

Supporting Document 1

RISK ASSESSMENT REPORT

APPLICATION A1024 EQUIVALENCE OF PLANT STEROLS, STANOLS AND THEIR FATTY ACID ESTERS

Risk Assessment Summary

The *Australia New Zealand Food Standards Code* (the Code) currently permits the addition of particular plant sterols to one or more of the following foods: edible oil spreads; low-fat milk; low-fat yoghurt and some breakfast cereals. In this assessment, the collective term 'plant sterols' is used for preparations that include phytosterols, phytostanols and their esterified forms; however more specific terms are used as appropriate.

This Application seeks approval of a generic permission and specifications for phytosterols, phytostanols and their corresponding esters as a replacement for the many specific permissions and specifications that were developed over time in response to previous applications. The requested generic permission encompasses plant sterols derived from any source, either in the free or esterified form. The originally requested specifications are those published by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 2008 and subsequently adopted by reference in the Code in 2009. Following consideration of submissions to the A1024 Assessment Report, these specifications are proposed to be modified to set a minimum requirement for 4-desmethyl sterols and to increase solvent residue limits. The Application does not seek to expand the range of plant-sterol-fortified foods currently approved.

This risk assessment has considered the technological suitability, safety, efficacy to lower LDL-cholesterol, and market impact of a potential permission in the Code for the addition of all plant sterols that conform to the modified JECFA specifications to the four currently approved foods.

The Risk Assessment Report provides a succinct summary of the evidence supporting the safety of plant sterols. Toxicity data, clinical studies and information from published literature supporting the safety of plant sterols have been comprehensively assessed previously by FSANZ and there was no new evidence that would change previous conclusions regarding the safety of plant sterols. FSANZ also investigated the suitability and stability of uniformly incorporating plant sterols that conform to the modified JECFA specifications into the four different food matrices. Technical issues or safety concerns arising from such addition were assessed.

FSANZ has had regard to the Ministerial Policy Guideline on Substances other than Vitamins and Minerals (2008) by considering the blood LDL-cholesterol lowering effects of commercial preparations of phytosterols, phytostanols, either free or esterified, when added to each of the four approved food matrices. Studies confirmed that consumption of around 2 g/day of plant sterols, including phytostanols and phytostanol esters not previously assessed, can be effective in lowering LDL-cholesterol levels.

Finally, the report draws substantially on previous assessments carried out by FSANZ that have estimated the cumulative dietary intake of plant sterols. The report also reviewed the literature on purchasing behaviours and consumption patterns, as well as considered market share and product substitution issues. No additional dietary intake assessment was required because this Application sought no new foods to be fortified.

Uncertainties in the risk assessment

The available data for all types and forms of plant sterols that conform to the modified JECFA specifications are sufficient to provide a high level of confidence in the conclusions of this report relating to the safety and suitability for purpose of these plant sterols in the four foods previously approved. However, one uncertainty identified in the report that arose from a lack of direct evidence relates to the cholesterol lowering effect of free phytosterols and phytostanols added to breakfast cereals if such plant sterols could not be uniformly distributed throughout the food matrix.

Conclusions of the risk assessment

- The properties of the plant sterols that conform to the modified JECFA specifications and the manufacturing processes by which such plant sterols are added to the four approved foods are generally suitable to deliver plant sterols on consumption.
- Such plant sterols are likely to remain stable in foods during storage under usual conditions.
- On the basis of the available safety data, the use of plant sterols in the four approved foods at the proposed levels does not raise any food safety concerns.
- Reduced carotenoid uptake associated with consumption of plant sterols is not a nutritional concern in adults as serum carotenoid levels fluctuate normally according to a number of dietary factors and environmental variables. It is not considered to be of nutritional significance and is partially compensated by additional fruits and vegetables in the diet.
- Consumption of plant sterols is not associated with any increase in cardiovascular disease risk.
- Consumption of the four approved foods containing plant sterols that conform to the modified JECFA specifications can potentially lower LDL-cholesterol levels in the blood.
- A small proportion of children is likely to consume plant-sterol-fortified foods, however this is not considered to raise a health concern.
- Broadening the specification and the associated permissions to include a wider variety of plant sterol preparations to the same foods does not change existing estimates of dietary intake.

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1. INTRODUCTION

The *Australia New Zealand Food Standards Code* (the Code) currently permits the addition of particular plant sterols as novel food ingredients to one or more of the following foods: edible oil spreads; low-fat milk; low-fat yoghurt and some breakfast cereals. To date, plant sterols derived from either a vegetable oil or tall oil source are permitted, each with its own set of specifications. Plant sterols esterified with fatty acids derived from vegetable oils are also permitted for addition to some of the above foods.

This Application seeks approval of a generic permission and specifications for phytosterols, phytostanols and their corresponding esters as a replacement for the many specific permissions and specifications that were developed over time in response to previous applications. The requested generic permission encompasses plant sterols derived from any source, either in the free or esterified form. The Application does not seek to expand the range of plant-sterol-fortified foods currently approved.

The Application originally requested that the specifications published by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) be incorporated into the Code. Through a separate process, independent of this Application, the JECFA specifications were adopted into the Code in August 2009. Submissions to this Application sought amendments to these specifications to clarify chemical composition of the plant sterols and to lessen FSANZ's proposed increase in solvent residue limits. Since FSANZ had proposed additional conditions to the JECFA specifications in the Assessment Report, this Risk Assessment Report at Approval stage refers to *modified* JECFA specifications.

The recent Ministerial Policy Guideline on Substances Other Than Vitamins and Minerals (2008) is relevant to novel food ingredients. Current permissions were approved prior to receipt of this Guideline to which FSANZ must now have regard.

