Batch 15.3.2013	Batch 18.11.2013	
Copper (mg/kg)	< 5.0	< 5.0	< 5.0	< 5.0	< 5.0	< 5.0	< 5.0	< 5.0	< 5.0	GB/T 5009.13-2003
Lead (mg/kg)	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	GB/T 5009.12-2010
Arsenic (mg/kg)	< 0.3	< 0.3	< 0.3	< 0.3	< 0.3	< 0.3	< 0.3	< 0.3	< 0.3	GB/T 5009.11-2003
Total colony forming unit (cfu/g)	10	10	< 10	10	< 10	< 10	< 10	< 10	5	FDA-BAM Chap3
<i>Salmonella</i> (cfu/25 g)	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	FDA-BAM Chap4 and 5
<i>E. coli</i> (MPN/100 g)	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	FDA-BAM Chap4
Yeast (cfu/g)	< 10	5	< 10	< 10	< 10	< 10	< 10	< 10	< 10	FDA-BAM Chap18
Mould (cfu/g)	< 10	5	5	5	< 10	< 10	5	< 10	15	FDA-BAM Chap18

XOS: xylo-oligosaccharides; MPN: most probable number.

### 3.4.1. Stability of the NF

The applicant proposed a shelf life of 12 months for XOS 70L and of 24 months for XOS 95P and XOS 70P.

Heat and pH stability of the NF in aqueous solutions were investigated in model experiments (Courtin et al., 2008).

To test the heat stability, XOS 95P was dissolved at a concentration of 15% (w/v) in buffers (pH 2.0, 3.0, 7.0 and 11.0), and each solution was kept at 100°C or in a heating block for 5, 10, 15, 20, 30 and 60 min, respectively. The NF did not show substantial decomposition (appr. 1–4% w/w) at low or neutral pH. In total, 21% and 9% of all glycosidic linkages were cleaved at pH 2.0 and 3.0, respectively; arabinose linkages were more susceptible to the acid-catalysed hydrolysis than xylose linkages.

Under alkaline conditions (pH 11.0), 73% of the NF was decomposed after 60 min of incubation at 100°C. The so-called 'alkaline peeling' type reaction leads to a cleavage of glycosidic bonds at the reducing end of the carbohydrate backbone; the reaction results in organic acids and a successive shortening of the saccharide chain by one carbohydrate unit.

At 121°C, faster decomposition and cleavage of glycosidic linkages via hydrolysis were reported by the authors (results not shown).

For shelf life stability measurements, XOS 95P solutions in the four buffers were stored at 4°C and at 37°C, and samples were analysed after 8 and 18 weeks (4°C) and after 1, 3, 7, 14, 30 and 60 days (37°C). There was no substantial decomposition of XOS 95P at low or neutral pH. At pH 11.0, decomposition between 67% and 16% (w/w) after 56 days of storage at 37°C and 18 weeks at 4°C, respectively, were observed.

These model experiments indicate that the NF is heat stable at low and neutral pH; in contrast, at high pH (11.0) substantial decomposition occurs even at low temperature.

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

### 3.4.2. Stability under the intended conditions of use

Liquid milk with 0.5–4.0 g/100 g XOS content was stored for 4 weeks and 83% of the initial XOS content was measured at the end of the storage period. The XOS content (2 g/100 g XOS) in yoghurt and powdered milk was reported to be maintained (more than 95%) after 16 days and 5 months, respectively (GRAS, 2013).

Upon EFSA's request of information on stability, the applicant provided data on the stability of the NF in different exemplary foods prepared in a pilot plant. There were no significant changes of the total contents of XOS 95P, XOS 70P and XOS 70L in the following foods under the indicated storage conditions:

- Yoghurt (pH 4.6) with XOS 95P (0.34 g/100 g), XOS 70P (0.36 g/100 g), XOS 70L (0.37 g/100 g), respectively; stored for 2 weeks at 4°C;
- Fruit jelly (pH 3.0) with XOS 95P (2.76 g/100 g), XOS 70P (2.73 g/100 g), XOS 70L (2.69 g/100 g), respectively; stored for 4 weeks at 20°C;
- Soy drink with XOS 95P (0.33 g/100 g), XOS 70P (0.35 g/100 g), XOS 70L (0.35 g/100 g), respectively; stored for 3 weeks at 4°C;
- Biscuits with XOS 95P (1.85 g/100 g), XOS 70P (1.85 g/100 g), XOS 70L (1.85 g/100 g), respectively; stored for 2 weeks at room temperature.

For some of the food examples selected by the applicant, e.g. fruit jelly and biscuits, the durations chosen for the storage experiments do not reflect the real conditions to be expected. However, taking into account the nature of the NF and the results of the stability experiments described in Section 3.4.1, the Panel considers that the data provided sufficient information with respect to the stability of the NF under the intended conditions of use.

## 3.5. Specifications of the NF

Upon request from EFSA, the applicant provided specifications for the NF in syrup (XOS 70L) and powder (XOS 70P and XOS 95P) forms, including information on the analytical method used for each parameter (Table 3).

The applicant indicated that analytical tests on the NF have been carried out in Shandong Centre for Disease Control and Prevention, which is accredited to ISO/IEO 17025:2005 General Requirements for the Competence of Testing and Calibration Laboratories (CNAS-CL01 Accreditation Criteria for the Competence of Testing and Calibration Laboratories) for the competence of testing.

