FOOD STANDARDS
Australia New Zealand
Te Mana Kounga Kai – Ahitereiria me Aotearoa

1 July 2022
207-22

Supporting document 1

Risk assessment – Application A1249
Addition of phytosterols, phytostanols or their esters as novel food to plant-based milk alternatives

---

Executive summary

This application seeks permission for the addition of phytosterols, phytostanols or their esters (plant sterols) as a novel food to plant-based milk alternatives, up to a maximum of 2.2 g per 250 mL. The purpose of the requested amendment is to improve public health by providing an alternative source of plant sterols in the food supply for consumers seeking to lower their blood cholesterol.

This risk and technical assessment considered the feasibility, potential for adverse effects, effects on blood cholesterol and other health outcomes, change in dietary exposure, and consumer behaviour, due to the addition of plant sterols to plant-based milk alternatives.

FSANZ has reviewed information and evidence about the incorporation of plant sterols into plant-based milk alternatives and their stability in these products, particularly in relation to heating. That information and evidence indicates that plant sterols can be incorporated into, and are stable in, plant-based milk alternatives.

Previous assessments by FSANZ concluded there are no toxicological concerns regarding consumption of plant sterol-fortified foods by the general population, and that there is no justification for establishing an acceptable daily intake (ADI) for plant sterols. A review of newly available information does not indicate a need to amend this conclusion.

As noted in previous FSANZ assessments, safety data for pregnant women, lactating women, and children under five years of age is relatively limited compared to the extensive data available for the target population. However, based on knowledge of the mechanisms of phytosterol action, the now extensive history of consumption of phytosterol-enriched foods in the general population, and the absence of effects in pregnant animals and their offspring in laboratory studies, there is unlikely to be an appreciable risk to these population sub-groups. No new data was identified that would change this conclusion. However, risk management measures may be required for individuals with the extremely rare inherited condition, phytosterolaemia[1], to enable them to identify foods containing plant sterols.

[1] Phytosterolaemia, also referred to as sitosterolaemia, is an extremely rare inherited metabolic disease. People with this condition absorb high levels of plant sterols which can lead to premature atherosclerosis and heart disease. People with phytosterolaemia should avoid foods with added plant sterols. Cases of phytosterolaemia are managed strictly under medical supervision.

A review of recent literature did not identify convincing evidence that the addition of plant sterols to soy-based drinks is associated with nutrition-related adverse effects in the general adult population. No studies were identified investigating the nutrition-related effects on children and other sub-groups such as pregnant or lactating women, and, therefore, the effects on these populations are unknown.

Efficacy studies demonstrate that consumption of soy-based drinks with added plant sterols is associated with lowered total and low density lipoprotein (LDL) blood cholesterol concentrations in adults with untreated hypercholesterolaemia[2] or hyperlipidaemia[3], but not in normocholesterolaemic[4] adults. The intake of plant sterols in these studies ranged from 1.6 to 2.7 g/day. The effect of plant sterols added to plant-based milk alternatives other than soy drinks was unable to be assessed due to a lack of evidence.

Dietary exposures to plant sterols for Australia and New Zealand populations were estimated, assuming the addition of plant sterols to plant-based milk alternatives at the maximum concentration of 2.2 g per 250 mL serving. Existing permissions were also considered. The resulting change in total dietary exposures to added plant sterols from baseline was an increase of 0.3 g/day or less at the mean and 90th percentile, expressed as plant sterol equivalents. For plant-based milk alternatives only, based on a concentration of 2.2 g per 250 mL serve and typical consumption patterns, mean dietary exposures for consumers would be around 2 grams on any given day and around double that for high consumers.

FSANZ has identified no substantial risks associated with potential changes to consumer behaviour as a result of the addition of plant sterols to plant-based milk alternatives on the basis of research that examined consumer understanding and use of a range of phytosterol-enriched products, including milk. Although it is possible that consumers may use multiple plant sterol-enriched products, which could lead to consumption of plant sterols beyond an adequate level of intake for cholesterol reducing effects, this does not raise public health and safety concerns. The availability of additional and diverse plant sterol-enriched products would benefit consumers by increasing the range of choice available, as well as increasing the likelihood of consumers reaching an adequate intake of plant sterols recommended for cholesterol reduction.

Overall, the available data provide a high level of confidence in the safety of plant sterol-enriched plant-based milk alternatives up to the proposed maximum concentration for the general population. The current literature provides evidence that consuming soy-based drinks with plant sterols added at levels similar to the proposed maximum concentration, is likely to lower the total and LDL-cholesterol of adults with untreated hypercholesterolaemia or hyperlipidaemia.

[2] High levels of cholesterol, such as low-density lipoprotein (LDL) and total cholesterol, in the blood.
[3] High levels of lipids (fats such as cholesterol and/or triglycerides) in the blood.
[4] Normal levels of cholesterol in the blood.

Table of contents

1 Introduction

The applicant, Sanitarium Health Food Company (Sanitarium), is seeking permission to vary the Australia New Zealand Food Standards Code (the Code) to include phytosterols, phytostanols or their esters (plant sterols) as novel food to be added to beverages derived from legumes, cereals, nuts, seeds, or a combination of those ingredients. These beverages are referred to as 'plant-based milk alternatives' in the application and in this report.

Plant sterols are already permitted in a range of foods by the Code and therefore this application is an extension of use to these existing permissions. The purpose of the requested amendment is to provide an alternative source of plant sterols in the food supply for consumers seeking to lower their blood cholesterol.

For the purpose of this report, phytosterols, phytostanols and their esters are collectively referred to as plant sterols. The term total plant sterol equivalents includes phytosterols and phytostanols (i.e. free form) as well as the hydrolysis products of their esters.

The requested concentration permitted to be added was up to 2.2 g of 'total plant sterol equivalents' per 250 mL of plant-based milk alternatives. A lower limit of no less than 0.8 g of total plant sterol equivalents per 250 mL serve was suggested by the applicant. Sanitarium proposed that only plant-based milk alternatives with at least 100 mg of calcium per 100 mL and that have no more than 0.75 g of saturated fat per 100 mL are permitted to contain added plant sterols.

The primary risk assessment question to be addressed is whether the addition of plant sterols pose a risk to public health and safety, and/or promote public health by lowering blood cholesterol.

1.1 Objectives of the assessment

The main objectives of this technical, risk and nutrition assessment were to:

- Assess if it is technologically feasible to add plant sterols to plant-based milk alternatives and whether they are stable once incorporated.
- Identify and evaluate potential public health and safety issues arising from the consumption of plant-based milk alternatives containing added plant sterols.
- Assess whether consumption of plant-based milk alternatives with added plant sterols lowers blood cholesterol concentration.
- Estimate the total plant sterol exposure from foods which may contain added plant sterols at baseline, and any potential increase in plant sterol exposure from the addition of plant sterols to plant-based milk alternatives.
- Determine whether any significant risks would arise from changes to consumer consumption behaviour as a result of permitting plant sterols to be added to plant-based milk alternatives.

2 Food technology assessment

Plant sterols have been comprehensively assessed by FSANZ in earlier applications (for example Applications A1134, A1019 and A1024) and are currently permitted to be added to certain foods as a novel food, by the Code. Their chemical and physical properties are provided in detail in section 2 of the supporting document for Application A1024 – Equivalence of plant sterols, stanols and their fatty acid esters (FSANZ 2010) and summarised in Supporting Document 1 for Application A1034 – Increased concentration of plant sterols in breakfast cereals (FSANZ 2017). A definition and the chemical names and structural formulae of major free phytosterols and phytostanols are also provided in the Joint FAO/WHO Expert Committee on Food Additives (JECFA) specification for Phytosterols, phytostanols and their esters (FAO and WHO 2008). That information is therefore not repeated here, however a summary of the key factors relevant to this application is provided below.

2.1 Characterisation of plant sterols

Phytosterols, phytostanols and their esters (collectively referred to as plant sterols hereafter, where relevant) are a group of steroid alcohols and esters that occur naturally in plants. Commercially, phytosterols and phytostanols are isolated from vegetable oils such as soybean oil, rapeseed oil, sunflower oil or corn oil or from tall oil (a by-product of the manufacture of wood pulp) (FAO and WHO, 2008). Synonyms are plant sterols/stanols, plant sterol/stanol esters, and phytosterol/phytostanol esters.

There are many different types of plant sterols. The most common free phytosterols and phytostanols and their corresponding CAS[5] numbers are:

Sitosterol: 83-46-5
Sitostanol: 83-45-4
Campesterol: 474-62-4
Campestanol: 474-60-2
Stigmasterol: 83-48-7
Brassicasterol: 474-67-9.

Phytosterols and phytostanols can be esterified by reacting with vegetable oil long chain fatty acids to form plant sterol esters, for example, sitostanyl oleate and campesteryl oleate.

Commercial products may be mixtures of phytosterols, phytostanols and their esters. They do not serve a technical function in food (FAO and WHO 2008).

2.2 Specifications

Specifications for plant sterols were comprehensibly considered in Application A1024 by FSANZ (FSANZ 2010). The Code was subsequently amended to include a specification for phytosterols, phytostanols and their esters in S3—24 of Schedule 3, to ensure only appropriate plant sterol preparations are approved for addition to food. Plant sterols added to food must comply with that specification. The specification requires compliance with either a primary source (section S3—2) or secondary source (section S3—3) of specifications, along with additional requirements relating to the concentration of hexane, isopropanol, ethanol, methanol and methyl ethyl ketone and the percentage of des-methyl sterols in the total plant sterol equivalents.

[5] Chemical Abstracts Service

There are relevant international specifications in the primary sources listed in section S3—2 of Schedule 3 of the Code. The JECFA has a specification titled *Phytosterols, Phytostanols and their Esters* in its Combined Compendium of Food Additive Specifications (FAO and WHO 2008). Food Chemicals Codex (FCC) has a specification titled Vegetable Oil Phytosterol Esters (FCC 2020).

Schedule 3 of the Code also includes specifications for arsenic, lead, cadmium and mercury (section S3—4) if they are not already detailed within specifications in sections S3—2 or S3—3. The JECFA specification has specifications for arsenic and lead and the FCC specification has specifications for lead.

There is also a specification in Schedule 3 specifically for tall oil phytosterol esters (S3—27). The applicant is not seeking permission to add tall oil phytosterol esters and therefore this specification is not relevant to this assessment.

2.3 Analytical method for detection

The analysis of the presence of, and the amounts of, added plant sterols in different food matrices has been well established and published in the scientific analytical literature (Laakso 2005, Guadalupe et al. 2021).

The applicant notes the following method from the AOCS, the Official Method Ce 12-16 – Sterols and Stanols in Foods and Dietary Supplements Containing Added Phtosterols (revised 2017). This method describes a procedure for the determination of plant sterols and stanols, collectively referred to as phytosterols, in foods and dietary supplements containing added phytosterols and in free sterol/stanol and steryl/stanol ester concentrates. It is appropriate for determining the content of the five major phytosterols (i.e., campesterol, campestanol, stigmasterol, beta-sitosterol, and sitostanol) that are the subject of the United States Food and Drug Administration's (FDA) health claim on the relationship between phytosterols and the reduced risk of coronary heart disease.

There is an ISO standard, *ISO 23349:2020(en) Animal and vegetable fats and oils — Determination of sterols and stanols in foods and dietary supplements containing added phytosterols* which provides a reference method for the determination of free sterols/stanols and steryl/stanol esters in foods containing added plant sterols and plant stanols and in plant sterols and plant stanols food additive concentrates.

2.4 Incorporating plant sterols into plant-based milk alternatives

FSANZ understands that the applicant intends to add plant sterols to beverages derived from legumes, cereals, nuts, seeds, or a combination of those ingredients. These products would be subject to similar processing and procedures as both conventional extended shelf-life plant-based milk alternatives and corresponding dairy equivalents, including ultra-high temperature (UHT) treatment. Examples of Sanitarium's extended shelf-life plant-based milk alternatives currently on the market are So Good Oat No Added Sugar, So Good Almond Unsweetened, and So Good Soy Regular.

Phytosterols and phytostanols are insoluble in water and poorly soluble in vegetable oils, thus hindering their incorporation into various foods. Plant sterols can also easily crystallise when added directly into food, causing an undesirable texture and affecting the quality of the food. Plant sterol esters however, have a higher solubility in fats and oils (Vaikousi et al. 2007, He et al. 2018). Esterification of the plant sterols and stanols with unsaturated fatty acids increases their lipid solubility, facilitating their incorporation into various foods (Vaikousi et al. 2007, Cantrill 2008). Formulation methods other than esterification can also be used to improve the incorporation of free phytosterols and stanols into foods (Soupas 2006).

Commercial products may be a mixture of phytosterols extracted from vegetable oils, a mixture of free phytosterols and phytostanols, phytosterol and phytostanol esters or phytostanol esters (Cantrill 2008). Additional substances used as food additives or processing aids to aid solubility and distribution in either the phytosterols/vegetable oil product or the food to which it is added may be necessary.

The applicant stated they would use a commercially available phytosterols product that has been demonstrated to be soluble and uniformly distributed in beverages similar to the intended finished product. Information about this product including its composition was provided to FSANZ as confidential commercial information (CCI).

There are various plant-based milk alternatives sold in other countries that contain plant sterols. There is therefore no reason to believe the applicant or other manufacturers of plant-based milk alternatives do not already have, or could not readily obtain, the expertise, and if needed, the equipment, to produce plant-based milk alternatives containing plant sterols as requested in the application. Food manufacturers are able to use the most appropriate plant sterol preparation suitable for their purpose, provided the product conforms to the specification (see above) and is able to be uniformly incorporated into the food to enable compliance with compositional limits.

2.5 Stability of plant sterols

Plant sterols are generally reported in the scientific literature to be stable compounds. Their resistance to oxidation and thus to formation of oxidation products is the main consideration from a safety aspect. Factors affecting plant sterol oxidation are their structure, temperature and heating time, and the composition of the lipid matrix in which they exist. At high temperatures (>100°C) in the presence of oxygen, oxidation of the phytosterol moiety may occur, in the same way as for cholesterol. Phytosterol esters are reported to be more susceptible to oxidation at elevated temperatures than free phytosterols. Phytostanols are generally heat stable and, being saturated compounds, less prone to oxidation than phytosterols. Phytostanol esters also show an oxidative stability (Cantrill 2008, Soupas 2006).

FSANZ has not identified any evidence relating specifically to the stability of plant sterols in plant-based milk alternatives. Various studies have however, been carried out on different food matrices including low fat/skim milk. Based on observations in food model studies (including phytosterol/stanol enriched heat treated dairy milk) commercially available phytosterol/stanol ingredients were stable during food processes such as UHT type heating, typically at a temperature range of 135 – 150°C (Burton 2012). Pan frying an oil at higher temperatures (160-200°C) did induce phytosterol oxidation however the degree of oxidation may have been influenced by other factors such as large surface to volume ratio and interaction with the pan itself (Soupas 2006, Boskou & Elmadfa 2016).

A study of the stability of plant sterols at different temperatures in phytosterol-enriched milk (1.8 g fat per 100 g) found the milk to be an adequate phytosterol source even when it is heated for consumption (1.5 minutes in a microwave), although there was some loss of phytosterol content following this treatment. There was additional losses when heat treatments were more drastic (Menéndez-Carrenõ et al. 2008). However, FSANZ does not consider this a significant issue for plant-based milk alternatives as it is unlikely the plant-based milk alternatives would be cooked in this manner or to such temperatures by consumers.

Plant sterols have also been found to be stable in liquid non-fat milk products during storage for up to 6 months including at 4, 24 and 37°C (Soupas 2006, Gonzalez-Larena et al. 2012). This may be influenced by antioxidants present in the product (for example vitamin E)

(Gonzalez-Larena et al. 2012).

The applicant stated that standard food manufacturing processes ensure the ingredients are present and intact throughout their shelf life. They also provided examples of both shelf-stable and chilled storage plant-based milk alternatives currently in the market overseas (as confidential information).

2.6 Food technology conclusion

Plant sterols are a group of steroid alcohols and esters that occur naturally in plants. Plant sterols have been comprehensively assessed by FSANZ in earlier applications (for example Applications A1134, A1019 and A1024) and are currently permitted to be added to certain foods as a novel food, by the Code.

Plant sterols added to food must comply with the specification for phytosterols, phytostanols and their esters in S3—24 of Schedule 3. The specification requires compliance with either a primary source (section S3—2) or secondary source (section S3—3) of specifications, along with additional requirements.

Esterification of phytosterols and phytostanols facilitates their incorporation into various foods and there are a number of commercial products available for fortifying foods that comprise different mixtures of esterified and free phytosterols and phytostanols. The applicant stated they would use a commercially available phytosterols product that has been demonstrated to be soluble and uniformly distributed in beverages similar to the intended finished product.

Plant sterols are generally reported to be stable compounds. FSANZ has not identified any evidence relating specifically to the stability of plant sterols in plant-based milk alternatives. Although heating at higher temperatures has been found to induce oxidation, this is associated with pan frying of oils. FSANZ considers that plant based milk alternatives would not be subject to the same conditions as occurring during pan frying of oils and this is therefore this is unlikely to affect the stability of plant sterols in plant-based milk alternatives. Plant-based milk alternatives containing plant sterols are likely to be subject to UHT (ultra-heat treatment) during their production. However, temperatures during UHT processing are lower than pan frying, at a range of 135 – 150°C typically. The conditions are also different during UHT processing compared to pan frying. These factors reduce the likelihood of plant sterol oxidation. The applicant stated they will apply food manufacturing processes to ensure plant sterols are present throughout their shelf life.