1.1 Objective of the assessment

In proposing to adopt a generic permission for phytosterols, phytostanols and their esters that conform to the modified JECFA specifications and that is applicable to all currently approved foods, the objectives of this risk assessment are to:

- assess the risk to public health and safety; and
- assess whether the relevant plant sterols lower blood LDL-cholesterol when consumed in each of the four approved foods.

1.2 Terminology

In this assessment, the collective term 'plant sterols' is used for preparations that include phytosterols, phytostanols and their esterified forms; however biological source is not necessarily specified. The following terms are used in this report:

Plant sterols	Collective term referring to free and esterified phytosterols and phytostanols, regardless of biological source.
Plant sterol equivalents	The total free (non-esterified) phytosterol and phytostanol content of the product/preparation/commercial mixture.
Phytosterols	Free (non-esterified) steroid alcohols occurring in plants, e.g. β -sitosterol, campesterol, stigmasterol.
Phytostanols	Free (non-esterified) fully saturated steroid alcohols occurring in plants or converted from phytosterols by catalytic hydrogenation e.g. sitostanol, campestanol.
Phytosterol esters	Phytosterols esterified with fatty acids derived from vegetable oils.
Phytostanol esters	Phytostanols esterified with fatty acids derived from vegetable oils.
Type	Refers to phytosterol or phytostanol chemical structure.
Form	Refers to free or esterified chemical structure.

1.3 Amendments to Risk Assessment Report at approval stage

This Risk Assessment Report at Approval stage has undergone small changes and minor edits since its release at Assessment stage. These amendments, listed below, clarify issues raised through submissions.

Report Section	Amendment
Throughout	Where relevant, reference to JECFA specifications were amended to <i>modified</i> JECFA specifications in light of additional conditions to the JECFA specifications proposed at Assessment stage
Section 1	Definition of plant sterol equivalents added
Section 2	Additional explanation of des-methyl sterols
Section 3	Simplification of question 2 related to nutritional safety
Section 6.2	Additional explanation of extraction solvent residues and 4-des-methyl sterols. Clarification of allergenicity section.
Section 7	Corrected Table 7.6
Section 8	Revision of term 'phytosterol equivalent' to 'plant sterol equivalent'

2. SOURCES, CHEMICAL AND PHYSICAL PROPERTIES OF PLANT STEROLS

Plant sterols are steroid alcohols and their esters that occur naturally in plants. Common edible vegetable oils can contain up to 0.9% plant sterols (Kochhar 1983). There are several bases on which plant sterols can be grouped.

Plant sterols can be divided on structural and biosynthetic grounds into three major classes: 4-desmethyl sterols, 4 α -methyl sterols and 4,4-dimethyl sterols. The 4-desmethyl sterols are the main components of plant sterols extracted from vegetable oil. The 4,4-dimethyl sterols

and 4 α -methyl sterols are plant sterol precursors and exist at lower levels than the terminal products 4-desmethyl sterols (Piironen *et al*, 2000).

Plant sterols also comprise two broad groups: phytosterols and phytostanols in which phytostanols are the saturated (hydrogenated) forms of phytosterols. Phytosterols are more abundant in nature than phytostanols; esterified forms of these two groups also occur naturally but in very small amounts. There have been more than 100 chemically distinct plant sterols reported. The most common are sitosterol, campesterol and stigmasterol (Fernandes and Cabral, 2007), whereas others in smaller proportions are brassicasterols and avenasterols (Brufau *et al*, 2008).

Because phytosterol and phytostanol esters consist of up to 45% fatty acids, this has to be taken into account when considering the amount of plant sterols added to meet the stated level of phytosterols and phytostanols in the final product.

2.1 Sources

Commercially, phytosterols and phytostanols are isolated from tall oil (a by-product of the manufacture of wood pulp) or vegetable oils such as soybean oil, rapeseed oil, sunflower oil or corn oil (JECFA, 2008). The different source oils have quantitative variations in their composition of the individual plant sterols depending on the variety of the crop, season, processing and supplier (EFSA, 2003). Thus each commercial preparation of plant sterols has its own typical composition. Although commercial preparations comprise varying proportions of individual sterols, they are generally referred to as phytosterols or phytostanols depending on the predominant type. The most abundant components of phytosterols are sitosterol, campesterol, stigmasterol and in certain raw material sources, brassicasterol. Phytosterols are monounsaturated compounds that can be converted to their corresponding phytostanols by a catalytic saturation (hydrogenation) process. During the catalytic saturation step, phytosterols with 29 carbon atoms (β -sitosterol, stigmasterol, avenasterols etc) are converted to sitostanol and all phytosterols with 28 carbon atoms (campesterol and brassicasterol) to campestanol (Raisio Nutrition Ltd, 2009). Table 2.1 shows the typical variation in plant sterol preparations from the different oil sources. The ranges are indicative rather than definitive. Currently the Code allows the phytostanol content of permitted vegetable oil phytosterol preparations to be as high as 8.5%, and as high as 48% in tall oil phytosterols (see the relevant specifications in Schedule 1 of Standard 1.3.4 – Identity and Purity).

Table 2.1: Typical composition of major plant sterols in commercial preparations according to their source

Source of plant sterol preparation	β -Sitosterol	Campesterol	Stigmasterol	β -Sitostanol	Campestanol
Tall oil phytosterols	36-79%	4-25%	-	6-34%	0-14%
Vegetable oil phytosterols	42-55%	20-29%	12-23%	0-2.5%	0-6%
Tall oil phytostanols	-	-	-	~ 90%	~ 10%
Vegetable oil phytostanols	-	-	-	68-75%	25-32%

Phytosterols compiled from Schedule to Standard 1.3.4 of the Code; phytostanols from Salo *et al*. 2002 and Soupas, 2006.

