SCIENTIFIC OPINION

EFSA Journal

ADOPTED: 5 May 2020

doi: 10.2903/j.efsa.2020.6135

Safety of the extension of use of plant sterol esters as a novel food pursuant to Regulation (EU) 2015/2283

EFSA Panel on Nutrition, Novel Foods and Food Allergens (NDA), Dominique Turck, Jacqueline Castenmiller, Stefaan De Henauw, Karen Ildico Hirsch-Ernst, John Kearney, 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, Rosangela Marchelli, Monika Neuhäuser-Berthold, Morten Poulsen, Josef Rudolf Schlatter, Henk van Loveren, Wolfgang Gelbmann 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 the safety of an extension of use of the novel food 'plant sterol esters' when added to vegetable fat spreads and to liquid vegetable fat-based emulsions for cooking and baking purposes pursuant to Regulation (EU) 2015/2283. Member States expressed concerns in relation to plant sterol oxidation products (POP) and consumption by non-target population groups. The median (0.5%) and P90 (2.28%) value of the oxidation rates of plant sterols determined by a wide range of cooking experiments were used together with exposure estimates for plant sterol when added and cooked with vegetable fat spreads and liquids. The no-observed adverse effect level (NOAEL) of a subchronic rat study and an applied default uncertainty factor of 200 served to derive levels (i.e. 0.64 mg POP/kg body weight (bw) per day) considered safe for humans. This safe level of exposure would be exceeded at the P95 by all age groups when considering the P90 oxidation rate and using EFSA's comprehensive food consumption database for assessing the potential exposure. When considering the median oxidation rate, the safe level of 0.64 mg POP/kg bw per day would be exceeded at the highest P95 intake estimates in children below 9 years of age. When considering an intake of the maximum authorised use level of 3 g plant sterols/person per day and oxidation rates of 0.5% and 2.28%, the resulting daily POP intakes per kg bw by an adult weighing 70 kg would be 0.21 and 0.98 mg/kg bw per day, respectively, the latter value exceeding 0.64 mg/kg bw per day. The Panel concludes that the safety of the intended extension of use of plant sterol esters under the proposed conditions of use has not been established.

Keywords: phytosterols, plant sterol esters, oxidation products, cholesterol, heating, cooking

Requestor: European Commission following an application by (originally) Unilever Research and Development Vlaardingen B.V (now Upfield Research and Development B.V.)

Question number: EFSA-Q-2014-00865

Correspondence: nda@efsa.europa.eu

Panel members: Dominique Turck, Jacqueline Castenmiller, Stefaan De Henauw, Karen Ildico Hirsch-Ernst, John Kearney, 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.

Suggested citation: EFSA NDA Panel (EFSA Panel on Nutrition, Novel Foods and Food Allergens), Turck D, Castenmiller J, De Henauw S, Hirsch-Ernst KI, Kearney J, Maciuk A, Mangelsdorf I, McArdle HJ, Naska A, Pelaez C, Pentieva K, Siani A, Thies F, Tsabouri S, Vinceti M, Cubadda F, Frenzel T, Heinonen M, Marchelli R, Neuhäuser-Berthold M, Poulsen M, Schlatter JR, van Loveren H, Gelbmann W and Knutsen HK, 2020. Scientific Opinion on the safety of the extension of use of plant sterol esters as a novel food pursuant to Regulation (EU) 2015/2283. EFSA Journal 2020;18(6):6135, 36 pp. https://doi.org/10.2903/j.efsa.2020.6135

ISSN: 1831-4732

The EFSA Journal is a publication of the European Food Safety Authority, an agency of the European Union.

Table of contents

1. Introduction

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

On 24 June 2013, the company Unilever Research and Development Vlaardingen B.V (now Upfield Research and Development B.V.) submitted a request in accordance with Article 4 of the Novel Food Regulation (EC) No[1] to extend the use of phytosterols (=plant sterols, PS) esters as a novel food ingredient to be used in spreads and liquid margarines for cooking and baking purposes.

On 2 April 2014, the competent authority of the United Kingdom forwarded to the Commission its initial assessment report, which came to the conclusion that the requested extension of use of plant sterol esters (PSE) meets the criteria for acceptance of a novel food defined in Article (3)1 of Regulation (EC) No 258/97.

On 3 April 2014, the Commission forwarded the initial assessment report to the other Member States. Several Member States submitted comments or raised objections.

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

- Heating plant sterol esters (PSE) during the production of food increases the formation of plant sterol oxidation products (POP). The formation of POP in the various food production processes is not adequately explained and discussed in the application documents. Information regarding the analytical methods applied in the stability studies and their suitability for analyses of specific POP and/or specific chemical substance classes should be provided.
- For spreads with added PSE for cooking and baking (CB), the applicant investigated POP oxidation using various different conditions to mimic the variety of cooking and frying practices at home. The information on the variation of the results obtained for each test condition is not presented in the dossier (this also holds for the vegetable oils used as controls), which makes it difficult to interpret these data.
- The oxidation stability studies of the applicant were reported in 2002. The applicant should review also recent relevant literature on this topic, with particular attention to the results published by Soupas et al. (2007). This report deals with a systematic investigation on the effect of parameters like temperature, duration of heating/frying, type of lipid matrix and water content on the formation of POP. The data presented by Soupas et al. (2007) seem to indicate a more extensive oxidation while using less severe heat-stress conditions compared to the applicant's tests.
- The requested extension of use can be expected to cause an exposure of the non-targeted population (children amongst others) to the product.
- While the applicant recommends that the product is not to be used for deep frying, fats offered for cooking and baking purposes cannot be excluded from also being used for roasting and frying.
- The report on post launch marketing (PLM) data by Europanel in 2013 shows that households consisting of two members have a much higher daily average of PS use compared with the average of all households. A more detailed analysis of the daily consumption by loyal consumers in the two member-households e.g. the 95th percentile in The Netherlands or in the United Kingdom is desirable.
- The PLM study mentioned did not cover the other product categories that have also been authorized, although more recently, i.e. sauces, cheese type products, soya drinks and rye bread. Apparently, such products seem not (yet) widely available, but it is recommendable to closely monitor consumption of phytosterol-enriched foods on a regular basis, because convincing scientific evidence that chronic exposure gives no cause for concern is still absent.
- Data are lacking on the intestinal absorption of POP from the consumption of heated foods with added PSE.
- An assessment of the toxicological tests involving the phytosterol oxide concentrate (POC) is only possible after the full study reports have been submitted.
- When a chronic toxicity study is not available, an additional uncertainty factor is normally included in the risk assessment, and therefore the safety margin for the expected absorption would decrease accordingly.

[1] 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.

- There are studies which showed undesirable effects of POP on endothelial functions in experimental systems. However, there is a lack of studies that determine the content of POP in human plasma after the consumption of heated foods added with PSE and their effects on vascular parameters. The data available to assess the atherogenic potential of POP is insufficient and inconsistent and such effects of PS and POP cannot be ruled out (Kelly et al., 2011; Weingärtner et al., 2008, 2009, 2011).

Experimental studies with animals:

Yang et al. (2013) showed an undesirable influence of POP on the endothelial function of rats. Tomoyori et al. (2004), on the other hand, did not discover any undesirable effects of POP on the endothelial function of rats.

A study by Liang et al. (2011) showed that the plaque-reducing effects of PSE were cancelled out by their oxidation products in apoE-deficient hamster.

Plat et al. (2014) found POP significantly increased the development of atherosclerotic lesions in LDL receptor deficient (LDLR+/−) mice, although oxysterols (oxidised cholesterol) were shown to cause a greater increase.

Studies in humans

POP can be identified in human serum; however, the question of whether the consumption of foods with added PSE increases the content of POP in plasma has not yet been answered. Baumgartner et al. (2013), for example, found no connection between POP concentration in plasma and the consumption of products with added PS, whereas Husche et al. (2011) found that plasma POP concentration doubled after consumption of these types of products.

Schött et al. (2014) analysed the PS and POP content of the plasma and aortic valve cusp tissue of patients with severe aortic stenosis. They discovered increased amounts of POP in the aortic tissue that did not correlate to the content found in the plasma. The authors discuss the possibility of POP developing locally from PS in the valve tissue, which could lead to increased inflammatory reactions with increased plaque formation.

On 27 November 2014 and in accordance with Article 29(1)(a) of Regulation (EC) No 178/2002[2], the Commission asked the European Food Safety Authority to provide a scientific opinion by carrying out the additional assessment for the NF in the context of Regulation (EC) No 258/97 and to consider the elements of a scientific nature in the comments raised by the other Member States.

According to Article 35 (1) of Regulation (EU) 2015/2283[3], any request for placing a novel food on the market within the Union submitted to a Member State in accordance with Article 4 of Regulation (EC) No 258/97, and for which the final decision has not been taken before 1 January 2018, shall be treated as an application under this Regulation. (Note: 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 the United Kingdom, the concerns and objections of a scientific nature raised by the other Member States and information submitted by the applicant in response to the Member States' comments and following an EFSA request for supplementary information as well as additional data identified by the Panel.

[2] Regulation (EC) 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.

[3] 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.

Administrative and scientific requirements for NF applications referred to in Article 10 of Regulation (EU) 2015/2283 are listed in Commission Implementing Regulation (EU) 2017/2469[4].

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.[5] 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.

This NF application includes a request for protection of proprietary data in accordance with Article 26 of Regulation (EU) 2015/2283. The applicant claims proprietary rights for a human pharmacokinetic study (i.e. Title: The effect of oxidised PS Intake on serum concentrations of PS oxidation products. Global Study Number: FDS-SCC-2838).

2.2. Methodologies

The assessment follows the methodology set out in the EFSA guidance on NF applications (EFSA NDA Panel, 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 Commission Implementing Regulation (EU) 2017/2469.

3. Assessment

3.1. Introduction

In 2000, the Scientific Committee on Food (SCF) assessed the safety of PSE in yellow fat spreads on the basis of an application from Unilever under Regulation (EU) No 258/97 (SCF, 2000). This application specified that this NF was not intended for use in cooking. The toxicological information provided in the application included a 13-week feeding study with rats, a two-generation feeding study with rats, studies on oestrogenic potential and tests on genotoxicity. In its assessment, the SCF noted the target population 'adults above 50 years of age, who try to control their elevated blood cholesterol', but considered that also children would not be expected to experience any adverse effect on metabolism when their blood cholesterol is lowered. The SCF noted also that PS absorption was found to be higher in children than in adults. The SCF concluded that PSE in yellow fat spreads at a maximum level corresponding to 8% free PS (FPS) are safe for human use. The SCF noted that ingestion of 20 g/day for 1 year of products containing 8% PS reduced plasma β-carotene concentrations in adults by 20% and that the reduced plasma β-carotene levels might become relevant when the vitamin A status is not optimal, which may be the case for pregnant and lactating women as well as younger children.

Consequently, in 2000, the European Commission adopted the Decision 2000/500/EC authorising the placing on the market of 'yellow fat spreads with added PSE' as a novel food or novel food ingredient under Regulation (EC) No 258/97 (European Commission, 2000). According to the specifications of that marketing authorisation, the margarine/vegetable oil spreads may contain up to 8% w/w of added PS (equivalent to 14% w/w PSE) with a relative content among PS of 10–40% campesterol, 6–30% stigmasterol, 30–65% β-sitosterol and 0–5% other PS. The marketing authorisation included labelling requirements specifying (1) the target population, i.e. 'people who want to lower their cholesterol levels', (2) that patients on cholesterol lowering medication should only consume the product under medical supervision, (3) that the product may not be nutritionally appropriate for pregnant and breastfeeding women and children under the age of 5 years and (4) that the product should be used as part of a healthy diet, including regular consumption of fruit and vegetables to help maintain carotenoid levels.

In 2002, the SCF assessed the long-term effects of the intake of elevated levels of PS from multiple dietary sources, with particular attention to the effects on β-carotene (SCF, 2002a). Noting that no

[4] 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.

[5] 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 HJ, 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(11):4594, 24 pp. https://doi.org/10.2903/j.efsa.2016.4594

additional effect on cholesterol levels is derived from an intake of PS above the 3 g per person per day and that the consequences of a persistent decrease of blood concentrations of β-carotene on human health are largely unknown, the SCF considered it prudent to avoid PS intakes exceeding 3 g/day.

In 2004, an extension of this marketing authorisation was authorised for the uses of PSE in milk- and yoghurt-type products by Commission Decision 2004/335/EC, (European Commission, 2004a). Also in 2004, Commission Regulation (EC) No 608/2004 concerning the labelling of foods and food ingredients with added phytosterols, phytosterol esters, phytostanols and/or phytostanol esters, laid down labelling requirements and limiting the consumption of added PS to a maximum of 3 g per person per day (European Commission, 2004b).

At their 69th meeting, JECFA established a group acceptable daily intake (ADI) of 0–40 mg PS/kg body weight. This corresponds to a daily intake of 2.8 g PS/day for a 70-kg individual (JECFA, 2009). This conclusion was based on an overall NOAEL derived from several subchronic (90-day) studies, supported by studies on reproductive toxicity. Noting the absence of a chronic toxicity study, JECFA found the application of an uncertainty factor of 100 sufficient taking into account the availability of a range of human studies including two 1-year studies.

In this application, the applicant seeks the authorisation to extend the uses of PSE, i.e. the addition to vegetable fat spreads and to liquid vegetable fat-based emulsions for cooking and baking purposes excluding deep-frying. The applicant indicated that the production process and specifications do not change. This assessment concerns the risks that might be associated with the consumption of the NF used for cooking purposes.

In accordance with Article 5(6) Commission Implementing Regulation (EU) 2017/2469, the safety of the NF is assessed for the general population. It is not an assessment of the efficacy of the NF with regard to any claimed benefit.

3.2. Identity of the NF

The NF concerns PSE added to vegetable fat spreads ('PS Spreads') and liquid vegetable fat-based emulsions ('PS Liquids') which meet the specifications laid down in Commission Decisions 2000/500/EC and 2004/335/EC and subsequently included in Commission Implementing Regulation (EU) 2017/2470 establishing the Union list of novel foods in accordance with Regulation (EU) 2015/2283.

3.3. Compositional data

The content of PSE is 12.5 g/100 g, corresponding to a content of PS of approximately 7.5 g/100 g. The applicant provided information on the compositions of PS-Spreads and PS-Liquids for CB. Both product categories (spreads and liquids) contain proteins, varying levels of emulsifiers, stabilisers, salt and vitamins.

3.3.1. Stability

Upon request of EFSA, the applicant provided data from a publication on the amounts of POP in 19 foods prepared by typical household cooking and baking methods using margarine without (control) and with 7.5% added PS (as 12.5% PSE; further named PS-margarine) (Lin et al., 2016a). Various foods, including vegetables (green beans, cabbage, onions), potatoes, meat (pork, beef and chicken), fish (cod, salmon and frozen fish fingers) and eggs, were subjected to shallow-frying, stir-frying, stewing, roasting and microwave cooking (details given in Annex 1). In addition, different baked foods (cookies, muffins, banana bread and sponge cake) were investigated for POP content (Annex 2). Methodologies for the quantification of PS and POP in these cooked and baked foods, in particular extraction procedures, have been developed and validated (Menéndez-Carreno et al., 2016).

The time and temperature conditions of the applied cooking and baking procedures are summarised in Annex 3. The portion sizes prepared varied between 100 and 250 g. Roast beef was prepared from 1 kg of meat, and the portion size was defined as 200 g roast (cooked) meat. The portion sizes of the baked products were defined as one typical slice of banana bread (80 g) or sponge cake (50 g), one piece of muffin (44 g) and one cookie (18 g).

The experimental data demonstrate that the increase of POP contents in cooked and baked foods, resulting from the use of the PS-margarine as compared to the control margarine, was heavily influenced by the applied cooking/baking procedures (Annex 2). The median contents of POP per portion size of cooked foods were 0.57 mg (range 0.05–1.11 mg) with control margarine and 1.42 mg (range 0.08–20.5 mg) with PS-margarine. The increase of POP in the cooked foods prepared with the

PS-margarine compared to those prepared with the control margarine ranged from 1.8-fold (shallow-fried egg, stewed beef and microwave-cooked codfish) to 21-fold (stir-fried chicken and shallow-fried potatoes).

In foods prepared with the control margarine, the oxidation rate of phytosterols (ORP) ranged from 0.51% (microwave-cooked codfish) to 9.82% (shallow-fried beefsteak) with a median of 3.66%. For foods prepared with the PS-margarine, the oxidation rates ranged from 0.02% (microwave-cooked codfish) to 3.45% (shallow-fried potatoes), with a median of 0.50%, the P90 oxidation rate across the whole range of cooking and baking experiments for all foods was 2.28%.

For all cooked foods, except for the stir-fried cabbage where all fat was absorbed, a variable amount of residual fat remained in the pan or the wok after cooking. When the control margarine was used, the median POP content of residual fat was 0.52 mg (range 0.04–1.88 mg) per amount of residual fat observed for the different foods and cooking methods. Using the PS-margarine, the median POP content of the residual fat was 6.98 mg (range 0.22–23.9 mg) per observed amount of residual fat.