Based on studies of the stability of plant sterols in liquid non-fat milk products during storage and the availability of plant-based milk alternatives currently in the market overseas, and FSANZ concludes that plant sterols are likely to be stable in plant-based milk alternatives over their shelf life.

3 Toxicological assessment

3.1 Previous FSANZ safety assessments of plant sterols

Previous safety assessments by FSANZ, other regulatory agencies, and international scientific committees have concluded that plant sterols are poorly absorbed from the gastrointestinal tract. Minor differences in the extent of absorption occur among individual sterol compounds, but less than 5% of dietary plant sterols enter the circulation. By contrast, approximately 80% of structurally related cholesterol in the diet enters the circulation. The major proportion of ingested plant sterols is excreted unchanged in the faeces. Plant sterols that are absorbed are transported to the liver, metabolised and excreted through the bile.

Based on the results of numerous short-term and sub-chronic toxicity studies showing no adverse effects associated with plant sterols administered to animals at high doses, FSANZ has previously concluded that consumption of plant sterol fortified foods raises no safety concerns and a reference health standard is not warranted. This conclusion was also reached by regulatory agencies in Europe and the USA.

In 2008, JECFA established an Acceptable Daily Intake (ADI) of 40 mg/kg bw for phytosterols, phytostanols and their esters, based on heart muscle degeneration in a 90-day oral toxicity study in rats. FSANZ re-evaluated this study as part of application A1019, and concluded there was no justification for establishing an ADI for plant sterols. The incidence of heart muscle changes was within the range of historical control data relevant for the strain of rats used in the study, and similar changes were not seen in other 90-day studies of phytosterols in the diet, so it was not considered to be treatment-related (FSANZ 2010).

FSANZ has previously stated that safety data for pregnant women, lactating women, and children under five years of age are relatively limited compared to the extensive data available for the target population. However, based on knowledge of the mechanisms of phytosterol action, the now extensive experience of use of phytosterol-enriched foods in the general population and the absence of effects in pregnant animals and their offspring, there was no basis for postulating a risk to these population subgroups (FSANZ 2012).

An updated literature review conducted as part of application A1134 found no evidence to alter FSANZ's conclusion that a specified ADI is not justified for plant sterols. It was concluded that there were no toxicological concerns regarding the addition of plant sterols to breakfast cereals up to the concentrations proposed for consumption by the general population. Occasional consumption of plant sterol-enriched breakfast cereal by young children or pregnant or lactating women was not considered to be of toxicological concern. However, it was concluded that appropriate risk management measures are required for individuals with phytosterolaemia (sitosterolaemia)[6] (FSANZ 2017).

3.2 Newly available data

The applicant conducted several literature searches for studies investigating the safety of plant sterols in humans or experimental animals published since 2016. In addition, the applicant submitted several clinical studies of plant sterols in soy drinks published prior to 2016, that had not previously been reviewed by FSANZ. FSANZ also conducted updated literature searches in February 2022.

[6] Phytosterolaemia is an extremely rare inherited metabolic disease. People with this condition absorb high levels of plant sterols which can lead to premature atherosclerosis and heart disease. People with phytosterolaemia should avoid foods with added plant sterols. Cases of phytosterolaemia are managed strictly under medical supervision.

3.2.1 Studies in laboratory animals

Plasma and tissue concentrations of phytosterols in mouse models of hypercholesterolaemia (Nunes et al. 2019) Regulatory status: Non-GLP; Non-guideline

Groups of male apolipoprotein E knockout (apoE-KO), low density lipoprotein receptor knockout (LDLR-HO) and wild type C57BL/6J mice (group size not reported) were administered 1624 µg/day phytosterols (1464 µg/day sitosterol; 160 µg/day campesterol) via the diet for 12 weeks following weaning. The authors reported that this dose would represent 4574 mg/day for an adult human of 70 kg (65 mg/kg bw/day). At the end of the study plasma cholesterol and concentrations of phytosterols in the plasma, whole body, liver and intestine were examined.

Body weights were similar in all three groups at the end of the study. Plasma cholesterol concentrations were significantly higher in LDLR-KO mice than wild-type, and concentrations in apoE-KO mice were significantly higher than both groups. In comparison to wild-type mice, lower campesterol concentrations were observed in the intestine in both KO mice, which was considered to indicate reduced campesterol uptake from the intestinal lumen. However, in apoE-KO mice campesterol concentrations were significantly higher in plasma and whole body, suggesting marked retention in the body. For sitosterol, intestinal concentrations were significantly lower than controls in apoE-KO mice, but plasma and whole body concentrations were significantly higher, suggesting reduced elimination of sitosterol in apoE-KO mice. Plasma sitosterol concentrations were significantly lower than controls in LDLR-KO mice. Liver concentrations of each phytosterol were similar in all three groups, but liver sitosterol concentrations were lower than those of campesterol despite sitosterol being present at higher concentrations in the diet and intestinal tissue. The authors considered that intestinal absorption of sitosterol may be higher than that of campesterol, but elimination of sitosterol is also greater.

Anti-inflammatory potential of phytosterols from Nicotiania tabacum (Akinloye et al. 2020) Regulatory status: Non-GLP; Non-guideline

Administration of phytosterols extracted from *Nicotiana tabacum* to male Wistar rats (5/group; age not reported) at 50 or 100 mg/kg bw/day for 8 days by oral gavage inhibited increased serum alanine aminotransferase and lipid peroxidation induced by administration of 0.15 M HCl/60% ethanol on day 8. Liver catalase activity was increased by phytosterol administration compared with basal controls and in animals administered HCl/ethanol alone. Cyclooxgenase-2 (COX-2) mRNA expression in the liver was inhibited by phytosterols, but COX-1 expression was unaffected. Histopathological analysis of gastric mucosal tissues showed no adverse changes in animals administered phytosterols alone (100 mg/kg bw/day), and phytosterols protected against degenerative changes induced by HCl/ethanol. In silico screening indicated that phytosterols from *N. tabacum* may be substrates of cytochrome P450 3A4 and did not show alerts for toxicity or carcinogenicity. The study authors concluded that phytosterols are selective inhibitors of COX-2, hepatoprotective, regenerate parietal cells and non-toxic.

3.2.2 Human studies

Clinical studies of the impact of plant sterols on cholesterol

A range of studies assessing the impact of plant sterols on cholesterol are available. Safety aspects from these studies are discussed below; effects on cholesterol concentrations are discussed in the nutrition assessment (section 4).

Clinical study of phytosterols in children and adolescents with dyslipidaemia (Tavares et al.

2021)

No adverse events were self-reported in a randomised, double-blind, crossover trial with children and adolescents (n = 31; aged 7 – 16 years; mean ± SD 9.0 ± 2.22 years) with dyslipidaemia who consumed capsules containing 1.5 g/day phytosterols for 8 weeks. No effects on blood pressure were observed during the study.

Clinical study of a phytosterol-enriched soya drink in normocholesterolaemic adults (Chau et al. 2020)

In a randomised, double-blind, single centre, placebo-controlled trial, groups of healthy normocholesterolaemic Chinese males and females aged > 18 years were assigned to consume a 250 mL soya drink containing phytosterols at a daily dose of either 0 g or 2 g for a period of three weeks. The intention-to-treat (ITT) analysis included 159 participants (82 treatment [46 male/36 female]; 77 placebo [39 male/38 female]). In the treatment group, 5/82 participants (6.1%) reported adverse events, while no adverse events were reported in the control group. The adverse events were nearly all gastrointestinal discomfort (3 diarrhoea; 1 slight hardening of stool; 1 flu). None were classed as serious adverse events and none led to withdrawal from the study. No significant effects on blood pressure were observed.

Clinical study of free-phytosterols nanoparticles in individuals with metabolic syndrome (Palmeiro-Silva et al. 2020)

In a parallel, randomised, double-blind, placebo-controlled trial, individuals from Chile aged 18 – 65 years diagnosed with metabolic syndrome were assigned to consume placebo (n = 100) or an aqueous dispersion containing 2 g free-phytosterols nanoparticles (n = 102; particle size not reported) derived from pine, surfactants and water daily for 6 months. Participants in the control group received an aqueous dispersion containing titanium dioxide, xanthan gum, carrageenan, surfactants, potassium sorbate, citric acid and water. Participants were assessed at baseline, 4, 12 and 24 weeks. General health questions, anthropometric measurements and blood parameters were analysed. Safety outcomes included adverse events (including diarrhoea), serious adverse events and blood concentrations of vitamin D.

At the end of the study median levels of vitamin D were lower than at baseline in both groups, but levels were similar among the placebo and intervention groups at baseline and at the end of the study. The study authors attributed the reduction to a seasonality effect, as recruitment started in summer with the third and fourth assessment visits taking place in autumn and winter. No effects on blood pressure were observed. No diarrhoea episodes were reported by either group during this study. Most of the participants in the phytosterol group (68%) self-reported an improvement in their bowel habits at visit 4 compared with baseline, compared with just 4% of the placebo group. No adverse events were reported. FSANZ notes that the relationship of the nanoparticles used in this study to phytosterols typically added to food is unclear.

Clinical study of phytosterol esters in healthy adults (Reaver et al. 2019)

In a randomised, placebo-controlled, double-blind crossover study, 32 healthy adults (9 females; 23 males; age 30 – 65) were assigned to receive a placebo or an experimental phytosterol ester emulsion (1.5 g/day phytosterol equivalents) for one month followed by a one month washout period then placebo or treatment for one month. The intervention was given via capsules.

The investigational product was well tolerated. Four adverse events were reported by participants. Three resolved on their own (funky after taste/dry mouth, photophobia,

constipation). A fourth participant reported statin-like symptoms such as cognitive and erectile dysfunction while on placebo, and withdrew from the study before receiving the phytosterols. No significant changes in haematology or clinical chemistry parameters, or in blood levels of vitamin D, folate, vitamin B12, calcium and magnesium, were observed.

Clinical study of plant sterols in individuals with or at risk of type-2 diabetes mellitus (Trautwein et al. 2018)

In a double-blind, randomised, placebo-controlled, parallel study in Australia, 161 individuals at increased risk of or with established type-2 diabetes mellitus consumed low-fat spreads without or with added phytosterols (2 g/d) for 6 weeks after a 2-week run-in period. Of the 161 participants randomised into the study, 151 completed all study visits, and 138 (59 women, 79 men; mean age 58 years) were considered as being compliant with the study protocol (70 placebo, 68 treatment).

A total of 143 adverse events were reported, of which 96% (n = 137) were classed as non-serious. One adverse event (loose stools) was scored as 'definitely related' to the study product, six were scored as being 'possibly related' and 14 were scored as 'unlikely to be related'. The remaining 116 non-serious AEs were scored as being 'not related' to the study product. Six serious adverse events were reported; two were scored as 'unlikely to be related' to the study product and four were scored as 'not related' to the study product. Postprandial lipid and glycaemic responses did not differ between phytosterols and placebo.

Clinical study of phytosterols alone or in combination with curcumin in hypercholesterolaemic individuals (Ferguson et al. 2018)

No adverse events were reported in a double-blind, randomised placebo controlled trial in which 70 Australian individuals with hypercholesterolaemia (mean age 50.7 ± 1.51 years; 57% female) were assigned to receive placebo, phytosterols (2 g/day), curcumin (200 mg/day) or a combination of phytosterols and curcumin for four weeks.

Clinical study of phytosterols from Diascorea alata in postmenopausal women (Hsu et al. 2017)

In a randomized, double-blind, placebo-controlled clinical investigation, 50 Taiwanese postmenopausal women were randomly assigned to receive placebo or two sachets of *Diascorea alata* (Taiwanese yam) extracts containing 12 mg *Diascorea* extract/sachet daily for 12 months. The mean age in the placebo and intervention groups was 53.08 and 51.92 years, respectively. The major phytosterol in the extract was β-sitosterol (concentration not reported). Safety was monitored by evaluation of haematology and clinical chemistry parameters at baseline, 6 months and 12 months.

No significant changes in blood pressure were observed. Baseline values of markers of liver and kidney function and haematology profiles were within normal limits and similar in both groups, with the exception of higher white blood cells counts in the treatment group compared with placebo. No adverse effects on haematology or clinical chemistry parameters were reported. White blood cell counts in the *Diascorea* group were significantly reduced compared with baseline at 6 and 12 months, and similar to the placebo group. The study authors were uncertain whether this change was treatment-related or linked to the relatively higher white blood cell count at baseline in this group. Differential white blood counts were not assessed. After 12 months treatment, elevations of hematocrit and mean corpuscular volume, as well as slight, non-significant increases in haemoglobin and red blood cell count, were noted in those receiving *Diascorea*. The study authors considered these changes indicative of beneficial effects on the haematological profile in these women.

Clinical study of phytosterol-enriched low-fat milk in Chinese adults (Cheung et al. 2017)

In a randomised, double-blind, placebo-controlled trial in Hong Kong, 221 Southern Chinese healthy adults (41 men, 189 women; age 24 – 79) were assigned to consume a low-fat milk enriched with phytosterols (1.5 g/day; n = 110) or the same milk without phytosterols (n = 111). The milk was consumed with breakfast and lunch daily for 3 weeks. The number of participants experiencing at least one adverse event was similar in the treatment and placebo groups (27.3% and 32.4%, respectively). The frequency and type of adverse event was similar in both groups. The most common adverse event was diarrhoea, followed by flatulence. No adverse effects on blood pressure were observed. Phytosterol consumption was associated with significantly decreased diastolic blood pressure compared with controls, although this was largely due to an increase in diastolic blood pressure in the placebo group at the end of the intervention period.

Clinical study of phytosterols in healthy individuals (Alphonse et al. 2017)

A randomised, double-blinded, crossover, placebo-controlled crossover clinical trial with three treatment phases of 4-weeks duration was conducted in normocholesterolaemic or slightly hypercholesterolaemic but otherwise healthy adults (n = 49; age 18 – 55 years) from a Manitoba Hutterite population. During each phase, participants consumed a milk shake containing one of the three treatments: 600 mg/day dietary cholesterol, 2 g/day plant sterols or placebo. Safety was assessed by analysis of plasma liver enzymes (alanine transaminase, aspartate transaminase, alkaline phosphatase, γ-glutamyltransferase, lactate dehydrogenase and total bilirubin).

No changes in any of the liver enzymes were observed following phytosterol consumption compared with placebo. The study authors state that adverse events due to treatment were monitored, but no information on whether any adverse events occurred is reported.

Clinical study of a soy milk drink supplemented with plant stanol esters in individuals with mild to moderate hypercholesterolaemica (Hallikainen et al. 2013)

In a randomised, double-blind, placebo-controlled trial with a parallel design, adults from Sweden aged 25 – 65 years with mild to moderate hypercholesterolaemia were asked to consume a soy-based mini-drink with or without plant stanols (2.8 g/day; 17.9% campestanol; 72.1% sitostanol) for four weeks. While 61 subjects were originally included in the intervention, two participants in the control group withdrew due to an adverse event and three in the intervention group withdrew due to an adverse event, unwillingness to continue or a major protocol violation (8 kg weight reduction). The nature of these adverse events was not reported. The control and intervention groups that completed the study comprised 29 (7 male/22 female) and 27 individuals (4 male/23 female), respectively. Blood samples were collected at baseline and at the end of the intervention. The study authors reported that the products did not cause any major side effects, with two participants in each group reporting gastrointestinal discomfort without withdrawing from the study. No adverse changes in haematology or clinical chemistry parameters were observed, with all values remaining within reference values.

Clinical study of plant stanol ester-fortified soy milk in mildly hypercholesterolaemic individuals (Kriengsinyos et al. 2011)

In a double-blind, placebo-controlled study, mildly hypercholesterolaemic adults aged 25 – 60 years were assigned to receive a strawberry flavoured soymilk containing 0 or 2 g plant stanol esters daily for 6 weeks. The study was completed by 60/60 volunteers (19 male/41 female) in the intervention group and 58/60 (19 male/39 female) in the control group. No side effects were reported in either group. Serum concentrations of lipid-soluble antioxidants were within normal reference limits in all participants, however after adjusting for total cholesterol, serum β-carotene and β-cryptoxanthin concentrations were significantly lower in the stanol group than controls. No adverse effects on serum testosterone (males), estradiol (females) or luteinising hormone (both sexes) were observed.

Clinical study of low- and moderate-fat plant sterol fortified soy milk in individuals with normal to high cholesterol concentrations (Rideout et al. 2009)

In two randomised crossover feeding trials, individuals from the USA (age 19 – 60 years) were assigned to consume 3 drinks of 1% dairy milk or plant sterol enriched soy milk (low fat in study 1; moderate fat in study 2) daily for 28 days. The total dose of plant sterols from the enriched soy milks was 1.95 g/day. In study 1, individuals were normal to hypercholesterolaemic (n = 33; gender not specified), in study 2 they were hypercholesterolaemic (n = 23; 10 male/13 female). No side effects associated with consumption of the fortified drink were reported.