From a composition study of 19 vegetable oil samples (not tall oil), sitosterol was found to be the dominant phytosterol (60%), followed by campesterol (24%), stigmasterol (7%) and

Δ^5 -avenasterol (5%). There were relatively small amounts of brassicasterol (3%), sitostanol (1%) and campestanol (<0.1%) (Normen, 2007). Figure 1 shows the proportions of the chemically distinct plant sterols in several oils.

Other minor components that could be found in phytosterol and phytostanol preparations include:

- Mono-, di- and triglycerides, which are natural constituents of edible vegetable oils
- Fatty acid methyl esters – these occur at similar low levels in other processed fats
- Steradienes – dehydrogenated by-products of processing
- Long chain aliphatic alcohols (C20-C26) – trace amount of plant wax from the leaves and fruits of edible plants, e.g. rice bran wax, sunflower wax and carnuba wax.

Further information on production of commercial preparations of plant sterols is given in Section 6.

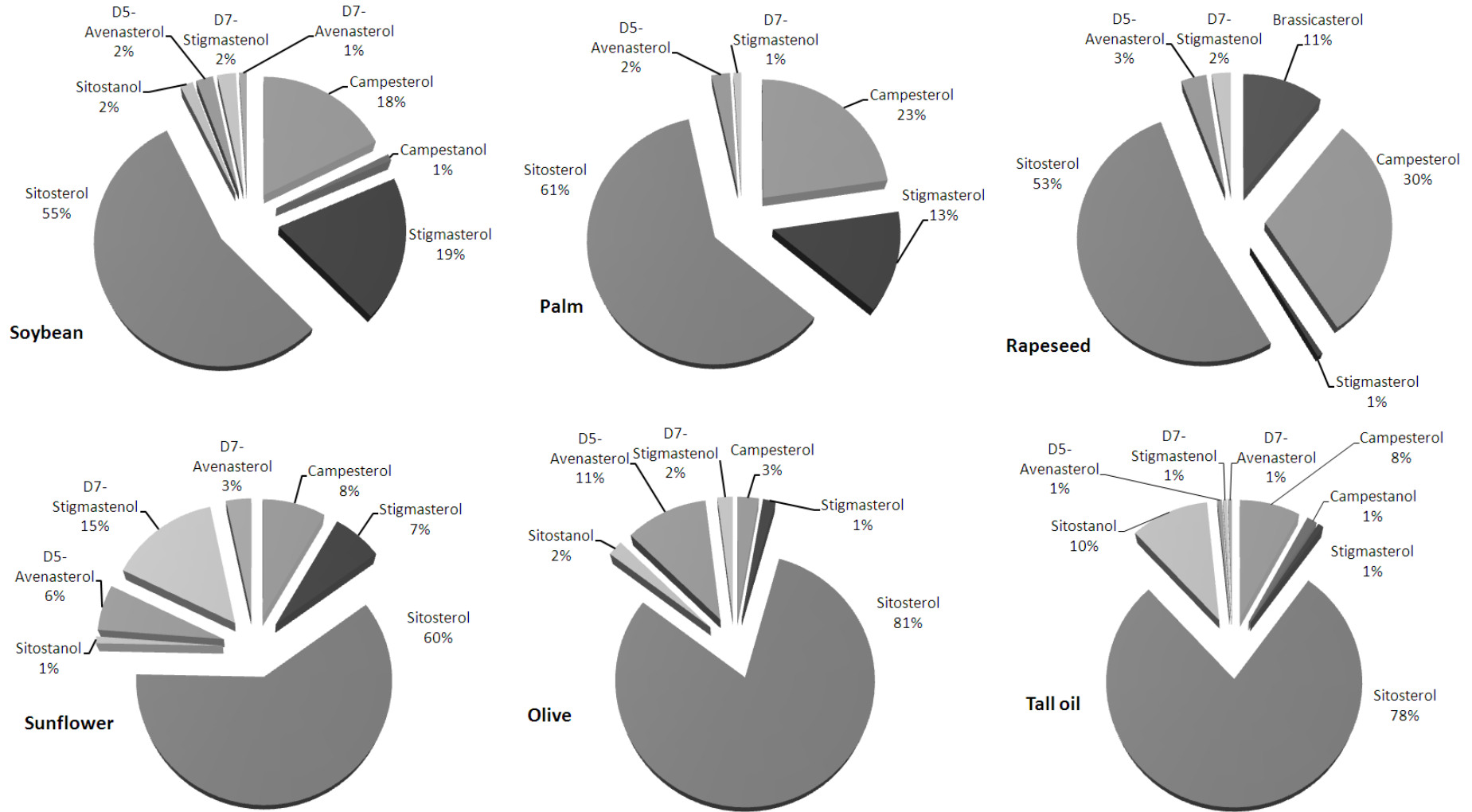


Figure 1: Plant sterol composition of selected vegetable oils and tall oil (Data source: modified from Kochhar 1983).

2.2 Chemical and physical properties

2.2.1 Chemical structures

Phytosterols and cholesterol share the same steroid skeleton (Figures 2 and 3), but differ in the structure of the side-chain at the C24 position. They both have a four-ring steroid nucleus, the 3 β -hydroxyl group and often a 5,6-double bond (Piironen *et al*, 2000). Phytosterols have roles in plants similar to that of cholesterol in mammals, e.g. stabilising phospholipid bilayers in cell membrane structures.