Regarding the sum of POP contents of foods plus residual fat, the median total POP content was 1.06 mg (range 0.15–2.85 mg) with the control margarine and 6.48 mg (range 0.30–44.4 mg) with the PS-margarine. The distribution of POP between the food itself and the corresponding residual fat widely ranged among the foods. Most of the cooked animal foods and fish, like pork fillet, steak, minced meat, salmon and codfish contained only about 3–41% of the total amount of POP, whereas in other foods, e.g. green beans, cabbage, onions but also fish fingers (which absorb more fat), 71–100% of the POP were found within the food matrix. Whenever the total amount of residual fat was higher than 5 g, more than 50% of the total amounts of POP were found in the residual fat.

The POP contents per portion size of baked products and the respective oxidation rates are presented in Annex 3. Median POP contents across cookies, muffins, banana bread and sponge cake were 0.12 mg per portion (range 0.11–0.21 mg) with the control margarine and 0.24 mg per portion (range 0.19–0.60 mg) with the PS-margarine. For baked foods, the POP content increase ranged from a factor 1.7 (for cookies) up to a factor 2.9 (for sponge cake) as compared to the control margarine.

The applicant also expressed the POP contents as mg per 100 g prepared food and residual fat (Annex 4). Using the control margarine, the median POP content of cooked foods was 0.47 mg/100 g food, while using the PS-margarine, the median POP content was 1.52 mg/100 g food. The median POP amounts in the residual fat were 7.12 mg/100 g fat and 76.68 mg/100 g fat, with the use of the control margarine and PS-margarine, respectively. The highest amount of POP was found in the residual fat (415.58 mg/100 g fat) from shallow-frying of potatoes with the PS-margarine.

The median amount of POP/100 g of baked products was 0.33 mg/100 g (range 0.15–0.66 mg/100 g) with the control margarine and 0.78 mg/100 g (range 0.34–1.20 mg/100 g) with the PS-margarine.

When control margarine was used, the median values from the distributions of individual POP compounds (expressed as percent of total POP) in the foods and in the residual fat, respectively were 47.2% and 45.1% for 7-keto-PS, 25.0% and 29.9% for 5,6-epoxy-PS, 22.1% and 21.3% for 7-hydroxy-PS and 5.6% and 3.9% for PS-triols, with 7-keto-PS being the dominant individual POP. When PS-margarine was used for cooking, 5,6-epoxy-PS and 7-keto-PS each accounted for 35.8–37.8% (median) of the total POP, with 7-hydroxy-PS and PS-triols accounting for 23.9% and 1.9%, respectively, in foods. In the residual fat, 7-hydroxy-PS, 5,6-epoxy-PS, 7-keto-PS and PS-triols accounted for about 38.4%, 30.7%, 28.1% and 1.2% of the total POP, respectively, with 7-hydroxy-PS being the dominant individual POP. For baked foods prepared with the control and PS-margarines, the distribution of individual POP was in the order of 7-keto-PS > 5,6-epoxy-PS > 7- hydroxy-PS > PS-triols.

The applicant also performed a series of frying experiments without the use of foods. Heating of the PS-Spread for CB in tub format at 180°C for 15 min resulted in amounts of POP ranging from 34.3 to 48.5 mg/100 g product.

In addition, two samples of the PS-Liquid for CB (containing 70% of total fat), with 12.5% PSE (equivalent to 7.5% plant sterols) were shallow-fried at 205°C ± 5°C for 30 min. On average 74.2 mg/100 g (range 70.4–80.0 mg/100 g) POP was formed.

The occurrence of POP in foods with added PSE has been recently been reviewed (Scholz et al., 2015). Heating temperature and time, the chemical form in which the phytosterols are added (free phytosterols, PSE or phytostanol esters), and the food matrix were shown to be critical parameters determining the formation of POP. For example, in heat-treated milk, the POP contents ranged from 0.2 mg/kg (milk with added plant stanol esters (PAE), corresponding to 0.5% free phytostanols; pasteurised at 127°C for 2 s) (Soupas et al., 2006) to 6.4 mg/kg (milk with added PSE, corresponding to 0.3% PS; microwave heated at 900 W for 1.5 min) (Menéndez-Carreno et al., 2008).

Pan-frying of a liquid spread (with added PSE corresponding to 5% PS) at 180°C for 5 and 10 min, resulted in an increase of the content of POP from 255 mg/kg to 291 mg/kg and 668 mg/kg POP, respectively (Soupas et al., 2007).

Storage of a dark chocolate with added PSE at 30° C for 5 months resulted only in a minor increase of POP from 68.6 to 71 mg/kg (Bothelo et al., 2014). On the other hand, in spread with added PAE, the contents of POP were reported to increase upon storage for 6 weeks at 4°C and 20°C from 255 mg/kg to 354 and 734 mg/kg, respectively (Rudzinska et al., 2014).

In a systematic review, the applicant evaluated 14 studies measuring POP contents of foods with added FPS, PSE and PAE (Lin et al., 2016b). In non-heated or stored foods, POP contents (medians) ranged from 0.03 to 3.6 mg/100 g with corresponding ORP of 0.03–0.06%. In fat-based products with 8% of added FPS, PSE or PAE pan-fried at 160–200°C for 5–10 min, median POP contents were 72.0, 38.1 and 4.9 mg/100 g, respectively, with median oxidation rates of 0.90%, 0.48% and 0.06%, respectively, indicating that the resistance to thermal oxidation was in the order of PAE > PSE > FPS. POP formation was highest in butter followed by margarine and rapeseed oil. In margarines with 7.5–10.5% of added PSE content, oven-heating at 140–200°C for 5–30 min resulted in a median POP content of 0.3 mg/100 g. Further heating at the same temperature conditions but for 60–120 min resulted in markedly further increased POP formation to 384.3 mg/100 g.

In a recent study, two types of margarine either with added PAE or with an added mixture of PSE/PAE were subjected to heating procedures representing typical ways of preparing foods in the home (Scholz et al., 2016). Complementary analyses were carried out to determine the loss of individual PSE/PAE as well as the concurrent formation of POP. Microwave-heating led to the lowest decreases (around 5%) of esters content in both margarines. Oven-heating of the two margarines in a casserole caused the strongest decreases, with respectively 68% and 86% of the esters remaining; the impact on individual esters was more pronounced with increasing degree of unsaturation of the esterified fatty acids. In the PSE/PAE – added margarine, approximately 20% of the ester losses could be explained by the formation of POP; in the PSE-added margarine, the POP accounted for less than 1% of the observed ester decreases. Another study by Raczyk et al. (2018) reported a 31–49% loss of PSE when pan-frying potato chips (with diameter of 0.5 cm) for 15 min and 180°C.

The Panel notes that the observed oxidation rates for PS varies substantially across a number of different cooking experiments carried out under different conditions. However, the experiments conducted by Lin et al. (2016a), represent a wide range of different foods cooked and baked under various different conditions, resulting in a median and a P90 oxidation rates of 0.50% and 2.28%, respectively. The Panel considers these values to be sufficiently conservative and appropriate for their use in the exposure calculation of POP from the NF. The Panel notes that cooking and baking of PSE results in oxidation of a relatively small proportion of PSE. However, the P90 oxidation rate is about 100 times higher than the oxidation rate for unheated PS added at 3 g to 20 g margarine, which was reported to be 0.02% by Baumgartner et al. (2013). The Panel also notes that the loss of only a small fraction of the PSE is due to the formation of POP.

3.4. Specifications

According to the specifications laid down by the Union List, the PS fraction contains 10–40% campesterol, 6–30% stigmasterol, 30–65% β-sitosterol and up to 5% other PS.

The applicant states that the production process, composition and specifications remain unchanged.

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

The phytosterols as well as the fatty acids used for esterification are obtained from commonly used edible plant oils with a safe history of food use.

3.6. Proposed uses and use levels and anticipated intake

3.6.1. Target population

The applicant refers to the currently applicable mandatory labelling requirements in place for foods and food ingredients with added FPS, PSE, plant stanols and/or PAE in accordance with the Union list (Commission Implementing Regulation (EU) 2017/2470). Regarding target and non-target population, the labelling requirements include a mandatory statement that the food is intended exclusively for

people who want to lower their blood cholesterol level and that it may not be nutritionally appropriate for pregnant or breastfeeding women and children under the age of 5 years.

The Panel notes that Article 5(6) of the Commission Implementing Regulation (EU) 2017/2469 stipulates that [citation: 'Where it cannot be excluded that a novel food intended for a particular group of the population would be also consumed by other groups of the population the safety data provided shall also cover those groups']. The Panel therefore considers all age groups of the general population above six months of age in this risk assessment.

3.6.2. Proposed uses and use levels

The NF is currently authorised for its use in milk-based products, products based on semi-skimmed and skimmed milk, products based on fermented milk such as yoghurt and cheese based products, soy drinks, salad dressings, mayonnaise, spicy sauces, and spreadable fats excluding cooking and frying fats and spreads based on butter and other animal fats (EU Union List NF). According to the authorisation, the products containing the NF shall be presented in such a manner that they can be easily divided into portions that contain either a maximum of 3 g (in case of one portion per day) or a maximum of 1 g (in case of three portions per day) of added phytosterols/phytostanols. The amount of phytosterols/phytostanols added to a container of beverages shall not exceed 3 g. Furthermore, products must be labelled with a statement that the consumption of more than 3 g/day of added PS should be avoided.

The applicant asks for an 'extension of use of phytosterols esters in spreads and liquid fats for home cooking as a direct replacement of other fats and oils'. These intended new uses include roasting and shallow-frying, but exclude deep-frying. The intended uses cover vegetable fat products that are liquid emulsions ('PS-Liquids'), as well as vegetable fat products ('PS-Spreads'), that are similar in composition to the authorised vegetable fat spreads with added plant sterols. The NF with the proposed extended use is intended to be presented as a multipurpose tub.

The proposed PSE level to be added to PS-Spreads/Liquids for cooking purposes is 12.5 g (corresponding to 7.5 g PS) per 100 g or 100 mL product. This is the same use level of plant sterols applied to spreads with added PS currently on the market. The applicant notes that the current conditions of use including the maximum use levels (i.e. 3 g per person per day) would also apply to this proposed extension of use and that the extension would provide an alternative to existing and authorised products with added PSE. Thus, according to the applicant, under the current labelling measures in place, the exposure to PSE would not increase with this extension of use.

3.6.3. Anticipated intake of the NF

3.6.3.1. Intake estimation of extended use of the NF

In order to estimate the possible exposure to PSE and POP from cooked and baked fats with added PSE, the applicant used different approaches: a) food consumption data of margarine and liquid vegetable-based emulsions, b) data from the applicant's PLM and c) published reports. In addition, EFSA performed a refined exposure assessment on the basis of individual consumption data and representative for the EU population (d).

a) Use of food consumption data bases

The applicant used three data sets on the consumption of margarine and liquid vegetable-based emulsions to estimate the intake of PS from PS-Spreads and PS-Liquids for intended CB: (i) the UK National Diet and Nutrition Survey (NDNS, 2011; UKDA 2012), (ii) the Dutch National Food Consumption Survey (RIVM, 2011; Ocké et al., 2008; van Rossum et al., 2011), and summary statistics of the EFSA's comprehensive data base (EFSA, 2011), respectively.

These intake estimates indicate that high (P95) percentile consumption may exceed 3 g per person per day (Table 1). The applicant notes that these intake estimates are conservative, most notably because it is assumed that all consumed margarines and liquid vegetable-based emulsions contain PS.

Table 1: Overview on daily intake estimates of plant sterols using margarine and vegetable fat liquid emulsions consumption data from the NL, UK and EFSA

	Plant sterol daily intakes (g) based on total margarine intake (all users) female/male								
	NL			UK			EFSA		
	Age	Median	P95	Age	Mean	P95	Age	Mean	P95
Infants	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	0–11 m	0.2	0.4
Young children or toddlers	2–6	0.8/1.0*	1.5/2.0	1–3	0.7	1.4	1–3	0.2/0.7	0.6/1.6
Other children	7–13	1.0/1.4	2.9/3.7	4–10	1.0	2.1	3–9	0.2/1.0	0.5/3.4
Adolescents	14–18	0.9/1.4	2.9/4.7	11–18	1.0	2.4	10–17	0.4/1.1	0.9/3.4
Adults	19–30	1.1/1.7	3.4/5.4	19–44	1.2	2.9	n.a.	n.a.	n.a.
Female Adults	19–50	1.1	3.4	19–44	1.1	2.5	n.a.	n.a.	n.a.
Adults	31–69	1.1/1.8	3.4/5.0	45+	1.4	3.2	18–64	0.6/1.7	1.4/6.4
Elderly	n.a.	n.a.	n.a.	n.a.			65–74	0.3/2.5	0.5/7.1

na = data not available for this population group.
*: Data on the Netherlands toddlers aged 2–6 are presented as mean intakes rather than median intakes.

b) Post launch monitoring (PLM)

The applicant used detailed quantitative market research based on purchase data to estimate the levels of consumption of a range of products (including spreads, milk drinks, yoghurts and dressings) with added PSE and PAE in five EU Member States (United Kingdom, Germany, France, Belgium and the Netherlands) (Willems et al., 2013). The study, carried out in 2011, used relatively large consumer panels (5,000–30,000 per country), and households used a barcode reader at home to continually log the food that they purchased. It was assumed that all products purchased were consumed and that no additional products were purchased and consumed outside the home. The households that purchased products with added PSE and/or PAE ranged from 4,700 households in the UK to 646 in the Netherlands. The results of this study are summarised in Table 2.

Table 2: Mean, median and 95th percentile daily PS plus phytostanol intakes per household (g/day) (Willems et al., 2013)

Country	Mean	Median	P95
UK	0.86	0.22	2.53
DE	0.40	0.17	1.53
FR	0.35	0.13	1.06
NL	0.82	0.26	3.70
BE	0.76	0.26	2.96

The applicant has broken down the data according to household size, with a separate category for households with children less than 5 years old. These results indicated that two-member households consume the highest levels of products with added PS, followed by 1 and 3 member households (Table 3).

Table 3: Mean daily PS plus phytostanol intakes per household (g/day), differentiated per household size (Willems et al., 2013)

	UK	NL	FR	DE	BE
All households	0.86	0.82	0.35	0.40	0.76
1 member HHs	0.75	0.53	0.28	0.34	0.67
2 member HHs	1.09	1.09	0.42	0.46	0.88
3 member HHs	0.63	0.79	0.41	0.45	0.78
4 member HH	0.71	0.65	0.28	0.28	0.56
5+ member HH	0.50	0.43	0.18	0.26	0.50
Households with children < 5 years	0.36	0.20	0.14	0.41	0.45

HHs: households.

According to the applicant's assumption, the result that 2-member households consume more than the national averages may be explained by the fact that 2-member households are more likely to be composed of individuals aged 45 years or older. In response to a Member State comment, the applicant provided the figures for the P95 consumption of the two-member households in the UK and the NL (i.e. 3.95 and 4.05 g/day).

Table 4 presents the highest mean single household (0.75 g/day), the highest mean among all households (0.86 g/day) and the highest P95 (3.70 g/day) among all households intake of PS from the five countries indicated in Willems et al. (2013). Corresponding exposure estimates to POP using different scenarios of applied oxidation rates as reported for non-heated and heated products with added PS are also presented. An exposure scenario to POP is also included which considers the same oxidation rates and the authorised maximum intake level of 3 g per person per day for PS.

Table 4: Intake scenarios for daily POP intakes (mg/day) based on different oxidation rates (ORP %) of added PS in non-heated and heat-treated foods

		ORP [%]		PLM survey of purchases of foods with added PS (Willems et al., 2013)			Maximum intake of PS (EU Union List)
PS intake scenarios (g/day)				0.75[(c)]	0.86[(d)]	3.70[(e)]	3
POP intakes (mg/day) depending on the ORP	**Non-heated foods[(a)]**	Median	0.06	0.5	0.5	2.2	1.8
		P90	0.38	2.9	3.3	14.1	11.4
	Non-heated Margarine	Ref[(f)]	0.023[(f)]	0.2	0.2	0.8	0.68
		Ref[(g)]	0.08[(g)]	0.6	0.6	3.0	2.4
	Heat-treated foods[(b)]	Median	0.50[(b)]	3.8	4.3	18.5	15.0
		P90	2.28[(b)]	17.1	19.6	84.4	68.4

POP: plant sterol oxidation products; ORP: oxidation rate of plant sterols; PS: plant sterols.
(a): Median and P90 oxidation rates (%) of non-heated foods with added PS reviewed in Lin et al. (2016b).
(b): Median and P90 oxidation rates (%) of heated foods with added PS reviewed in Lin et al. (2016a).
(c): Mean daily phytosterol intake per one-person household in the UK, which was the highest value in five European countries.
(d): Mean daily phytosterol intake per household in the UK, which was the highest value in five European countries.
(e): P95 of phytosterol intake per household in the Netherlands, which was the highest value among five European countries.
(f): Unheated margarine with added plant sterols, containing 3 g plant sterols/20 g margarine and approximately 34 ng oxyphytosterols per mg margarine (Baumgartner et al., 2013).
(g): Commercially available spread with 8% added phytosterols (47% sitostanol, 25% campesterol, 18% stigmasterol, 3.5% brassicasterol) in conformity with the specifications of the novel food (Grandgirard et al., 2004a).