Clinical study of plant sterol enriched soy drink in individuals with moderate hypercholesterolaemia (Weidner et al. 2008)

In a randomised, placebo-controlled double blind study in France, 50 individuals (19 male/31 women; age 19 – 65 years) with moderate hypercholesterolaemia were assigned to consume a soy drink with or without 2.6 g plant sterol esters (1.6 g free plant sterol equivalents) daily for 8 weeks. The drink was well tolerated and there was no difference in reported adverse events between the intervention and control groups.

Other studies in humans

Meta-analysis of effects of phytosterol supplementation on blood pressure (Ghaedi et al. 2020)

A systematic review and meta-analysis of 19 randomised controlled trials involving 1567 adult participants found that phytosterol supplementation led to reductions in systolic and diastolic blood pressure compared with controls. Weighted mean differences (95% confidence intervals) were – 1.55 mmHg (- 2.67, - 0.42; p = 0.007) and – 0.84 (- 1.60, - 0.08; p = 0.03), respectively. Subgroup analysis based on duration showed no significant effects of phytosterols on blood pressure with interventions ≥ 12 weeks, while a significant effect on systolic blood pressure and a borderline significant effect on diastolic blood pressure were observed in studies of < 12 weeks duration. Additional subgroup analysis found that reductions in systolic blood pressure were significant with doses ≥ 2 g/day but not < 2 g/day. For diastolic blood pressure, significant reductions were observed with doses < 2 g/day but not doses ≥ 2 g/day.

Clinical study of phytosterols alone or in combination with phytosterol oxidation products (Lin et al. 2019)

In a double-blind, randomised, placebo controlled pilot study healthy individuals recruited in in Germany were assigned to one of four groups (n =15 per group, aged 42 – 72 years). The control group received products with no added phytosterols or phytosterol oxidation products (POP) and treatment groups received daily portions of margarine with added phytosterols and two cookies containing POPs for 42 days. The POPs were produced by shallow-frying potatoes with a margarine containing 22.5% added phytosterols (in the form of 37.5% phytosterol esters) in a canteen setting. The cookies also contained phytosterols. The average phytosterol intake was 3 g/day in the intervention groups, while POP intakes were 8.7, 15.2, or 37.2 mg/day in the low, medium and high dose groups, respectively. Serum POP and cholesterol oxidation products (COP) were monitored at intervals, and adverse events were monitored throughout the study. The aim of this study was to generate data on plasma/serum POP concentrations after the consumption of foods that are prepared by cooking.

Daily intake of increasing POP doses increased serum POP concentrations nonlinearly and reached a steady state in < 7 days. Serum POP concentrations remained below serum COP concentrations in all groups. The authors reported that the study products were well tolerated by the study participants, and no serious adverse events occurred. In total, 16 adverse events were reported in 12 study participants; 10 events (in 8 individuals) occurred across the groups that received phytosterols, and six (in 4 individuals) occurred in the control group. All adverse events were considered not or unlikely to be related to the study product. The most common adverse events were common cold and headache.

Association of plasma phytosterols and cardiovascular events (Fuhrmann et al. 2018)

A group of 376 patients who underwent elective coronary angiography were monitored for cardiovascular events (cardiovascular death, myocardial infarction, stroke or revascularisation of a vessel and amputation of limbs above the ankle) for a mean observation period of 4.2 ± 1.8 years. Plasma levels of phytosterols and oxidised phytosterols, as well as cholesterol and lipoproteins, were compared between the 82 patients who experienced a cardiovascular event (cases) and the 294 who did not (controls).

No significant differences in campesterol and sitosterol concentrations were observed between cases and controls. Median levels of absolute and cholesterol-corrected 7α-OH-campesterol were significantly higher in cases than controls, but no differences were observed for the other oxidised phytosterols assessed. Hazard ratios per one standard deviation suggested an increased risk of a cardiovascular event for absolute and cholesterol-corrected 7α-OH-campesterol, although they did not reach statistical significance (HR (95% confidence interval) 1.19 (0.95-1.48) and 1.18 (0.94-1.48), respectively). Kaplan-Meier failure curves suggested a significantly increased risk of cardiovascular events for patients with cholesterol corrected 7α-OH-campesterol levels above the median, but not for absolute 7α-OH-campesterol or any other oxidised phytosterols. Multivariate logistic Cox regression found no significant associations between any of the absolute or cholesterol corrected oxidised phytosterols and occurrence of cardiovascular events.

Association of plasma phytosterols with cardiovascular disease status (Baumgartner et al. 2019)

In a re-analysis of samples from a previous study of the Framingham Offspring Study cohort which reported positive associations between plasma total cholesterol-standardised sitosterol and campesterol concentrations and increased cardiovascular disease risk (Matthan et al. 2009), potential associations between oxidised phytosterols and cardiovascular disease were

examined. Plasma phytosterols and oxidised phytosterols were analysed in 144 cases with cardiovascular disease and 383 matched controls from the Framingham Offspring Study cohort. Due to limited plasma availability, this re-analysis used samples from slightly fewer cases and controls than were analysed in the original study (155 cases and 414 controls).

As found in the previous study, positive associations were observed between plasma total cholesterol-standardised campesterol and higher sitosterol and cardiovascular disease. Hazard ratios per standard deviation of 1.52 (95% confidence interval: 1.18-1.96) and 2.43 (1.72-3.41) were observed for sitosterol and campesterol, respectively. No association was found between plasma oxidised phytosterols and cardiovascular disease risk.

Impact of genetic variability of dietary sterol absorption on the risk of coronary artery disease (Helgadottir et al. 2020a)

The contribution of genetic variability in the intestinal sterol transporters ABCG5/8 (some variants are associated with increased risk of cardiovascular disease and increased cholesterol absorption) on plasma cholesterol and phytosterol concentrations as well as risk of coronary artery disease, was assessed among individuals from Iceland, Denmark and the UK. Several variants were identified that were associated with increased levels of non-HDL cholesterol, phytosterols and increased coronary artery disease risk. Genetic risk scores for non-HDL cholesterol were calculated to determine whether ABCG5/8 variants confer a greater risk of coronary artery disease than that predicted by their effect on non-HDL cholesterol concentrations. It was considered that 38% of the risk could not be explained by increased non-HDL cholesterol. The study authors hypothesised that absorption of dietary phytosterols may also contribute to the risk of coronary artery disease, but noted that the data did not demonstrate a risk of adverse effects of phytosterol supplementation.

Association between dietary phytosterol consumption and colorectal cancer (Huang et al. 2017)

A case control study investigated the association between dietary phytosterol intake and colorectal cancer risk in the Chinese population. In total 1802 eligible colorectal cancer cases plus 1813 age (5-year interval) and gender frequency-matched controls were included. Controls were either recruited from hospital in-patients or from residents of the community. Dietary information was collected by a validated food frequency questionnaire, and the odds ratio (OR) and 95 % confidence intervals (CI) for colorectal cancer were calculated by multivariable logistic regression models.

A higher total intake of phytosterols was found to be associated with a 50 % reduction in colorectal cancer risk. After adjusting for various confounders, the OR of the highest quartile intake compared with the lowest quartile intake was 0·50 (95 % CI 0·41, 0·61, P_{trend} <0·01) for total phytosterols. An inverse association was also found between the consumption of β-sitosterol, campesterol, campestanol and colorectal cancer risk. However, stigmasterol intake was related to an increased risk of colorectal cancer. No statistically significant association was found between β-sitostanol and colorectal cancer. Stratified analysis by gender showed that the positive association of stigmasterol intake with colorectal cancer was found only among women. Subgroup analysis by cancer site showed that stigmasterol and β-sitostanol intake was positively associated with colon cancer but not rectal cancer. When the analysis was restricted to either the hospital or community derived controls, the positive association of stigmasterol intake with colorectal cancer was only observed in comparison with community-derived controls, while the positive association of β-sitostanol intake with colorectal cancer was found only in comparison with hospital-derived controls. The study authors suggested that these positive associations may be a chance finding. It was concluded that the consumption of total phytosterols, β-sitosterol, campesterol and campestanol is inversely associated with colorectal cancer risk in a Chinese population.

3.3 Assessments by other agencies

One report published by an overseas agency since FSANZ's last evaluation of plant sterols was identified. In 2020, the European Food Safety Authority (EFSA) Panel on Nutrition, Novel Foods and Food Allergens (NDA) published 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 (EFSA 2020). The Panel concluded that the safety of the intended extension of use of plant sterol esters under the proposed conditions of use has not been established.

The conclusions of this report are not considered relevant to the current application given the differences in proposed use conditions. FSANZ is not aware of any proposed changes to current European regulations which permit the addition of plant sterols to plant-based milk alternatives (soy drinks and rice drinks specifically) and other foods.

3.4 Key findings of the toxicological assessment

Previous assessments by FSANZ concluded there are no toxicological concerns regarding consumption of plant sterol fortified foods, and no justification for establishing an ADI given the absence of adverse effects in short-term and sub-chronic toxicity studies. Information newly available since FSANZ's last assessment of plant sterols does not indicate a need to amend this conclusion.

A short-term study of phytosterols in rats found no evidence of adverse effects on body weight or gastric mucosal tissues. A study involving administration of phytosterols in mouse models of hypercholesterolaemia found evidence suggesting that higher plasma concentrations compared to wild-type mice may be due to reduced elimination rather than enhanced intestinal absorption. The relevance of this finding beyond individuals with the rare human genetic condition of phytosterolaemia (sitosterolaemia) is uncertain.

No adverse events were reported in a clinical study of a small group of children with dyslipidaemia aged 7 – 16 years given 1.5 g/day phytosterols for 8 weeks. In a range of clinical studies investigating the impact of dietary phytosterol supplementation up to 2 g/day on blood lipids in adults, the intervention was generally well tolerated and no serious treatment-related adverse events were reported.

The assessment of application A1134 concluded there was no robust evidence to support concerns that consuming plant sterols will increase the risk of cardiovascular disease or that the oxidation products of dietary plant sterols pose a risk to consumers. At that time, FSANZ noted that while some epidemiological studies have observed a positive association between moderate elevation of plasma plant sterol concentrations and increased cardiovascular disease risk, other studies have observed an inverse association or no association.

New studies published since 2017 similarly report conflicting findings. One study found no association between cholesterol-adjusted plasma campesterol and cardiovascular events. A positive or no association with cardiovascular events was observed for the oxidised phytosterol 7α-OH-campesterol depending on the statistical approach taken, but no associations were found with other oxidised phytosterols (Fuhrmann et al. 2018). In a second study, no association was observed between plasma oxidised phytosterols and cardiovascular disease risk. A positive association between cholesterol-adjusted sitosterol and campesterol and cardiovascular disease was reported, but this finding is not new data as it was based on reanalysis of samples from a study which previously reported the same association in 2009 (Baumgartner et al. 2019; Matthan et al. 2009).

While elevations in serum plant sterol concentrations in individuals with certain genetic variants in the ATP binding cassette transporter G5 and G8 (ABCG5/8) or ABO genes show a correlation with risk of cardiovascular disease, these individuals also have increased cholesterol absorption and in particular, high serum concentrations of LDL cholesterol. High serum LDL cholesterol is a well-recognised marker of increased cardiovascular risk. A recent study of the impact of genetic variability in ABCG5/8 on circulating cholesterol and phytosterol concentrations and risk of coronary artery disease hypothesised that increased phytosterol absorption may contribute to the increased risk. However, as the study authors acknowledged, the study did not demonstrate a causal association between phytosterols and atherosclerotic disease and other mechanisms may be involved (Helgadottir et al. 2021a,b; Plat et al. 2021).

A systematic review and meta-analysis reported that phytosterol supplementation led to reductions in systolic and diastolic blood pressure compared with controls, depending on the dose and duration of consumption (Ghaedi et al. 2020).

Based on the available data, there is no convincing evidence of a causal association between phytosterol consumption and cardiovascular disease.

One study reported serum concentrations of plant sterol oxidation products (POP) after 42 days consumption of oxidized plant sterols produced by frying potatoes with margarine supplemented with phytosterols. Serum POP concentrations increased non-linearly with increasing dose, reaching stable levels in less than 7 days and remaining below concentrations of cholesterol oxidation products. The relevance of this study to the intended use pattern currently under evaluation is uncertain.

A case-control study reported that total dietary phytosterol consumption was inversely associated with colorectal cancer risk in a Chinese population (Huang et al. 2017).

As noted in previous FSANZ assessments safety data for pregnant women, lactating women, and children under five years of age is relatively limited compared to the extensive data available for the target population. However, based on knowledge of the mechanisms of phytosterol action, the now extensive history of consumption of phytosterol-enriched foods in the general population and the absence of effects in pregnant animals and their offspring in laboratory studies, there is unlikely to be an appreciable risk to these population subgroups. No new data was identified that would change this conclusion.

FSANZ has no toxicological concerns regarding the addition of plant sterols to plant-based milk alternatives at the proposed levels, for consumption by the general population. However, risk management measures may be required for individuals with the extremely rare inherited condition, phytosterolaemia, to enable them to identify foods containing plant sterols.

4 Nutrition assessment

4.1 Effect on blood cholesterol

4.1.1 Objective

The objective was to assess whether consumption of plant-based milk alternatives with added plant sterols lowers blood cholesterol concentration.

4.1.2 Previous FSANZ assessments

FSANZ assessed whether any effects on blood cholesterol can be expected from the intake of plant sterols in a systematic review (FSANZ 2014) and for two applications: A1019 - Exclusive use of phytosterol esters in lower-fat cheese products (FSANZ 2010) and A1134 - Increased concentration of plant sterols in breakfast cereals (FSANZ 2017).

The purpose of the FSANZ systematic review was to assess the currency of the pre-approved high level health claim that plant sterols 'reduce blood cholesterol'. FSANZ reviewed two published reviews by Demonty et al. (2009) and Ras et al. (2013), and primary research identified via literature searches conducted in March and April 2014 and restricted to a start date of either 2012 or June 2012. FSANZ (2014) states that the meta-analyses by Demonty et al. (2009) and Ras et al. (2013) estimated that an intake of 1.6 g to 2.2 g phytosterols per day resulted in an approximately 0.33 mmol/L decrease in LDL-cholesterol concentration and a 0.36 mmol/L decrease in total cholesterol concentration. Further, a dose response curve by Demonty et al. (2009) predicted that a daily intake of 2 g/day would reduce LDL-cholesterol by 0.35 mmol/L, with effects plateauing at doses above 3 g/day (FSANZ 2014). FSANZ (2014) concluded that an intake of 2 g/day is adequate to reduce blood total and LDL-cholesterol concentrations in adults, with a high degree of certainty for a causal relationship, and that the pre-approved high level health claim remained current. The relationship between phytosterol intake and reduced blood cholesterol concentrations was applicable to adults with normal cholesterol concentrations as well as those with elevated cholesterol concentrations.

As reported by FSANZ (2014), one plausible mechanism explaining how phytosterol intake may affect blood cholesterol is the structure and absorption of phytosterols. Phytosterols and cholesterol are similar in structure, however phytosterols have a methyl or ethyl group in their side chains which reduces their absorption. Phytosterols and phytostanols are poorly absorbed from the intestine, at less than 10% and 1% the rate of cholesterol absorption, respectively. Phytosterols compete with cholesterol for intestinal absorption due to their structural similarity, leading to reduced cholesterol uptake in the gut, reduced blood cholesterol concentrations, and increased faecal cholesterol excretion (FSANZ 2014).

The most widely used foods with added phytosterols/stanols are low-fat spread/margarine, followed by dairy products (milk and yoghurt), and cereal products (Fardet et al. 2017). Other food products include dairy-free drinks, chocolate bars, orange juice, and biscuits (Fardet et al. 2017; Gylling et al. 2014). FSANZ's systematic review included trials using a variety of foods, including bread, milk, margarine, yoghurt and soy drinks, and concluded that the evidence supports the efficacy of phytosterols in a variety of foods (FSANZ 2014). As reviewed by FSANZ (2014), Demonty et al. (2009) found no difference in absolute changes in LDL-cholesterol between fat and non-fat foods, dairy and non-dairy foods, or solid and liquid foods. However, solid foods were found to have a greater effect on LDL-cholesterol reduction in the relative dose-response curve, compared to liquid food formats (Demonty et al. 2009). We note that Trautwein et al. (2018) has queried whether liquid foods enable faster gastric emptying rates in comparison to solid foods and therefore, may have lesser efficacy.

However, as the scope of the current assessment is limited to plant-based milk alternatives, the relative effect of liquid versus solid forms has not been evaluated.

We have not summarised the FSANZ assessment of application A1019 (FSANZ 2010) because it is superseded by FSANZ's systematic review. The assessment of application A1134 (FSANZ 2017) was conducted after FSANZ's systematic review, however it did not include evidence on the effect of plant sterols added to plant-based milk alternatives. These assessments are, therefore, not relevant to the current assessment.

4.1.3 Impact on blood cholesterol

We reviewed the evidence published since the FSANZ (2014) systematic review, which searched the literature up to March 2014. In March 2022, we searched PubMed using (phytosterols[MeSH Terms] OR plant sterol* OR plant stanol* OR phytosterol* OR phytostanol* OR sitosterol* OR sitostanol* OR campesterol* OR campestanol* OR stigmasterol* OR brassicasterol*) AND (blood* OR plasma OR serum) AND ("randomized controlled trial"[Publication Type] OR "controlled clinical trial"[Publication Type] OR randomized[Title/Abstract] OR randomised[Title/Abstract] OR placebo[Title/Abstract] OR randomly[Title/Abstract] OR trial[Title/Abstract] OR groups[Title/Abstract]), and filtered results by studies in humans, published in English from 2014 to March 2022, and available in full text. We identified primary research by screening individual publications against inclusion criteria (Table 1) and included systematic reviews or meta-analyses of such primary research.