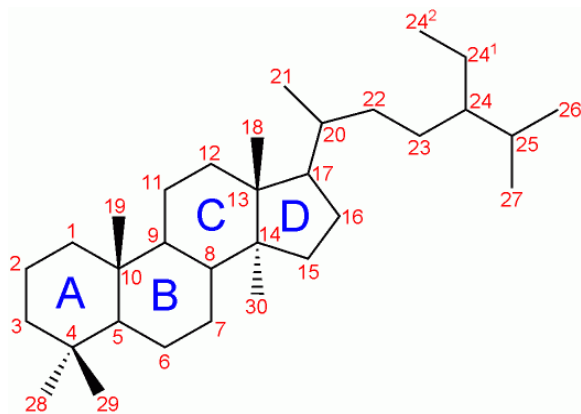


Figure 2: Steroid skeleton (JECFA 2008)

Phytosterols can be divided into three major chemical classes: 4-desmethyl sterols (no methyl group at position 4), 4 α -methyl sterols (one methyl group) and 4,4-dimethyl sterols (two methyl groups). The 4-desmethyl sterols are the main components of plant sterols extracted from vegetable oil. The 4 α -methyl sterols and 4,4-dimethyl sterols are precursors of 4-desmethyl sterols and exist at lower levels than the final products of the biosynthetic pathways (Piironen *et al.*, 2000).

The 4-desmethyl sterols may be categorised into Δ^5 -sterols, Δ^7 -sterols and $\Delta^{5,7}$ -sterols according to the position and number of double bonds in the B ring (Piironen *et al*, 2000). The most abundant phytosterols, sitosterol and campesterol, have a Δ^5 bond and an additional one-carbon or two-carbon substituent in the side chain at C-24. Phytostanols are less abundant in nature but can be produced by 5 α hydrogenation of the corresponding phytosterols (e.g. sitostanol in Figure 3 below).

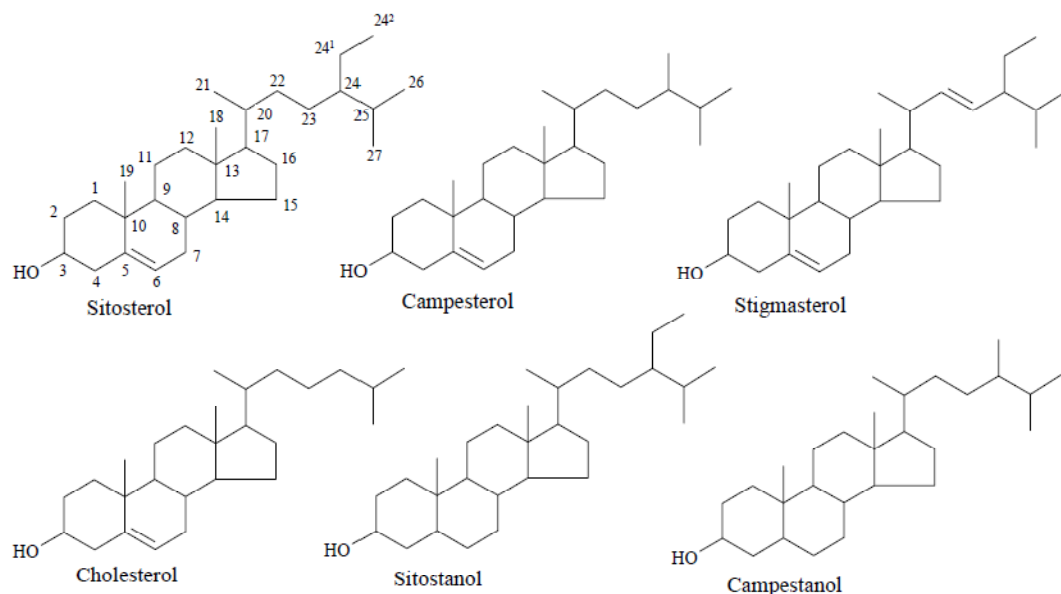


Figure 3: Examples of some plant sterols (from Soupas, 2006).

Figure 3 shows examples of structures of major plant Δ^5 -sterols with a similar sterol nucleus to cholesterol. They differ from cholesterol in:

- the additional alkyl substituents at C-24 (sitosterol, campesterol)
- an alkene group (Δ^5 -avenasterol) and/or a double bond at C-22 (stigmasterol).

An example of a phytostanol with a saturated bond between carbon 5 and 6 is sitostanol, shown in Figure 3. The 3-hydroxyl group of phytosterols/phytostanols may be esterified by a fatty acid from vegetable oils to form phytosterol/phytostanol esters, e.g. sitostanol esterified with oleic acid to form sitostanyl oleate (Figure 4).

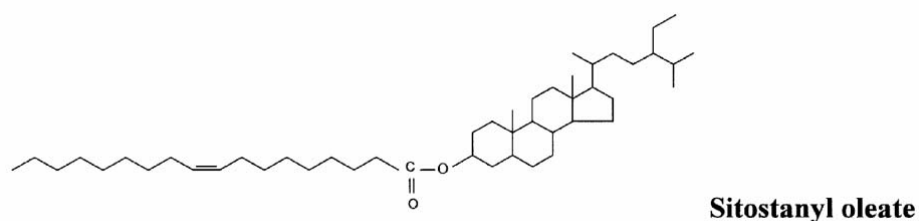


Figure 4: Example of a phytostanol ester

2.2.2 Physical properties

Phytosterols and phytostanols exist as inert crystalline structures and are solid at room temperatures. Sitosterol, campesterol and stigmasterol have melting points of 140, 157-158 and 170 °C respectively. They also have poor solubility in oil. The bigger its side chain, the more hydrophobic a sterol becomes. Thus, phytosterols with 28 or 29 carbon atoms are more hydrophobic and have lower micellar solubilities than cholesterol with 27 carbon atoms. However, free plant sterols and their esters are soluble in non-polar solvents such as hexane. Because of the high melting points of phytosterols and phytostanols, processes such as cryogenic (frozen state at temperatures below -150°C) grinding may be required to ensure an even distribution of the plant sterols in aqueous-based liquid foods.