**Table 3:** Specifications of XOS 95P, XOS 70P and XOS 70L as proposed by the applicant

Parameter	XOS-95P	XOS-70P	XOS-70L	Method
Moisture (%)	≤ 5.0	≤ 5.0	70–75	GB 5009.3-2016
Protein (g/100 g)		< 0.2		GB 5009.5-2010
Ash (%)		≤ 0.3		GB 5009.4-2016
pH		3.5–5.0		GB/T 20884-2007
Total carbohydrate content (g/100 g)	≥ 97	≥ 95	≥ 70	GB/Z 21922-2008
XOS content (dry basis) (g/100 g)	≥ 95	≥ 70	≥ 70	HPLC
Other carbohydrates (g/100 g) <sup>(a)</sup>	2.5–7.5	2–16	1.5–31.5	
Monosaccharides total (g/100 g)	0–4.5	0–13	0–29	
Glucose (g/100 g)	0–2	0–5	0–4	
Arabinose (g/100 g)	0–1.5	0–3	0–10	
Xylose (g/100 g)	0–1.0	0–5	0–15	
Disaccharides total (g/100 g)	27.5–48	25–43	26.5–42.5	
Xylobiose (XOS DP2) (g/100 g)	25–45	23–40	25–40	
Cellobiose (g/100 g)	2.5–3	2–3	1.5–2.5	
Oligosaccharides total (g/100 g)	41–77	36–72	32–71	
Xylotriose (XOS DP3) (g/100 g)	27–35	18–30	18–30	
Xylotetraose (XOS DP4)(g/100 g)	10–20	10–20	8–20	
Xylopentaose (XOS DP5) (g/100 g)	3–10	5–10	3–10	
Xylohexaose (XOS DP6) (g/100 g)	1–5	1–5	1–5	
XOS DP ≥ 7 (g/100 g)	0–7	2–7	2–6	
Maltodextrin (g/100 g) <sup>(b)</sup>	0	20–25	0	–

DP: degree of polymerisation; XOS: xylo-oligosaccharides; HPLC: high-performance liquid chromatography.

(a): Other carbohydrates include monosaccharides (glucose, xylose and arabinose) and cellobiose.

(b): Maltodextrin content is calculated according to the amount added in the process.

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

The Panel notes that the source of the NF, corncob from *Z. mays* subsp. *mays*, is used as feed, but it has no history of food use.

#### 3.6.2. History of use of the NF

The applicant indicated that XOS have been used as food ingredients (e.g. in dairy products, beverages, bread, cereal products, and confectionary) and as food supplement in Asia (China, Korea, Taiwan, Japan). In Japan, XOS are on the market since 1993. XOS 95P and XOS 70P were subject to a GRAS Notification in 2013 in the USA (GRAS, 2013).

### 3.7. Proposed uses and use levels and anticipated intake

#### 3.7.1. Target population

The target population as proposed by the applicant is the general population above 1 year of age.

### 3.7.2. Proposed uses and use levels

The applicant intends to add the NF to a variety of foods: bakery and dairy products, fruit jelly, chocolates and soy-drinks. Table 4 presents the uses and maximum use levels of the NF as proposed by the applicant.

**Table 4:** Uses and maximum use levels of the NF as proposed by the applicant

Food category <sup>(a)</sup>	Proposed use	Maximum use level of the NF
Breads and rolls	White bread	1.4%
	Whole meal bread	1.4%
Breakfast cereals	Breakfast cereals	1.4%
Fine bakery wares	Biscuit	1.4%
Milk and milk product imitates	Soy-drink	0.35%
Fermented milk products	Yoghurt	0.35%
Jams, marmalades and fruit spreads	Fruit jelly	3%
Chocolate	Chocolate	3%

(a): Food categories used for the estimation of the anticipated daily intake of the NF.

### 3.7.3. Anticipated intake of the NF

The applicant provided estimates of the anticipated daily intake of the NF for each food category indicated in Table 4, for adults only, based on the summary statistics from the EFSA Comprehensive European Food Consumption Database.

An additional intake assessment of the NF was performed by EFSA based on the individual European Union (EU) data from the EFSA Comprehensive Food Consumption Database (EFSA, 2011). For the estimation of the intake of the NF, EFSA considered the food categories and the maximum use levels proposed by the applicant in Table 4.

The ranges of the estimated daily intake of the NF (i.e. lowest and highest means and 95th percentiles among surveys in EU) from foods added with the NF are presented in Table 5 (on a g per day basis).

**Table 5:** Anticipated daily intake of the NF on a g per day basis: lowest and highest means and 95th percentiles anticipated daily intake of the NF among the EU surveys based on the EFSA Comprehensive European Food Consumption Database

Population group	Range of means g per day	Range of 95th percentiles g per day
Infants ( $\leq 11$ months)	0.1–0.9	0.6–2.6
Toddlers (12–35 months)	0.4–2.6	1.2–4.2
Children (3–9 years)	0.6–3.6	1.5–5.9
Adolescents (10–17 years)	0.6–3.9	1.7–7.4
Adults (18–64 years)	1.9–3.7	4.2–7.4
Elderly and very elderly ( $\geq 65$ years)	2.2–3.8	4.0–7.7

### 3.7.4. Precautions and restrictions of use

The applicant indicated that consumers will be informed about the exact amount of the NF added to foods and that high consumption might result in gastrointestinal effects.