Table 1 PICOTS criteria for study selection

Population	All apparently healthy populations.
Intervention	Intake of a plant sterol enriched plant-based milk alternative (liquid form).
Comparator	Placebo control that enables any difference in effects to be attributed to the addition of plant sterols.
Outcome	Total or LDL cholesterol.
Time	Minimum two week duration.
Study design	Controlled trials.

The search retrieved 241 results of which four studies met the inclusion criteria (Dong et al. 2016; Ho et al. 2016a; Chau et al. 2020; Oliveira Godoy Ilha et al. 2020). With respect to the outcome, blood cholesterol, the application provided seven publications of which two were captured by our search (Dong et al. 2016; Chau et al. 2020). The remaining five were published prior to 2014 and, therefore, not captured by our search (Hallikainen et al. 2013; Weidner et al. 2008; Rideout et al. 2009; Gylling et al. 2010; Kriengsinyos et al. 2011). Hallikainen et al. (2013) and Weidner et al. (2008) were included in the FSANZ (2014) systematic review and we provide a summary of the previous evaluation here. We excluded Rideout et al. (2009) and Gylling et al. (2010). Rideout et al. (2009) compares a plant sterol enriched soy milk intervention to a dairy milk comparator. Gylling et al. (2010) tested the effect of consuming a vegetable oil-based spread and oat-based drink enriched with plant stanol ester together and does not identify the relative contribution of plant stanol ester from the test drink. In both studies, the effect cannot be attributed to plant sterols added to a plant-based milk alternative and were excluded from our review. Kriengsinyos et al. (2011) was not included as primary research in the FSANZ (2014) systematic review, or in the reviews by Demonty et al. (2009) and Ras et al. (2013) and thus, was included.

A summary of the seven included studies is provided in Appendix 1: Impact on blood cholesterol and other health outcomes: study summaries (Weidner et al. 2008; Kriengsinyos et al. 2011; Hallikainen et al. 2013; Dong et al. 2016; Ho et al. 2016a; Chau et al. 2020; Oliveira Godoy Ilha et al. 2020). All studies were randomised although the method varied or was not stated. All were parallel trials except two crossover studies (Ho et al. 2016a; Oliveira

Godoy Ilha et al. 2020). Six studies were industry-funded and the funding source of the remaining study was unclear (Weidner et al. 2008). Study duration ranged from 3 weeks to 6 months. Sample sizes ranged from 18 to 159 participants. All the included studies were conducted on adults of both genders, most of whom were female. Participants in four studies' had mild or moderate hypercholesterolaemia, one study used a sample with the broader criteria of mild or moderate hyperlipidaemia (Dong et al. 2016), and participants in two studies were normocholesterolaemic (Ho et al. 2016a; Chau et al. 2020).

All studies specify that participants taking lipid-lowering medication were excluded, except for one study of healthy normocholesterolaemic participants where lipid medication use was not mentioned (Ho et al. 2016a). Participants were French (Weidner et al. 2008), Thai (Kriengsinyos et al. 2011), Swedish (Hallikainen et al. 2013), Chinese (Dong et al. 2016; Chau et al. 2020), Singaporean (Ho et al. 2016a), and Brazilian (Oliveira Godoy Ilha et al. 2020). Thus, their habitual diets may differ substantially to that of Australia and New Zealand populations. This level of indirectness (the population of the studies not matching the population of interest, i.e. Australia and New Zealand populations) reduces the certainty that we have in the evidence. Participants from all seven studies' intervention and comparator conditions consumed a soy drink.

Plant sterols were added to the soy drink consumed by participants in the intervention condition, with doses as follows: 2.6 g sterol esters/day, equivalent to 1.6 g free sterols/day (Weidner et al. 2008); 2 g stanol esters/day (Kriengsinyos et al. 2011); 2.8 g plant stanols in test drink with a mean consumption of 2.7 g stanols/day, taking into account compliance (Hallikainen et al. 2013); 3.4 g phytostanol ester-enriched soy milk powder (2 g/day free phytosterols in 30 g soy milk powder; Dong et al. 2016); 2.0 g free plant sterols/day, administered as palmitates (Ho et al. 2016a); 2 g phytosterols/day with phytosterols being mainly sterol esters (>90%) (Chau et al. 2020); and, 1.6 g phytosterols/day (Oliveira Godoy Ilha et al. 2020). In summary, the dose of plant sterols ranged from 1.6 to 2.7 g/day, with a mean intake of 2.1 g/day (a mean intake of 2.2 g/day in the five studies using participants with hypercholesterolaemia or hyperlipidaemia and a mean intake of 2 g/day in the two studies with normocholesterolaemic adult participants).

Effect on LDL-cholesterol

In hypercholesterolaemic or hyperlipidaemic adults, LDL-cholesterol was significantly reduced in four parallel studies' intervention conditions over the study duration compared to the comparator condition: $P<0.05$ (Weidner et al. 2008); $P<0.05$ (Kriengsinyos et al. 2011); $P<0.001$ (Hallikainen et al. 2013); and, $P=0.036$ (Dong et al. 2016). In the fifth study, a crossover study, LDL-cholesterol was significantly lower ($P=0.001$) in the intervention condition compared to the comparator at follow-up (Oliveira Godoy Ilha et al. 2020). The absolute change in mean LDL-cholesterol across time in the intervention condition ranged from -0.23 to -0.56 mmol/L (Dong et al. 2016 and Kriengsinyos et al. 2011, respectively). The change in LDL-cholesterol cannot be calculated from Oliveira Godoy Ilha et al. (2020) because baseline data were not reported. This range is not adjusted for the difference in the comparator condition across time (in some studies the comparator's LDL-cholesterol also decreased over time).

Of the two studies with a sample of normocholesterolaemic adults, neither reported a significant difference in LDL-cholesterol concentration between conditions over time. Ho et al. (2016a) did not observe a difference in LDL-cholesterol between conditions after baseline adjustment. Chau et al. (2020) reported an almost significant reduction in LDL-cholesterol in the intervention condition over the study duration compared to the comparator condition: a least square mean change (mmol/L; 95% confidence interval, CI) of -0.12 (-0.23, 0.00) and $P=0.048$. The absolute change in mean LDL-cholesterol across time in the intervention condition was +0.01 mmol/L (Ho et al. 2016a) and -0.18 mmol/L (change in least square

mean; Chau et al. 2020). In both studies, when adjusted for the difference in the comparator condition, the treatment effect was not significant.

Effect on total cholesterol

In hypercholesterolaemic or hyperlipidaemic adults, total cholesterol was significantly reduced in four parallel studies' intervention conditions over the study duration compared to the comparator condition: $P<0.05$ (Weidner et al. 2008); $P<0.05$ (Kriengsinyos et al. 2011); $P<0.001$ (Hallikainen et al. 2013); and, $P=0.011$ (Dong et al. 2016). In the fifth study, a crossover study, total cholesterol was significantly lower ($P<0.001$) in the intervention condition compared to the comparator at follow-up (Oliveira Godoy Ilha et al. 2020). The absolute change in mean total cholesterol across time in the intervention condition ranged from -0.26 to -0.58 mmol/L (Weidner et al. 2008 and Dong et al. 2016, respectively). Similarly to LDL-cholesterol, data from Oliveira Godoy Ilha et al. (2020) is not considered in this range, as baseline data were not reported. This range is not adjusted for the difference in the comparator condition; about half the studies' comparator's total cholesterol also decreased over time.

Of the two studies with a sample of normocholesterolaemic adults, neither reported a significant difference in total cholesterol concentrations between conditions over time. Ho et al. (2016a) did not observe a difference in total cholesterol between conditions after baseline adjustment. Chau et al. (2020) reported no difference in total cholesterol in the intervention condition over the study duration compared to the comparator condition: a least square mean change (mmol/L; 95% CI) of -0.10 (-0.24, 0.03) and $P=0.141$. The absolute change in total cholesterol across time in the intervention condition was -0.33 mmol/L (change in median; Ho et al. 2016a) and -0.12 mmol/L (change in least square mean; Chau et al. 2020). In both studies, when adjusted for the decreased concentration in the comparator condition, the treatment effect was not significant.

4.1.4 Conclusions of the nutrition assessment on blood cholesterol

The current assessment evaluates seven parallel or crossover controlled trials in humans, where the intervention and comparator groups consumed a soy drink and which, for the intervention group only, contained added plant sterols. No studies were identified investigating the effect of plant sterols added to plant-based milk alternatives other than soy drinks.

The eligible studies were mainly randomised trials. All but one study was industry funded (funding for one study was unclear). Participants' ethnicity and habitual diets are likely to differ to that of Australia and New Zealand and the sub-group of Australian and New Zealanders who consume plant-based milk alternatives. A summary of the limitations of our assessment is provided below. Effects on other health outcomes are described in section 4.2 and Appendix 1: Impact on blood cholesterol and other health outcomes: study summaries.

Our narrative review found a statistically lower total and LDL cholesterol was observed after consuming soy drinks with added plant sterols in hypercholesterolaemic or hyperlipidaemic, but not normocholesterolaemic, adults. In this body of evidence, the dose of plant sterols ranged from 1.6 to 2.7 g/day, with a mean intake of 2.1 g/day. A dose-response analysis was not conducted due to the small number of studies.

4.1.5 Limitations of the nutrition assessment on blood cholesterol

The main limitations of the assessment on blood cholesterol include:

- Included studies did not investigate the use of plant-based milk alternatives other than soy drinks and only two studies used a sample of normocholesterolaemic participants.
- Included studies did not investigate the efficacy in vegetarians or vegans, who may be both common consumers of plant-based milk alternatives and more likely to be normocholesterolaemic.
- The small number of trials and lack of studies without industry associations precluded any subgroup analysis to examine potential bias in the findings.
- The level of indirectness (i.e. the studies' populations not matching the population of interest, i.e. Australia and New Zealand populations) reduces the certainty that we have in the evidence.
- We did not quantify the pooled effect via meta-analysis, of consumption of plant sterols on blood cholesterol.
- We did not assess the level of certainty in the evidence, as was conducted in the FSANZ (2014) systematic review.
- Given the limited number of studies, a meta-regression or subgroup analysis was not conducted which would otherwise be necessary to determine the minimal dose of plant sterols at which a statistically and/or meaningful reduction in blood cholesterol might be observed.
- We did not assess any new published research on foods other than plant-based milk alternatives.

4.2 Effect on other health outcomes

4.2.1 Objective

The objective was to evaluate if there are any nutrition-related adverse effects from consuming plant-based milk alternatives with added plant sterols. If adverse effects are identified, evaluate at what level of intake they occur.

A summary of the previous FSANZ safety assessments is provided above (see section 3). The FSANZ assessments of applications A1019 and A1134 did not review evidence on the potential adverse effects of plant sterols when added to plant-based milk alternatives.

4.2.2 Impact on other health outcomes

We used the search strategy listed in section 4.1.3 to identify relevant publications. We identified primary research by screening individual publications against inclusion criteria (Table 2) and included systematic reviews or meta-analyses of such primary research.

Table 2 PICOTS criteria for study selection

Population	All apparently healthy populations.
Intervention	Intake of a plant sterol enriched plant-based milk alternative (liquid form).
Comparator	Placebo control that enables any difference in effects to be attributed to the addition of plant sterols.
Outcome	Outcomes other than total and LDL-cholesterol, and outcomes considered in the toxicological assessment (Section 3).
Time	Any duration including single-dose, acute studies.
Study design	Controlled trials.

The search retrieved 241 results of which five publications reporting on four studies met the inclusion criteria (Dong et al. 2016; Ho et al. 2016a; Ho et al. 2017; Chau et al. 2020; Oliveira Godoy Ilha et al. 2020). Ho et al. (2016a) and Ho et al. (2017) report on the same trial. Most publications were excluded as they did not evaluate the effect of plant sterol-enriched plant-based milk alternatives (if primary research) or in a separate subgroup analysis (if a

systematic review or meta-analysis), in isolation to other food sources to which plant sterols have been added.

Due to limited data, the systematic reviews and meta-analyses captured by our search strategy were unable to conduct analyses of potential adverse nutrition-related health effects of plant-based milk alternatives with added plant sterols.

With regards to adverse effects, the application provides 28 studies in humans from a search spanning 2016 to May 2021. Of these, four evaluated the effect of plant sterol-enriched plant-based milk alternatives but one was excluded due to being a conference abstract (Ho et al. 2016b) and the remaining three had already been captured by our search (Dong et al. 2016; Ho et al. 2017; Oliveira Godoy Ilha et al. 2020). We also considered any adverse effects reported by additional studies provided by the application in relation to a beneficial effect on blood cholesterol (Weidner et al. 2008; Kriengsinyos et al. 2011; Hallikainen et al. 2013; see Appendix 1: Impact on blood cholesterol and other health outcomes: study summaries).

Ho et al. (2016a) and Ho et al. (2017) report on different outcomes from the same trial. In a randomised, double-blind, crossover study, 18 healthy Singaporean adults consumed soy milk powder dissolved in water resulting in an intake of 2.0 g free plant sterols per day (intervention) or without plant sterols (comparator) for four weeks.

Ho et al. (2016a) investigated if phytosterol intake protects against oxidative damage, lipid peroxidation, and inflammation. A range of biomarkers were measured including: 5-lipoxygenase, 12-lipoxygenase, and myeloperoxidase activities; blood plasma and urine F_2-isoprostanes, leukotriene B4 concentrations; and, high-sensitivity C-reactive protein, tumor necrosis factor-α, and lipoxin A4 concentrations in blood serum. The role of phytosterols in the pathogenesis of atherosclerosis via lipid peroxidation and inflammation is unclear. The measures reported by Ho et al. (2016a) from one study of 18 participants, does not provide sufficient evidence to conclude if the intake of phytosterols or the observed differences in some physiological outcomes pose a risk to nutritional imbalance or public health.

Ho et al. (2017) measured the effect on urinary and blood plasma nitrite and nitrate, blood plasma arginine, and blood plasma asymmetric dimethylarginine. The role of these outcomes in public health, such as increasing the risk of carcinogenicity and methemoglobinemia, or decreasing the risk of cardiovascular disease, is not sufficiently well-established for these outcomes to be used as criteria to evaluate public health risk. It has not been established if these clinical endpoints are mediated, in part, through the effect of phytosterol intake (with or without plant-based milk alternatives) on intermediary physiological outcomes such as those measured by Ho et al. (2017). It is, therefore, not possible to evaluate the overall risk-benefit to public health and attribute this to the intake of phytosterols when added to plant-based milk alternatives. Based on the available data from one study of 18 participants, there is no convincing evidence that the intake of phytosterols nor the observed differences in physiological outcomes found by Ho et al. (2017) pose a risk to nutritional imbalance or public health.

All seven included studies reported HDL-cholesterol and triglyceride levels. Dong et al. (2016) reported that the intervention condition's HDL-cholesterol decreased over the study duration, in comparison to the comparator (P=0.0001). Oliveira Godoy Ilha et al. (2020) reported that the intervention condition's triglyceride's level was lower at follow-up, in comparison to that of the comparator (P=0.08). The remaining studies reported non-significant differences in HDL-cholesterol and/or triglyceride levels.

A range of other biomarkers were measured, as summarised in Appendix 1: Impact on blood cholesterol and other health outcomes: study summaries. Kriengsinyos et al. (2011) reported that the intervention condition's serum α-tocopherol and lycopene (unadjusted), and β-

cryptoxanthin and β-carotene (with and without adjustment for total cholesterol) decreased over the study duration, in comparison to the comparator (P<0.05) and suggest that the stanol-ester consumers may be at risk of fat-soluble vitamin deficiencies if the vitamin intake from foods is inadequate. None of the remaining studies investigated these outcomes. Oliveira Godoy Ilha et al. (2020) reported that plasma endothelin-1, which may impact the development of endothelial dysfunction, was beneficially lower in the intervention condition at follow-up (P=0.02).

We conclude that, due to the insufficient and/or inconsistent data on these physiological outcomes, there is no convincing evidence that the intake of plant sterols poses a risk of nutritionally-related adverse effects.

4.2.3 Conclusions of the assessment on other health outcomes

The current assessment evaluates seven parallel or crossover, controlled trials in humans, where the intervention and comparator groups consumed a soy drink and which, for the intervention group only, contained added plant sterols. No other types of plant-based milk alternatives have been assessed. The eligible studies were mainly randomised trials. All but one study was industry funded (one study's funding was unclear). Participants' ethnicity and habitual diets are likely to differ to that of Australia and New Zealand and the subgroup of Australian and New Zealanders who consume plant-based milk alternatives. A summary of the limitations of our assessment is provided below.

Our narrative review found no convincing evidence that the consumption of plant-based milk alternatives with added plant sterols led to nutritionally-related adverse effects in the general adult population. We did not identify evidence investigating the effects on children and other sub-groups such as pregnant women, and, therefore, the effects on these populations are unknown. In this body of evidence, the dose of plant sterols ranged from 1.6 to 2.7 g/day, with a mean intake of 2.1 g/day.