Based on the median and high percentile (P90) oxidation rates (0.5% and 2.28%) of PS added to spreads used for cooking reported by Lin et al. (2016a), POP intakes from 3 g cooked PS would result to an exposure of 15 and 68 mg, respectively. When considering a default body weight of 70 kg for an adult person, such intake would correspond to 0.22 and 0.97 mg POP/kg bw. The corresponding exposure to POP from an intake of 3.7 g (P95 household exposure) would be 0.26 and 1.21 mg POP/kg bw.

c) Published reports on PS intakes

The applicant also summarised findings from two EFSA references (EFSA, 2008; EFSA ANS Panel, 2012) and referred to a Belgian study (Sioen et al., 2011a,b). In 2008, EFSA issued a report which covered an earlier PLM study by the applicant (Lea and Hepburn, 2006), a report on Irish consumption (Poulsen, 2007), a report issued by the German BfR (Niemann et al., 2007), and a report on British consumption pattern of products with added PS (Kemplay and Nordfjord, 2006). In this report from 2008, EFSA noted that the median and the high (95th or 97.5th) percentile intakes by adults of PS from products with added PS ranged from 1.0 to 1.9 and 2.2 to 3.6 g/day, respectively, for the five countries (i.e. the United Kingdom, Germany, France, Belgium and the Netherlands) considered by Lea and Hepburn (2006) (EFSA, 2008). Of these, 1–4% were identified as having consumption in excess of 3 g/day. More than 60% of the consumers of such products had high blood cholesterol and the large majority belonged to the over 45 years age group. Consumption among children was up to 8% and less than 1% in children below 5 years of age. The market share among spreads and margarines for products with added PS was less than 10%.

In 2012, EFSA's opinion on the use of stigmasterol-rich PS as a food additive noted that the average daily intake of PS from all sources was 2,770 mg/day in adults (EFSA ANS Panel, 2012).

According to a survey of phytosterol intakes in Belgium, the mean intake was 1.5 g/day and the 95th percentile 4.2 g/day. Twenty-one per cent of preschool children (2.5–7 year old) included in the survey were identified as consumers (mean intake 0.7 g/day, range 0.01–2.1 g/day) (Sioen et al., 2011a,b).

d) EFSA Comprehensive Food Consumption Data

In order to get a refined exposure estimate on the basis of individual food consumption data and representative for the EU population, EFSA consulted its EFSA Comprehensive Food Consumption Database (EFSA, 2011). Considering the subject of this application, i.e. the use of multipurpose vegetable fat spreads and liquid vegetable fat-based emulsions with added PSE for cooking and baking purposes (excluding deep-frying), the Panel considered the food categories presented in Table 5 for its exposure assessment. The use level indicated by the applicant is 7.5 g PS (corresponding to 12.5 g PSE) per 100 g or 100 mL product. Also for this exposure assessment of POP, oxidation rates of 0.5 and 2.28% of the phytosterols were used. The resulting occurrence levels of POP in the oils/fats would be 0.0375% (7.5 × 0.005) and 0.171% (7.5 × 0.0228), respectively.

Table 5: Food categories and, use level of PS (i.e. 7.5%) and occurrence of POP used for the intake estimates

CODE	FoodEx2_Level	FoodEx2_Name	Use level of the NF (%) in the oils/fats	Occurrence of POP (%) in the oils/fats
A0F1G	3	Margarines and similar	7.5*	0.0375** 0.171***
A039F	4	Butter and margarine/oil blends		
A039G	4	Shortening and similar baking fats		
A039J	4	Blended frying oil/fats		
A036N	3	Vegetable fats and oils, edible		

NF: novel food; POP: plant sterol oxidation products.
*: 12.5 g plant sterol esters per 100 g correspond to approximately 7.5 g free plant sterol equivalent.
**: When considering an oxidation rate of 0.5% of the cooked phytosterols (see Section 3.3 'Stability').
***: When considering an oxidation rate of 2.28% of the cooked phytosterols.

Tables 6 and 7 provide the summary results for the intake estimates of the NF (as plant sterol equivalents) and for POP, per person and day (Table 6) and per kg bw and day (Table 7).

Table 6: Ranges among EU surveys of the estimated daily intake of the PS (g per day)* and POP (mg per day), based on the individual data from the EFSA Comprehensive Food Consumption Database and considering oxidation rates of 0.5 and 2.28% of the PS. ('All subjects' means all participants of an age group of the consumption surveys)

Age groups	Number of EU dietary surveys	Estimated daily intake of the NF – all subjects in g per day (Estimated daily intake of POP – all subjects in mg per day)	
		Range of means (lowest and highest) among EU dietary surveys*	Range of 95th percentile (lowest and highest) among EU dietary surveys*
Infants (up to 11 months)	13	0.03–0.61* (0.1–3.0)** (0.6–13.9)***	0.14–1.44 (0.7–7.2) (3.3–32.8)
Young children or toddlers (12–35 months)	16	0.05–1.08 (0.2–5.4) (1.0–24.7)	0.24–1.84 (1.2–9.2) (5.6–42.0)
Other children (3–9 years)	19	0.10–2.18 (0.5–10.9) (2.3–49.8)	0.54–3.83 (2.7–19.1) (12.3–87.4)
Adolescents (10–17 years)	20	0.10–2.67 (0.5–13.2) (2.4–60.1)	0.46–4.48 (2.3–22.4) (10.5–102.1)

Age groups	Number of EU dietary surveys	Estimated daily intake of the NF – all subjects in g per day (Estimated daily intake of POP – all subjects in mg per day)	
		Range of means (lowest and highest) among EU dietary surveys*	Range of 95th percentile (lowest and highest) among EU dietary surveys*
Adults (18–64 years)	22	0.17–2.81 (0.9–14.0) (3.9–64.0)	0.81–5.77 (4.1–28.9) (18.5–132)
Elderly (≥ 65 years)	20	0.26–2.78 (1.3–13.9) (6.0–63.4)	1.16–7.06 (5.8–35.3) (26.5–161)

NF: novel food; POP: plant sterol oxidation products: PS: plant sterols.

*: The ranges for the mean and the P95 in the first line of each of the age groups refer to the estimated intake of free PS equivalent when PSE were added at 12.5% to the food categories in Table 4. The second line of each of the age group refers to the consumption of POP when considering an oxidation rate of 0.5% of the cooked PS and the third line refers to the consumption of POP when considering an ORP of 2.28% of the cooked PS (see Section 3.3 'Stability').

The highest intakes were estimated for elderly above 65 years of age. The Panel notes that the intake estimates for phytosterols presented in Table 6 derived by EFSA are of similar magnitude as the estimates provided by the applicant presented in Table 1, e.g. while the applicant considered 5.4 g/day as a 'theoretical worst-case estimation' for adult consumers on the basis of a the P95 consumption data from the NL, EFSA derived 5.8 g/day as the highest P95 among 22 surveys.

Table 7: Ranges among EU surveys of the estimated daily intake of the PS (mg per kg bw and day)* and POP (mg per kg bw and day), based on the individual data from the EFSA Comprehensive Food Consumption Database and considering oxidation rates of 0.5 and 2.28% of the PS

Age groups	Number of EU dietary surveys	Estimated daily intake of the NF and POP – all subjects in mg per kg bw and day	
		Range of means (lowest and highest) among EU dietary surveys*	Range of 95th percentile (lowest and highest) among EU dietary surveys*
Infants (up to 11 months)	13	2.9–67.9 (0.01–0.3 (0.07–1.55)	14.8–176.2 (0.07–0.88) (0.34–4.02)
Young children or toddlers (12–35 months)	16	4.2–85.6 (0.02–0.43 (0.10–1.95)	22.4–155.0 (0.11–0.77) (0.51–3.53)
Other children (3–9 years)	19	4.3–89.3 (0.02–0.44) (0.10–2.03)	26.6–168.7 (0.13–0.84) (0.61–3.84)
Adolescents (10–17 years)	20	2.0–53.0 (0.01–0.26) (0.05–1.21)	9.7–88.6 (0.05–0.44) (0.22–2.02)
Adults (18–64 years)	22	2.3 – 40.3 (0.01–0.20) (0.05–0.91)	11.3–77.1 (0.06–0.38) (0.26–1.76)
Elderly (≥ 65 years)	20	3.3–38.8 (0.02–0.19) (0.076–0.89)	18.0–101.6 (0.09–0.51) (0.41–2.31)

NF: novel food; POP: plant sterol oxidation products: PS: plant sterols.

*: The ranges for the mean and the P95 in the first line of each of the age groups refer to the estimated intake of free PS equivalent when PSE were added at 12.5% to the food categories in Table 4. The second line of each of the age group refers to the consumption of POP when considering an oxidation rate of 0.5% of the cooked PS and the third line refers to the consumption of POP when considering an ORP of 2.28% of the cooked PS (see Section 3.3 'Stability').

The Panel notes that this exposure assessment includes the conservative assumption that all the consumed oils and spreads listed in Table 4 are added with PS and that all these products are cooked which results in oxidation rates of 0.5% or 2.28% of the added PS. Furthermore, despite the mandatory labelling requirements for the existing authorisation of phytosterols, this exposure scenario also takes into account the requirements of Article 5(6) of Commission Implementing Regulation (EU) 2017/2469 which stipulate that the safety assessment cannot be limited to the target population, where it cannot be excluded that a NF food could be consumed also by non-target population groups. This exposure assessment does also not consider existing labelling requirements to limit the consumption to not more than 3 g per person per day for the target population.

3.6.3.2. Estimates for POP intakes from other sources

Low oxidation rates of 0.023 and 0.08% of added PS in unheated spreads have been reported in two articles (Baumgartner et al., 2013; Grandgirard et al., 2004a). The resulting POP intake is up to 100 times lower than when considering an oxidation rate of 2.28% of heated PS, assuming the same PS intake levels .

POP from unheated PS are not to be added to the exposure assessment because the exposure assessment in the Section 3.6.3.1d considers that all consumed spreads and oils would undergo heat treatment. If spreads with unheated PS is consumed as an alternative source to cooked spreads with added phytosterols, this would result to lower POP exposure.

Typical intake levels of phytosterols from the western diet have been reported to be in the range of about 200–400 mg/day (Andersson et al., 2004; Chan et al., 2006). When considering an oxidation rate of 0.48% (suggested by the applicant), the resulting intake would be about 1–2 mg POP per day which would correspond to 0.014–0.028 mg/kg bw. POP intake from the background diet would therefore be only a small fraction of what could be consumed from PS added to spreads and oils for cooking purposes.

3.6.4. Precautions and restrictions of use

The applicant refers to the applicable conditions of use laid down by the Union list of NF, which are:

1) Products containing the NF shall be presented in such a manner that they can be easily divided into portions that contain either a maximum of 3 g (in case of one portion per day) or a maximum of 1 g (in case of three portions per day) of added phytosterols/phytostanols.
2) The amount of phytosterols/phytostanols added to a container of beverages shall not exceed 3 g.
3) Salad dressings, mayonnaise and spicy sauces shall be packed as single portions.

Furthermore, the applicant also refers to the following mandatory labelling requirements laid down in Annex III.5 of Regulation (EU) No 1169/2011 for foods with added PSE:

1) 'with added plant sterols' in the same field of vision as the name of the food;
2) the amount of added PSE content (expressed in % or as g of free plant sterols (FPS) per 100 g or 100 mL of the food) shall be stated in the list of ingredients;
3) a statement that the product is not intended for people who do not need to control their blood cholesterol level;
4) a statement that patients on cholesterol lowering medication should only consume the product under medical supervision;
5) an easily visible statement that the food may not be nutritionally appropriate for pregnant or breastfeeding women and children under the age of 5 years;
6) advice that the food is to be used as part of a balanced and varied diet, including regular consumption of fruit and vegetables to help maintain carotenoid levels;
7) in the same field of vision as the statement required under point (3) above, a statement that the consumption of more than 3 g/ day of added plant sterols/plant stanols should be avoided;
8) a definition of a portion of the food or food ingredient concerned (preferably in g or ml) with the amount of the plant sterol/plant stanol that each portion contains.

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

Absorption, distribution, metabolism and excretion of PSE have been assessed from the pre-marketing studies by the SCF (2000, 2002a,b). The absorption rates of campesterol, sitosterol and stigmasterol

have been reported ranging from 1.9% to 15%, 0.5% to 5% and up to 4%, respectively (Heinemann et al., 1993; Lutjohann et al., 1995; Jones et al., 1997; Ostlund et al., 2002b; Sanders et al., 2000). In 2000, the SCF noted that PS absorption was found to be higher in children than in adults and referred to each one study with children (Mellies et al., 1976) and adults (Tilvis and Miettinen, 1986), respectively.

The essential precondition for the low-density lipoprotein (LDL)-cholesterol (LDL-C)-lowering properties of PSE added to foods is the intestinal cleavage of the ester bonds (Brown, 2011; de Jong et al., 2003; Garcia-Llatas and Rodriguez-Estrada, 2011). The impact of oxidation on the activity of pancreatic cholesterol esterase has been studied *in vitro* using sitosteryl oleate and the oxidation products 7-keto-sitosteryl oleate and sitosteryl-9,10-dihydroxystearate as substrates (Julien-David et al., 2009). As shown for 7-keto-sitosteryl oleate, the oxidation of the sterol moiety led to an increased affinity to the cholesterol esterase and a faster hydrolysis when compared to sitosteryl oleate. In contrast, the oxidative modification of the fatty acid moiety leading to sitosteryl-9,10-dihydroxystearate resulted in an almost complete loss of hydrolytic enzyme activity. In addition, in the presence of sitosteryl-9,10-dihydroxystearate the hydrolysis rate of sitosteryl oleate was significantly decreased. These data indicate potential interactions between the various oxidised 'species' that may be formed during the oxidation process, their impact on the hydrolysis rate of intact PSE and consequently their possible impact on the LDL-C-lowering effect. Data on the impact of POP on the active intestinal transport of cholesterol, e.g. via the Niemann-Pick C1 like protein, are however limited (DeSmet et al., 2012; Alemany et al., 2013).

In a study with 12 male hamsters per group fed for 6 weeks a control diet or one of four experimental diets containing 0.1% of β-sitosterol, stigmasterol or their respective oxidation products, the latter two groups of hamsters fed with oxidised PS had significantly higher total cholesterol (TC), LDL-C and triglyceride plasma values as compared to those hamsters fed with non-oxidised PS. However, no significant difference of these parameters were reported for the comparison between hamsters fed with oxidised PS and the control hamsters receiving no added PS (Liang et al., 2011). Despite this limitation, the experimental data available suggest that the oxidation of PS results in a loss of their LDL-C-lowering properties.

Regarding the absorption of POP, the applicant compared the median plasma concentration of POP (0.03 μmol/L) in healthy individuals consuming habitual diets among six human studies (range 0.011–0.263 μmol/L) with the 6 times higher median plasma concentration of cholesterol oxidation products (COP) (0.189 μmol/L) in healthy individuals consuming habitual diets (among fifteen human studies, range 0.034–0.709 μmol/L) (Table 8). The applicant argued that based on the estimated upper POP intake (the applicant considered an oxidation rate of 1.59% and resulting POP intake of 48 mg/day when 3 g phytosterols are consumed), POP plasma concentrations would not increase above levels of reported COP plasma concentrations.

Table 8: Estimates for intakes of dietary sterols and human plasma sterol concentrations

Sterols	Sterol intakes, in mg/day	Plasma sterol concentration	
		Median or mean values μmol/L	Range μmol/L
Total cholesterol	200–550 (776)[a]	5,880[b]	
Plant sterols	200–400[c] (1,000)[d]	Campesterol: 14.3[e]	Campesterol: 6.9–27.9[e]
		Sitosterol 7.9[e]	Sitosterol: 2.8 – 16.1[e]
COP	3[f]	0.189[g]	0.034–0.709[h]
POP	0.96–1.92[i]	0.030[j]	0.011 – 0.263[k]
	[j]		
POP intake from 3 g non-heated PS added to spreads in 2 human studies	0.68[l],[m] (ORP: 0.02)	Baseline 0.012[l] End of treatment: 0.016[l], 0.011[m]	–
POP intake from heated PS – Unilever study (2018)	0.3 8.7 15.1 37.2	0.039 0.091 0.144 0.203	–

COP: cholesterol oxidation products; POP: plant sterol oxidation products: PS: plant sterols.

(a): Average intakes in adults (EFSA NDA Panel, 2010), value in bracket refers to the highest P95 intake among EU countries by adults aged 35–64.
(b): Mean value for intakes from the normal diet reported by 45 studies reviewed and reported by Chan et al. (2006).
(c): Range of typical PS intake levels for normal western diets (Klingberg et al. 2008; Sioen et al., 2011a,b).
(d): Value in brackets represents high intakes reported for vegetarian & vegan diets (Jenkins et al., 2001).
(e): Mean and ranges for the predominant PS plasma concentrations based on 45 studies reviewd & reported by Chan et al. (2006).
(f): van de Bovenkamp et al. (1988).
(g): Median and (h) range COP plasma concentrations based on fifteen studies reviewed by the applicant (Bjorkhem et al., 1988; Kudo et al., 1989; Breuer and Bjorkhem, 1990; Dzeletovic et al., 1995; Breuer, 1995; Kuroki et al., 1995; Salonen et al., 1997; Mol et al., 1997; Babiker and Diczfalusy, 1998; Zieden et al., 1999; Murakami et al., 2000; Plat et al., 2001; Iuliano et al., 2003; Guardiola et al., 2007; Helmschrodt et al., 2013).
(i): Typical intake of 200–400 mg PS multiplied by an ORP of 0.48% estimated by the applicant.
(j): Median.
(k): Range POP plasma concentrations based on six studies reviewed by the applicant (Plat et al., 2001; Grandgirard et al., 2004a; Menendez-Carreno et al., 2012; Husche et al., 2011; Baumgartner et al., 2013; Luister et al., 2015).
(l): Husche et al. (2011).
(m): Baumgartner et al. (2013).