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

*In vitro* and animal studies on degradation of xylobiose or XOS were provided. In addition, animal and human studies were provided on effects of mixtures of XOS on bacterial counts, short-chain fatty acids (SCFA) formation and pH in faeces.

The monomer D-xylose is absorbed up to 95% in the small intestine in different species (Huntley and Patience, 2018) after supplementation in the diet. The absorption decreases with increasing doses of D-xylose indicating saturation of uptake. In a study with volunteers, 70% of the dose was absorbed

after oral administration of 25 g D-xylose (Craig and Ehrenpreis, 1999). A part of D-xylose also reaches the colon, where it is metabolised by gut bacteria (Huntley and Patience, 2018).

After intravenous infusion of 20 g <sup>14</sup>C-xylose to one human, 13.5% of the dose was detected in expired CO<sub>2</sub>. About 50% of the dose was excreted unchanged in the urine (Wyngaarden et al., 1957). Similar values were obtained in another study after infusion of 7.5 mg <sup>14</sup>C-xylose (Segal and Foley, 1958).

The metabolism of xylobiose was investigated *in vitro* and *in vivo* (Okazaki et al., 1991). No degradation was found in artificial saliva (containing α-amylase, lysozyme, acid and alkaline phosphatase and lipase), gastric juice (0.1 N HCl and pepsin), pancreas extract and intestine brushborder homogenate (both from pigs). Ten Sprague–Dawley rats received xylobiose (100 or 300 mg/kg body weight (bw)) via stomach tube. No xylobiose was detected in urine or faeces 24 h after application.

Incubation of different species of faecal bacteria with xylose, xylobiose, xylotriose or a mixture of XOS (obtained from birch wood xylan hydrolysed by *Trichoderma*-derived xylanase; 22% xylose, 58% xylobiose, 13% xylotriose and 7% other saccharides) showed that bifidobacteria could degrade the mixture to a greater extent than other intestinal bacteria (demonstrated by a decrease in pH in the culture medium) (Okazaki et al., 1990).

In a study a diet containing 6% XOS (Xylooligo 95<sup>®</sup>, obtained from birch wood hydrolysed by *Trichoderma*-derived xylanase, containing mainly xylobiose, xylotriose, xyloetraose, 6% corresponds to 2.4 g/kg bw per day) and 5% cellulose, was fed for 14 days to 10 male Sprague–Dawley rats (Campbell et al., 1997). As a control, either normal diet without cellulose or diet supplemented with 5% cellulose was used. Compared to the cellulose control, treatment with XOS plus cellulose resulted in an increased number of overall anaerobic bacteria including bifidobacteria in the faeces, while the number of aerobic bacteria was decreased. Levels of SCFA in the faeces were increased; in particular, levels of acetate were statistically significantly higher. Faecal and caecal pH were decreased.

Nine healthy men (50–60 years) ingested daily 5 g XOS (obtained from birch wood xylan hydrolysed by *Trichoderma*-derived xylanase; containing mainly xylobiose (58%), xylose (22%) and xylotriose (13%)) for 3 weeks (Okazaki, 1990). The overall count of intestinal bacteria remained the same over the duration of the experiment. Faecal pH decreased from 6.16 to 6.29 before administration to 5.95–6.08 during administration. In five subjects, who showed markedly lowered pH, SCFA were higher at the end of 3 weeks as compared to the beginning of the study.

In the double-blind study by Finegold et al. (2014), 32 healthy subjects (n = 21 women with age range of 21–49 years and mean body mass index (BMI) of 24.1 kg/m<sup>2</sup>; n = 11 men with age range of 23–34 years and mean BMI of 25.6 kg/m<sup>2</sup>) were randomised to consume daily capsules containing either 175 mg XOS, 350 mg XOS (produced by the applicant) or maltodextrin (placebo) for 8 weeks. Bifidobacterium counts increased in both XOS groups compared to the placebo group. Total anaerobic counts and *Bacteroides fragilis* group counts were significantly higher in the 350 mg XOS group as compared to placebo. There were no significant differences in the counts of *Lactobacillus*, Enterobacteriaceae and *Clostridium* between the three groups. XOS intervention had no significant effect on stool pH, SCFA or lactic acid. The Panel noted that this study was performed with low doses of the NF.

Fourteen healthy young women were administered with 1.4 or 2.8 g XOS (no specification on the components) per day for 28 days in the study by Na and Kim (2001). The authors reported a reduction of faecal pH and an increase in the faecal lactic acid amount in the 2.8 g/day group at day 28 as compared to baseline.

The information provided indicates that D-xylose is readily absorbed and partially metabolised, while a major part is excreted unchanged in the urine. In contrast, XOS reach the colon unchanged, where they are degraded by bacteria to SCFA.

### 3.9. Nutritional information

The Panel notes that the main components of the NF are oligosaccharides, which are resistant to human digestive enzymes and are fermented by colonic bacteria. Based on the nature of the NF, the Panel considers that consumption of the NF under the conditions of use is not nutritionally disadvantageous.

### 3.10. Toxicological information

The applicant provided several toxicological studies (genotoxicity, subacute, acute, subchronic and chronic studies) which have been described in the sections below.

In reply to MS's comments, the applicant indicated that the toxicological studies were carried out in Shandong Centre for Disease Control and Prevention, which is accredited by CNAS (China National Accreditation Service for Conformity Assessment). The applicant indicated that all toxicity tests were performed in accordance with China State Food and Drug Administration Good Laboratory Practices standards. The applicant provided the National Chinese Standards according to which the toxicological studies were performed (GB 15193.3/4/5/7/8-94; GB15193.13-2003; GB15193.13-94).