4.2.4 Limitations of the assessment on other health outcomes

The main limitations of the assessment on other health outcomes include:

- Identification of the potential adverse effects of plant sterols added to plant-based milk alternatives does not include research published prior to 2014.
- The small number of trials and lack of studies without industry associations precluded any subgroup analysis to examine potential bias in the findings.
- Previous assessments by FSANZ (FSANZ 2010; FSANZ 2017) did not review evidence on the potential adverse effects of plant sterols when added to plant-based milk alternatives.
- In the current assessment, we exclude the identification of potential adverse effects of plant sterols added to foods other than plant-based milk alternatives.
- We did not evaluate the effects of inherent characteristics of plant-based milk alternatives, such as liquid versus solid form.

5 Dietary exposure assessment

Previous assessments for A1019, A1024 and A1134 have estimated the dietary exposure to plant sterols[7] if added to edible oil spreads, breakfast cereals, low fat milk, low fat yoghurt and reduced fat cheese.

This application requests that plant sterols be permitted to be added to plant-based milk alternatives, in amounts from 0.8 g to 2.2 g of total plant sterol equivalents per 250 mL serving of plant-based milk alternatives. The consumption of plant-based milk alternatives is increasing. The Australian Bureau of Statistics (ABS) recently reported that between 2019-20 and 2020-21, apparent consumption of dairy milk substitutes (unflavoured) increased by 14.6% or by 2.2 g/person/day to 17.3 g/person/day (ABS 2022). The applicant also provided confidential information that supports the data showing the consumption increase for plant-based milk alternatives. The confidential data shows the increase in sales over recent years for this food group.

5.1 Objective and approach to the dietary exposure assessment

Dietary exposure assessments require data on the concentration of the chemical of interest in the foods requested, and consumption data for the foods that have been collected through a national nutrition survey.

The objectives of this dietary exposure assessment were to:

- estimate the total plant sterol exposure from foods which may contain added plant sterols at baseline,
- estimate any potential increase in plant sterol exposure from the addition of plant sterols to plant-based milk alternatives,
- to determine whether normal consumption patterns of plant-based milk alternatives would result in dietary exposure to plant sterols of 2.2 g, and
- estimate if other individual foods to which plant sterols can be added provide similar dietary exposures, or meet daily target intakes, through typical consumption patterns compared to plant-based milk alternatives.

Baseline dietary exposure was estimated using (1) the consumption of foods containing added plant sterols, and other foods as reported in the Australian and New Zealand national nutrition surveys for which there are current permissions for the addition of plant sterols; and (2) maximum permitted levels (MPL) for added plant sterols in the Australia New Zealand Food Standards Code (the Code). The application requests permission to add plant sterols to plant-based milk alternatives at a content of no less than 0.8 g and no more than 2.2 g per 250 mL serving. Therefore, dietary exposures of added plant sterols from plant-based milk alternatives were calculated using 2.2 g per 250 mL serving in a conservative estimate.

The dietary exposure assessment was undertaken using FSANZ's dietary modelling computer program Harvest[8]. A summary of the general FSANZ approach to conducting dietary exposure assessments for applications is on the FSANZ website. A detailed discussion of the FSANZ methodology and approach to conducting dietary exposure assessments is set out in *Principles and Practices of Dietary Exposure Assessment for Food Regulatory Purposes* (FSANZ 2009).

[7] The term plant sterols has been used in the DEA to include the total amount of phytosterols, phytostanols and phytosterols, and phytostanols following hydrolysis of any phytosterol esters and phytostanol esters, as defined as 'total plant sterol equivalents' in section 1.1.2—2 in the Code.
[8] Harvest is FSANZ's custom-built dietary modelling program that replaced the previous program, DIAMOND, which does the same calculations just using a different software program.

5.2 Dietary exposure methodology

5.2.1 Food consumption data used

The permissions contained in the Code apply to foods sold in both Australia and New Zealand, therefore dietary exposure assessments were undertaken for both countries.

The food consumption data used for the dietary exposure assessment were:

- 2002 New Zealand National Children's Nutrition Survey (2002 NZNNS), one 24-hour recall covering 3,275 New Zealand school children aged 5-14 years (Ministry of Health 2005).
- 2008/09 New Zealand Adult Nutrition Survey (2008 NZANS), one 24-hour recall covering 4,721 New Zealanders aged 15 years and above (Ministry of Health 2011a & 2011b).
- 2011-12 Australian National Nutrition and Physical Activity Survey (2011-12 NNPAS), one 24 hour food recall survey of 12,1253 Australians aged 2 years and above, with a second 24-hour recall undertaken for 64% of respondents (n=7,735) (ABS 2015).

For Australia, two days of consumption data were averaged to represent longer term exposures, whereas the assessments for New Zealand were based on a single day of data. All results were weighted to make them representative of the respective populations. The design of these nutrition surveys vary and the key attributes of each, including survey limitations are on the FSANZ website.

5.2.2 Population groups assessed

The hazard assessment did not identify any population sub-groups or at-risk groups for which there were specific safety considerations or where separate dietary exposure estimates were needed. Therefore, the whole survey population from each of the nutrition surveys were used for the dietary exposure assessment.

5.2.3 Food categories and plant sterol concentrations

Plant sterols are currently permitted in the Code to be added to breakfast cereals, yoghurt, milk, edible oil spread including margarine, and cheese (see Table 3). There are different forms of plant sterols that are permitted to be added to foods, i.e. esters or tall oil plant sterols. The amounts of plant sterols permitted to be added to foods are defined in terms of total plant sterol equivalents and not the plant sterol preparations actually added to foods. Adjustment factors for plant sterol concentrations in the foods may need to be applied to the MPLs to determine plant sterol equivalent concentrations. For example, for cheese and processed cheese where the permission is for tall oil phytosterol esters, on a plant sterol equivalents basis would be 54 g/kg (using a 0.6 conversion factor based on the molecular weights).

Plant sterols also occur naturally in foods. In a study of the intake of phytosterols and risk of cardiovascular disease in 35,597 Dutch adults, the mean intake of naturally occurring plant sterols was approximately 300 mg/day (Ras et al. 2015). Therefore in a similar approach to previous FSANZ assessments (FSANZ 2017), this dietary exposure assessment did not include the contribution of intrinsic plant sterols naturally occurring in foods.

Table 3 Foods currently permitted to contain added plant sterols in Australia and New Zealand

Food[1]	Standard	Prescribed form	Maximum Permitted Level	Conditions for plant sterol addition
Breakfast cereals	Schedule 25	Total plant sterol equivalents	2.2 g/serving	The total fibre content is no less than 3 g/50 g and contains no more than 30 g/100 g of total sugars
Yoghurt	2.5.3-5	Total plant sterol equivalents	1.0 g/ package (capacity no more than 200 g)	Contains no more than 1.5 g total fat/100 g
Milk	2.5.1-6	Total plant sterol equivalents	4 g/L	Contains no more than 1.5 g total fat/100 g
Edible oil spread (including margarine)	2.4.2-2	Total plant sterol equivalents	82 g/kg	The total saturated and trans fatty acids are no more than 28% of the total fatty acid content of the food
Cheese and processed cheese	2.5.4-4	Tall oil phytosterol esters	90 g/kg	Contains no more than 12 g total fat/100 g

[1] See Appendix 2 for the corresponding Harvest food classification names and codes.

Only foods that are permitted in the Code to contain added plant sterols (breakfast cereals, yoghurt, milk, edible oil spread including margarine and cheese) and foods proposed in this application (plant-based milk alternatives) were included in this dietary exposure assessment. The food classifications and concentrations used in this dietary exposure assessment are in Appendix 2.

Specific consumption data for foods containing added plant sterol were collected in each of the national nutrition surveys. In the 2011-12 NNPAS added plant sterol-containing spreads, unflavoured milks and processed cheese were reported to be consumed. In the New Zealand surveys only added plant sterol containing spreads were reported to be consumed. Consumption of breakfast cereals and yoghurts containing added plant sterols were not reported in the national nutrition surveys, presumably because these foods were not available at the time.

As there was no reported consumption of breakfast cereals containing plant sterols in the national nutrition surveys, all raw and cooked oat cereals, muesli, flake and biscuit type breakfast cereals were included in the dietary exposure assessment in a conservative approach. The criteria around the fibre and sugar content was not applied to the cereals to include in the assessment which is a worst case scenario. The MPL of 2.2 g/serving was used as the plant sterol concentration for breakfast cereals, with the weight of a serving calculated using label data from breakfast cereals containing plant sterols currently available in Australia and New Zealand. Because consumption amounts of oats and porridge was reported as either uncooked or cooked amounts, the concentration used in the Harvest modelling was adjusted by taking a hydration factor into account (Table 4).

Similarly, as there was no reported consumption of yoghurt containing added plant sterols in the national nutrition surveys and few consumers of cheese containing added plant sterols in the 2011-12 NNPAS, all low fat/skim milk yoghurts and reduced/low fat cheese (including processed cheese) were included in the dietary exposure assessment in a conservative approach.

Table 4 Plant sterol concentrations used in the dietary exposure assessment for breakfast cereals

Cereal types	MPL[1]	Assumed serving weight[2] (g)	Concentration used in DEA (g/kg)
Breakfast cereals, biscuit type	2.2 g/serving	36	61
Breakfast cereals, flake type	2.2 g/serving	50	44
Breakfast cereals, oats, raw	2.2 g/serving	45	49
Breakfast cereals, oats, cooked	2.2 g/serving	295[3]	7

[1] As specified in Schedule 25.
[2] Based on label data for foods containing added plant sterols currently available in Australia and New Zealand.
[3] Cooked weight = 45 g dry weight with 250 mL liquid added.

5.2.4 Scenarios

FSANZ used three scenarios to estimate added plant sterol exposures:

> **'***Baseline'* included plant sterol exposures from foods in which the addition of plant sterols is currently permitted in the Code (see Table 3). These foods included breakfast cereals, yoghurt, milk, edible oil spread including margarine and cheese.
>
> **'***Plant-based milk alternatives only'* included plant sterol exposures from all plant-based milk alternatives (cereal beverages, nut- or seed-based beverages and soy beverage) based on the requested maximum permitted level of 2.2 g plant sterols per 250 mL serving. This scenario was to assist in determining the amount of exposure to plant sterols that would result if consumers used plant-based milks as they would typically use the product, as opposed to strictly adhering to one serve of 250 mL.
>
> *'Baseline plus plant-based milk alternatives'* included plant sterol exposures from both the '*Baseline*' and '*Plant-based milk alternatives only*' scenarios.

All three scenarios included foods consumed 'as is' (e.g. glasses of plant-based milk alternatives), and when used in mixed dishes (e.g. added to coffee or porridge), based on FSANZ's recipe database as used in the Harvest Food Additive model.

5.2.5 Assumptions and limitations of the dietary exposure assessment

The aim of the dietary exposure assessment was to make the most realistic estimation of dietary exposure of plant sterols as possible. However, where significant uncertainties in the data existed, conservative assumptions were generally used to ensure that the estimated dietary exposure was not an underestimate of exposure.

Assumptions made in the dietary exposure assessment included:

- Unless otherwise specified, the included breakfast cereals, low fat yoghurts, edible oil spreads (including margarine) and low fat cheese contained plant sterols at the concentrations listed in Appendix 2.
- 1 serving of breakfast cereal, biscuit type = 36 g
- 1 serving of muesli or flake type breakfast cereal = 50 g
- 1 serving raw oats = 45 g
- 1 serving cooked porridge = 295 g
- 1 serving of plant-based milk alternative = 250 g
- 1 litre of milk = 1000 g

- Milk ‘not further defined’, ‘not further specified’, or ‘not specified kind’ were assumed not to contain added plant sterols.
- Cereal bars were not included in the assessment as breakfast cereal bars are excluded from the permissions in the Code.
- Naturally occurring plant sterols were not included in the assessment.
- Where a food or food category was not included in the dietary exposure assessment, it was assumed to contain a zero concentration of plant sterols.
- Although Schedule 25 in the Code states that ‘foods to which phytosterols, phytostanols or their esters have been added must not be used as ingredients in other foods’ for food manufacturing purposes, uses by consumers following purchase of the product are different. Therefore, where a concentration was assigned to a food category, this concentration was carried over to all mixed dishes where foods in this category have been used as an ingredient to capture all possible exposure from foods in the diet in a conservative approach.

In addition to the specific assumptions made in relation to this dietary exposure assessment, there are a number of limitations associated with the nutrition surveys from which the food consumption data used for the assessment are based. A discussion of these limitations is included in Section 6 of the *Principles and Practices of Dietary Exposure Assessment for Food Regulatory Purposes* (FSANZ 2009).

5.3 Dietary exposure results

5.3.1 Estimated dietary exposures to added plant sterols

The estimated mean and 90th percentile (P90) exposures to added plant sterols for the three scenarios for Australian and New Zealand consumers, assuming added plant sterols at the MPL, for all plant sterol containing foods reported as being consumed in the national nutrition surveys (milk and edible oil spreads), all other foods for which there is permission to contain added plant sterols in the Code (breakfast cereals, yoghurt and cheese), in addition to plant-based milk alternatives, are shown in Table 5.

Australia

For Australian consumers (aged 2+ years), the estimated mean and P90 exposures to added plant sterols at *Baseline* are 2.2 g/day and 4.3 g/day expressed as plant sterol equivalents. For the *Plant-based milk alternatives only* scenario estimated mean and P90 exposures are 1.2 g/day and 2.8 g/day, and for the *Baseline plus plant-based milk alternatives* scenario estimated mean and P90 exposures are 2.2 g/day and 4.6 g/day respectively, expressed as plant sterol equivalents.

New Zealand

For New Zealand children (aged 5-14 years), the estimated mean and P90 exposures for consumers at *Baseline* are 2.3 g/day and 4.2 g/day expressed as plant sterol equivalents. For the *Plant-based milk alternatives only* scenario estimated mean and P90 exposures are 2.1 and 4.4 g/day, and for the *Baseline plus plant-based milk alternatives* scenario estimated mean and P90 exposures are 2.4 g/day and 4.2 g/day respectively, expressed as plant sterol equivalents.

For New Zealand adults (15 years and over), the estimated mean and P90 exposures for consumers at *Baseline* are 2.7 g/day and 5.2 g/day expressed as plant sterol equivalents. For the *Plant-based milk alternatives only* scenario estimated mean and P90 exposures are 2.0 and 4.5 g/day, and for the *Baseline plus plant-based milk alternatives* scenario estimated

mean and P90 exposures are 2.7 g/day and 5.3 g/day respectively, expressed as plant sterol equivalents.

Table 5 Estimated dietary exposure to added plant sterols from baseline and extension of use to plant-based milk alternatives assuming plant sterols added at the MPL

Country	Nutrition Survey	Age group	Scenario	Proportion of plant sterol consumers	Estimated dietary exposure, consumers only (g/day)*	
					Mean	P90
Australia	2011-12 NNPAS	2 years and above	*Baseline*	58	2.2	4.3
			Plant-based milk alternatives only	7**	1.2	2.8
			Baseline plus plant-based milk alternatives	60	2.2	4.6
New Zealand	2002 NZ CNS	5-14 years	*Baseline*	42	2.3	4.2
			Plant-based milk alternatives only	1**	2.1	4.4
			Baseline plus plant-based milk alternatives	42	2.4	4.2
	2008 NZ ANS	15 years and above	*Baseline*	45	2.7	5.2
			Plant- based milk alternatives only	3**	2.0	4.5
			Baseline plus plant-based milk alternatives	46	2.7	5.3

* Expressed as plant sterol equivalents. Two day average for Australia; day 1 only data for New Zealand.
** Indicative of the proportion of consumers at the time of the nutrition survey, and included here for illustrative and discussion purposes only. Based on evidence of increased sales and consumption of this product in recent years noted at the start of section 5, these proportions are now likely to be higher in reality.

Discussion

Further information and explanations are provided below to assist with the interpretation of the results.

The estimated dietary exposures from *Baseline* and *Plant-based milk alternatives only* combined do not equal the sum of the results from the separate *Baseline* and *Plant-based milk alternatives only* results. This is because the results are presented on a 'consumers only' basis and there are a different proportion of consumers of plant sterols in the population at *Baseline* when many foods with added plant sterols are considered, compared to a much smaller proportion of consumers who consume only plant-based milk alternatives.

The difference in the estimated dietary exposures from *Baseline* to when exposure from *Plant-based milk alternatives only* are added to *Baseline* is small. The increase in mean and P90 exposures were estimated to be 0.1 g/day or less, and 0.3 g/day or less respectively. This is due to the exposures from the small number of plant-based milk consumers being included in the distribution of exposures from all other foods and the additional exposures not substantially changing the exposure distribution from which the mean and P90 are derived.

If consumers of plant-based milk alternatives consume the product in accordance with proposed use (a 250 mL serve), the estimated dietary exposure would result in an exposure of 2.2 grams per day. If the consumer is adding this amount from one serve to exposures from other foods in the diet, the dietary exposures would be 2.2 grams per day higher than

the estimated baseline dietary exposures. Consumers of plant sterol containing products could increase their current exposure up to around 7 g/day for high consumers if one serve of plant-based milk alternatives providing 2.2 g/day was included in their current daily diet.