Incorporation of phytosterols and phytostanols into some foods is difficult because of their high melting point and tendency to form insoluble crystals. This may be overcome by esterification of the phytosterols and phytostanols with polyunsaturated fatty acids, which increases their solubility.

Phytosterol and phytostanol esters exist as viscous, semi-solid and light-yellow materials. These esters have much lower melting point temperatures than their respective free forms. These esters may be melted completely at 40°C. Above 40°C the esters behave like liquid oil and it has been suggested that they can be homogenised in an aqueous-based drink to provide an even distribution.

Phytosterol and phytostanol esters are insoluble in water but soluble in non-polar solvents and vegetable fats and oils. The solubility of phytosterol and phytostanol esters increases more than 10-fold compared to that of free phytosterols and phytostanols (Engel and Schubert 2005). The esters with their increased solubility in oil allow the incorporation of phytosterols and phytostanols into various processed foods by dissolving or suspending in fat matrices (AbuMweis and Jones 2008).

2.2.3 Stability

Plant sterols are basically very stable compounds and experience only limited damage during oil processing (Ferrari et al 1997). Under specific conditions, such as high temperatures (>100 °C) and in the presence of air, some phytosterols may oxidise in the same way as cholesterol (Yanishlieva-Maslrova and Marinova 1985).

Plant sterol oxidation depends on temperature and heating time, as well as the composition of the lipid matrix. Plant sterols were reported to be more stable when added to soybean oil than in high-oleic sunflower oil during high temperature heating, which indicates that the more unsaturated matrix of soybean oil provided better protection for the plant sterols (Winkler and Warner 2008).

Phytosterols are monounsaturated compounds (double bond in the B-ring), but because of steric hindrance by their ring structure, they are much more stable than the monounsaturated fatty acids (e.g. oleic acid) (Dutta et al 1996). Therefore, even under severe conditions, such as during deep frying, sterol oxidation products are formed only in parts per million concentrations (Johnsson and Dutta, 2006). Phytostanols (as saturated compounds) are more stable under all conditions studied than unsaturated phytosterols. In addition, esterification makes the plant sterol esters more reactive than phytosterols and phytostanols at low temperatures, while at high temperatures the situation is reversed (Soupas, 2006).

Microwave and conventional heating were found to have no effect on plant sterols in edible fats (Albi et al 1997). In the study, the edible oils were heated in a microwave oven at half power for 120 min to remain at 170°C, or in an electric oven at 180°C for 120 min, or they were exposed to microwave energy for 60 min at intervals of 50s below 40°C.

It should be noted that most spreads on the market that contain added plant sterols are lower-fat spreads (< 40% fat) and they are intended for spreads and not for frying.

2.2.4 Stability during storage

Based on the stability of the plant sterols during food processing conditions, no significant changes in total sterol contents are likely to take place in most practical situations (Piironen

2000). However, after prolonged storage, some oxidation products may be found. For example, potato chips fried in cottonseed oil and stored for 150 days at 23°C contained no detectable sitosterol oxidation products but those kept at 40°C for 95 days contained oxidised products.

Phytosterols and phytostanols are not affected microbiologically through the fermentation process used to produce yoghurt and during their storage in this medium (JECFA 2008).

The esters were also demonstrated to be stable at different pHs during long term storage (over a year) (JECFA 2008).

3. KEY RISK ASSESSMENT QUESTIONS

The risk assessment questions relevant to this Application were developed under Section 18 Objectives of the *Food Standards Australia New Zealand Act 1991*, and also with regard to the Ministerial Policy Guidelines for the Addition of Substances other than Vitamins and Minerals.

The following key questions are addressed in the Risk Assessment Report:

1. Are plant sterols (conforming to the modified JECFA specifications) safe for human consumption at the levels of use currently specified in the Code?
2. Are plant sterols (conforming to the modified JECFA specifications) nutritionally safe?
3. Do the chemical properties of phytosterols, phytostanols and their esters (conforming to the modified JECFA specifications) make them technologically suitable for addition to the four approved foods?
4. Do plant sterols (conforming to the modified JECFA specifications) lower blood LDL-cholesterol when consumed in each of the four approved foods?
5. Does dietary intake, understanding of the product or purchasing behaviour differ according to the type and form of plant sterols?
6. Would a permission to add plant sterols (conforming to the modified JECFA specifications) to approved foods be likely to:
 - a) increase the number of brands available in the market?
 - b) result in flow-on changes in consumption patterns?

This Risk Assessment Report is structured to address the above questions in order.

Assessment of a health claim for plant sterols is not part of the assessment of this Application.

4. SAFETY ASSESSMENT

Toxicity data, clinical studies and information from published literature supporting the safety of plant sterols have been assessed previously in the context of a number of applications to FSANZ (Applications A410, A417, A433, A434 and A508). An updated and comprehensive risk assessment of plant sterols has recently been completed for another application

(A1019)¹. Collectively, these assessments have considered all new information, including epidemiological data (see Section 5), relevant to the safety of plant sterols that has become available since they were first assessed. No new evidence has been presented that would indicate the need to change previous conclusions regarding the safety of plant sterol-fortified foods, and a reference health standard is not warranted.