In response to EFSA asking for data on the human plasma/serum after consumption of heated PS spreads or PS liquids with an oxidation rate representative for the P90 estimated by the applicant (1.5–2%), the applicant conducted a double-blind randomised controlled trial (RCT) with 60 healthy individuals (Unilever, 2018; Lin et al., 2019). In this study, 4 groups of each 15 subjects received 20–25 g of margarine and two cookies (2 × 14 g) per day for 6 weeks. The control group received products with no added PS and POP. The three other groups received margarine with added PS and cookies resulting in a POP intake of 8.7 (low-dose), 15.2 (medium-dose) or 37.2 (high-dose) mg/day. The intake of non-oxidised PS from the margarine and cookies was 2.34 and 0.62 g, respectively, for the low-dose group (∑ 3 g), 1.87 and 1.30 g for the mid-dose group (∑ 3.2 g), and 0.03 and 2.69 g for the high-dose group (∑ 2.7 g). The intake of 37.2 mg/d in the high-dose group resulted in POP plasma concentrations of 0.2 μmol/L, which is about five times higher than reported for the control group and about seven times higher than the median among six studies reviewed by the applicant (Table 8).

3.8. Nutritional information

The kinetic study by the applicant (Unilever, 2018; Lin et al., 2019) also examined as secondary objectives plasma concentrations of TC, LDL-C and high-density lipoprotein (HDL)-cholesterol (HDL-C), and triglycerides. There were no statistically significant effects on these endpoints compared to the control. According to the study report, the study was not sufficiently powered to demonstrate effects on TC and LDL-C. Since this is the only study available with heat-treated PSE added to foods, there is no evidence available showing that heat-treated PSE have an effect on TC and LDL-C as demonstrated for unheated PS.

The Panel notes that studies consistently report that a decrease in cholesterol absorption resulting in low serum LDL-C triggers an increase in cholesterol synthesis, thus maintaining adequate cellular cholesterol levels (Goodman et al., 1983; Ho et al., 1977; Olsson et al., 2017; Santosa et al., 2007; Wong et al., 1993). This has also been observed in children. The Panel has therefore no concerns regarding potential low serum cholesterol levels in normocholesterolaemic subjects, both for adults and for children.

Concerns have been raised regarding the potential of phytosterols to impair absorption and status of fat-soluble vitamins. Particular consideration is given to the observed lowering effect of PS on plasma concentrations of carotenoids, especially of β-carotene (e.g. the latter in the range of up to 20% decrease, observed with ingestion over one year of 20 g/day of products containing 8% PS (Unilever 1998, cited in SCF, 2000); the question arises as to whether reduced β-carotene levels due to long-term consumption PS might become relevant when the vitamin A status is not optimal.

In this context, the applicant has provided a meta-analysis of 41 RCTs investigating the plasma concentrations of fat-soluble vitamins and carotenoids in a total of 3,306 subjects after the consumption of non-heated PS and stanol products (Baumgartner et al., 2017). The median study duration was 28 days (range: 21–364 days) with an average PS or stanol intake of 2.5 g/day (range: 0.45–9.0 g/day), and 80% of the studies were performed with esterified plant sterols or plant stanols.

β-carotene plasma concentrations (μmol/L) expressed as means (95% CI) were at baseline 0.60 (0.54; 0.67) and 0.10 (0.09; 0.11) when adjusted for TC; after PS intervention the plasma concentrations were 0.52 (0.46; 0.57) and 0.09 (0.08; 0.10) when TC-adjusted. Non-adjusted and

TC-adjusted serum concentrations for lycopene, α-carotene and β-carotene, were significantly ($p < 0.0001$) lowered after consumption of foods with added PS or plant stanols. β-Carotene concentrations decreased on average by 0.08 μmol/L (−16.3%; −10.1% when TC-adjusted). Non-adjusted tocopherol concentrations significantly ($p < 0.0001$) decreased after PS or plant stanol consumption (α-tocopherol on average by 2.43 μmol/L (−7.1%) and γ-tocopherol by 0.17 μmol/L (−6.9%)). However, when adjusted for TC concentrations, α- and γ-tocopherol concentrations were no longer different compared with baseline values. Concentrations of retinol and vitamin D were not changed after PS or plant stanol consumption.

Total cholesterol, LDL-C and triglycerides (TAG) concentrations significantly ($p < 0.0001$) decreased by on average 0.39 mmol/L (−6.5%), 0.35 mmol/L (−9.0%) and 0.06 mmol/L (−4.6%) after PS or plant stanol consumption, respectively. However, HDL-C concentrations remained unchanged. The covariate analyses done in this study revealed the following results: baseline concentrations did not have a significant impact on the observed changes after PS or plant stanol consumption; the PS or plant stanol dose did not significantly affect TC-adjusted absolute or relative changes in β-carotene, lutein and α-tocopherol; study duration (≤ 4 vs. > 4 weeks) resulted in larger TC-adjusted absolute and relative reductions in lutein (0.000 vs. −0.002 μmol/mmol TC and 1.0 vs. −5.6%, respectively), whereas only a trend for such effect was observed for β-carotene. Consumption of plant stanols seemed to have a stronger effect on lowering relative TC-adjusted β-carotene concentrations than plant sterols (−14.2 vs. −8.9%, respectively), while changes in α-tocopherol concentrations showed a trend toward larger reductions after plant stanol vs. sterol consumption. Changes in TC concentrations were also larger after consumption of plant stanols vs. PS (−8.0 vs. −6.1%, respectively), which is probably related to a higher dose in the plant stanol compared to the PS studies (3.2 vs. 2.1 g/day). In addition, changes in TC concentrations after PS or plant stanol consumption were significantly affected by baseline concentrations (only absolute TC changes) and by the dose of PS or plant stanol intake.

The Panel notes that in this study, consumption of PS and stanols and their esters in amounts of 2.5 g/d (0.45–9.0 g/day) significantly lower β-carotene concentration by 16% or 10% when TC-adjusted, while the decrease in other carotenoids is between 7.4 and 14.4% or between zero and –7.8% when adjusted for TC. Respective α- and γ-tocopherol concentrations dropped by 7.1 and 6.9%, but not when TC adjusted. Thus, a part of the decrease in carotenoid concentrations can be attributed to the decrease in TC concentrations, as LDL- and very low-density lipoprotein (VLDL) are the main lipoprotein fractions that carry carotenoids as well as tocopherols in the circulation. While baseline concentrations and dose did not impact plasma concentrations of carotenoids, the consumption of plant sterols and stanols for more than 4 weeks led to a greater decrease in some carotenoids. Plasma concentrations of vitamin D and retinol were not affected. However, the retinol concentration in plasma is not a sensitive marker for vitamin A status, as this will decline only, when liver stores are almost depleted.

The Panel notes that the mean concentrations of β-carotene in this meta-analysis were in the higher end of average or median ranges reported in the literature. Average fasting blood concentrations of β-carotene in the range 0.2–0.7 μmol/L have been reported in adult European populations (Al-Delaimy et al., 2004; Hercberg et al., 2004) and medium serum concentration assessed in NHANES III were 0.22 and 0.28 μmol/L for men and women, respectively, with the 5th–95th percentiles being 0.09–0.91 μmol/L (Olsen JA, in Modern Nutrition in Health and Disease, 9th edition, Eds. Shils, Olson, Pike, Ross. Williams & Wilkins 1999). With the drop of β-carotene from 0.60 μmol/L (95% CI 0.54; 0.67) to 0.52 μmol/L (95% CI 0.46; 0.57) as observed in the study of Baumgartner et al. (2017), plasma concentrations were still in the upper/middle range of values and thus would not raise concern.

The carotenoids α-carotene, β-carotene and β-cryptoxanthin function as provitamins A. β-Carotene is the major vitamin A precursor in the diet, contributing for 30–35% of vitamin A intake in Western countries (Weber and Grune, 2011; Strobel et al., 2007). Although carotenoids, and β-carotene in particular, are not considered essential nutrients, the intake becomes important in the absence of adequate preformed vitamin A in the diet.

In European and other developed countries, vitamin A intake generally complies with Dietary reference values (DRVs). Nevertheless, some children, adolescents, pregnant and lactating women, population groups with low educational background and/or low income, and those practicing highly restrictive diets or avoiding certain types of foods have been identified at potential risk of not meeting the DRVs for vitamin A. In such cases, i.e. when long-term vitamin A intake is insufficient, the prolonged intake of PS, plant stanols, either in free or esterified form, may be disadvantageous. However, the Panel notes that overall the absorption of β-carotene appears to be highly variable (5–

65%), depending on food- and diet-related factors, genetic characteristics and the health status of the subject (Haskell, 2012).

Besides the provitamin A activity, carotenoids function as antioxidants in the body and have been suggested to play a role in disease prevention (Bohn, 2017). In addition, some studies suggest that low serum carotenoid concentrations predict mortality (Shardell et al., 2011, Buijsse et al., 2005). However, the evidence from these studies is insufficient to draw conclusions regarding recommendations for intake or status. Although there are no studies available on the effect of heated PS on β-carotene plasma concentrations, the Panel considers that heated PS would not decrease β-carotene plasma concentrations to a higher degree than unheated PS.

The Panel considers that the extension of use of the NF at an intake of about 3 g per day is not nutritionally disadvantageous in the context of a balanced diet.

Considering the necessity to produce a test material which allows to study high doses of POP in toxicological studies, the Panel considers the test material appropriate to study the toxicity of POP and acceptable to study the toxicity of the NF for the intended extension of use, despite the described shortcoming of the representativeness of the test material.

3.9. Toxicological information

The safety of the PS and their esters, which are the subject of the present application, has been assessed in the context of a previous application (SCF, 2000; *Opinion on PEs in yellow fat spreads, Unilever*). In its general view on the long-term effects of the intake of elevated levels of PS from multiple dietary sources (SCF, 2002a), the SCF noted that blood carotenoids' concentrations, especially β-carotene, were decreased after daily consumption of 3 g PS or plant stanols, and that there is no evidence for an additional cholesterol lowering effect at intakes above 3 g/day. Therefore, the SCF advised that the intake of PS and plant stanols should not exceed 3 g/day (SCF, 2002a).

Given the proposed uses that are described in this application, the applicant has conducted toxicological studies in order to assess potential effects of POP which may be produced during cooking or baking.

Test compound used for the toxicological studies

The PS oxide concentrate (POC) used in the toxicological studies was produced by heating PSE at 150–200°C for several hours in the presence of air. The composition of the starting material (approximate distribution of sterols: 47% sitosterol, 25% campesterol, 19% stigmasterol and 3% brassicasterol) was consistent with material that was previously tested and evaluated by Hepburn et al. (1999) and the SCF (2000), respectively. The oxidised material was subjected to alkaline hydrolysis to obtain the unsaponifiable fraction and further purified by recrystallisation. The resulting concentrate consisted of approximately 31% plant sterol oxides and 19% FPS plant sterols. The plant sterol oxide fraction was composed of approximately 26–27% polar (i.e. POP) and approximately 4% apolar phytosterol oxides (e.g. steradienes). Approximately 50% of the concentrate remained unknown; according to the applicant, this unknown fraction corresponded to sterol fragments.

The alkaline hydrolysis and the subsequent purification applied in the production of the test material result in a pronounced increase of the content of POP. The content of POP achieved in the test material corresponded to approximately 30% ORP. The direct use of a thermally treated PSE ester mixture would not have allowed achieving such test concentrations of POP.

Owing to the applied hydrolysis step, the fatty acid moieties are cleaved from the oxidised PSE and removed in the subsequent purification process. PSE are not only prone to oxidations in the steroid moiety but also in the unsaturated fatty acid moieties. Such oxidised acyl chains are cleaved off and removed in the course of the process employed to produce the POC. The Panel notes a study demonstrating that only approximately 20% of the ester losses observed upon heating of a margarine with added PSE/PAE could be explained by the formation of POP (Scholz et al., 2016). Therefore, the test material used for the toxicological studies does not fully represent the spectrum of oxidation products to be expected from thermal treatments of PS-Spreads and PS-Liquids for CB.

The metabolic fate of PSE oxidised in the fatty acid moiety is largely unknown. One *in vitro* study demonstrated that the oxidative modification of sitosteryl oleate leading to sitosteryl-9,10-dihydroxystearate resulted in an almost complete loss of hydrolysis (Julien-David et al., 2009). If PSE were hydrolysed, the metabolism and the reactivity of the liberated oxo-, keto-, and hydroxy-acids are expected to be similar to those released by enzyme-catalysed cleavage of the corresponding oxidised triglycerides resulting from the heat-treatment of normal edible vegetable oils. Considering the high

molecular weight and the hydrophobicity of PSE and that the intestinal cleavage of the ester bonds is a precondition for the micellisation and absorption, the Panel considers that the potential for adverse effects of PSE oxidised in their fatty acid moiety is low.

3.9.1. Genotoxicity

Bacterial reverse mutation tests using *Salmonella* Typhimurium strains TA98, TA100, TA1535, TA1537 and TA102 were conducted according to OECD Guideline 471 and compliant with good laboratory practice (GLP) (Beevers, 2000; Lea et al., 2004). The POC was dissolved in dimethylformamide and tested in independent experiments at doses up to 5,000 μg/plate using the plate incorporation method and up to 2,000 μg/plate using the pre-incubation method. There was no indication of a mutagenic response in any of the strains tested, neither in the presence nor in the absence of a metabolic activation system (rat liver S9).

An *in vitro* mammalian chromosomal aberration study using human peripheral blood lymphocytes was conducted according to OECD guideline 473 and in compliance with GLP (Lloyd, 2000; Lea et al., 2004). Preliminary solubility experiments showed that slight precipitation occurred when the test material (dissolved in dimethylformamide) was added to the culture medium at a concentration of approximately 178 μg/mL. The concentrations evaluated for cytogenetic damage in the first experiment were: 500, 256 and 131.1 μg/mL for the 3-h treatment in the absence of S9 (plus 17 h further incubation); the mitotic inhibition was 42, 12 and 0%, respectively. The concentrations evaluated for the 3 h treatment in the presence of S9 (plus 17 h in the absence of S9) were 500, 400 and 256 μg/mL; mitotic inhibition was 28, 14 and 0%, respectively. In the second experiment, the concentrations evaluated for the 20-h continuous treatment in the absence of S9 were: 204.8, 131.1 and 67.11 μg/mL; mitotic inhibition was 53, 34 and 3%, respectively. The concentrations evaluated for the 3-h treatment in the presence of S9 (plus 17 h in the absence of S9) were 625, 500 and 320 μg/mL; mitotic inhibition was 33, 22 and 0%, respectively. Treatment of cultures with POC in the absence and presence of S9 did not induce a statistically significant increase in the number of cells with structural chromosome aberrations (excluding gaps). Thus, there are no indications that the POC is clastogenic. However, in the first experiment dose-related increases in the frequencies of polyploid and endoreduplicated cells were observed following the 3-h treatment in the absence and presence of S9. According to the author, there were no increases in the second experiment (but the frequencies were highest for the highest concentrations tested after 20 h and 3 h incubation in the absence or presence of S9, respectively). The Panel notes that the purpose of the *in vitro* mammalian chromosome aberration test is to identify substances that cause structural aberrations. Polyploidy (including endoreduplication) can arise in this test. While aneugens can induce polyploidy, polyploidy alone does not indicate aneugenic potential, and can simply indicate cell cycle perturbation or cytotoxicity. In this study, the occurrence of precipitation and cytotoxicity might thus have influenced the outcome with regard to the occurrence of polyploidy.

An *in vitro* micronucleus test using human peripheral blood lymphocytes was conducted (Lloyd, 2001; Lea et al., 2004). The design of the GLP-complying study was largely in accordance with the current OECD guideline 487. The *in vitro* mammalian micronucleus test can efficiently detect both clastogens and aneugens (EFSA, 2011). The cytokinesis-block proliferation index (CBPI) was determined in order to assess whether cell cycle delay or cytotoxicity had occurred. Based on the CBPI, doses selected for analysis were 400, 320 and 163.8 μg/mL for the 3-h treatment in the absence of S9; cytotoxicity was 44, 44 and 20%, respectively. Doses selected for the 3-h treatment in the presence of S9 were 625, 500 and 320 μg/mL; cytotoxicity was 47, 45 and 25%, respectively. The doses selected for the 20-h treatment in the absence of S9 were 104.9, 67.11 and 42.95 μg/mL; cytotoxicity was 48, 13 and 20%, respectively. Treatment of cultures with the POC in the absence and presence of S9 did not induce a statistically significant increase in the number of cells containing micronuclei at any concentration tested. The frequencies of cells containing micronuclei fell within the appropriate historical control ranges. The study thus provides no evidence that POC induces structural or numerical chromosome aberrations.

The Panel considers that the POC has been adequately tested *in vitro* and additional *in vivo* studies are not required. It is concluded that there are no concerns with regard to genotoxicity.