#### 3.10.1. Genotoxicity

The applicant provided an Assay Inspection Report (summary of unpublished study reports, 2001a; Fu et al., 2012) on XOS, which were produced by the applicant according to the procedure described in Section 3.3.

A bacterial reverse mutation test (Ames test) with *Salmonella* Typhimurium TA97, TA98, TA100, TA102 with or without S-9-mix with five concentrations of XOS (250, 500, 1,000, 2,500 or 5,000 µg/plate) was negative in two repetitions in the presence and absence of S-9-mix (from rat liver, induced with polychlorinated biphenyls). The positive control provided positive results. The Panel noted that the study was not in line with the current standard (i.e. OECD, 1997; TG 471) since one *S. Typhimurium* strain (TA1535) in the test was missing.

In a bone marrow micronucleus test, five male and five female [Grade II] Kunming mice were given XOS doses of 2.5, 5.0 and 10.0 g/kg bw via gavage twice with an interval of 24 h. Animals were sacrificed 6 h after the second administration. There were no significant differences in the micronucleus rate between test and control groups. The group given cyclophosphamide (positive control) provided a positive result. The ratio of polychromatic to normochromatic cells was not provided.

In a sperm abnormality test, groups of five male [Grade II] Kunming mice received 0, 2.5, 5 and 10 g/kg bw of XOS in distilled water via gavage on five consecutive days. Animals were sacrificed 35 days after the first application and epididymal sperm were obtained. While the positive control (cyclophosphamide) led to increased counts of abnormal sperm, no increase compared to controls was detected in sperm from animals treated with XOS.

In a testis chromosome aberration test, groups of five male [Grade II] Kunming mice received 0, 2.5, 5 and 10 g/kg bw of XOS in distilled water via gavage on five consecutive days. Animals were sacrificed 13 days after the first application; spermatocytes were obtained and processed for cytogenetic investigation. No increase in the chromosome aberration rate compared to the control was detected in cells from mice receiving XOS in contrast to the positive control (cyclophosphamide).

The Panel notes that for all genotoxicity studies the results were negative. The Panel notes that (i) the Ames test was carried out with only four strains, (ii) no *in vitro* micronucleus assay was provided and (iii) for all *in vivo* tests, based on the ADME data, it is not expected that XOS reach the target tissue. Nonetheless, taking into account also the nature, the source and the production process of the NF, the Panel considers that there are no concerns with respect to genotoxicity of the NF.

#### 3.10.2. Acute toxicity studies

Acute toxicity studies in rats, mice, and dogs were performed using XOS, produced by the applicant (according to the procedure described in Section 3.3), at dose levels up to 32 g/kg bw. Soft stools and vomiting were observed at single doses of XOS of 6, 9, 14 g/kg bw (but not at the dose of 4 g/kg bw) in dogs; dogs recovered 48 h after administration (summary of unpublished study report, undated). Soft stool and diarrhoea were observed in rats 2–5 h after taking oral XOS doses of 5 and 10 g/kg bw; symptoms disappeared after 1 day (Park et al., 1999). Watery stools were also observed in mice taking a single oral dose of 32 XOS g/kg bw (Gao et al. (2012).

No mortalities occurred up to dose levels of 32 g/kg bw.

#### 3.10.3. Subacute toxicity studies

Wistar rats (10/sex per group) were fed 0, 1, 2 or 4 g/kg bw of XOS (produced by the applicant according to the procedure described in Section 3.3) for 30 days (summary of Unpublished study



reports, 2001a). No statistically significant differences between XOS and the control group with regard to the parameters investigated (body weights, feed intake, haematology and clinical biochemistry, organ weights, histopathological examination) were reported.

A diet-containing 6% XOS (from a different producer than the applicant; derived from birch wood hydrolysed by *Trichoderma*-derived xylanase, containing mainly xylobiose, xylotriose, xylotetrose, 6% corresponds to 2.4 g/kg bw per day) and 5% cellulose was fed for 14 days to 10 male Sprague Dawley rats (Campbell et al., 1997). As a control, either normal diet without cellulose or diet supplemented with 5% cellulose were used. Both full and empty colon and caecum weights were increased, with the effect being more pronounced in the caecum, in the XOS group as compared to the cellulose control group. Caecum enlargement is a frequent finding in rats after feeding of non-digestible substances. Caecum and colon weights were not determined in the repeated dose toxicity studies described below. For effects on caecum content, see Section 3.8.

#### 3.10.4. Subchronic toxicity studies

In a study (Inspection report, 2009; Gao et al. (2012), five groups (10/sex per group) of Wistar rats were fed diets containing 0% (control), 0.9%, 2.9%, 8.8% and 10% of XOS for 13 weeks, corresponding to average intakes of 1.1, 3.6, 9.8 and 11.5 g/kg bw per day for females and 1.4, 4.7, 13.8 and 15 g/kg bw per day for males. The test product was produced by the applicant in accordance with the procedure described in Section 3.3. The test material was composed of xylobiose (29%), xylotriose (30%), xylotetraose (16%), xylopentaose (8%) and xylohexaose (4%), complying with the specifications described in Table 4.