The estimates of dietary exposure to plant sterols from *Plant-based milk alternatives only* shown in Table 5 are around half as much for Australians compared to New Zealanders. This is because the results for Australia are a two day average and will take into consideration that some respondents may not have consumed the product on both days of the nutrition survey. Consumption patterns and daily use of plant-based milk alternatives may have changed since the time the nutrition survey data were collected.

As mentioned previously, consumption amounts and sales of plant-based milk alternatives has increased in recent years. Therefore the proportion of consumers of plant-based milk alternatives would be expected to be higher now compared to when the latest nutrition survey data were collected. Despite this, consumption amounts used for this assessment (see Appendix 3) are typical of daily uses, therefore estimates of plant sterol exposures from this food would be indicative of daily exposures.

5.3.2 Estimated consumption of foods which may contain added plant sterols

Consumption of edible oil spreads, milk, and cheese containing added plant sterols were reported in one or more of the national nutrition surveys. In the absence of national nutrition survey consumption data for breakfast cereals and yoghurt containing added plant sterols, conservative estimates of consumption were used. Conservative estimates were also calculated for cheese as there was an insufficient number of consumers of processed cheese containing added plant sterols in the 2011-12 NNPAS to provide robust data. A summary of the mean and P90 food consumption amounts from this assessment are shown in Appendix 3: Estimated baseline mean and P90 consumption of foods containing added plant sterols and plant sterol dietary exposures for Australian and New Zealand consumers.

5.3.3 Estimated dietary exposure to plant sterols for individual foods

Also shown in Appendix 3, estimated baseline mean and P90 consumption of foods containing added plant sterols and plant sterol dietary exposures for Australian and New Zealand consumers are estimates of dietary exposure to plant sterols for individual foods. These results show that on any single day, consumers of plant-based milk alternatives, based on typical use patterns for such products, would consume a mean of around 2 grams of plant sterols per day and around double that for high consumers.

Other individual foods permitted to contain plant sterols that also provide mean exposures of around 2 g/day based on usual consumption patterns for the food include breakfast cereals for both Australian and New Zealand consumers. This is also the case for cheese for Australians and New Zealand children, with New Zealand adults having about half of this amount. Other individual foods providing a mean of around 1 g/day were edible oil spreads and yoghurts for both countries, and milk for Australia (plant sterol containing milk was only reported as being consumed in Australia).

Over a longer period of time, taking into account where daily consumption of plant-based milk alternatives may not occur (based on the two day average data for Australia), estimates of dietary exposure are estimated to be lower, around half a single day of exposure. However given the changes in the market of plant-based milk alternatives in recent years, the proportion of daily consumers may be higher and longer term exposures not as low as half a single day.

5.4 Dietary exposure assessment conclusions

Plant sterol dietary exposures from foods which may contain added plant sterols were estimated using a conservative approach, whereby all foods reported in the national nutrition surveys to contain plant sterols and all other foods for which there is permission to contain added plant sterols in the Code were included in the assessment, in addition to plant-based milk alternatives.

Baseline dietary exposures to plant sterols (as plant sterol equivalents) from current permitted uses are around 2-3 g/day at the mean and around 5 g/day for high consumers in Australia and New Zealand. Overall, the addition of plant sterols to plant-based milk alternatives is predicted to make little difference to estimated exposures for Australian and New Zealand consumers of plant sterol containing products in the context of the whole diet. Expressed as plant sterol equivalents, the increase in mean and P90 exposures for the *Baseline plus plant-based milk alternatives* scenario compared to the *Baseline* scenario were estimated to be 0.1 g/day or less, and 0.3 g/day or less respectively. On top of baseline dietary exposures, a serve of plant-based milk alternatives could result in dietary exposures of up to around 7 g/day for high consumers. For plant-based milk alternatives only, based on a concentration of plant sterols of 2.2 g/serve and typical consumption patterns, mean dietary exposures for consumers would be around 2 grams on any given day and around double that for high consumers. Some other individual foods permitted to contain plant sterols can also provide mean daily exposures of 2 g/day whereas other foods provide less.

6 Social science assessment

6.1 Objectives of the social science assessment

The objective of the social science assessment is to determine whether any substantial risks would arise from changes to consumer consumption behaviour as a result of permitting plant sterols to be added to plant-based milk alternatives. This involves determining whether:

a) Dietary intake, understanding of the product, or purchasing behaviour differs according to the product to which plant sterols are added;
b) Extending the permission for plant sterols would result in flow-on changes in consumption patterns (e.g. substitution, addition, or avoidance behaviours);
c) It is possible that consumers would consume this product in addition to other products that contain plant sterols (as may already be their habit).

6.2 Social science research specific to plant sterols in plant-based milk alternatives

A FSANZ search of bibliographic databases for research regarding consumers' understanding of plant sterols specifically in plant-based milk alternatives has found no specific research on consumer response to plant sterols in plant-based milk alternatives.

6.3 Does dietary intake, understanding of the product or purchasing behaviour differ according to the product the plant sterols are added to?

A qualitative study by Weiner and Will (2015) that looked at consumer understanding and use of phytosterol-enriched products, including milk, found that consumers tended to use and understand them in similar ways to their non-enriched equivalents. Although mandatory statements on product packaging advise consumers about the adequate intake levels of phytosterols, they found that few consumers tracked the amounts of phytosterol-enriched products that they consumed, or their cumulative consumption across different products. Instead, these products were consumed normally as foods in line with existing habits established in respect of their non-enriched equivalents. This is particularly the case where the recommended serving size differs from the portion provided in the package. The study authors suggest that this behaviour is because these types of phytosterol-enriched products are interpreted within the frame of being a food, rather than as being more like a medicine with a specific dosage. Advisory statements did not motivate consumers to change existing habits of consumption.[9] In contrast, single-serve packages were more likely to be interpreted by consumers as providing a specific 'dose' that should be consumed in line with the written advice (e.g. once a day). These findings suggest that consumers are most likely to consume the proposed plant sterol-enriched plant-based milk alternatives in the way suggested by the applicant (via a single 250 mL serve) if that was already the consumers' habit regarding plant-based milk alternatives.

[9] A FSANZ assessment conducted in 2006 found that adults who used plant sterol enriched spreads used them differently to those who used their non-enriched equivalents, tending to use less spread on bread and toast. This is consistent with Weiner and Will's (2015) finding that some consumers of phytosterol-enriched spread, although desiring to lower cholesterol, also tried to reduce its use due to a competing health-based motivation to reduce consumption of fat. This is less likely to be a competing motivation in plant-based milk alternative consumption due to the applicant's proposed limitation of only allowing phytosterols to be added to plant-based milk alternatives that are low in saturated fat. This suggests that their usage patterns may more closely follow their normal consumption of the non-enriched equivalent.

In-home usage data supplied by the applicant suggests that a substantial proportion of plant-based milk alternative users have the potential to consume plant-based milk alternatives in the servings that the applicant recommends, therefore reaching an adequate intake level (as determined in FSANZ [2014]) in one serve. However, the higher price point associated with the plant sterol-enriched version of plant-based milk alternatives (as noted by the applicant) may discourage equivalent use in contexts where a plant-based milk alternative is the predominant ingredient.

6.4 Would extending the permission for plant sterols result in flow-on changes in consumption patterns? (E.g. substitution, addition, or avoidance behaviours)

Weiner and Will's (2015) qualitative research suggests that consumers tend to understand and use phytosterol-enriched products in similar ways to their non-enriched counterparts. This suggests that consumers may substitute plant sterol-enriched plant-based milk alternatives for its non-enriched equivalent. However, the high price point and mandatory advisory statements are likely to discourage both non-target consumers and excessive consumption. It is possible that consumers may reduce their intake of other plant sterol-enriched products due to the greater concentration of plant sterols within the applicant's proposed product, which allows an adequate intake to be consumed in a single serve, given both the mandatory advisory warnings and the generally higher price point of plant sterol-enriched products compared to their non-enriched counterparts. However, no data is available on this point.

6.5 Is it possible that consumers would consume this product in addition to other products that contain plant sterols (as may already be their habit)?

As noted above, a qualitative study by Weiner and Will (2015) suggests that people tend to understand and use food enriched with phytosterols in similar ways to their non-enriched counterparts, without calculating their cumulative consumption. This suggests that people may consume multiple plant sterol-enriched products, such as spread, yoghurt, cheese, and/or milk as part of their habitual consumption without regard to the amount of plant sterols each provides. This could potentially result in consumption beyond an adequate intake level, particularly when one product provides an adequate intake of phytosterols in a single serve and multiple plant sterol-enriched foods are already part of the consumers' usual consumption habits. However, FSANZ's toxicological assessment suggests that this does not raise public health and safety concerns, and would be more a matter of consumers potentially wasting their money without receiving any additional benefit. In addition, the high price point of plant sterol-enriched products in combination with the mandatory advisory statements may discourage consumers from purchasing multiple products if their needs can be met through the consumption of only one product.

6.6 Conclusions of the social science assessment

It is not anticipated that any substantial risks will arise from changes to consumer consumption behaviour associated with an extension of the permission of plant sterols to be added to plant-based milk alternatives. Although target consumers may treat plant sterol-enriched plant-based milk alternatives in similar ways to their non-enriched equivalents, both non-target consumers and excessive consumption are discouraged by the combination of a high price point and mandatory advisory statements that educate consumers on the adequate level of intake. It is possible that consumers may use multiple plant sterol-enriched products, which could lead to consumption of plant sterols beyond an adequate level of

intake for cholesterol reducing effects. However, even if this were the case, FSANZ's toxicological assessments of plant sterols have concluded that this does not raise public health and safety concerns. On the other hand, the availability of additional and diverse plant sterol-enriched products will benefit consumers by increasing the range of choice available, as well as increasing the likelihood of consumers reaching an adequate intake of plant sterols recommended for cholesterol reduction.

6.7 Limitations of the social science assessment

The social science assessment relies on research provided by the applicant, additional published research available through bibliographic databases, and previous related literature reviews prepared by FSANZ.

Plant sterols have been comprehensively assessed by FSANZ in earlier applications, and plant sterol-enriched foods have been available on the market for the last two decades. Plant sterols are currently permitted to be added to edible oil spreads, breakfast cereals, reduced fat dairy milk, and yoghurt. This social science assessment does not revisit these permissions.

7 Risk characterisation and conclusion

This risk assessment comprises a hazard assessment which considered the potential adverse effects associated with increased plant sterol intake in animal and human studies, and an assessment of the dietary exposure to plant sterols.

The hazard assessment found no convincing evidence of toxicological or nutritional adverse effects attributed to the consumption of plant-based milk alternatives with added plant sterols at the proposed levels, by the general population. No new information was identified that would indicate a need to revise FSANZ's previous conclusion that there is no justification for establishing an acceptable daily intake (ADI) for plant sterols. However, risk management measures may be required for individuals with phytosterolaemia to enable them to identify foods containing plant sterols.

Plant sterol dietary exposures for Australia and New Zealand populations were estimated, assuming the addition of plant sterols to plant-based milk alternatives at the maximum concentration of 2.2 g per 250 mL serving. Existing permissions were also considered. The resulting change in total dietary exposures to added plant sterols from baseline was an increase of 0.3 g/day or less at the mean and 90th percentile, expressed as plant sterol equivalents. For plant-based milk alternatives only, based on a concentration of 2.2 g per 250 mL serve and typical consumption patterns, mean dietary exposures for consumers would be around 2 grams on any given day and around double that for high consumers.

We conclude that consumption of plant-based milk alternatives with added plant sterols at the proposed level is unlikely to pose a risk to the health of Australian and New Zealand populations.

8 References

ABS (2015) National Nutrition and Physical Activity Survey, 2011-12, Basic CURF. Australian Government, Canberra. http://www.abs.gov.au/AUSSTATS/abs@.nsf/Latestproducts/4324.0.55.002Main%20Features652011-12?opendocument&tabname=Summary&prodno=4324.0.55.002&issue=2011-12&num=&view=

ABS (2022) Apparent consumption of selected foodstuffs, Australia. Australian Government, Canberra. https://www.abs.gov.au/statistics/health/health-conditions-and-risks/apparent-consumption-selected-foodstuffs-australia/2020-21

Akinloye OA, Akinloye DI, Onigbinde SB, Metibemu DS (2020) Phytosterols demonstrate selective inhibition of COX-2: in-vivo and in-silico studies of Nicotiana tabacum. Bioorg Chem 102:104037.

Alphonse PA, Ramprasath V, Jones PJ (2017) Effect of dietary cholesterol and plant sterol consumption on plasma lipid responsiveness and cholesterol trafficking in healthy individuals. Br J Nutr 117(1):56-66.

Baumgartner S, Ras RT, Trautwein EA, Konings MCJM, Mensink RP, Plat J (2019) Plasma oxyphytosterol concentrations are not associated with CVD status in Framingham Offspring Study participants. J Lipid Res 60(11):1905-1911.

Boskou D, Elmadfa I (2016) Frying of food: oxidation, nutrient and non-nutrient antioxidants, biologically active compounds and high temperatures, Chapter 9 Phytosterols and Frying Oils, P225, Second Edition CRC Press

Burton, H. (1994) Types of UHT Processing Plant. In: Ultra-High-Temperature Processing of Milk and Milk Products. Springer, Boston, MA. https://doi.org/10.1007/978-1-4615-2157-0_4

Cantrill R (2008) JECFA Chemical and technical assessment on phytosterols, phytostanols and their esters. Prepared for the 69th JECFA meeting.

Chau YP, Cheng YC, Sing CW, Tsoi MF, Cheng VK, Lee GK, Cheung CL, Cheung BMY (2020) The lipid-lowering effect of once-daily soya drink fortified with phytosterols in normocholesterolaemic Chinese: a double-blind randomized controlled trial. Eur J Nutr 59(6):2739-2746.

Cheung CL, Ho DK, Sing CW, Tsoi MF, Cheng VK, Lee GK, Ho YN, Cheung BM (2017) Randomized controlled trial of the effect of phytosterols-enriched low-fat milk on lipid profile in Chinese. Sci Rep 7:41084.

Demonty I, Ras RT, van der Knaap HC, Duchateau GS, Meijer L, Zock PL, Geleijnse JM, Trautwein EA (2009) Continuous dose-response relationship of the LDL-cholesterol-lowering effect of phytosterol intake. J Nutr 139(2):271–284.

Dong S, Zhang R, Ji Y, Hao J, Ma W, Chen X, Xiao R, Yua H (2016) Soy milk powder supplemented with phytosterol esters reduced serum cholesterol level in hypercholesterolemia independently of lipoprotein E genotype: a random clinical placebo-controlled trial. Nutr Res 36:879-884.

EFSA (2020) 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). EFSA Journal 2020;18(6):6135, 36 pp.

FAO and WHO (2008) Phytosterols, phytostanols and their esters. In: *Combined Compendium of Food Additive Specifications [Online Edition].* Joint FAO/WHO Expert Committee on Food Additives (JECFA) 69th Meeting 2008 FAO JECFA Monographs 5. Rome. Available at: https://www.fao.org/food/food-safety-quality/scientific-advice/jecfa/jecfa-additives/detail/en/c/500/

Fardet A, Morise A, Kalonji E, Margaritis I, Mariotti F (2017) Influence of phytosterol and phytostanol food supplementation on plasma liposoluble vitamins and provitamin A carotenoid levels in humans: an updated review of the evidence. Crit Rev Food Sci Nutr 57(9):1906-1921.

FCC (2020). Vegetable oil Phytosterol Esters. In: Food Chemicals Codex, Twelfth Edition. Rockville (MD): United States Pharmacopeial Convention, pp. 1208.

Ferguson JJA, Stojanovski E, MacDonald-Wicks L, Garg ML (2018) Curcumin potentiates cholesterol-lowering effects of phytosterols in hypercholesterolaemic individuals. A randomised controlled trial. Metabolism 82:22-35.

FSANZ (2006) Second Review Report: Applications A433, A434, and A508. Report prepared by Food Standards Australia New Zealand, Canberra. Second Review Report: Applications A433, A434, and A508.

FSANZ (2009) Principles and practices of dietary exposure assessment for food regulatory purposes. Report prepared by Food Standards Australia New Zealand, Canberra. http://www.foodstandards.gov.au/publications/Pages/Principles-and-Practices-of-Dietary.aspx

FSANZ (2010) Application A1024 – Equivalence of plant sterols, stanols and their fatty acid esters. Approval Report. Supporting Document 1: Risk Assessment. Report prepared by Food Standards Australia New Zealand, Canberra. Application A1024 - Equivalence of Plant Stanols, Sterols & their Fatty Acids Esters (foodstandards.gov.au). Accessed 23 March 2022

FSANZ (2010) Application A1019 – Exclusive use of phytosterol esters in lower-fat cheese products. Supporting Document 1: Risk Assessment Report prepared by Food Standards Australia New Zealand, Canberra. A1019 - Exclusive use of phytosterol esters in lower-fat cheese products. Accessed 21 March 2022.

FSANZ (2012) Application A1065 – Packaging Size for Phytosterol-enriched Milk.Supporting document 1 – Risk assessment report. Report prepared by Food Standards Australia New Zealand, Canberra. Application A1065 – Packaging Size for Phytosterol-enriched Milk (foodstandards.gov.au). Accessed 13 April 2022

FSANZ (2014) Systematic review of the evidence for a relationship between phytosterols and blood cholesterol. Report prepared by Food Standards Australia New Zealand, Canberra. Systematic review phytosterols and cholesterol.pdf (foodstandards.gov.au). Accessed 21 March 2022.