3.9.2. Subchronic toxicity

A subchronic oral toxicity study was conducted according to OECD Guideline No 408 and in compliance with GLP (Appel, 2001; Lea et al., 2004). Groups of 20 male and 20 female Wistar rats

were administered diets containing the POC at 0.2%, 0.6% or 1.6% (w/w) for 90 days. The respective doses were 139, 425 and 1,133 mg/kg bw per day for males and 160, 481 and 1,269 mg/kg bw per day for females. All three test diets as well as a diet controlling for PSE ('PSE control') contained also 5.67% of the respective non-heated PSE. The Panel notes that the oxidation rate (POP/non-oxidised phytosterols) of the feed in the low- and mid-dose groups (about 1 and 3%, respectively) is comparable to the median and P90 oxidation rate of added phytosterols in heated foods reported by Lin et al. (2016a) and used for the intake estimate of POP in humans. The ORP in the feed of the high-dose group was approximately 8%. An additional control group was fed a standard rodent diet ('standard control') without added phytosterols. Results are presented in Annex 5.

Four rats died or were euthanised *in extremis* during the treatment period; none of these deaths is considered treatment-related. Daily observations for clinical signs and ophthalmoscopy conducted towards the end of the treatment period did not reveal treatment-related changes.

Females of the high-dose group showed statistically significant lower body weight compared to both control groups throughout the treatment period. Total body weight gain was approximately 13% lower compared with the PSE control group and approximately 3% lower compared with the standard control group. Observed significant differences in feed consumption and feed conversion efficiency between groups were considered incidental and not relevant by the Panel.

Haematology analyses at the end of the treatment period showed several statistically significant differences between the test groups and the control groups: Red blood cell counts (RBC) were lower in males of the high- and mid-dose groups (not dose-related) when compared with the PSE control group; the mean values were close to the mean value of the standard control group, which also showed a significantly lower RBC compared with the PSE control group. There was no statistically significant difference at the mid- and high-dose compared to the standard control diet. There was also no effect of PEs on RBC compared with the standard control group in another 90-day study (Hepburn et al., 1999) using higher dose levels of PEs (up to 8.1%).

Haemoglobin (Hb) was significantly lower in females administered the high dose compared with both control groups (dose-related). Packed cell volume (PCV) was significantly lower in males of the high- and mid-dose groups (not dose-related) compared to the PSE control group but not significantly different from the standard control group (which was also significantly lower compared with the PSE control group). PCV was significantly lower in females administered the high dose compared with both control groups and in the mid-dose group compared with the PSE control group (dose-related). Mean corpuscular volume (MCV) was significantly lower in females administered the high dose (dose-related) compared with both control groups. Mean corpuscular haemoglobin (MCH) was significantly higher in males of the high- and mid-dose groups (not dose-related) compared with the PSE control group; the mean values were close to that of the standard control group. In females, MCH was significantly lower (not dose-related) in the mid- and high-dose groups compared with the standard control group. Mean corpuscular haemoglobin concentration (MCHC) was significantly higher in females of the high-dose group (dose-related) compared with the PSE control group, but not significantly different from the standard control group. Compared with the PSE control group, thrombocyte counts were significantly higher in males of all dose groups and in females of the high-dose group (dose-related). Compared to the standard control group, only platelet counts for males of the high-dose group were significantly higher (+10%).

A thrombocyte count lowering effect of PSE (in relation to the standard control group) was also observed in the study by Hepburn et al. (1999). It seems that POP counteract this effect and even may lead to higher counts in comparison to the standard control diet without added PSE. Prothrombin time was lower in males of all groups (not dose-related) compared with the standard control group.

Although not all changes were related to the dose and some changes only appeared in one sex, these findings indicate possible effects of the test material POC, especially the findings on thrombocyte counts. There were no relevant findings in white blood cell parameters.

Clinical chemistry analysis showed significantly lower serum glucose concentrations in males of the high-dose group compared with both control groups. Gamma-glutamyl transferase (γ-GT) activity was significantly higher in females (1.2 U/L) administered the high-dose compared with both control groups (0.3 U/L). The albumin level was significantly higher in males of the high-dose group, and the albumin/globulin (A/G) ratio was significantly higher in males of the high- and mid-dose groups (the latter dose-related) compared with both control groups.

Total cholesterol concentrations were significantly lower in males administered the high-dose compared to the PSE control group (compared with the standard control group, cholesterol concentrations were significantly increased in the PSE control group). In females of all three dose

groups, TC concentrations were significantly higher (not dose-related) compared with the standard control group (concentrations were higher in the PSE control group). Non-HDL-C concentrations were significantly lower in males administered the high-dose compared to both control groups and in females of the mid- and high-dose groups compared to the PSE control group. Thus, it seems that in this study the PE effect on TC and non-HDL-C concentrations (i.e. an increase) was partially reversed in the presence of POP. However, PEs had no effect on cholesterol levels in the study by Hepburn et al. (1999).

Triglyceride levels were significantly lower in males and females of the high-dose group (seemingly dose-related) compared with both control groups. Phospholipid levels were significantly lower in males administered the high dose (dose-related) compared to the PSE control group; the value was close to that of the standard control group, which was also significantly lower than in the PSE control group. In females of the high- and mid-dose groups phospholipid levels were significantly lower compared with both control groups (dose-related). Urinalysis findings were unremarkable.

Macroscopic examinations at necropsy revealed no findings associated with treatment. Organ weight determinations showed significantly higher absolute (about 12–13%) and relative (about 15–16%) liver weights in females of the high-dose group compared with both control groups. Microscopic examinations of tissues showed no differences in the incidence and severity of histopathological findings between groups.

Based on the findings in the high-dose group (receiving a diet containing 1.6% POC plus 5.67% of unheated phytosterols) in comparison to the standard diet control, which are considered treatment-related (higher γ-GT activity, and higher absolute and relative liver weights in females, and higher thrombocyte counts in males and females, respectively), the Panel regards the mid dose of 0.6% POC in feed, corresponding to 425 mg POC (plus 4 g PSE)/kg bw per day as the 'no-observed adverse effect level' (NOAEL).

Noting that the POC consists of approximately 30% POP, the Panel considers 128 mg per kg bw per day to be the NOAEL of POP in this study.

3.9.3. Other toxicity studies

In their comments, Member States referred to some experimental *in vitro* and *in vivo* studies (Yang et al., 2013; Tomoyori et al., 2004; Liang et al., 2011; Plat et al., 2014).

Yang et al. (2013) investigated *ex vivo* the effects of β-sitosterol oxidation products (30 mg/mL) and (non-oxidised) b-sitosterol on vasorelaxation of an endothelium-intact preparation of aortic rings obtained from Sprague–Dawley rats. β-Sitosterol oxidation products, but not β-sitosterol, attenuated endothelium-dependent relaxation by increased NADPH oxidase-derived reactive oxygen species.

Tomoyori et al. (2004) found no significant differences in atherosclerotic lesion area after 9 weeks of feeding plant sterol or plant sterol oxide (β-sitosterol and campesterol oxides) at concentrations of 0.02% in the diet to apoE-deficient mice. The phytosterol and oxyphytosterol diets did not affect the body weight gain, food intake, serum cholesterol levels, or liver weight. The authors reported also that dietary oxyphytosterol did not affect the amount of urinary 8-iso-PGF2α excreted.

In another study, 12 male hamsters per group were fed for 6 weeks a control diet or one of four experimental diets containing 0.1% of β-sitosterol, stigmasterol or their respective oxidation products (Liang et al., 2011). At the end of the study, the two groups of hamsters fed with oxidised plant sterols had significantly higher TC, LDL-C and triglyceride plasma values, higher relative liver weight, higher formation of aortic plaques and greater endothelium-dependent contractions in response to acetylcholine as compared to those hamsters fed with non-oxidised plant sterols. No significant difference in these parameters were reported for the comparison between hamsters fed oxidised plant sterols and the control hamsters receiving no added plant sterols. However, in both hamster groups fed with oxidised sitosterol and stigmasterol products, accumulation of these products was found in the liver which was not observed for their non-oxidised forms.

Plat et al. (2014) studied the formation of atherosclerotic lesions and inflammatory markers in female LDL receptor-deficient (LDLR+/−) mice for 35 weeks. One group (n = 9) of mice received an atherogenic high-fat diet containing 0.25 g cholesterol/100 g, a second group (n = 12) received the same atherogenic control diet except that 10% of the added cholesterol was replaced by oxidised cholesterol (0.025 g/100 g diet). A third group of mice (n = 12) was also fed the same atherogenic diet as the control group, but with oxyphytosterols replacing 10% of the cholesterol added to the control diet. The proportion of severe atherosclerotic lesions among atherosclerotic lesions was significantly higher after oxidised cholesterol (41%; p = 0.004) and oxyphytosterol (34%; p = 0.011)

diet consumption than after control diet consumption (26%). There was no difference in inflammatory markers (monocyte chemoattractant protein-1, and tumour necrosis factor-α) among the groups.

In the response to an EFSA's request, the applicant provided a review on the available literature on phytosterols and/or its oxidised forms and cardiovascular risk. This review contained, in addition to the above four experimental studies cited by Member States, two additional animal studies (Grandgirard et al., 2004b; Bang et al., 2008).

In a study in hamsters by Grandgirard et al. (2004b), which were fed diets containing different amounts of POP (0.01%, 0.05% or 0.25%) for 2 weeks, plasma POP concentrations were 32.6, 264.3 and 806.3 ng/mL in the control, 0.05% POP and 0.25% POP diet groups, respectively (data for the 0.01% group were not reported in the publication). Aorta endothelial POP concentrations were 3.2, 10.4, 13.1 and 75.7 ng per mg aortic lipids in the control, 0.01%, 0.05% and 0.25% POP diet groups. In this study, POP added to feed dose-dependently increased the POP contents in plasma and aorta. As lipid concentrations were not reported, the POP concentration in the aortas could not be presented as μg/g aortic tissue.

In 2008, Bang et al. (2008) fed C57BL/6J mice for 4 weeks with diets without (control) or with added 0.2 g/kg of POP. Serum POP concentrations were 271 and 897 μg/dL (6.38 and 21.11 μmol/L) in the control and POP group, respectively. Liver POP contents were 10.9 and 24.3 μg/g (0.026 and 0.057 μmol/g), respectively. These results indicated that feeding mice with a diet containing POP may significantly increase POP contents in serum and liver.

The Panel notes that the experimental studies which raised concerns by Member States reported that the POP had either (1) no different effects on atherosclerotic lesion and inflammatory markers at a concentration of 0.02% as compared to non-oxidised plant sterols in apoE deficient mice (Tomoyori et al., 2004), (2) no effects at concentrations of 0.1% compared to control diets but attenuating effects of non-oxidised phytosterols in hamster (Liang et al., 2011) or (3) attenuated endothelium-dependent relaxation (Yang et al., 2013). In one study by Plat et al. (2014), POP resulted to a significantly higher proportion of severe atherosclerotic lesions as compared to the (non-oxidised) cholesterol control diet. The atherosclerotic effect observed in this study was smaller, albeit statistically not significantly different in comparison to oxidised cholesterol.

Regarding *in vitro* cytotoxicity studies with POP and COPs, the available information reports that the *in vitro* effects (cytotoxicity, effect on cell viability, apoptosis induction) of the two groups of oxidised sterols are similar, albeit often higher concentrations of POP are required for the same effect size (Garcia-Llatas and Rodriguez-Estrada, 2011; Maguire et al., 2003; Vejux et al., 2012; Ryan et al., 2005; Kenny et al., 2012).

With regard to the relevance of these findings in experimental studies for human, the Panel notes that *in vitro* studies and one animal study (Plat et al., 2014) suggest that POP may have similar, albeit often reported as weaker, effects in *in vitro* and *in vivo* studies as compared to COPs.

3.9.4. Human studies

With reference to three human studies, concerns were also raised by Member States on inconsistent results on whether the consumption of foods with added PSE would increase the content of POP in plasma. Two of these studies are related to human POP plasma concentrations after consumption of PS added in margarine (Baumgartner et al., 2013; Husche et al., 2011). An additional study (Baumgartner et al., 2015) on this endpoint was provided in the applicant's response to EFSA. This response included an updated review of the available literature on POP and cardiovascular disease (CVD) risk.

In an RCT with a cross-over design, 43 subjects (18–70 years) received 20 g margarine with 3 g added plant sterols (containing 0.68 mg oxyphytosterols) or control margarine for 4 weeks. There was no statistically significant effect on plasma concentrations of 12 different POP (Baumgartner et al., 2013).

In a second RCT of these authors, a subgroup of volunteers (N = 10) randomly selected from the double-blind placebo-controlled cross-over intervention study by Baumgartner et al. (2015) received a shake (± 400 mL with 50 g of fat, 12 g of protein and 67 g of carbohydrates), containing no or 3.0 g of PSE (with 0.68 mg oxyphytosterols) followed by a second shake 4 h later without added plant sterols. Blood samples were taken before and up to 8 h after consumption of the first shake. Fasting plasma POP concentrations at the start of the postprandial test meal and after consuming plant sterol added margarine for 4 weeks were not different between the control and the plant sterol group. The maximal postprandial plasma 7β-hydroxy-campesterol and 7β-hydroxy-sitosterol concentrations were

slightly higher (each approximately +10% increase corresponding to about each 0.05 μg/L). 7-Keto-sitosterol and 7-keto-campesterol concentrations (major POP measured in plasma) were not different between the plant sterol and control group during the 8 h of postprandial follow-up.

An uncontrolled study with 16 subjects (25–41 years) reported an increase (+87%) in serum 7β-hydroxy-sitosterol concentration after 28 days of daily consumption of margarine with 3 g added, not commercially available blend of campesterol and sitosterol (in a 1:4 ratio, oxidation rate approx. 0.02%) as compared to baseline (Husche et al., 2011), while five other POP of campesterol and sitosterol and the sum of all POP showed no statistically significant increase. The authors also reported a significant increase of serum PS levels (approx. +74%).

The Panel notes the low oxidation rate (≈ 0.02%) of the consumed plant sterols in the three studies (Baumgartner et al., 2013, 2015; Husche et al., 2011) and the resulting intakes of POP which were approximately 25- to 100-fold lower than estimated intakes based on the median and the P90 oxidation rate of plant sterols added to foods undergoing cooking as determined by Lin et al. (2016a). The intake of POP from the test margarines was only about 0.68 mg/day, which is below the range of POP intake from a typical European diet as estimated by the applicant (1–2 mg/day, Table 8).

The Panel therefore considers that due to the low oxidation rates of unheated plant sterols and resulting low POP intake levels, these three studies (Baumgartner et al., 2013, 2015; Husche et al., 2011) are not pertinent to assess possible changes of plasma POP concentrations in human resulting from the consumption of heated (cooked) plant sterols added to foods.

Phytosterols and oxidised plant sterols in human plasma and tissue and cardiovascular disease risk

In a study with 104 subjects who underwent elective aortic valve replacement due to severe aortic stenosis, there was a high correlation between plant sterols (campesterol, sitosterol) in the plasma and valve cusps tissue, as well as a high correlation between plant sterols and oxyphytosterols and oxyphytosterols themselves within the valve cusps tissue, but there was little or no any correlation between different oxyphytosterols in plasma and valves. According to the authors, the latter could be explained by local oxidation of the plant sterols which may induce local inflammatory processes. The highest concentration of each individual POP (7α-hydroxy-, 7β-hydroxy- and 7-keto-derivatives of sitosterol and campesterol) in dry aortic valve cusp tissues was maximally 0.175 μg/g tissue among all patients (Schött et al., 2014). Mean oxy-campesterol, oxy-sitosterol and total POP concentrations in aortic valves in subjects without coronary artery disease were 0.35, 0.40 and 0.75 μg/g, respectively (Luister et al., 2015). Average dry aortic valve cusp concentrations of cholesterol, plant sterol and total POP were 22,680 μg/g, 85 μg/g (Schött et al., 2014) and 0.75 μg/g (Luister et al., 2015), respectively.

The applicant also provided an abstract on a study by Baumgartner et al. (2018) who measured plasma POP concentrations in 155 cases with CVD and 414 controls of the Framingham Offspring Study and related POP concentrations to CVD status. Multivariate analysis showed that higher campesterol concentrations (OR: 2.36, 95% CI: 1.60–3.50) and higher sitosterol concentrations (95% CI: 1.09–1.97) were significantly associated with an increased CVD risk as was previously reported by Matthan et al. (2009) for this cohort. In contrast, higher plasma POP concentrations (sum of all POP or any individual POP) were not associated with CVD risk. Furthermore, circulating POP concentrations correlated weakly with their respective non-oxidised PS precursors.

Fuhrmann et al. (2018) prospectively studied CVD endpoints (such as myocardial infarction, stroke, cardiovascular death) and plasma concentrations of COP and POP, in a cohort of 376 patients with suspected coronary artery disease who underwent coronary angiography as part of the HOM sweet HOMe study. Elevated absolute and cholesterol-adjusted 7α-hydroxy-campesterol plasma concentrations were found to be associated with a higher occurrence of cardiovascular (CV) events.

The Panel notes that the evidence from these studies does not allow conclusions regarding a causal relationship between POP and CVD risk and that the reported associations may be biased given the chemical similarity of PS, POP and cholesterol.

4. Discussion

As shown by the applicant's PLM data covering five European countries, high (P95) percentile household consumption of already authorised (non-heated) products with added plant sterols was below 3 g/day in four of the countries and exceeded 3 g in one of the countries. Although these PLM consumption data refer to households, it can be assumed that products containing added plant sterols may be consumed by only one person in some households. Among the non-target population, children have been identified to be among consumers: up to 8% of children were consumers, while in children

below 5 years of age, less than 1% were consumers. Any additional consumption of PS coming from new uses, if not consumed as an alternative to already authorised uses, could therefore result to intakes in excess of levels considered to be safe for adults (i.e. 3 g per person per day). Furthermore, the intended extension of use could increase consumption by non-target population groups. This safe intake level of 3 g/day for adults established by the SCF has been supported by the ADI established by JECFA in 2008.