Clinical observations were recorded daily and body weights and food consumption were measured weekly. Ophthalmic examinations were performed at pre-test and just prior to termination. Blood samples were obtained at days 46 and 91 for analysis of haematology, coagulation and clinical chemistry parameters. At the end of the study, urine samples were collected for urinalysis, and all animals were euthanised for necropsy. Selected organs (i.e. brain, heart, lungs, liver, spleen, adrenals, kidneys, ovary and testes) were weighed. Histological examinations were carried out on several organs.

Transient statistically significant changes in food consumption and in food conversion efficiency, in different directions, in males and females, were reported in the XOS groups as compared to the control groups. Starting in week 3, body weights were slightly decreased only in males (dose dependent, no information on statistical significance was provided).

Statistically significant changes in few clinical chemistry parameters were noted in some XOS groups as compared to the control groups. A dose-related decrease in triglycerides was observed in the 2.9%, 8.8% and 10% female groups and in the 10% male group. This effect is treatment related but is considered not to be adverse. Other statistically significant changes (i.e. a decrease in neutrophil counts only in the male 0.9% group; a decrease in lymphocytes counts only in the male 2.9% group) were considered incidental.

The no-observed adverse effect level (NOAEL) in this study is the highest dose tested, i.e. 10% in the diet, which corresponded to 11.5 and 15 g/kg bw per day in females and males, respectively.

In the study by Park et al. (2000), four groups of 10 male and female SPF SD rats were given, via gavage, either 0, 0.3, 1.0 or 3.0 g/kg bw of XOS dissolved in water daily for 13 weeks. Upon EFSA's request for clarifications on the test material, the applicant indicated that XOS was obtained from a different producer. The test material was also produced using an enzyme-catalysed hydrolysis of xylan from corncob; the test material contained xylobiose, xylotriose and 30% water. Ophthalmic investigations and urinalysis were conducted at the end of the treatment period. During the 13-week of intervention and the 4-week of recovery, changes in body weights, food and water consumption were investigated. At week 13 and at the end of the 4-week recovery period, haematological parameters, organ weights and histopathological findings were investigated. Statistically significant changes in several parameters were reported. These changes were not dose related and the Panel considered these as incidental findings. The NOAEL in this study is the highest dose tested (i.e. 3 g/kg bw).

A total of 32 Beagle dogs (7–9 months of age; bw range: 8.1–10.0 kg in females and 8.0–11.0 kg in males) were randomly divided into four groups (4/sex per group) which were orally administered pills of XOS for 26 weeks, at doses of 0 (control), 1.25, 2.5 and 5 g/kg bw, once a day for 6 days and then stopped for one day (Unpublished study report, undated a,b; Gao et al., 2017). One dog/sex per group was sacrificed after 13 weeks of exposure, two dogs/sex per group after 26 weeks of exposure, and one dog/sex per group after a 28-day recovery period.



The test product was produced by the applicant in accordance with the procedure described in Section 3.3. XOS was composed of 29% xylobiose, 66% xylose-based total fibre (a combination of 30% xylotriose, 16% xylotetraose, 10% xylopentaose, 4% xylohexaose, and 6% xyloglucan), and 5% monosaccharides (such as glucose, arabinose and xylose).

Number of deaths were recorded throughout the study; dogs were regularly observed for appearance of signs (e.g. around the eyes, nose, mouth, urethra, etc.), for behavioural activity, for breathing difficulties, for appetite. Individual body weight and temperature were recorded at the beginning of the study, at weekly intervals and just prior to sacrifice.

Serum biochemistry, haematology, ophthalmoscopy, electrocardiogram examinations, urine and stools examinations were carried out at weeks 0, 13, 26, and at the end of the 4-week recovery period.

Animals were examined for macroscopic morphology, organ weights were determined and histopathology was carried out at weeks 13, 26 and at the end of the recovery period.

During the study, no animals died. As compared with the control group, all animals in the 5 g/kg bw group had vomiting and loose stools at the beginning of the study and at some days throughout the study. Neither vomiting nor loose stools were reported in dogs in the high-dose recovery group. In the mid-dose group (2.5 g/kg bw), all dogs had loose stools, especially in the beginning of the study. Neither vomiting nor loose stools were reported in dogs in the mid-dose recovery group. Throughout the study and after the recovery period, neither vomiting nor loose stools were reported in any of the animals in the low-dose group (1.25 g/kg bw).

During the 26-week period up to the 4-week recovery period, weekly body weights in the XOS groups were not statistically significantly different than those in the control group. However, weight change rate was statistically significantly lower in the high-dose group at weeks 28 and 29 (recovery period) as compared to the control group, while for the mid- and low-dose group weight was higher (but not statistically significant) as compared to the control group.

No statistically significant differences between the XOS and the control groups were reported in haematological and clinical biochemistry parameters and in relative organ weights at weeks 13 and 26 and at the end of the 4-week recovery period. Statistically significant changes were reported in some electrocardiogram parameters in the recovery period in the high-dose group and in the low-dose group as compared with placebo, which were not judged as adverse.

Histopathological examinations did not show treatment related effects.

The Panel notes that vomiting which was observed in the high-dose group can be attributed to consumption of very high doses of a non-digestible substance. Dogs have a similar sensitivity to the induction of vomiting as humans (Holmes et al., 2009).

The Panel considers that the occurrence of loose stools, which were observed in the high-dose and in the mid-dose groups, were effects which can be expected from the intake of non-digestible carbohydrates.