Evidence which underpins the safety of the existing permissions for plant sterols is summarised in the following:

1. Studies in laboratory animals with different phytosterol/phytostanol preparations indicate that plant sterols are poorly absorbed from the gastrointestinal tract, show no oestrogenic activity, are not genotoxic and demonstrate no reproductive or developmental toxicity.
2. In humans, there is no evidence of adverse effects associated with consumption of phytosterols and phytostanols, and their esterified forms, up to a level of 6.6 g phytosterols/day. Daily consumption of 1.6 g phytosterols for one year is not associated with any adverse health outcomes.
3. Clinical studies indicate that consumption of phytosterols, phytostanols and their esters can reduce plasma levels of carotenoids, particularly β -carotene, without resulting in any overt vitamin deficiency. Carotenoid absorption is not a well-regulated process in humans and plasma levels are known to fluctuate widely according to physiological and environmental factors. As the observed levels of β -carotene remain within the broad natural range for this nutrient, there is no evidence in the literature that consumption of plant sterol-fortified foods will result in adverse health outcomes.

4.1 Bioequivalence of phytosterols and phytostanols

Phytosterols and phytostanols share a common molecular structure similar to cholesterol, and differ from one another by a double bond (see Section 2.2). This close structural relationship results in similar pharmacokinetics in rats, and causes molar equivalent cholesterol lowering effects in humans (for reviews see Law *et al.* 2000 and Katan *et al.* 2003). A more detailed discussion of the LDL-cholesterol lowering efficacy of plant sterols is in Section 7.

Esterification of plant sterols with long chain fatty acids derived from vegetable oils (such as sunflower oil) is not a new process, and studies demonstrating equivalent nutritional and physiological effects of the esterified and free plant steroid compounds have already been reviewed (FSANZ, 2000). Several studies in humans and *in vitro* experiments with porcine cholesterol esterase or pancreatic lipase preparations show that both phytosterol esters and phytostanol esters are likely to be effectively hydrolysed in the proximal part of the intestine, resulting in the corresponding free phytosterol/phytostanol and free fatty acids (FSANZ 2000). While the greater proportion of plant sterol esters is enzymatically hydrolysed and eliminated in the faeces, the esterified forms are also eliminated, suggesting that some incomplete hydrolysis and some esterification of sterols occurs in the gut *in vivo*.

4.2 Absorption of phytosterols and phytostanols

The most abundant phytosterols and phytostanols in commercial preparations of plant sterols include β -sitosterol, campesterol, β -sitostanol and campestanol which are present in differing proportions in a range of commercial products. While minor differences in the extent

¹ See supporting documents to Application A1019 Exclusive Use of Phytosterol Esters in Lower-fat Cheese: Risk Assessment Report.

of absorption of individual phytosterol and phytostanol compounds have been reported, the major proportion (at least 95%) of all ingested plant sterols is excreted unchanged in the faeces, irrespective of their source. Absorption rates of β -sitostanol and campestanol from a phytostanol-fortified diet have even been reported as low as 0.2% (Ostlund *et al.* 2002; Gylling & Miettinen, 2005), indicating that the phytostanols are practically not absorbed at all.

4.3 Nutritional safety

Consumption of plant sterols is known to lead to a reduction in concentrations of some fat soluble vitamins, and serum carotenoids (α - and β -carotenes and lycopene), but not in the serum concentrations of retinol. These reductions associated with consumption of phytosterol-fortified foods were previously evaluated by FSANZ (FSANZ, 2005; FSANZ, 2006a). Previous evaluations concluded that, as the carrier LDL-cholesterol levels decrease by whatever means (i.e. cholesterol-lowering medication or diet), serum carotenoids are concomitantly decreased. Decreases in tocopherol, vitamin K, and vitamin D levels are also not significant once adjusted for LDL decreases. It was also concluded that levels of serum carotenoids in the blood fluctuate widely as a consequence of many dietary and environmental factors and a decrease of 20-25% as reported in studies falls within a broad natural range.

Several studies published since 2006 support previous conclusions as they reported no significant differences in mean serum levels or percentage changes in serum levels of β -carotene, α -tocopherol, and plasma fat soluble vitamins compared to controls after standardisation for LDL-cholesterol reductions (Korpela *et al.*, 2006; Hansel *et al.*, 2007; Plana *et al.*, 2008; Rudkowska *et al.*, 2008; De Jong *et al.*, 2008a). Noakes *et al.* (2002) found that increased consumption of one serve of fruit or vegetables (especially varieties rich in carotenoids) during phytosterol interventions resulted in compensatory increases in serum carotene levels (α - and β -carotene, lycopene). Several studies have since supported the conclusion that there are no overt nutritional issues associated with the reductions in serum carotenoid levels with the use of plant sterol-fortified foods (Jula *et al.*, 2002; Colgan *et al.*, 2004; Brufrau *et al.*, 2008).

The current evidence has shown that serum concentrations of carotenoids and tocopherol remain within the normal range, and an increase in the dietary intake of carotenoids can mediate the lower absorption effects seen with consumption of plant sterols. There is no change to the previous conclusion that the reductions in serum carotenoid levels do not pose a health risk to the adult population.

However, children and pregnant or lactating women in general do not need to lower cholesterol levels and, in addition, are considered to have increased growth or physiological requirements compared with other adults. Therefore cholesterol lowering food products are not considered to offer any benefits for these groups.

4.4 Conclusion

Based on extensive evidence showing similar physiological properties *in vivo*, phytosterols, phytostanols and their esters may be considered equivalent in terms of food safety. At the levels of use corresponding to the current permissions, consumption of plant-sterol-fortified foods raises no safety concerns.

The response to risk assessment questions 1 and 2 are shown following section 5.