FSANZ (2017) Application A1134 – Increased concentration of plant sterols in breakfast cereals. Approval Report. Supporting Document 1: Risk Assessment (at Approval). Report prepared by Food Standards Australia New Zealand, Canberra. A1134 – Increased Concentration of Plant Sterols in Breakfast Cereals (foodstandards.gov.au). Accessed 23 March 2022

Fuhrmann A, Weingärtner O, Meyer S, Cremers B, Seiler-Mußler S, Schött HF, Kerksiek A, Friedrichs S, Ulbricht U, Zawada AM, Laufs U, Scheller B, Fliser D, Schulze PC, Böhm M, Heine GH, Lütjohann D (2018) Plasma levels of the oxyphytosterol 7α-hydroxycampesterol are associated with cardiovascular events. Atherosclerosis 279:17-22.

Ghaedi E, Foshati S, Ziaei R, Beigrezaei S, Kord-Varkaneh H, Ghavami A, Miraghajani M (2020) Effects of phytosterols supplementation on blood pressure: a systematic review and meta-analysis. Clin Nutr 39(9):2702-2710.

Gonzalez-Larena M, Cilla A, Garc´ ía-Llatas G, Barbera R, Lagarda´ MJ (2012) Plant Sterols and Antioxidant Parameters in Enriched Beverages: Storage Stability J. Agric. Food Chem. 60, 4725-4734

Gylling H, Hallikainen M, Nissinen MJ, Miettinen TA (2010) The effect of a very high daily plant stanol ester intake on serum lipids, carotenoids, and fat-soluble vitamins. Clin Nutr 29:112-118.

Gylling H, Plat J, Turley S, Ginsberg HN, Ellegård L, Jessup W, Jones PJ, Lütjohann D, Maerz W, Masana L, Silbernagel G, Staels B, Borén J, Catapano AL, De Backer G, Deanfield J, Descamps OS, Koyanen PT, Riccardi G, Tokgözoglu L, MJ Chapman (2014) Plant sterols and plant stanols in the management of dyslipidaemia and prevention of cardiovascular disease. Atherosclerosis 232(2):346-360.

Guadalupe G, Amparao A, Reyes B, Antonio C (2021) Current methodologies for phytosterol analysis in foods. Microchem J 168.

Hallikainen M, Olsson J, Gylling H (2013) Low-fat nondairy minidrink containing plant stanol ester effectively reduces LDL cholesterol in subjects with mild to moderate hypercholesterolemia as part of a Western diet. Cholesterol 192325.

He W, Zhu H, Chen H (2018) Plant sterols: chemical and enzymatic structural modifications and effects on their cholesterol-lowering activity. J Agric Food Chem 66(12):3047-3062.

Heart Foundation (2009) Summary of evidence: phytosterol/stanol enriched foods PRO-095 (updated 2017). National Heart Foundation of Australia, Canberra. https://heartfoundation.org.au/getmedia/b466322b-c4ea-400b-b1ae-cffa98ec60df/Heart_Foundation_Summary_of_Evidence_Phytosterol_Stanol_enriched_foods_2009.pdf Accessed 25 February 2022

Helgadottir A, Thorleifsson G, Stefansson K (2021) Increased absorption of phytosterols is the simplest and most plausible explanation for coronary artery disease risk not accounted for by non-HDL cholesterol in high cholesterol absorbers. Eur Heart J 42(3):283-284.

Helgadottir A, Thorleifsson G, Alexandersson KF, Tragante V, Thorsteinsdottir M, Eiriksson FF, Gretarsdottir S, Björnsson E, Magnusson O, Sveinbjornsson G, Jonsdottir I, Steinthorsdottir V, Ferkingstad E, Jensson BÖ, Stefansson H, Olafsson I, Christensen AH, Torp-Pedersen C, Køber L, Pedersen OB, Erikstrup C, Sørensen E, Brunak S, Banasik K, Hansen TF, Nyegaard M, Eyjolfssson GI, Sigurdardottir O, Thorarinsson BL, Matthiasson SE, Steingrimsdottir T, Bjornsson ES, Danielsen R, Asselbergs FW, Arnar DO, Ullum H, Bundgaard H, Sulem P, Thorsteinsdottir U, Thorgeirsson G, Holm H, Gudbjartsson DF, Stefansson K (2020) Genetic variability in the absorption of dietary sterols affects the risk of coronary artery disease. Eur Heart J 41(28):2618-2628.

Ho XL, Liu JJ, Loke WM (2016a) Plant sterol-enriched soy milk consumption modulates 5-lipoxygenase, 12-lipoxygenase, and myeloperoxidase activities in healthy adults - a

randomized-controlled trial. Free Radic Res 50(12):1396-1407.

Ho XL, Liu JJH, Loke WM (2016b) Plant sterol-enriched soy milk consumption alleviates 5-lipoxygenase and myeloperoxidase reactivities in healthy individuals [conference abstract]. In: Insights to Emerging Trends in Food Science and Technology, September 14-16 2016, Singapore.

Ho XL, Loke WM (2017) Dietary plant sterols supplementation increases in vivo nitrite and nitrate production in healthy adults: a randomized, controlled study. J Food Sci 82(7):1750-1756.

Hsu CC, Kuo HC, Huang KE (2017) The effects of phytosterols extracted from Diascorea alata on the antioxidant activity, plasma lipids, and hematological profiles in Taiwanese menopausal women. Nutrients 9(12):1320.

Huang J, Xu M, Fang YJ, Lu MS, Pan ZZ, Huang WQ, Chen YM, Zhang CX (2017) Association between phytosterol intake and colorectal cancer risk: a case-control study. Br J Nutr 117(6):839-850.

Kriengsinyos W, Sumriddetchkajorn K, Yamborisut U (2011) Reduction of LDL-cholesterol in mildly hypercholesterolemic Thais with plant stanol ester-fortified soy milk. J Med Assoc Thai 94(11):1327-1336.

Laakso P (2005) Analysis of sterols from various food matrices. Eur J Lipid Sci Technol 107(6):402-410.

Lin Y, Koppenol WP, Knol D, Vermeer MA, Hiemstra H, Friedrichs S, Lütjohann D, 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(10):2319.

Matthan NR, Pencina M, LaRocque JM, Jacques PF, D'Agostino RB, Schaefer EJ, Lichtenstein AH (2009) Alterations in cholesterol absorption/synthesis markers characterize Framingham offspring study participants with CHD. J Lipid Res 50(9):1927-35.

Menéndez-Carrenõ M, Ansorena D, Astiasara´N I (2008) Stability of Sterols in Phytosterol-Enriched Milk under Different Heating Conditions. J. Agric. Food Chem., 56, 9997–10002.

Ministry of Health (2005) 2002 National Children's Nutrition Survey: National Confidentialised Unit Record File (CURF) User Document. Ministry of Health, Wellington

Ministry of Health (2011a) Methodology report for the 2008/09 New Zealand Adult Nutrition Survey. Ministry of Health, Wellington

Ministry of Health (2011b) A focus on nutrition: Key findings of the 2008/09 New Zealand Adult Nutrition Survey. Ministry of Health, Wellington

Nunes VS, Cazita PM, Catanozi S, Nakandakare ER, Quintão ECR (2019) Phytosterol containing diet increases plasma and whole body concentration of phytosterols in apoE-KO but not in LDLR-KO mice. J Bioenerg Miomembr 51(2):131-136.

Oliveira Godoy Ilha A, Sutti Nunes V, Silva Afonso M, Regina Nakandakare E, da Silva Ferreira G, de Paula Assis Bombo R, Rodrigues Giorgi R, Marcondes Machado R, Carlos Rocha Quintão E, Lottenberg AM (2020) Phytosterols supplementation reduces endothelin-1 plasma concentration in moderately hypercholesterolemic individuals independently of their

cholesterol-lowering properties. Nutrients 12(5)*:*1507.

Palmeiro-Silva YK, Aravena RI, Ossio L, Parro Fluxa J (2020) Effects of daily consumption of an aqueous dispersion of free-phytosterols nanoparticles on individuals with metabolic syndrome: a randomised, double-blind, placebo-controlled clinical trial. Nutrients 12(8):2392.

Plat J, Strandberg TE, Gylling H (2021) Intestinal cholesterol and phytosterol absorption and the risk of coronary artery disease. Eur Heart J 42(3):281-282.

Ras RT, Hiemstra H, Lin Y, Vermeer MA, Duchateau GSMJ, Trautwein EA (2013) Consumption of plant sterol-enriched foods and effects on plasma plant sterol concentrations - a meta-analysis of randomized controlled studies. Atherosclerosis 230(2):336–346.

Ras RT, van der Schouw YT, Trautwein EA, Sioen I, Dalmeijer GW, Zock PL, Beulens JWJ (2015) Intake of phytosterols from natural sources and risk of cardiovascular disease in the European Prospective Investigation into Cancer and Nutrition-the Netherlands (EPIC-NL) population. Eur J Prev Cardiol 22(8):1067-1075.

Reaver A, Hewlings S, Westerman K, Blander G, Schmeller T, Heer M, Rein D (2019) A randomized, placebo-controlled, double-blind crossover study to assess a unique phytosterol ester formulation in lowering LDL cholesterol utilizing a novel virtual tracking tool. Nutrients 11(9):2108.

Rideout TC, Chan YM, Harding SV, Jones PJ (2009) Low and moderate-fat plant sterol fortified soymilk in modulation of plasma lipids and cholesterol kinetics in subjects with normal to high cholesterol concentrations: report on two randomized crossover studies. Lipids Health Dis 8:45.

Soupas L (2006) Oxidative stability of phytosterols in food models and foods (academic dissertation), University of Helsinki, Helsinki, ISBN 952-10-3423-8.

Tavares AKMM, Ribas SA, Paravidino VB, Sgambato MR, Rodrigues RDRM, da Rocha CMM, Sichieri R, Cunha DB (2021) Effect of phytosterol capsule supplementation associated with the National Cholesterol Education Program Step 2 diet on low-density lipoprotein in children and adolescents with dyslipidemia: a double-blind crossover trial. Nutrition 82:111051.

Trautwein EA, Vermeer MA, Hiemstra H, Ras RT (2018) LDL-cholesterol lowering of plant sterols and stanols-which factors influence their efficacy? Nutrients 10(9):1262.

Vaikousi H, Lazaridou A, Biliaderis CG, Zawistowski J (2007) Phase transitions, solubility, and crystallization kinetics of phytosterols and phytosterol−oil blends. J Agric Food Chem 55(5):1790-1798.

Weidner C, Krempf M, Bard JM, Cazaubiel M, Bell D (2008) Cholesterol lowering effect of a soy drink enriched with plant sterols in a French population with moderate hypercholesterolemia. Lipids Health Dis 7:35.

Weiner K, Will C (2015) Materiality matters: blurred boundaries and the domestication of functional foods. BioSocieties 10(2):194–212.

Appendix 1: Impact on blood cholesterol and other health outcomes: study summaries

First author (year), study design, randomisation, blinding, trial duration	Intervention (I) and comparator (C) conditions	Sample	Outcome: total- and LDL-cholesterol	Outcomes: HDL-cholesterol, triglycerides, and other outcomes.	Other
Weidner (2008)[1] Parallel Randomised (unclear method) Double-blind (participants and personnel) 2 week run-in, followed by 8 week trial.	I: 200 mL soy drink once each morning with added plant sterols (2.6 g sterol esters/day, equivalent to 1.6 g free sterols/day). C: same drink as I but without added plant sterols. I+C: dietary recommendations during run-in and trial to avoid plant sterol enriched foods.	50 French participants with untreated moderate hypercholesterolaemia. 49 completed trial (38% male, 19-65 y). Intention to treat analysis used.	Significant reduction in total and LDL-c (I vs. C over time). LDL-c (mmol/L, mean ± SD): C (B/L): 4.10 ± 0.64 C (F/U): 4.09 ± 0.73 I (B/L): 4.21 ± 0.64 I (F/U): 3.92 ± 0.61 LDL-c (change): C: 0.84% I: -6.76% or -0.29 mmol/L C vs. I: *P*<0.05 Total-c (mmol/L, mean ± SD): C (B/L): 6.24 ± 0.77 C (F/U): 6.16 ± 0.79 I (B/L): 6.42 ± 0.68 I (F/U): 6.16 ± 0.79 Total-c (change): C: -1.05% I: -3.91% or -0.26 mmol/L C vs. I: *P*<0.05	HDL-c (change over time, I vs. C): NSD. Triglycerides (change over time, I vs. C): NSD. Other: see section 3.2.2.	High risk of reporting bias: dietary intake data not reported. Compliance recorded by counting empty packets. Compliance was >96%. Dietary intake recorded by food survey at baseline and of intervention (but not reported). Power calculation for LDL-c performed. Funding source unclear.
Kriengsinyos	I: habitual diet plus one	120 Thai participants	Significant decrease in total	HDL-c (change over	Compliance (method of

(2011) Parallel Randomised (method not specified) Double-blind 6 weeks	daily dose (postprandially, within 30 minutes after lunch or dinner) of plant stanol esters in a strawberry flavoured soymilk (2 g stanol esters/day). C: habitual diet plus same drink as I but without added plant stanol esters.	with untreated mild hypercholesterolemia. 118 completed the trial (C: n=58 and I: n=60): 32% male, age (C; mean ± SD): 40.1 ± 8.4 y.	and LDL-c (%; I vs. C over time): I decreased, *P*<0.05. LDL-c (mean change, %): C: -4.6 I: -13.5 Total-c (mean change, %): C: 0.6 I: -8.2 LDL-c (mmol/L, mean ± SD): C (B/L): 4.16 ± 0.70 C (F/U): 3.97 ± 0.70 I (B/L): 4.16 ± 0.71 I (F/U): 3.60 ± 0.74 C (B/L vs. F/U): *P*<0.05 I (B/L vs. F/U): *P*<0.05 (Note: no between-groups *P* values for statistical difference were provided.) Total-c (mmol/L, mean ± SD): C (B/L): 6.30 ± 0.80 C (F/U): 6.26 ± 0.72 I (B/L): 6.33 ± 0.87 I (F/U): 5.81 ± 0.85 C (B/L vs. F/U): NSD I (B/L vs. F/U): *P*<0.05 (Note: no between-groups *P* value for statistical difference were provided.)	time, I vs. C): NSD. Triglycerides (change over time, I vs. C): NSD. Serum retinol (adjusted for total cholesterol) (change over time, I vs. C): I increased, *P*<0.05. Serum α-tocopherol and lycopene (unadjusted), and β-cryptoxanthin and β-carotene (unadjusted and adjusted for total cholesterol) (change over time, I vs. C): I decreased, *P*<0.05. Serum α-tocopherol (unadjusted): I (change from B/L to F/U): -3.85 ± 5.84 C (change from B/L to F/U): -0.20 ± 4.50 I vs. C: *P*<0.05. Serum lycopene (unadjusted): I (change from B/L to F/U): -0.03 ± 0.09 C (change from B/L to F/U): 0.03 ± 0.15 I vs. C: *P*<0.05. β-cryptoxanthin	recording not specified). Compliance was good (C: 98.7% and I: 99.1%). Dietary intake recorded before and during (biweekly) via 4-day food records. The sample size was determined to be n=50 per arm. This was based on a different study which found “a mean reduction of 10% of LDL-c with plant sterols with α error at the 5% level and β error 0.20.” Industry funded study.

				(unadjusted): I (change from B/L to F/U): -0.10 ± 0.59 C (change from B/L to F/U): 0.20 ± 0.38 I vs. C: *P*<0.05. β-carotene (unadjusted): I (change from B/L to F/U): -0.11 ± 0.22 C (change from B/L to F/U): 0.09 ± 0.31 I vs. C: *P*<0.05. β-cryptoxanthin (adjusted for total cholesterol): I (change from B/L to F/U): -0.007 ± 0.09 C (change from B/L to F/U): 0.03 ± 0.06 I vs. C: *P*<0.05. β-carotene (adjusted for total cholesterol): I (change from B/L to F/U): -0.01 ± 0.03 C (change from B/L to F/U): 0.02 ± 0.05 I vs. C: *P*<0.05. Serum retinol (unadjusted), Y-tocopherol, lutein + zeaxanthin, and α-carotene (unadjusted and adjusted), and α-tocopherol and	

				lycopene (adjusted) (change over time, I vs. C): NSD. Other outcomes: see section 3.2.2.	
Hallikainen (2013)[1] Parallel Randomised (by independent statistician) Double-blind (participants and personnel) 2 week run-in, followed by 4 week trial.	I: regular diet plus one daily dose of plant stanol esters in a soy-based mini-drink after lunch or dinner (2.8 g plant stanols in test drink; mean of 2.7 g stanol consumed per day, taking into account compliance). C: regular diet plus same drink as I but without added plant stanols.	61 Swedish participants with untreated mild to moderate hypercholesterolaemia. 56 completed trial (20% male, 30-66 y). C: n=29; I: n=27 (lipid data reported for 26).	Significant decrease in total and LDL-c (mmol/L; I vs. C over time): I decreased, *P*<0.001. LDL-c (mmol/L, mean ± SE): C (B/L): 4.44 ± 0.16 C (F/U): 4.54 ± 0.17 I (B/L): 4.35 ± 0.18 I (F/U): 3.94 ± 0.13 LDL-c (relative change[2], %), I: -8.5, -11.1[3]. LDL-c (relative change, %) I vs. C over time): *P*<0.05. Total-c (mmol/L, mean ± SE): C (B/L): 6.54 ± 0.16 C (F/U): 6.64 ± 0.18 I (B/L): 6.49 ± 0.20 I (F/U): 6.03 ± 0.14 Total-c (relative change[2], %), I: -6.4, -8.0[3].	HDL-c (change over time, I vs. C): NSD. Triglycerides (change over time, I vs. C): NSD. Other: see section 3.2.2.	Compliance recorded by daily study diary. Dietary intake recorded by 2x 3 day food records. Industry funded study.
Dong (2016) Parallel Randomised ("randomly assigned to [I or	I: usual dietary habits plus 3.4 g phytostanol ester-enriched soy milk powder (2 g/day free phytosterols in 30 g soy milk powder).	Chinese participants with untreated mild or moderate hyperlipidaemia. 170 (25% male; age (C; mean ± SD): 61.7	LDL-c (mmol/L, mean ± SD): C (B/L): 3.04 ± 0.61 C (F/U): 3.33 ± 0.76 I (B/L) 3.18 ± 0.59 I (F/U): 2.95 ± 0.78 Treatment effect (I vs. C):	HDL-c: I (B/L): 1.48 ± 0.49 I (F/U): 1.33 ± 0.30 C (B/L): 1.40 ± 0.34 C (F/U): 1.30 ± 0.25 Change over time, I vs. C: I decreased,	The sample size was determined to be n=78 per arm but the outcome this was based on is not stated. Industry funded study.