### 3.10.5. Chronic toxicity of D-xylose

The Panel notes that a 2-year carcinogenicity study on D-xylose has been cited in the GRAS dossier (Kuroiwa et al., 2005). Upon request, the applicant provided the publication by Kuroiwa et al. (2005).

In this 2-year carcinogenicity study, performed according to the Japanese guideline for food additives (MHLW, 1996), groups of 50 male and 50 female F344 rats received 0% (control), 2.5% and 5% D-xylose in feed, corresponding to 1,000 and 2,200 mg/kg bw per day in male, and 1,200 and 2,500 mg/kg bw per day in female rats. Soft faeces were observed in male and female rats of the 5% group. In the 5% group, terminal body weight was reduced by 8% in male and by 6% in female rats (statistically significant). A statistically significant decrease in absolute and a statistically significant increase in relative weight of the brain was found in male rats, a decrease of absolute kidney weight was observed in female rats in the 5% group. There were no histopathological changes reported attributable to the treatment in these organs. Statistically significantly lower absolute and relative testes weights in the high-dose group were attributed by the authors to the lower occurrence of interstitial cell tumours in the testes in xylose-treated animals (72%), compared to controls (92%). No statistically significant increase in the incidence of any type of neoplastic lesion was found for either sex in the treated groups.

No statistically significant or biologically relevant differences from the controls were noted in the XOS groups with regard to clinical signs, mortality and haematological findings.

Based on this study, the Panel considers that there is no concern for carcinogenicity of D-xylose. In addition, the Panel considers that there is no concern for toxicity of D-xylose at dose levels equal to or below 2.5%.

### 3.10.6. Human studies

XOS produced by the applicant in accordance with the procedure described in Section 3.3 was investigated in four human intervention studies (unpublished study report, 2001b; Xiao et al., 2012; Finegold et al., 2014; Yang et al. 2015). The effects of XOS on gastrointestinal parameters, at doses up to 2 g/day for 8 weeks or sequential weekly doses up to 12 g/day for 3 weeks were assessed. The only relevant findings were noted in the study by Xiao et al. (2012). This study reported that diarrhoea occurrence on the first day of consumption of 10–12 g/day XOS was 18–20% as compared to 6% in controls. No diarrhoea episodes occurred on the first day of consumption of 3–5 g/day of XOS. After 1 week consumption of 10–12 g/day of XOS diarrhoea ratios went back to normal. No other adverse effects were reported by participants.

The applicant also provided several human intervention studies which were carried out with XOS from other producers, and which were derived from corncobs or other starting materials. In these studies, doses of XOS up to 4 g/day for 8 weeks or up to 10 g/day for 5 days were investigated. The Panel notes that in the uncontrolled study by Kobayashi et al. (1991) (reviewed by Fu et al., 2012), an increased incidence in diarrhoea (18%) was associated with the consumption of 10 g of XOS at day one. After 5 days of consumption of 10 g of XOS, the incidence of diarrhoea went back to the initial incidence of 8%.

The Panel notes that the acute and transient gastrointestinal effects, which were reported with the consumption of 10–12 g/day XOS in some human intervention studies, have also been associated with the consumption of other non-digestible carbohydrates. Therefore, under the proposed condition of use the Panel considers that the available human data do not raise safety concerns in relation to the NF.

### 3.11. Allergenicity

The NF is obtained from corncob (*Z. mays* subsp. *mays*).

The production process involves the use of a xylanase (EC 3.2.1.8), which is produced with *T. reesei*, for the hydrolysis step.

According to the specifications (Table 4), the amount of protein in the XOS products is below the limit of detection (0.2 g/100 g) of the employed analytical technique. The activity of the xylanase in the NF was also below the limit of detection (10 U/g) of the applied assay.

The Panel considers that the likelihood of allergic reactions to the NF is low.

## 4. Discussion

The NF is a mixture of XOS which are obtained from corncobs (*Z. mays* subsp. *mays*) via enzyme-catalysed hydrolysis and subsequent purification. The main components of the NF, the oligosaccharides, are resistant to human digestive enzymes and are fermented by colonic bacteria. The intention is to add the NF to a variety of foods such as bakery and dairy products, fruit jelly, chocolates and soy-drinks.

The information provided on composition, specifications, production process and stability of the NF, does not raise safety concerns.

The Panel considers that there are no concerns with respect to genotoxicity of the NF.

There were effects observed in the animal studies with the NF or with other XOS which were considered by the Panel to be expected from the intake of non-digestible carbohydrates.

Human intervention studies, which were carried out with the NF or with other XOS, indicated the occurrence of acute and transient gastro-intestinal effects at the beginning of the consumption of XOS at doses of 10–12 g/day. The Panel notes that these effects have also been associated with the consumption of other non-digestible carbohydrates. Therefore, under the proposed condition of use, the Panel considers that the available human data do not raise safety concerns in relation to the NF.

The 95th percentile anticipated daily intake of the NF among the EU surveys ranges from 0.6 to 2.6 g/day for infants, 1.2 to 4.2 g/day for toddlers, 1.5 to 5.9 g/day for children, 1.7 to 7.4 for adolescents and 4.0 to 7.7 for adults. The Panel notes that the anticipated daily intake of the NF is based on the assumption that a person would consume all proposed food products containing the maximum added amount of the NF.