In order to demonstrate the safety of the intended extension of use, the applicant has investigated the subchronic toxicity in rats of heat-treated PS which contained up to 8% of POP in the test substance. Based on findings in the high-dose group (i.e. higher thrombocyte counts in males and females, higher γ-GT activity and higher liver weights in females), the Panel identified a NOAEL of 128 mg/kg bw (mid dose, male rats). The Panel applied an uncertainty factor (UF) of 200 accounting for intra- (10x) and interspecies (10x) differences and the absence of a chronic toxicity study (2x) (EFSA Scientific Committee, 2012). The Panel also considers that there is no history of consumption of such POP from heat-treated foods with added PS and that POP appear to have a different toxicological profile from that of non-oxidised PS. Furthermore the Panel notes that, unlike for non-heated foods with added PS for which a large number of human studies are available (albeit mostly investigating the efficacy of PS to lower LDL-C), there is only one human study available investigating the kinetics of POP from heat-treated foods with added PS. For the SCF and JECFA, the availability of a large number of human studies with unheated PS was supportive for deriving the safe level of about 3 g per person per day for adults. The kinetic study conducted by the applicant shows that consumption of heat-treated PS would increase human plasma levels of POP to levels which are not reached following consumption of unheated foods with added PS or with a normal diet without consumption of foods with added PS. The application of an UF of 200 to the NOAEL of the sub-chronic rat study results in 0.64 mg POP/kg bw per day to be considered safe for humans. This safe level of exposure would be exceeded at the P95 by all age groups when considering the P90 oxidation rate (i.e. 2.28%) reported for cooking experiments by Lin et al. (2016a) and using EFSA's comprehensive food consumption database for assessing the potential exposure. When considering the median oxidation rate (i.e. 0.5%) in these cooking experiments, the safe level of 0.64 mg POP/kg bw per day would be exceeded at the highest P95 intake estimates in children below 9 years of age.

When considering an intake of the maximum authorised use level of 3 g PS per person per day and oxidation rates of 0.5% and 2.28%, the resulting daily POP intakes per kg bw by an adult weighing 70 kg would be 0.21 mg/kg bw ($3{,}000 \times 0.005/70$) and 0.98 mg/kg bw ($3{,}000 \times 0.0228/70$), respectively, the latter value exceeding 0.64 mg/kg bw.

5. Conclusions

The Panel concludes that the safety of the intended extension of use of PSE under the proposed conditions of use has not been established.

6. Steps taken by EFSA

1) On 27 November 2014, EFSA received a letter from the European Commission with the request for a scientific opinion on the safety of phytosterol esters (extension of use, cooking oils). Ref. Ares(2014)3953385-26/11/2014.
2) On 22 January 2015, a valid application on phytosterol esters (extension of use, cooking oils), which was submitted by name of the company, was received from European Commission and the scientific evaluation procedure was initiated.
3) On 26 June 2015, 30 June 2016 and 04 March 2019, EFSA requested the applicant to provide additional information to accompany the application and the scientific evaluation was suspended.
4) On 11 December 2015, 20 September 2018 and 13 May 2019, additional information was provided by the applicant and the scientific evaluation was restarted.
5) During its meeting on 05 May 2020, the NDA Panel, having evaluated the data, adopted a scientific opinion on the safety of phytosterol esters (extension of use, cooking oils) as a novel food ingredient pursuant to Regulation (EU) 2015/2283.

References

Al-Delaimy WK, van Kappel AL, Ferrari P, Slimani N, Steghens J, Bingham S, Johansson I, Wallström P, Overvad K, Tjønneland A, Key TJ, Welch AA, Bueno-de-Mesquita HB, Peeters PHM, Boeing H, Linseisen J, Clavel-Chapelon F, Guibout C, Navarro C, Quirós JR, Palli D, Celentano E, Trichopoulou A, Benetou V, Kaaks R and Riboli E, 2004. Plasma levels of six carotenoids in nine European countries: report from the European Prospective Investigation into Cancer and Nutrition (EPIC). Public Health Nutrition, 7, 713–722. https://doi.org/10.1079/phn2004598

Alemany L, Laparra J, Baberá R and Alegría A, 2013. Relative expression of cholesterol transport-related proteins and inflammation markers through the induction of 7-ketosterol-mediated stress in Caco-2 cells. Food and Chemistry Toxicology, 56, 247–253.

Andersson SW, Skinner J, Ellegård L, Welch AA, Bingham S, Mulligan A, Andersson H and Khaw KT, 2004. Intake of dietary plant sterols is inversely related to serum cholesterol concentration in men and women in the EPIC Norfolk population: a cross-sectional study. European Journal of Clinical Nutrition, 58, 1378–1385.

Appel MJ, 2001. Unpublished. TNO Report, unpublished. Sub-chronic (13-week) oral toxicity study with CF636 in rats. 29 June 2001.

Babiker A and Diczfalusy U, 1998. Transport of side-chain oxidized oxysterols in the human circulation. Biochimica et Biophysica Acta, 1392, 333–339.

Bang HJ, Arakawa C, Takada M, Sato M and Imaizumi K, 2008. A comparison of the potential unfavorable effects of oxycholesterol and oxyphytosterol in mice: different effects, on cerebral 24S-hydroxychoelsterol and serum triacylglycerols levels. Bioscience, Biotechnology, and Biochemistry, 72, 3128–3133.

Baumgartner S, Mensink RP, Husche C, Lütjohann D and Plat J, 2013. Effects of plant sterol- or stanol-enriched margarine on fasting plasma oxyphytosterol concentrations in healthy subjects. Atherosclerosis, 227, 414–419.

Baumgartner S, Mensink RP, Konings M, Schott HF, Friedrichs S, Husche C, Lutjohann D and Plat J, 2015. Postprandial plasma oxyphytosterol concentrations after consumption of plant sterol or stanol enriched mixed meals in healthy subjects. Steroids, 99(Pt B), 281–286.

Baumgartner S, Ras TR, Trautwein EA, Mensink RP and Plat J, 2017. Plasma fat-soluble vitamin and carotenoid concentrations after plant sterol and plant stanol consumption: a meta-analysis of randomized controlled trials. European Journal of Nutrition, 56, 909–923.

Baumgartner S, Ras RT, Trautwein EA, Konings MCJM, Mensink RP and Plat J, 2018. Plasma oxyphytosterol concentrations are not associated with CVD status in framingham offspring study participants. Atherosclerosis, 275, e97.

Beevers C, 2000. Unpublished. CF 636: Reverse Mutation in five Histidine-requiring strains of Salmonella typhimurium, November 2000.

Bjorkhem I, Breuer O, Angelin B and Wikstrom SA, 1988. Assay of unesterified cholesterol-5,6-epoxide in human serum by isotope dilution mass spectrometry. Levels in the healthy state and in hyperlipoproteinemia. Journal of Lipid Research, 29, 1031–1038.

Bohn T, 2017. Carotenoids, Chronic Disease Prevention and Dietary Recommendations. International Journal for Vitamin and Nutrition Research, 87, 121–130. https://doi.org/10.1024/0300-9831/a000525

Bothelo PB, Galasso M, Dias V and Mandrioli M, 2014. Oxidative stability of functional phytosterol-enriched dark chocolate. LWT Food and Science Technology, 55, 444–451.

van de Bovenkamp P, Kosmeijer-Schuil TG and Katan MB, 1988. Quantification of oxysterols in Dutch foods: egg products and mixed diets. Lipids, 23, 1079.

Breuer O, 1995. Identification and quantitation of cholest-5-ene-3 beta,4 beta-diol in rat liver and human plasma. Journal of Lipid Research, 36, 2275–2281.

Breuer O and Bjorkhem I, 1990. Simultaneous quantification of several cholesterol autoxidation and monohydroxylation products by isotope-dilution mass spectrometry. Steroids, 55, 185–192.

Brown AW, 2011. "Mechanisms of decreased cholesterol absorption mediated by phytosterols in the intestinal lumen". Nutrition & Health Sciences Dissertations & Theses. Paper 19. Available online: http://digitalcommons.unl.edu/nutritiondiss/19

Buijsse B, Feskens EJ, Schlettwein-Gsell D, Ferry M, Kok FJ, Kromhout D and de Groot LC, 2005. Plasma carotene and alpha-tocopherol in relation to 10-y all-cause and cause-specific mortality in European elderly: the Survey in Europe on Nutrition and the Elderly, a Concerted Action (SENECA). American Journal of Clinical Nutrition, 82, 879–886.

Chan YM, Varady KA, Lin Y, Trautwein E, Mensink RP, Plat J and Jones PJ, 2006. Plasma concentrations of plant sterols: physiology and relationship with coronary heart disease. Nutrition Reviews, 64, 385–402.

DeSmet E, Mensink RP and Plat J, 2012. Effects of plant sterols and stanols in intestinal cholesterol metabolism: suggested mechanisms from the past to present. Molecular Nutrition & Food Research, 56, 1058–1072.

Dzeletovic S, Breuer O, Lund E and Diczfalusy U, 1995. Determination of cholesterol oxidation products in human plasma by isotope dilution-mass spectrometry. Analytical Biochemistry, 225, 73–80.

EFSA (European Food Safety Authority), 2008. Consumption of Food and Beverages With Added Plant Sterols in the European Union (EFSA/DATEX 03): a Report from the Data Collection and Exposure Unit in Response to a Request from the European Commission [European Food Safety Authority]. EFSA Journal 2008;133, 21 pp. Available online: http://www.efsa.europa.eu/en/scdocs/scdoc/133r.htm

EFSA (European Food Safety Authority), 2011. The EFSA Comprehensive European Food Consumption Database. European Food Safety Authority (EFSA), Parma, Italy. Available online: http://www.efsa.europa.eu/en/datexfoodcdb/datexfooddb.htm

EFSA ANS Panel (EFSA Panel on Food additives and Nutrient Sources added to Food), 2012. Scientific Opinion on the safety of stigmasterol-rich plant sterols as food additive. (Question no EFSA-Q-2011-00428, adopted on 19 April 2012 by European Food Safety Authority). EFSA Journal 2012;10(5):2659, 39 pp. https://doi.org/10.2903/j.efsa.2012.2659. Available online: http://www.efsa.europa.eu/en/efsajournal/doc/2659.pdf

EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition, and Allergies), 2010. Scientific Opinion on Dietary Reference Values for fats, including saturated fatty acids, polyunsaturated fatty acids, monounsaturated fatty acids, trans fatty acids, and cholesterol. EFSA Journal 2010;8(3):1461, 107 pp. https://doi.org/10.2903/j.efsa.2010.1461. Available online: www.efsa.europa.eu

EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition and Allergies), 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

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

European Commission, 2000. Commission Decision 24 July 2000 authorising the placing on the market of yellow fat spreads with added phytosterol esters as novel food ingredients under Regulation (EC) No 258/97 of the European Parliament and of the Council (2000/500/EC). Available online: http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32000D0500&from=EN

European Commission, 2004a. Commission Decision of 31 March 2004 authorising the placing on the market of milk type products and yoghurt type products with added phytosterol esters novel food ingredients under Regulation (EC) No 258/97 of the European Parliament and of the Council (2004/335/EC). Available online: http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32004D0335&from=EN

European Commission, 2004b. Commission Regulation (EC) No 608/2004 of 31 March 2004 concerning the labelling of foods and food ingredients with added phytosterols, phytosterol esters, phytostanols and/or phytostanol esters. Available online: http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32004R0608&from=EN

Fuhrmann A, Weingartner O, Meyer S, Cremers B, Seiler-Mussler S, Schott HF, Kerksiek A, Friedrichs S, Ulbricht U, Zawada AM, Laufs U, Scheller B, Fliser D, Schulze PC, Bohm M, Heine GH and Lutjohann D, 2018. Plasma levels of the oxyphytosterol 7alpha-hydroxycampesterol are associated with cardiovascular events. Atherosclerosis, 279, 17–22. https://doi.org/10.1016/j.atherosclerosis.2018.10.010

Garcia-Llatas G and Rodriguez-Estrada MT, 2011. Current and new insights on phytosterol oxides in plant sterol-enriched food. Chemistry and Physics of Lipids, 164, 607–624.

Goodman DS, Deckelbaum RJ, Palmer RH, Dell RB, Ramakrishnan R, Delpre G, Beigel Y and Cooper M, 1983. Cholesterol turnover and metabolism in two patients with abetalipoproteinemia. Journal of Lipid Research, 24, 1605–1611.

Grandgirard A, Martine L, Demaison L, Cordelet C, Joffre C, Berdeaux O and Semon E, 2004a. Oxyphytosterols are present in plasma of healthy human subjects. British Journal of Nutrition, 91, 101–106.

Grandgirard A, Demaison-Meloche J, Cordelet C and Demaison L, 2004b. Incorporation of oxyphytosterols in tissues of hamster. Reproduction, Nutrition, Development, 44, 599–608.

Guardiola F, Tres A, Codony R, Addis PB, Bergmann SD and Zavoral JH, 2007. Lack of effect of oral supplementation with antioxidants on cholesterol oxidation product concentration of human plasma, as revealed by an improved gas chromatography method. Analytical and Bioanalytical Chemistry, 389, 277–289.

Haskell MJ, 2012. The challenge to reach nutritional adequacy for vitamin A: β-carotene bioavailability and conversion–evidence in humans. American Journal of Clinical Nutrition, 96, 1193S–1203S. https://doi.org/10.3945/ajcn.112.034850

Heinemann T, Axtmann G and von Bergmann K, 1993. Comparison of intestinal absorption of cholesterol with different plant sterols in man. European Journal of Clinical Investigation, 23, 827–831.

Helmschrodt C, Becker S, Schroter J, Hecht M, Aust G, Thiery J and Ceglarek U, 2013. Fast LC-MS/MS analysis of free oxysterols derived from reactive oxygen species in human plasma and carotid plaque. Clinica Chimica Acta, 425C, 3–8.

Hepburn PA, Horner SA and Smith M, 1999. Safety evaluation of phytosterol esters. Part 2. Subchronic 90-day oral toxicity study on phytosterol esters - a novel functional food. Food and Chemical Toxicology, 37.

Hercberg S, Galan P, Preziosi P, Bertrais S, Mennen L, Malvy D, Roussel AM, Favier A and Briancon S, 2004. The SU.VI.MAX Study: a randomized, placebo-controlled trial of the health effects of antioxidant vitamins and minerals. Archives of Internal Medicine, 164, 2335–2342.

Ho YK, Faust JR, Bilheimer DW, Brown MS and Goldstein JL, 1977. Regulation of cholesterol synthesis by low density lipoprotein in isolated human lymphocytes. Comparison of cells from normal subjects and patients with homozygous familial hypercholesterolemia and abetalipoproteinemia. Journal of Experimental Medicine, 145, 1531–1549. https://doi.org/10.1084/jem.145.6.1531

Husche C, Weingärtner O, Pettersson H, Vanmierlo T, Böhm M, Laufs U and Lütjohann D, 2011. Validation of an isotope dilution gas chromatography-mass spectrometry method for analysis of 7-oxygenated campesterol and sitosterol in human serum. Chemistry and Physics of Lipids, 164, 425–431. https://doi.org/10.1016/j.chemphyslip.2011.04.009

Iuliano L, Micheletta F, Natoli S, Ginanni CS, Iappelli M, Elisei W, Giovannelli L, Violi F and Diczfalusy U, 2003. Measurement of oxysterols and alpha-tocopherol in plasma and tissue samples as indices of oxidant stress status. Analytical Biochemistry, 312, 217–223.

JECFA, 2009. Toxicological Evaluation of Certain Food Additives. Prepared by the Sixty-ninth meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), June 17-26-29, 2008, Rome, Italy. (WHO Food Additives Series, no 60). World Health Organization (WHO), Geneva, Switzerland, 2009. Available online: http://whqlibdoc.who.int/publications/2009/9789241660600_eng.pdf

Jenkins DJ, Kendall CW, Popovich DG, Vidgen E, Mehling CC, Vuksan V, Ransom TP, Rao AV, Rosenberg-Zand R, Tariq N, Corey P, Jones PJ, Raeini M, Story JA, Furumoto EJ, Illingworth DR, Pappu AS and Connelly PW, 2001. Effect of a very-high-fiber vegetable, fruit, and nut diet on serum lipids and colonic function. Metabolism, 50, 494–503.

Jones PJ, MacDougall DE, Ntanios F and Vanstone CA, 1997. Dietary phytosterols as cholesterol-lowering agents in humans. Canadian Journal of Physiology and Pharmacology, 75, 217–227.

de Jong A, Plat J and Mensink RP, 2003. Metabolic effect of plant sterols and stanols. Review. Journal of Nutrional Biochemistry, 14, 362–369.

Julien-David D, Ennahar S, Miesch M, Geoffroy P, Raul F, Aoude-Werner D, Lessinger JM and Marchioni E, 2009. Effects of oxidation on the hydrolysis by cholesterol esterase of sitosteryl esters as compared to a cholesteryl ester. Steroids, 74, 832–836.