C] according to the baseline serum TC levels") Double-blind 6 months	C: usual dietary habits plus same drink as I but without added phytostanol esters. I+C: timing of soy milk consumption or details regarding single or split dose were not provided.	± 6.9 y) were randomised but 137 (C: n=68 and I: n=69) completed the study. Unclear what sample size was analysed but appears to be n=137 (no intention to treat analysis).	*P*=0.036 (significant decrease in LDL-c in I compared to C). Total-c (mmol/L, mean ± SD): C (B/L): 5.96 ± 0.80 C (F/U): 5.92 ± 1.04 I (B/L): 5.95 ± 0.67 I (F/U): 5.37 ± 0.92 Treatment effect (I vs. C): *P*=0.011 (significant decrease in total-c in I compared to C).	*P*=0.0001. Triglycerides (change over time, I vs. C): NSD.	Measurement of compliance or diet: not stated.
Ho (2016a & 2017) Crossover Randomised (method not stated) Double-blind 1 week run-in, followed by 4 week trial. Washout: 1 week.	I: usual dietary habits plus soy milk powder (20 g) dissolved in 300 mL of warm water per day, containing 2.0 g free plant sterols equivalent of their palmitates (BASF Vegapure® 67WDP [palmityl esters of β-sitosterol, max 55% (w/w); campesterol, max 29% (w/w) and stigmasterol, max 23% (w/w)]). C: usual dietary habits plus soy milk powder manufactured by Medispec (M) Sdn Bhd (Malaysia). The nutritional compositions of the I	18 Singaporean healthy and normocholesterolaemic participants. The sample size analysed varied by outcome (33% male, age (mean ± SD): 35.3 ± 9.5 y).	LDL-c (mmol/L, mean ± SD): C (B/L): 1.50 ± 0.45 C (F/U): 1.62 ± 0.41 I (B/L): 1.73 ± 0.59 I (F/U): 1.74 ± 0.54 I vs. C after B/L adjustment: NSD. Total-c (mmol/L, median (25% and 75% percentiles)): C (B/L): 4.79 (3.98, 5.42) C (F/U): 4.67 (4.01, 5.23) I (B/L): 4.77 (0.31, 0.46) I (F/U): 4.44 (3.92, 5.20) I vs. C after B/L adjustment: NSD.	HDL-C (I vs. C after B/L adjustment): NSD. Triglycerides (I vs. C after B/L adjustment): NSD. Remaining outcomes.[4]	Compliance: "participants were told to return the remaining intervention material at the ends of the two crossover arms. Only results from the participants who met the minimum intake compliance of 80% were analyzed. Compliance to treatment was also verified with the change in the circulating concentrations of the total plant sterols." Power calculation for the outcome, leukotriene B_4, performed. Industry funded study. Measurement of compliance or diet: not

	and C powders were analysed by a commercial food laboratory and were found to be comparable with the exception of phytosterol contents.				stated.
Chau (2020) Parallel Randomised (block randomisation to balance the study groups' size, sex and age, via a web-based randomisation system) Double-blind (investigators and participants) 3 weeks	I: usual dietary habits plus one daily dose (during their main meal) of 250 mL of a phytosterol-enriched soya drink (2 g phytosterol/day; Vitasoy Calci-Plus Hi-calcium plant sterol soya milk). Phytosterols were mainly sterol esters (>90%) and the free sterols included were mainly β-sitosterol, campesterol, and stigmasterol. C: usual dietary habits plus same drink as I but without added phytosterols. Note: the nutrient profiles of the two drinks were the same except higher total fat in phytosterols-enriched soya drink (2.8 g/100 mL) compared with the	Chinese normocholesterolaemic participants, who were not on regular medication known to interfere with lipid profiles. 201 were randomised but 159 were analysed (C: n=77 and I: n=82; 53% male; aged 19-79 y; age (C; mean ± SD): 42 ± 17 y). Efficacy analysis was performed on both intention to treat (n=159) and per protocol (n=156; participants with 80% compliance) samples.	Change in LDL-c (mmol/L, least square mean change, 95% CI): C: -0.05 (-0.13, 0.03) I: -0.18 (-0.26, -0.09) Treatment effect (I vs. C): -0.12 (-0.23, 0.00) and *P*=0.048 LDL-c (mmol/L, least square mean, SE): I (B/L): 2.80 (0.08) I (F/U): 2.62 (0.08) C (B/L): 2.69 (0.08) C (F/U): 2.64 (0.09) Change in total-c (mmol/L, least square mean change, 95% CI): C: -0.06 (-0.16, 0.04) I: -0.12 (-0.22, -0.03) Treatment effect (I vs. C): -0.10 (-0.24, 0.03) and *P*=0.141 Results are based on intention to treat analysis.	HDL-c, treatment effect: NSD. Triglycerides, treatment effect: NSD. Creatinine, fasting glucose, body temperature, weight, body mass index, waist circumference, and hip circumference: treatment effect, NSD. Other: see section 3.2.2.	Compliance was measured by participants' recording product consumption and the return of unused packs returned to investigators. Compliance was good (C: 97.7% and I: 97.9%). "ANCOVA was used to estimate the sample size with baseline serum LDL-c level as the independent covariate, assuming a power of 0.8 according to a previous study, an attrition rate of 25% and a maximum tolerable false positive rate of 5%." Food consumption not recorded. Industry funded study.

	placebo (1.2 g/100 mL).				
Oliveira Godoy Ilha (2020) Crossover Randomised (simple random sampling performed by a statistician) Double-blind 3 week run-in, followed by 4 week trial. Washout: not stated. Treatment order effects: not stated.	I: normocaloric diet based on the NCEP-ATPIII[5] recommendation plus 400 mL/day of soy milk with 1.6 g phytosterols/day (represented as β-sitosterol-ester (78%), sitostanol-ester (13%), campesterol-ester (5.3%), and campestanol-ester (0.5%)). Drink (a 200 mL portion) was consumed twice a day, during lunch and dinner. C: normocaloric diet based on the NCEP-ATPIII recommendation plus plus same drink as I but without added phytosterols.	40 Brazilians with untreated moderate hypercholesterolaemia. n=38 were analysed (18% male, age (mean ± SD): 58 ± 12 y).	LDL-c at F/U (mg/dL; mean ± SE): I: 169 ± 5.2 C: 183 ± 5.9 I vs. C: *P*=0.001 Total-c at F/U (mg/dL; mean ± SE) I: 244 ± 5.8 C: 261 ± 7.1 I vs. C: *P*<0.001	HDL-c at F/U (mg/dL; mean ± SE), I vs. C: NSD. Triglycerides at F/U (mg/dL; n=38; mean ± SE): I: 133 ± 7 C: 154 ± 10 I vs. C: *P*=0.008. Weight, body mass index, fibrinogen, high-sensitivity C-reactive protein, serum amyloid, interleukin-6, tumor necrosis factor-α, vascular cell adhesion molecule-1: NSD. Plasma endothelin-1 at F/U (pg/mL; n=24; mean ± SE): I: 1.13 ± 0.09 C: 1.31 ± 0.09 I vs. C: *P*=0.02.	Industry funded study. Sample size calculation: not stated. Adherence to the prescribed diet and estimation of food intake was conducted by a registered dietitian using a 24 h recall. Frequency and further details of recalls: not provided. Results of recalls: not provided. Compliance was not recorded (but authors note that higher plasma concentrations of campesterol and β-sitosterol in I vs. C).

C, comparator arm; I, intervention arm; n/a, not available; y, years; LDL-c, low density lipoprotein cholesterol; B/L, baseline; F/U, follow-up; NSD, not statistically different (*P*≥0.05); 95% CI, 95% confidence interval; SE, standard error.

[1]Information in the Table was taken from FSANZ (2014), except outcome data which were sourced directly from the publications.

[2]Relative change was usually reported as change from baseline, not the difference in change between intervention and placebo groups, except where indicated.

[3]Change adjusted for difference in control group.

[4](Non-)significant differences of several outcomes reported by Ho et al. (2016a) and Ho et al. (2017) are not reported in Table 3, as their role as an adverse physiological effect is unclear (see section 4.2).

[5]NCEP-ATPIII, the US National Cholesterol Education Program Adult Treatment Panel III.

Appendix 2: Concentration data and classification systems used in the dietary exposure assessment

Table A2.1 Classification names and codes and concentrations used in the dietary exposure assessment

Food and use details			Harvest	
Existing Permissions – Food Standards Code				
Food category	**Standard**	**MPL**	**Classification Name**	**Code**
Yoghurt	Section 2.5.3-5	1.0 g/200 g package (5 g/kg)	Ferm & renn milk, unflav, low/skim	1.2.1.4
			Ferm & renn milk prod, flavoured, low/skim	1.2.2.4
Milk	Section 2.5.1-6	4 g/L	Liquid milk, phytosterol esters (PSE)	1.1.2.5
			Liquid milk products and flavoured liquid milk, reduced fat, unflavoured, phytosterols	1.1.2.3.1.1
Edible oil spread (including margarine)	Section 2.4.2-2	82 g/kg	Edible oil spread, standard fat (60-80%), regular salt, phytosterols	2.2.2.1.1.4.1
			Edible oil spread, standard fat (60-80%), reduced salt, phytosterols	2.2.2.1.1.5.1
			Edible oil spread, reduced fat (<60%), reduced salt, phytosterols	2.2.2.1.2.5.1
Cheese	Section 2.5.4-4	90 g/kg*	Unripened cheese, red fat	1.6.1.2
			Unripened cheese, low fat (<=3%)	1.6.1.3
			Ripened cheese, red/low fat (<15%)	1.6.2.2
			Processed cheese, whole fat (=>15%), PSE added	1.6.3.1.2
			Processed cheese, red/low fat (<15%)	1.6.3.2
Breakfast cereals	n/a	61 g/kg	Breakfast biscuits	6.3.5
			Breakfast cereals, ready to eat, biscuit type	20.2.2.5.1.4
		44 g/kg	Breakfast flakes	6.3.6
			Breakfast cereals, ready to eat, flaky type	20.2.2.5.1.3
		49 g/kg	Oats, raw	6.1.1.1
			Breakfast cereals, porridge, dry mix	20.2.2.6.1
		7 g/kg	Oats, cooked	6.1.1.2
			Breakfast cereals, porridge	20.2.2.6
Requested use as included in the application				
Plant-based milk alternatives	MPL proposed in this application	2.2 g/ 250 mL serving (9 g/kg)	Nut- or seed-based beverages	14.1.9
			Cereal beverages	14.1.8
			Soy beverage	14.1.7

Abbreviations: PSE = phytosterols esters, ferm = fermented, renn = rennetted, prod= products, unflav = unflavoured, red=reduced, MPL= maximum permitted level (concentration used in DEA estimation if converted from a per serve to a per kg basis); na = not applicable.

* The concentration of 90 g/kg is the MPL expressed as tall oil phytosterol esters, and is the concentration used in the dietary exposure assessment. This would correspond to 54 g/kg of total phytosterol equivalents so therefore what is used in the assessment is a worst case scenario.

Appendix 3: Estimated baseline mean and P90 consumption of foods containing added plant sterols and plant sterol dietary exposures for Australian and New Zealand consumers for individual foods

Table A3.1 Estimated baseline mean and P90 consumption of foods containing added plant sterols, and plant sterol dietary exposures for Australian consumers (two day average)*

Country	Age group	Food	Estimated consumption of foods containing added plant sterols (g/day)		Estimated plant sterol dietary exposure (g/day)	
			Mean	P90	Mean	P90
Australia	2 years and above	Breakfast cereals, uncooked (includes raw oats, breakfast biscuits, muesli, and flake type cereals)	42	83	2.1	4.2
		Breakfast cereals, cooked (includes porridge and other cooked cereals)	168	338	1.3	2.5
		Edible oil spread (including margarine), containing added plant sterols	7	14	0.6	1.2
		Milk, containing added plant sterols	91	187	0.4	0.7
		Low fat/skim yoghurt	66	150	0.3	0.8
		Reduced/low fat cheese and processed cheese	22	45	2.0	4.0
		Plant-based milk alternatives$^{\Delta}$	133	320	1.2	2.8

*2011-12 Australian National Nutrition and Physical Activity Survey. Based on consumption data from respondents with two days of data only.

$^{\Delta}$ Estimated food consumption and dietary exposure for the *plant-based milk alternatives only* scenario.

Table A3.2 Estimated baseline mean and P90 consumption of foods containing added plant sterols, and plant sterol dietary exposures for Australian consumers (day 1 only)*

Country	Age group	Food	Estimated consumption of foods containing added plant sterols (g/day)		Estimated plant sterol dietary exposure (g/day)	
			Mean	P90	Mean	P90
Australia	2 years and above	Breakfast cereals, uncooked (includes raw oats, breakfast biscuits, muesli, and flake type cereals)	55	104	2.8	5.2
		Breakfast cereals, cooked (includes porridge and other cooked cereals)	257	468	1.9	3.5
		Edible oil spread (including margarine), containing added plant sterols	11	24	0.9	1.9
		Milk, containing added plant sterols	163	374	0.7	1.5
		Low fat/skim yoghurt	111	200	0.6	1.0
		Reduced/low fat cheese and processed cheese	39	96	3.5 (2.1**)	8.6 (5.1**)
		Plant-based milk alternatives$^{\Delta}$	217	478	1.9	4.2

*2011-12 Australian National Nutrition and Physical Activity Survey. Based on consumption data from respondents with on day 1 consumption data from all respondents.

$^{\Delta}$ Estimated food consumption and dietary exposure for the *plant-based milk alternatives only* scenario.

** Exposure deterministically estimated using the concentration of 54 g/kg plant sterol equivalents.

Table A3.3 Estimated baseline mean and P90 consumption at baseline of foods containing added plant sterols and plant sterol dietary exposures for New Zealand consumers (day 1 only)*

Country	Age group	Food	Estimated consumption of foods containing added plant sterols (g/day)		Estimated plant sterol dietary exposure (g/day)	
			Mean	P90	Mean	P90
New Zealand*	5-14 years	Breakfast cereals, uncooked (includes raw oats, breakfast biscuits, muesli, and flake type cereals)	43	77	2.3	4.1
		Breakfast cereals, cooked (includes porridge and other cooked cereals)	272	424	2.0	3.2
		Edible oil spread (including margarine), containing added plant sterols	NR	NR	NR	NR
		Milk, containing added plant sterols	NC	NC	NC	NC
		Low fat/skim yoghurt	149	173	0.7	0.9
		Reduced/low fat cheese and processed cheese	33	79	3.0 (1.7**)	7.1 (4.3**)
		Plant-based milk alternatives$^{\Delta}$	242	504	2.1	4.4
New Zealand*	15 years and above	Breakfast cereals, uncooked (includes raw oats, breakfast biscuits, muesli, and flake type cereals)	53	101	2.7	5.0
		Breakfast cereals, cooked (includes porridge and other cooked cereals)	266	390	2.0	2.9
		Edible oil spread (including margarine), containing added plant sterols	15	30	1.2	2.5
		Milk, containing added plant sterols	NC	NC	NC	NC
		Low fat/skim yoghurt	117	202	0.6	1.0
		Reduced/low fat cheese and processed cheese	20	40	1.8 (1.1**)	3.6 (2.2**)
		Plant-based milk alternatives$^{\Delta}$	225	510	2.0	4.5

*2002 New Zealand National Children's Nutrition Survey and the 2008/09 New Zealand Adult Nutrition Survey. Based on day 1 consumption data from all respondents.
$^{\Delta}$ Estimated food consumption and dietary exposure for the *plant-based milk alternatives only* scenario.
NR – results not reported as <10 consumers.
NC – food not reported as consumed in the national nutrition survey.
** Exposure deterministically estimated using the concentration of 54 g/kg plant sterol equivalents.