In order to assess the anticipated daily intake of the NF, the Panel considers the current DRV and intake data (in the case of infants) of dietary fibre, as a proxy for non-digestible carbohydrates. The DRVs are 10 g/day for toddlers, 14–16 g/day for children, 19–21 g/day for adolescents and 25 g/day for adults (EFSA NDA Panel, 2010). The Panel notes that the highest 95th percentile anticipated daily intake of the NF is below the DRV for dietary fibre in these population groups. Regarding infants, no DRV for dietary fibre has been determined for this population group (EFSA NDA Panel, 2010). The Panel notes that the highest 95th percentile anticipated daily intake of the NF for infants is similar to or below the average intake of dietary fibre for this population group (i.e. 0.3 g/day for a 3 months old infant and 3.9–8.7 g/day for 6–12 months age infants; Hilbig, 2005).

The Panel considers that the consumption of the NF in the intended foods and at the intended use levels, in addition to the background dietary exposure of fibre, does not raise safety concerns.

## 5. Conclusions

The Panel concludes that the NF, a mixture of XOS, is safe under the proposed uses and use levels. The target population is the general population.

### Steps taken by EFSA

- 1) Letter from the European Commission to EFSA with the request for a scientific opinion on the safety of xylo-oligosaccharide as a NF. Ref. Ares(2017)4346773, dated 6/9/2017.
- 2) On 14 September 2017, EFSA received the following documentation: technical dossier on xylo-oligosaccharide (XOS), submitted by Longlive Europe Food Division Ltd; initial assessment report ('Opinion on an application under the novel foods regulation for xylo-oligosaccharide (XOS)' carried out by the Food Safety Authority of Hungary; Member States' comments and objections; responses by the applicant to the initial assessment report and to the Member States' comments and objections.
- 3) On 25 October 2017, EFSA requested the applicant to provide missing information.
- 4) On 29 November 2017, EFSA received the missing information as submitted by the applicant.
- 5) On 6 December 2017, the application was considered valid and the scientific evaluation procedure started.
- 6) On 24 January 2018, EFSA requested the applicant to provide additional information to accompany the application and the scientific evaluation was suspended.
- 7) On 12 April 2018, additional information was provided by the applicant and the scientific evaluation was restarted.
- 8) During its meeting on 27 June 2018, the NDA Panel, having evaluated the data, adopted a scientific opinion on the safety of xylo-oligosaccharide (XOS) as a NF pursuant to Regulation (EC) No 258/97.

### References

- Blumenthal CZ, 2004. Production of toxic metabolites in *Aspergillus niger*, *Aspergillus oryzae*, and *Trichoderma reesei*: justification of mycotoxin testing in food grade enzyme preparations derived from the three fungi. *Regulatory Toxicology and Pharmacology*, 39, 214–228.
- Campbell JM, Fahey Jr GC and Wolf BW, 1997. Selected indigestible oligosaccharides affect large bowel mass, cecal and fecal short-chain fatty acids, pH and microflora in rats. *Journal of Nutrition*, 127, 130–136.
- Courtin K, Verjans P and Delcour JA, 2008. Heat and pH stability of prebiotic arabinoxylooligosaccharides, xylooligosaccharides and fructooligosaccharides. *Food Chemistry*, 112, 831–837.
- Craig RM and Ehrenpreis ED, 1999. D-xylose testing. *Journal of Clinical Gastroenterology*, 29, 143–150.
- EFSA (European Food Safety Authority), 2011. Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment. *EFSA Journal* 2011;9(3):2097. <https://doi.org/10.2903/j.efsa.2011.2097>
- EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), 2013. Scientific Opinion on the maintenance of the list of QPS biological agents intentionally added to food and feed (2013 update). *EFSA Journal* 2013;11(11):3449, 107 pp. <https://doi.org/10.2903/j.efsa.2013.3449>
- EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition, and Allergies), 2010. Scientific Opinion on Dietary Reference Values for carbohydrates and dietary fibre. *EFSA Journal* 2010;8(3):1462, 77 pp. <https://doi.org/10.2903/j.efsa.2010.1462>.
- Finegold SM, Li Z, Summanen PH, Downes J, Thames G, Corbett K, Dowd S, Krak M and Heber D, 2014. Xylooligosaccharide increases bifidobacteria but not lactobacilli in human gut microbiota. *Food and Function*, 5, 436–445.