Kenny O, O'Callaghan Y, O'Connell NM, McCarthy FO, Maguire AR and O'Brien NM, 2012. Oxidized derivatives of dihydrobrassicasterol: cytotoxic and apoptotic potential in U937 and HepG2 cells. Journal of Agriculture and Food Chemistry, 60, 5952–5961.

Kelly ER, Plat J, Mensink RP and Berendschot TT, 2011. Effects of long term plant sterol and - stanol consumption on the retinal vasculature: a randomized controlled trial in statin users. Atherosclerosis, 214, 225–230.

Kemplay G and Nordfjord H, 2006. Consumer research on the consumption of phytosterols. TNS, London, United Kingdom.

Klingberg S, Andersson H, Mulligan A, Bhaniani A, Welch A, Bingham S, Khaw KT, Andersson S and Ellegård L, 2008. Food sources of plant sterols in the EPIC Norfolk population. European Journal of Clinical Nutrition, 62, 695–703.

Kudo K, Emmons GT, Casserly EW, Via DP, Smith LC, St Pyrek J and Schroepfer Jr GJ, 1989. Inhibitors of sterol synthesis. Chromatography of acetate derivatives of oxygenated sterols. Journal of Lipid Research, 30, 1097–1111.

Kuroki S, Okamoto S, Naito T, Oda H, Nagase S, Sakai H, Nawata H, Yamashita H, Chijiiwa K and Tanaka M, 1995. Serum 7 alpha-hydroxycholesterol as a new parameter of liver function in patients with chronic liver diseases. Hepatology, 22(4 Pt 1), 1182–1187.

Lea LJ and Hepburn PA, 2006. Safety evaluation of phytosterol-esters. Part 9: results of a European post-launch monitoring programme. Food Chemistry Toxicology, 44, 1213–1222.

Lea LJ, Hepburn PA, Wolfreys AM and Baldrick P, 2004. Safety evaluation of phytosterol esters. Part 8. Lack of genotoxicity and subchronic toxicity with phytosterol oxides. Food and Chemical Toxicology, 42, 771–783.

Liang YT, Wong WT, Guan L, Tian XY, Ma KY, Huang Y and Chen Z-Y, 2011. Effect of phytosterols and their oxidation products on lipoprotein profiles and vascular function in hamster fed a high cholesterol diet. Atherosclerosis, 219, 124–133.

Lin Y, Knol D, Menéndez-Carreno M, Blom W, Matthee J, Janssen HG and Trautwein EA, 2016a. Formation of plant sterol oxidation products in foods during baking and cooking using margarine without and with added plant sterol esters. Journal of Agriculture and Food Chemistry, 64, 653–662.

Lin Y, Knol D and Trautwein EA, 2016b. Phytosterol oxidation products (POP) in foods with added phytosterols and estimation of their daily intake: a literature review. European Journal of Lipid Science and Technology, 118, 1423–1438.

Lin Y, Koppenol WP, Knol D, Vermeer MA, Hiemstra H, Friedrichs S, Lütjohann D and Trautwein EA, 2019. Serum Concentration of Plant Sterol Oxidation Products (POP) Compared to Cholesterol Oxidation Products (COP) after Intake of Oxidized Plant Sterols: a Randomised, Placebo-Controlled, Double-Blind Dose–Response Pilot Study. Nutrients, 11, 2319, https://doi.org/10.3390/nu11102319

Lloyd M, 2000. Unpublished. Final report, CF 636: Induction of Chromosome Aberrations in Cultured Human Peripheral Blood Lymphocytes. December 2000.

Lloyd M, 2001. Unpublished. Final report, CF 636: Induction of micronuclei in cultured human peripheral blood lymphocytes. March 2001.

Luister A, Schött HF, Husche C, Schafers HJ, Bohm M, Plat J, Graber S, Lütjohann D, Laufs U and Weingärtner O, 2015. Increased plant sterol deposition in vascular tissue characterizes patients with severe aortic stenosis and concomitant coronary artery disease. Steroids, 99(Pt B), 272–280.

Lutjohann D, Bjorkhem I, Beil UF and von Bergmann K, 1995. Sterol absorption and sterol balance in phytosterolemia evaluated by deuterium-labeled sterols: effect of sitostanol treatment. Journal of Lipid Research, 36, 1763–1773.

Maguire J, Konoplyannikov M, Ford A, Maguire AR and O'Brien NM, 2003. Comparison of the cytotoxic effects of beta-sitosterol oxides and a cholesterol oxide, 7beta-hydroxycholesterol, in cultured mammalian cells. British Journal of Nutrition, 90, 767–775.

Matthan NR, Pencina M, LaRocque JM, Jacques PF, D'Agostino RB, Schaefer EJ and Lichtenstein AH, 2009. Alterations in cholesterol absorption/synthesis makers characterize Framingham Offspring study participants with CHD. Journal of Lipid Research, 50, 1927–1935.

Mellies M, Glueck CJ, Sweeney C, Fallat RW, Tsang RC and Ishikawa TT, 1976. Plasma and dietary phytosterols in children. Pediatrics, 57, 60–67.

Menendez-Carreno M, Steenbergen H and Janssen HG, 2012. Development and validation of a comprehensive two-dimensional gas chromatography-mass spectrometry method for the analysis of phytosterol oxidation products in human plasma. Analytical and Bioanalytical Chemistry, 402, 2023–2032.

Menéndez-Carreno M, Ansorena D and Astisarán I, 2008. Stability of sterols in phytosterol-enriched milk under different heating conditions. Journal of Agriculture and Food Chemistry, 56, 9997–10002.

Menéndez-Carreno M, Knol D and Janssen HG, 2016. Development and validation of methodologies for the quantification of phytosterols and phytosterol oxidation products in cooked and baked foods. Journal of Chromatography A, 1428, 316–325.

Mol MJ, de Rijke YB, Demacker PN and Stalenhoef AF, 1997. Plasma levels of lipid and cholesterol oxidation products and cytokines in diabetes mellitus and cigarette smoking: effects of vitamin E treatment. Atherosclerosis, 129, 169–176.

Murakami H, Tamasawa N, Matsui J, Yasujima M and Suda T, 2000. Plasma oxysterols and tocopherol in patients with diabetes mellitus and hyperlipidemia. Lipids, 35, 333–338.

NDNS, 2011. National Diet and Nutrition Survey, 2008-2010 [computer file], 3rd ed. (SN: 6533). UK Data Archive (UKDA) [distributor], Colchester, Essex; 2012. https://doi.org/10.5255/ukda-sn-6533-2

Niemann B, Sommerfeld C, Hembeck A and Bergmann C, 2007. Lebensmittel mit Pflanzensterinzusatz in der Wahrnehmung der Verbraucher. Bundesinstitut für Risikobewertung, Berlin, Germany. pp. 1–62. Available online: https://bfr.bund.de/cm/350/lebensmittel_mit_pflanzensterinzusatz_in_der_wahrnehmung_der_verbraucher.pdf

Ocké MC, van Rossum CTM, Fransen HP, Buurma EM, de Boer EJ, Brants HAM, Niekerk EM, Van der Laan JD, Drijvers JJMM and Ghameshlou Z, 2008. Dutch National Food Consumption Survey Young Children 2005/2006. [Voedselconsumptiepeiling bij peuters en kleuters 2005/2006]. (RIVM Report 350070001/2008). Rijksinstiuut Voor Volksgezondheid en Milieu (RIVM), Bilthoven, The Netherlands. Available online: http://www.rivm.nl/bibliotheek/rapporten/350070001.html

Olsson AG, Angelin B, Assmann G, Binder CJ, Björkhem I, Cedazo-Minguez A, Cohen J, von Eckardstein A, Farinaro E, Müller-Wieland D, Parhofer KG, Parini P, Rosenson RS, Starup-Linde J, Tikkanen MJ and Yvan-Charvet L, 2017. Can LDL cholesterol be too low? Possible risks of extremely low levels. Journal of Internal Medicine, 281, 534–553. https://doi.org/10.1111/joim.12614

Ostlund Jr RE, McGill JB, Zeng CM, Covey DF, Stearns J, Stenson WF and Spilburg CA, 2002. Gastrointestinal absorption and plasma kinetics of soy Delta(5)-phytosterols and phytostanols in humans. American Journal of Physiology. Endocrinology and Metabolism, 282, E911–E916.

Plat J, Brzezinka H, Lutjohann D, Mensink RP and von Bergmann K, 2001. Oxidized plant sterols in human serum and lipid infusions as measured by combined gas-liquid chromatography-mass spectrometry. Journal of Lipid Research, 42, 2030–2038.

Plat J, Theuwissen E, Husche C, Lütjohann D, Gijbels MJ, Jeurissen M, Shiri-Sverdlov R, van der Made I and Mensink RP, 2014. Oxidised plant sterols as well as oxycholesterol increase the proportion of severe atherosclerotic lesions in female LDL receptor+/– mice. British Journal of Nutrition, 111, 64–70.

Poulsen M, 2007. Noforisk - Quantitative risk assessment strategies for novel foods. 3rd periodic activity report. National Food Institute, Technical University, Denmark. pp. 150–156.

Raczyk M, Bonte A, Matthaeus B and Rudzinska M, 2018. Impact of added phytosteryl/phytostanyl fatty acid esters on chemical parameters of margarines upon heating and pan-frying. European Journal of Lipid Science and Technology, 120, n/a.

RIVM, 2011. Nederlands Voedingsstoffenbestand (NEVO) [Dutch Food Composition Database]. Rijksinstiuut Voor Volksgezondheid en Milieu (RIVM), Bilthoven, The Netherlands. Available online: http://nevo-online.rivm.nl/

van Rossum CTM, Fransen HP, Verkaik-Kloosterman J, Buurma-Rethans EJM and Ocké MC, 2011. Dutch National Food Consumption Survey 2007–2010: Diet of children and adults aged 7 to 69 years. [Nederlandse voedselconsumptiepeiling 2007–2010: Voeding van kinderen en volwassenen van 7 tot 69 jaar]. (RIVM rapport 350050006). Rijksinstiuut Voor Volksgezondheid en Milieu (RIVM), Bilthoven, The Netherlands. Available from: http://www.rivm.nl/bibliotheek/rapporten/350050006.html

Rudzinska M, Przybylski R and Wasowicz E, 2014. Degradation of phytosterols during storage of enriched maragarines. Food Chemistry, 142, 294–298.
Ryan E, Chopra J, McCarthy F, Maguire AR and O'Brien NM, 2005. Qualitative and quantitative comparison of the cytotoxic and apoptotic potential of phytosterol oxidation products with their corresponding cholesterol oxidation products. British Journal of Nutrition, 94, 443–451.
Salonen JT, Nyyssonen K, Salonen R, Porkkala-Sarataho E, Tuomainen TP, Diczfalusy U and Bjorkhem I, 1997. Lipoprotein oxidation and progression of carotid atherosclerosis. Circulation, 95, 840–845.
Sanders DJ, Minter HJ, Howes D and Hepburn PA, 2000. The safety evaluation of phytosterol esters. Part 6. The comparative absorption and tissue distribution of phytosterols in the rat. Food and Chemical Toxicology, 38, 485–491.
Santosa S, Varady KA, Abumweis S and Jones PJ, 2007. Review. Physiological and therapeutic factors affecting cholesterol metabolism: does a reciprocal relationship between cholesterol absorption and synthesis really exist? Life Sciences, 80, 505–514.
SCF (Scientific Committee on Food), 2000. Opinion on a request for the safety assessment of the use of phytosterol esters in yellow fat spreads. Opinion adopted by the Scientific Committee, 6 April 2000. Available online: http://europa.eu.int/comm/food/fs/sc/scf/out56_en.pdf
SCF (Scientific Committee on Food), 2002a. General view of the Scientific Committee on Food on the long-term effects of the intake of elevated levels of phytosterols from multiple dietary sources with particular attention to the effects on ß-carotene (expressed on 26 September 2002). Available online: http://ec.europa.eu/food/fs/sc/scf/out143 en.pdf
SCF (Scientific Committee on Food), 2002b. Opinion of the Scientific Committee on Foodon applications for approval of a variety of plant sterol-enriched foods (expressed on 5 March 2003). Available online: http://ec.europa.eu/food/fs/sc/scf/out174_en.pdf
Scholz B, Guth S, Engel K-H and Steinberg P, 2015. Phytosterol oxidation products in enriched foods: occurrence, exposure, and biological effects. Molecular Nutrition & Food Research, 59, 1339–1352.
Scholz B, Menzel N, Lander V and Engel K-H, 2016. Heating two types of enriched margarine: complementary analysis of phytosteryl/phytostanyl fatty acid esters and phytosterol/phytostanol oxidation products. Journal of Agriculture and Food Chemistry, 64, 2699–2708.
Schött HF, Luister A, Husche C, Schäfers HJ, Böhm M, Plat J, Lütjohann D, Laufs U and Weingärtner O, 2014. The relationships of phytosterols and oxyphytosterols in plasma and aortic valve cusps in patients with severe aortic stenosis. Biochemical and Biophysical Research Communications, 446, 805–810.
Shardell MD, Alley DE, Hicks GE, El-Kamary SS, Miller RR, Semba RD and Ferrucci L, 2011. Low-serum carotenoid concentrations and carotenoid interactions predict mortality in US adults: the Third National Health and Nutrition Examination Survey. Nutritional Research, 31, 178–189. https://doi.org/10.1016/j.nutres.2011.03.003
Sioen I, Matthys C, Huybrechts I, Van Camp J and De Henauw S, 2011a. Consumption of plant sterols in Belgium: estimated intakes abd sources of naturally occurring plant sterols and beta-carotene. British Journal of Nutrition, 105, 960–966.
Sioen I, Matthys C, Huybrechts I, Van Camp J and De Henauw S, 2011b. Consumption of plant sterols in Belgium: consumption patterns of plant sterol-enriched foods in Flanders, Belgium. British Journal of Nutrition, 105, 911–918.
Soupas L, Huikko L, Lampi A-M and Piironen V, 2006. Oxidative stability of phytosterols in some food applications. European Food Research Technology, 222, 266–273.
Soupas L, Huikko L and Lampi A-M, 2007. Piironen V. Pan-frying may induce phytosterol oxidation. Food Xhem, 101, 286–297.
Strobel M, Tinz J and Biesalski HK, 2007. The importance of beta-carotene as a source of vitamin A with special regard to pregnant and breastfeeding women. European Journal of Nutrition, 46(Suppl 1), I1–I20. https://doi.org/10.1007/s00394-007-1001-z
Tilvis RS and Miettinen TA, 1986. Serum plant sterols and their relation to cholesterol absorption. American Journal of Clinical Nutrition, 43, 92–97.
Tomoyori H, Kawata Y, Higuchi T, Ichi I, Sato H, Sato M, Ikeda I and Imaizumi K, 2004. Phytosterin oxidation products are absorbed in the intestinal lymphatics in rats but do not accelerate atherosclerosis in apolipoprotein e-deficient mice. Journal of Nutritional Biochemistry, 134, 1690–1696.
UKDA, 2012. National Diet and Nutrition Survey, 2008-2010 [computer file], 3rd ed. (SN:6533). UK Data Archive (KDA) [distributor], Colchester, Essex. Available online: https://doi.org/10.5255/ukda-sn-6533-2
Unilever, 1998. Phytosterol esters-use in Yellow Fat Spreads. Application for market approval under Regulation 258/97, and additional information submitted in October 1999 (doc. ref. D99/047) and of January 2000 (doc. ref.D00/004).
Unilever, 2018. The effect of oxidized plant sterol intake on serium concentrations of plant sterol oxidation products (POP) and cholesterol oxidation products (COP). Yuguang Lin, Wieneke P. Koppenol, Diny Knol, Mario A. Vermeer, Harry Hiemstra, Elke A. Trautwein, Unilever R&D Vlaardingen, The Netherlands prepared for Upfield Europe B.V., Nassaukade 5, 3071JL Rotterdam, Netherlands.

Vejux A, Montange T, Martine L, Zarrouk A, Riedinger JM and Lizard D, 2012. Absence of oxysterol-like side effects in human monocytic cells treated with phytosterols and oxyphytosterols. Journal of Agriculture and Food Chemistry, 60, 4060–4066.

Weber D and Grune T, 2012. The contribution of β-carotene to vitamin A supply of humans. Molecular Nutrition & Food Research, 56, 251–258. https://doi.org/10.1002/mnfr.201100230

Weingärtner O, Lütjohann D, Ji S, Weisshoff N, List F, Sudhop T, von Bergmann K, Gertz K, König J, Schäfers HJ, Endres M, Böhm M and Laufs U, 2008. Vascular effects of diet supplementation with plant sterols. Journal of the American College of Cardiology, 51, 1553–1561.

Weingärtner O, Ulrich C, Lütjohann D, Ismail K, Schirmer SH, Vanmierlo T, Böhm M and Laufs U, 2011. Differential effects on inhibition of cholesterol absorption by plant stanol and plant sterol esters in apoE-/- mice. Cardiovascular Research, 90, 484–492.

Weingärtner O, Weingärtner N, Scheller B, Lütjohann D, Gräber S, Schäfers HJ, Böhm M and Laufs U, 2009. Alterations in cholesterol homeostasis are associated with coronary heart disease in patients with aortic stenosis. Coronary Artery Disease, 20, 376–382.

Willems JI, Blommaert MA and Trautwein EA, 2013. Results from a post-launch monitoring survey on consumer purchases of foods with added phytosterols in five European countries. Food and Chemical Toxicology, 62, 48–53.