5. EPIDEMIOLOGICAL STUDIES- SERUM PLANT STEROL LEVELS AND HEART DISEASE

Cardiovascular disease (CVD) includes all diseases and conditions of the heart and blood vessels (AIHW, 2008). Coronary heart disease (CHD) is one of the most common forms of CVD (AIHW, 2009). Although investigations into the potential toxicity of plant sterols have demonstrated no adverse effects in either animals or humans, evidence from sitosterolaemia patients with markedly increased plasma sterol levels and premature development of CVD has raised a question about whether modest increases in plasma plant sterols in consumers of plant-sterol-fortified foods might be a potential risk factor for CVD. It also raises the question of whether plant sterols are more atherogenic than cholesterol (Kratz, 2007).

FSANZ has reviewed and summarised the relevant types of evidence in humans. FSANZ undertook a literature search to identify human studies that examined the relationship between serum plant sterol levels and the risk of some forms of CVD. A total of 32 articles were identified from the search strategy. Of these, only 12 were selected for further examination, based on information in their abstracts. After full papers were read, three cohort or nested case control studies were identified and are summarised below. All prevalence case control studies were excluded as the cases in these studies had already experienced some form of CVD, either CHD or atherosclerosis, and thus dietary and lifestyle behavior may have changed. Cross-sectional studies were excluded for this same reason.

5.1 Absorption of plant sterols

Cholesterol absorption and metabolism were previously reviewed in detail and reported in the Final Assessment Report for Application A434 (FSANZ, 2004a).

Plant sterols and cholesterol are absorbed by the same principal mechanism however the absorption rates for plant sterols are much lower than those for cholesterol. This results in much higher serum concentrations of cholesterol than of plant sterols (Lee et al., 2001; Ostlund, 2002; Kratz et al., 2007). Western-style diets are estimated to provide around 150-360 mg plant sterols per day (Chan et al., 2006), similar to the intake of cholesterol, but typical serum plant sterol levels amount to 0.025 mmol/L compared to around 5 mmol/L for cholesterol (see Table 5.1). The increases in serum plant sterols in response to consumption of plant-sterol-fortified foods result in levels of less than 1% of total serum sterols, even with high dietary intake of plant sterols (Windler et al., 2009).

Table 5.1: Comparison of typical intakes of sterols and serum sterol concentrations

Sterol	Average intake (mg/day)	Average serum concentration in healthy adults
Cholesterol	298*	<5.5 mmol/L in populations not at increased risk of CVD
Plant sterols (from unfortified foods)	~150-360	Campesterol 6.9-27.9 µmol/L** (mean 14.2 ± 5.0 µmol/L) Sitosterol 2.8-16.0 µmol/L** (mean 7.9 ± 2.7 µmol/L)

* Mean intake of Australians aged 19 years and above, 1995 National Nutrition Survey (McLennan & Podger, 1998)

**Based on review of 45 studies published 1986-2005 in Chan *et al.*, 2006

Consumption of plant-sterol-fortified foods will increase serum plant sterol levels. Several factors influence both cholesterol and plant sterol absorption and plasma lipid responses i.e. their levels in plasma. These include:

- genetic factors such as apolipoprotein E phenotype (Uusitupa et al., 1997; Sehayek, 2003; Sanchez-Muniz et al., 2009)
- type of diet (Miettinen et al., 1990)
- gender (Sutherland et al., 1998)
- use of statins (Miettinen et al. 2003)

5.2 Sitosterolaemia

Sitosterolaemia (also known as phytosterolaemia) is a rare genetic (autosomal recessive) disorder² of lipid absorption in which affected individuals experience increased intestinal phytosterol absorption, decreased biliary excretion of dietary sterols and low endogenous cholesterol synthesis (Berge *et al.*, 2000). Sitosterolaemia is caused by mutations in both the ABC transporters, ABCG5 and ABCG8, expressed in the liver and intestine (Lee *et al.*, 2001). This change in plant sterol metabolism in sitosterolaemia sufferers results in a progressive accumulation of plant sterols in plasma and tissues which can lead to premature atherosclerosis and early cardiovascular death (Salen *et al.*, 1992; Salen *et al.*, 2004; Kratz *et al.*, 2007).

In adults, the characteristic indicator of sitosterolaemia is substantially elevated levels of serum plant sterols, predominantly sitosterol; however cholesterol levels are not necessarily high. The plasma levels of plant sterols in sitosterolaemia patients are reportedly 10-100 times higher than in individuals with normal lipid absorption (Lee *et al.*, 2001; Chan *et al.*, 2006). However, the magnitude of the difference in serum plant sterol levels between otherwise normal individuals who consume plant-sterol-fortified foods on a daily basis, and patients with sitosterolaemia, is large.

5.3 Link between elevated serum plant sterols and cardiovascular disease

To further investigate the association between elevated serum plant sterol levels and CVD, three case-control studies nested within existing cohort studies were identified. These studies investigated whether levels of plasma plant sterols are related to an increase in risk of CVD or adverse cardiac events using stored blood from the baseline examination. The blood of participants who developed a CVD outcome, as defined within each study, and the blood of a set of controls was thawed and analysed for plant sterol level after some years of follow-up of the total cohort. Study details are shown in Table 5.2.

Pinedo *et al.*, (2007) assessed the incidence of fatal and non-fatal coronary artery disease by tertiles of sitosterol and campesterol concentrations (based on levels in controls). They found no difference in plasma sitosterol and campesterol levels between cases and controls over a follow-up period of six years. The median sitosterol concentration in both cases and controls was 2.1 µg/mL, while the median campesterol concentration in cases was 3.1 µg/mL, and in controls was 3.2 µg/mL. The baseline sitosterol:cholesterol ratio was significantly lower in cases than in controls, whereas the campesterol:cholesterol ratio did not differ significantly between cases and controls. The odds ratio for highest versus lowest tertile of sitosterol level was 0.79 (95% CI: 0.56-1.13) after adjustment for a range of CVD risk factors (age, sex, systolic blood pressure, total cholesterol, HDL-C, body mass index

² The first two diagnosed cases of sitosterolaemia were described in 1974. Only 45 diagnosed cases have been reported in the literature worldwide.