- Fu A, Lee AW, Case IL and Cho SC, 2012. Efficacy and safety of xylooligosaccharides. In: Cho SC and Almeida N (eds.). *Dietary Fiber and Health*. CRC Press, Boca Raton, FL. pp. 497–517.
- Gao Y, Zhang S, Li C, Xiao L, Shen J and Yin J, 2012. Acute and subchronic toxicity of xylo-oligosaccharide in mice and rats. *Toxicology Mechanisms and Methods*, 22, 605–610.
- Gao Y, Wang Y, Li Y, Han R, Li C, Xiao L, Cho S, Ma Y, Fang C and Lee AW, 2017. Repeated sub-chronic oral toxicity study of xylooligosaccharides (XOS) in dogs. *Regulatory Toxicology and Pharmacology*, 86, 379–385.
- GRAS (Generally Recognised as Safe), 2013. GRN No. 458: Notification for Shangdong Longlive Biotechnology, Ltd.'s Xylooligosaccharides. Available on: <https://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=458&sort=Substance&order=ASC&startrow=1&type=basic&search=458>
- Hilbig A, 2005. Längerfristige Trends bei der Ernährung von Säuglingen und Kleinkindern der DONALD Studie im Zeitraum 1989 – 1999 [Long-term trends in the nutrition of infants and young children of the DONALD study from 1989-1999]. Inaugural dissertation at the Justus-Liebig-Universität Gießen.
- Holmes AM, Rudd JA, Tattersall FD, Aziz Q and Andrews PL, 2009. Opportunities for the replacement of animals in the study of nausea and vomiting. *British Journal of Pharmacology*, 157, 865–880.
- Huntley NF and Patience JF, 2018. Xylose: absorption, fermentation, and post-absorptive metabolism in the pig. *Journal of Animal Science and Biotechnology*, 9, 4.
- Inspection Report, 2009. Report No: LuJiKongDuJianZiNO.2009042.
- JEFCA (Joint FAO/WHO Expert Committee on Food Additives), 2006. Combined Compendium of Food Additive Specifications. Available online: <http://www.fao.org/3/a-a0691e.pdf>
- Kobayashi TOM, Fujikawa S and Koga K, 1991. Effect of Xylooligosaccharides on Feces of Men. *Japan Society for Bioscience, Biotechnology and Agrochemistry*, 65, 1651.
- Kuroiwa Y, Nishikawa A, Imazawa T, Kitamura Y, Kanki K, Umemura T and Hirose M, 2005. Lack of carcinogenicity of D-xylose given in the diet to F344 rats for two years. *Food and Chemical Toxicology*, 43, 1399–1404.
- MHLW (Ministry of Health, Labor and Welfare of Japan), 1996. Guidelines for Designation of Food Additives, and for Revision of Standard for Use of Food Additives, Article No. 29 of the Life and Sanitation Bureau.
- Na MH and Kim WK, 2001. Effects of xylooligosaccharide intake on fecal bifidobacterium, lactic acid and lipid metabolism in Korean young women. *Korean Journal of Nutrition*, 40, 154–161.
- Nevalainen H, Suominen P and Taimisto K, 1994. On the safety of *Trichoderma reesei*. *Journal of Biotechnology*, 37, 193–200.
- OECD (Organisation for Economic Co-operation and Development), 1997. Test No. 471: Bacterial reverse mutation test. In: *OECD guidelines for the testing of chemicals, Section 4: Health effects*, 11 pp.
- Okazaki M, Fujikawa S and Matsumoto N, 1990. Effect of xylooligosaccharide on the growth of Bifidobacteria. *Bifidobacteria Microflora*, 9, 77–86.
- Okazaki M, Koda H, Izumi R, Fujikawa S and Matsumoto N, 1991. In vitro digestibility and in vivo utilization of xylobiose. *Journal of Japanese Society of Nutrition and Food Science*, 44, 41–44.
- Park YJ, Gyun H, Taek LU, Wan LJ, Seung LC, Kyoung RB, Kun YC, Wang YS and Hyun KB, 1999. Acute oral toxicity of xylooligosaccharide in rats. *Journal of Food Hygiene and Safety*, 14, 255–258.
- Park YJ, Taek LU, Wan LJ, Seung LC, Kyoung RB, Kun YC, Wang YS and Hyun KB, 2000. Subacute toxicity of xylooligosaccharide in rats. *Journal of Food Hygiene and Safety*, 15, 151–166.
- Segal S and Foley JB, 1958. The metabolic fate of C14 labeled pentoses in man. *Clinical Endocrinology Branch, National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Md.*
- Unpublished study report, 2001b. Function Test Centre of Health food Institute for Nutrition and Food Safety of the Chinese Center for Disease Control and Prevention. Test Report No: Yingweigongjian No.20010093.
- Unpublished study report, undated. Title: acute toxicity of xylo-oligosaccharide in dog by feeding.
- Unpublished study report a), undateda. Pathology report 26wk long-term toxicity trial of xylo-oligosaccharide in Beagle by oral administration.
- Unpublished study report b), undatedb. 26wk long-term toxicity trial of xylo-oligosaccharide in Beagle by oral administration.
- Unpublished study reports, 2001a. Assay Inspection Report, Shandongspa, No. 20011D073.
- Wyngaarden JB, Segal S and Foley JB, 1957. Physiological disposition and metabolic fate of infused pentoses in man. *Journal of Clinical Investigation*, 36, 1395–1407.
- Xiao L, Ning J and Xu G, 2012. Application of Xylo-oligosaccharide in modifying human intestinal function. *African Journal of Microbiology Research*, 6, 2116–2119.
- Yang J, Summanen PH, Henning SM, Hsu M, Lam H, Huang J, Tseng CH, Dowd SE, Finegold SM, Heber D and Li Z, 2015. Xylooligosaccharide supplementation alters gut bacteria in both healthy and prediabetic adults: a pilot study. *Frontiers in Physiology*, 6, 1–10.

## Abbreviations

ADME	absorption, distribution, metabolism and excretion
BMI	body mass index
bw	body weight
cfu	colony forming units

CICC	China Center of Industrial Culture Collection
CNAS	China National Accreditation Service for Conformity Assessment
DP	degree of polymerisation
DRV	dietary reference value
EC	Enzyme Commission
GLP	good laboratory practice
MPN	most probable number
MS	Member States
NDA	EFSA Panel on Dietetic Products, Nutrition and Allergies
NF	novel food
NOAEL	no-observed adverse effect level
OECD	Organisation For Economic Co-Operation and Development
QPS	Qualified presumption of safety
SCFA	short-chain fatty acids
XOS	xylo-oligosaccharides