Wong WW, Hachey DL, Insull W, Opekun AR and Klein PD, 1993. Effect of dietary cholesterol on cholesterol synthesis in breast-fed and formula-fed infants. Journal of Lipid Research.

Yang C, Chen ZY, Wong SL, Liu J, Liang YT, Lau CW, Lee HK, Huang Y and Tsang SY, 2013. ß-Sitosterol oxidation products attenuate vasorelaxation by increasing reactive oxygen species and cyclooxygenase-2. Cardiovascular Research, 97, 520–532.

Zieden B, Kaminskas A, Kristenson M, Kucinskiene Z, Vessby B, Olsson AG and Diczfalusy U, 1999. Increased plasma 7 beta-hydroxycholesterol concentrations in a population with a high risk for cardiovascular disease. Arteriosclerosis, Thrombosis, and Vascular Biology, 19, 967–971.

Abbreviations

ADI	acceptable daily intake
ADME	absorption, distribution, metabolism and excretion
A/G	albumin/globulin
bw	body weight
CB	cooking and baking
CBPI	cytokinesis-block proliferation index
COP(s)	cholesterol oxidation product(s)
CV(D)	cardiovascular (disease)
DRV	Dietary reference value
FPS	free plant sterols
GLP	good laboratory practice
γ-GT	gamma-glutamyl transferase
Hb	haemoglobin
HDL	high-density lipoprotein
HDL-C	HDL-cholesterol
HH	household
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LDL	low-density lipoprotein
LDL-C	LDL-cholesterol
LDLR	LDL receptor
MCH	mean corpuscular haemoglobin
MCHV	mean corpuscular haemoglobin concentration
MCV	mean corpuscular volume
NDA Panel	EFSA Panel on Nutrition, Novel Foods and Food Allergens
NF	novel food
NOAEL	no observed adverse effect level
NDNS	UK National Diet and Nutrition Survey
OECD	Organisation for Economic Co-operation and Development
ORP	oxidation rate of plant sterols
PAE	plant stanol esters
PCV	packed cell volume
PGF2α	prostaglandin F2 alpha
PLM	post launch monitoring

POC	plant sterol oxide concentrate
POP	plant sterol oxidation product(s)
PS	plant sterols
PSE	plant sterol esters
RBC	red blood cell counts
RCT	randomised controlled trial
RIVM	Dutch National Food Consumption Survey
SCF	Scientific Committee on Food
TAG	triglycerides
TC	total cholesterol
UF	uncertainty Factor
VLDL	very low-density lipoproteins

Annexes

Annex 1: POP contents (means ± standard deviation) in cooked foods (per portion size) and in residual fat (RF), and derived ORP for different cooking methods

Cooking methods	Foods (typical portion; g)*	Control margarine					PS-margarine				
		PS^ (A)	POP in food (B)	POP in RF (C)	Total POP (B + C)	ORP (B + C)/ A × 100)	PS (D)	POP in food (E)	POP in RF (F)	Total POP (E + F)	ORP (E + F)/ D × 100)
		Mg	mg	mg	mg	%	mg	mg	mg	mg	%
Stir-frying	Green beans (134)	29	0.55 ± 0.08	0.23 ± 0.04	0.78	2.69	1,286	2.27 ± 1.21	0.41 ± .0.47	2.68	0.21
	Cabbage (119)	29	0.67 ± 0.11	–	0.67	2.32	1,286	6.48 ± 1.50	–	6.48	0.50
	Chicken (120)	29	0.53 ± 0.15	0.53 ± 0.15	1.06	3.66	1,286	11.78 ± 3.3	16.48 ± 2.79	28.26	2.20
Shallow-frying	Egg (88)	29	0.29 ± 0.09	0.25 ± 0.14	0.54	1.86	1,286	0.55 ± 0.08	3.08 ± 1.06	3.63	0.28
	Onions (60)	29	0.30 ± 0.04	0.04 ± 0.004	0.34	1.17	1,286	0.92 ± 0.17	0.32 ± 0.08	1.24	0.10
	Codfish (130)	29	0.21 ± 0.05	0.46 ± 0.08	0.67	2.30	1,286	1.11 ± 0.23	10.87 ± 3.01	11.98	0.93
	Fish fingers (142)	29	1.11 ± 0.46	0.29 ± 0.05	1.40	4.82	1,286	4.51 ± 1.11	1.04 ± 0.42	5.52	0.43
	Pork fillet (124)	29	0.05 ± .0.03	0.93 ± 0.28	0.98	3.37	1,286	0.66 ± 0.16	20.44 ± 4.29	21.10	1.64
	Steak (beef) (110)	29	0.97 ± 0.39	1.88 ± 0.47	2.85	9.82	1,286	7.34 ± 0.55	22.74 ± 2.36	30.08	2.34
	Salmon (121)	29	0.57 ± 0.15	1.01 ± 0.17	1.58	5.45	1,286	1.15 ± 0.51	19.41 ± 2.70	20.56	1.60
	Potatoes (207)	29	0.99 ± 0.14	1.35 ± 0.06	2.34	8.05	1,286	20.50 ± 1.3	23.90 ± 2.83	44.40	3.45
	Minced meat (124)	29	0.74 ± 0.46	1.06 ± 0.33	1.80	6.19	1,286	1.88 ± 0.51	13.86 ± 3.27	15.74	1.22
Stewing	Beef (128)	29	0.75 ± 0.25	0.71 ± 0.36	1.46	5.05	1,286	1.32 ± 0.22	2.16 ± 0.28	3.48	0.27
Roasting**	Beef (200)	22	0.60 ± 0.18	0.52 ± 0.06	1.12	5.09	964	1.42 ± 0.48	2.51 ± 0.59	3.93	0.41

Cooking methods	Foods (g)*	Control margarine					PS-margarine				
		PS^ (A)	POP in food (B)	POP in RF (C)	Total POP (B + C)	ORP (B + C)/ A × 100)	PS (D)	POP in food (E)	POP in RF (F)	Total POP (E + F)	ORP (E + F)/ D × 100)
		mg	mg	mg	mg	%	mg	mg	mg	mg	%
Microwave cooking	Codfish (116)	29	0.05 ± 0.01	0.10 ± 0.04	0.15	0.51	1286	0.08 ± 0.01	0.22 ± 0.10	0.30	0.02
Median			0.57	0.52	1.06	3.66		1.42	6.98	6.48	0.50
Range			0.05–1.11	0.04–1.88	0.15–2.85	0.51–9.82		0.08–20.50	0.22–23.9	0.30–44.40	0.02–3.45

ORP: oxidation rate of plant sterols; POP: plant sterol oxidation products; PS: plant sterols; RF: residual fat.

*: Weights of the cooked foods are indicated in brackets, except for roasted beef (see**).

**: 200 g of roasted beef was defined as portion size, corresponding to about 21.6% of the total weight (925 g, Table 2) of the roasted beef after cooking. Accordingly, the corresponding amount of RF was calculated by multiplying the total amount of RF with 21.6% (59.1 g × 21.6% =12.8 g), from which the amount of POP was calculated.

^: Amount of PS measured from the used margarine (20 g), except for roasted beef, in which the amount (15 g) of margarine used was calculated according to 70 g × 21.6%.

Annex 2: POP contents (means ± standard deviation) per portion size of baked products and oxidation rates

	Foods (g)*	Control margarine			PS-margarine		
		PS**	POP in food	ORP (POP/PS × 100)	PS**	POP in food	ORP (POP/PS × 100)
		mg	mg	%	mg	mg	%
Baking	Cookies (18)	7.2	0.12 ± 0.02	1.67	321.5	0.20 ± 0.06	0.06
	Muffins (44)	5.4	0.11 ± 0.01	2.04	237.9	0.19 ± 0.07	0.08
	Banana bread (80)	12.7	0.12 ± 0.03	0.94	565.8	0.27 ± 0.13	0.05
	Sponge cake (50)	19.4	0.21 ± 0.02	1.08	861.5	0.60 ± 0.06	0.07
Median value across experiments		–	0.12	1.38	–	0.24	0.06
Range		–	0.11–0.21	0.94–2.04	–	0.19–0.60	0.05–0.08

ORP: oxidation rate of plant sterols; POP: plant sterol oxidation products; PS: plant sterols.
*: Weights per portion size of baked product in brackets.
**: Amount of PS calculated from the amount of margarine used for a portion of baked foods (Table 3).

Annex 3: Time/temperature conditions of cooking and baking experiments (Lin et al., 2016a)

Cooking methods	Ingredient	Cooking times (min)		Mean temperature* ± SD (°C)	
		Preheating	Main cooking	With control margarine	With PS-margarine
Stir-frying	Green beans	3	3	200.4 ± 15.0	199.5 ± 16.9
	Cabbage	3	3	248.6 ± 35.5	260.0 ± 16.2
	Chicken	3	3	228.4 ± 13.6	241.1 ± 16.4
Shallow-frying	Egg	5	4	170.8 ± 6.4	167.8 ± 5.9
	Onions	5	6	161.4 ± 7.2	161.5 ± 3.6
	Codfish	5	6	173.2 ± 5.8	175.3 ± 4.0
	Fish fingers	5	8	184.9 ± 4.7	188.9 ± 7.6
	Pork fillet	5	8	177.0 ± 4.8	177.1 ± 3.3
	Steak (beef)	5	10	168.9 ± 3.2	171.2 ± 6.2
	Salmon	5	10	192.9 ± 5.5	194.3 ± 7.7
	Potatoes	5	15	195.2 ± 6.2	198.5 ± 3.9
	Minced meat	5	18	168.8 ± 12.0	170.9 ± 8.6
Stewing^	Beef	5	4 shallow-frying	155.2 ± 21.6	149.5 ± 20.0
			90 stewing	85.4 ± 6.7	84.3 ± 2.7
Roasting^	Beef	5	9 shallow-frying	174.3 ± 10.7	185.4 ± 8.8
			30 roasting	140	140
Microwave cooking	Codfish	–	5	600 watt	600 watt
Baking		Baking times (min)		Fixed oven temperature (°C)	
	Cookies	12		170	170
	Muffins	25		140	140
	Banana bread	60		155	155
	Sponge cake	60		155	155

SD: standard deviation.
*: Mean temperature refers to the average temperature of three recorded temperatures taken during the main cooking (i.e. just before adding the ingredient into the pan, halfway through and at the end of the cooking) in five repeated cooking procedures.
^: Stewing and roasting had a pre-shallow-frying step.

Annex 4: POP contents in mg per 100 g prepared food and residual fat (RF)

Cooking methods	Foods	Control margarine		PS-margarine	
		in food	in RF	in food	in RF
Stir-frying	Green beans	0.41 ± 0.06	8.52 ± 1.58	1.68 ± 0.89	11.74 ± 1.34
	Cabbage	0.55 ± 0.09	–	5.58 ± 1.29	–
	Chicken	0.45 ± 0.13	10.80 ± 3.60	9.66 ± 2.71	272.35 ± 46.17
Shallow-frying	Egg	0.33 ± 0.10	4.14 ± 2.39	0.61 ± 0.09	46.23 ± 12.93
	Onions	0.50 ± 0.06	4.71 ± 0.45	1.54 ± ± 0.28	35.85 ± 9.41
	Codfish	0.16 ± 0.04	7.03 ± 1.15	0.87 ± 0.18	140.36 ± 38.87
	Fish fingers	0.77 ± 0.32	7.20 ± 1.18	3.21 ± 0.79	20.76 ± 8.67
	Pork fillet	0.04 ± 0.02	10.40 ± 3.20	0.50 ± 0.10	197.34 ± 41.42
	Steak (beef)	0.87 ± 0.35	10.40 ± 2.60	6.75 ± 0.51	116.40 ± 12.10
	Salmon	0.47 ± 0.12	6.18 ± ± 1.05	0.95 ± 0.42	117.78 ± 16.39
	Potatoes	0.48 ± 0.07	26.70 ± 1.10	9.83 ± 0.62	415.58 ± 49.18
	Minced meat	0.59 ± 0.37	8.83 ± 2.79	1.52 ± 0.41	107.50 ± ± 25.41
Stewing	Beef	0.59 ± 0.20	4.08 ± 2.04	1.03 ± 0.17	10.77 ± 1.38
Roasting	Beef	0.30 ± 0.09	4.10 ± 0.50	0.71 ± 0.24	19.58 ± 4.63
Microwave cooking	Codfish	0.04 ± 0.01	0.52 ± 0.20	0.07 ± 0.01	0.96 ± 0.45
	Median	0.47	7.12	1.52	76.86
	Range	0.04–0.87	0.52–26.70	0.07–9.83	0.96–415.58
Baking					
	Cookies	0.66 ± 0.11	–	1.13 ± 0.31	–
	Muffins/cupcakes	0.24 ± 0.02	–	0.43 ± 0.16	–
	Banana bread	0.15 ± 0.03	–	0.34 ± 0.16	–
	Sponge cake	0.42 ± 0.03	–	1.20 ± 0.12	–
	Median	0.33		0.78	–
	Range	0.15–0.66		0.34–1.20	

RF: residual fat.

Annex 5: Findings in the subchronic oral toxicity study (Appel, 2001; Lea et al., 2004)

Parameter	Sex	Group (mean ± standard error)				
		SDC Control	PSE control	0.2% POC	0.6% POC	1.6% POC
Body weight (g) (end of study)	m	420.4 ± 5.9	410.9 + 6.8	423.6 ± 8.8	425.2 ± 8.4	419.2 ± 6.4
	f	226.9 ± 4.0	226.9 ± 4.1	229.7 ± 5.5	233.6 ± 3.6	220.1 ± 3.9
Absolute liver weight (g)	m	15.38 ± 0.31	14.72 ± 0.41	14.85 ± 0.42	15.38 ± 0.41	15.83 ± 0.30
	f	7.34 ± 0.17	7.41 ± 0.17	7.52 ± 0.17	7.86 ± 0.19	8.29 ± $0.18^{b,d}$
Relative liver weight (g/kg bw)	m	36.6 ± 0.5	35.8 ± 08	35.0 ± 0.6	36.1 ± 05	37.8 ± 0.5
	f	32.3 ± 0.5	32.7 ± 0.5	32.8 ± 0.6	33.6 ± 0.5	37.6 ± $0.3^{b,d}$
γ-GT (U/L)	m	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.1 ± 0.0
	f	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	1.2 ± $0.2^{b,d}$
ALP	m	119 ± 5^{b}	150 ± 6^{d}	150 ± 7^{d}	148 ± 6^{d}	155 ± 4^{d}
	f	122 ± 6^{b}	158 ± 6^{d}	154 ± 8^{c}	155 ± 9^{c}	164 ± 10^{d}
Albumin	m	42 ± 0^{a}	43 ± 0^{c}	43 ± 0^{b}	43 ± 0	44 ± $0^{b,c}$
	f	46 ± 0	48 ± 0	48 ± 0	48 ± 0^{a}	48 ± 0^{a}
Thrombocyte count (× 10^{9}/L)	m	794 ± 13	767 ± 20	831 ± 16^{a}	827 ± 15^{a}	876 ± $15^{b,d}$
	f	844 ± 18	812 ± 19	825 ± 16	828 ± 17	894 ± 23^{b}
PTT (s)	m	39.7 ± 0.5^{b}	37.1 ± 0.4^{d}	37.4 ± 0.5^{d}	37.2 ± 0.4^{d}	37.3 ± 0.4^{d}
	f	35.4 ± 0.4	34.0 ± 0.3	34.4 ± 0.4	34.1 ± 0.4	34.0 ± 0.6
Total cholesterol (mmol/L)	m	2.15 ± 0.08^{b}	2.60± 0.06^{d}	2.60 ± 0.10^{d}	2.52 ± 0.05^{d}	2.28 ± 0.08^{a}
	f	1.76 ± 0.05^{b}	2.18 ± 0.07^{d}	2.06 ± 0.05^{d}	2.04 ± 0.06^{d}	2.05 ± 0.05^{d}

Parameter	Sex	Group (mean ± standard error)				
		SDC Control	PSE control	0.2% POC	0.6% POC	1.6% POC
HDL	m	1.41 ± 0.06[b]	1.72 ± 0.05[d]	1.75 ± 0.07[d]	1.73 ± 0.05[d]	1.69 ± 0.06[d]
(mmol/L)	f	1.36 ± 0.04[b]	1.63 ± 0.06[d]	1.55 ± 0.05[c]	1.60 ± 0.03[d]	1.74 ± 0.05[d]
Non-HDL	m	0.74 ± 0.04	0.87 ± 0.03	0.85 ± 0.06	0.79 ± 0.04	0.59 ± 0.03[b,c]
(mmol/L)	f	0.40 ± 0.03[a]	0.55 ± 0.04	0.52 ± 0.04[c]	0.44 ± 0.05[a]	0.31 ± 0.02[b]
Triglycerides	m	2.36 ± 0.19	2.10 ± 0.20	2.04 ± 020	1.85 ± 0.15	1.25 ± 0.07[b,d]
(mmol/L)	f	1.32 ± 0.16	1.40 ± 0.17	1.37 ± 0.16	1.14 ± 0.12	0.71 ± 0.04[b,c]

POC: plant sterol oxidation concentrate.
(a): $p < 0.05$ (vs. PSE = plant sterol esters control).
(b): $p < 0.01$ (vs. PSE control).
(c): $p < 0.05$ (vs. SDC = standard diet control).
(d): $p < 0.01$ (vs. SDC diet).