(BMI), smoking (never, past, current) and diabetes). There was a non significant inverse association with sitosterol:cholesterol ratio and CHD (after adjustment).

Assmann *et al.* (2006) investigated the risk of myocardial infarction or sudden death in a cohort of men. They found that those with higher sitosterol levels had a 1.8-fold increased risk of myocardial infarction or sudden coronary death over ten years compared with subjects with low sitosterol levels (control groups matched for age, smoking and body mass index). However, the analysis was not adjusted for a range of known CVD risk factors, even though the cases had a more adverse profile than the controls. Thus it is unclear whether the significant positive association between serum sitosterol levels and coronary events is due to confounding by other risk factors.

Fassbender *et al.* (2008) reported a reduction in risk (odds ratio 0.78, 95% CI: 0.62-0.98) for coronary heart disease related to sitosterol levels after adjustment for age, gender, cholesterol, diabetes, smoking and hypertension. The ratio of sitosterol (as a marker of total plant sterols) to cholesterol was also lower in those with coronary heart disease (Fassbender *et al.*, 2008).

The studies discussed above do not directly address the issue of whether increased levels of serum phytosterols resulting from consumption of plant-sterol-fortified foods could be contributing factors in coronary artery disease (CAD). All three studies collected their baseline blood samples in the mid to late 1990s and so pre-date the introduction of plant-sterol-fortified foods. The two studies that adjusted for known risk factors found that a higher plasma phytosterol level was associated with less CVD, whereas the study which did not adjust for known risk factors found the opposite effect. The number of studies is limited at present, which is presumably due to the recency of interest in the topic, and each study used a slightly different set of outcomes.

Although consumption of phytosterol-fortified products increases the serum levels of phytosterols, the increments seen in studies result in levels much lower than the serum phytosterol levels observed in sitosterolaemia. Studies also show baseline levels of serum phytosterols have a wide variation (Clifton *et al.*, 2004) and are affected by ApoE phenotype and ABCG5 and ABCG8 polymorphisms in the same manner as cholesterol. These factors were not adjusted for in the studies relating serum plant sterol levels to coronary disease.

Cholesterol homeostasis regulates whole body cholesterol content by balancing input (intestinal absorption of dietary and biliary cholesterol) and output (hepatic and extra-hepatic synthesis) (Matthan *et al.*, 2009). Changes in cholesterol homeostasis have been linked to increased risk of CAD and atherosclerosis (Sudhop *et al.*, 2002; Weingarter *et al.*, 2008; Silbernagel *et al.*, 2009). Despite this, many studies, which suggest elevated serum plant sterol levels are associated with an increased risk of CVD, have not considered the possible impact of cholesterol homeostasis (Glueck *et al.*, 1991; Rajaratnam *et al.*, 2000; Sudhop *et al.*; 2002, Miettinen *et al.*; 2005, Assmann *et al.*, 2006).

Silbernagel *et al.*, (2009) investigated the association between severity of CAD (diagnosed by angiogram) and cholesterol metabolism. The authors measured markers of cholesterol absorption and cholesterol synthesis in 2440 LURIC study participants who were grouped according to severity of CAD. The authors concluded that there was evidence of a small association of high cholesterol absorption and low cholesterol synthesis with increased severity of CAD in the study participants. Matthan *et al.*, (2009) investigated the association between CVD status and circulating indicators of cholesterol homeostasis. They measured markers of cholesterol absorption and synthesis in participants of the Framingham Offspring Study with established CVD and/or >50% carotid stenosis (not on lipid-lowering medication). Associations between the cholesterol homeostasis markers and CVD risk factors were

established by calculating correlation coefficients. The authors concluded that individuals with CVD or >50% carotid stenosis have higher cholesterol absorption and lower cholesterol synthesis relative to matched control subjects with comparable cholesterol concentrations. Also for the study population cholesterol homeostasis markers were highly significant predictors of CVD risk relative to established risk factors (Matthan et al., 2009). These studies both concluded that slightly elevated plasma plant sterol levels is a consequence of elevated cholesterol absorption; therefore the studies do not distinguish between cholesterol and plant sterols in CVD risk. Thus the associations found in other studies between elevated plasma levels of plant sterols and CVD risk would not provide evidence of a causal relationship between elevated plasma levels of plant sterols and promotion of atherosclerosis.

Studies which have measured levels of plant sterols in tissue and lesions suggest that plant sterols do not accumulate in plaques disproportionately to cholesterol. As noted above, the two better analysed nested case-control studies suggest reduced risk of CVD among those with higher serum phytosterol levels. FSANZ further notes that serum phytosterol levels are lower by several orders of magnitude than total cholesterol (e.g. less than 0.025 mmol/L versus 5 mmol/L) and so doubling of plant sterol levels in serum would have an immeasurable effect on cardiovascular risk even if it had the same risk as total cholesterol (Silbernagel *et al.*, 2009).

FSANZ concludes that, based on the limited available data, higher plant sterol consumption within the usual range of intake is more likely to decrease risk of CVD than to increase it. Weighed against any potential (and unproven) small increase in CVD risk associated with consuming plant-sterol-fortified foods, is the reduction in CVD risk associated with reductions in LDL-cholesterol levels due to plant sterol consumption which is assumed to also reduce CVD. Therefore the benefit of these foods is almost certainly far greater than any possible risk from them. Section 7 of this report investigates the cholesterol lowering action of plant sterols.

Table 5.2: Cohort and nested case control studies investigating the relationship between plasma plant sterols and risk of CVD

Author, year and study objective	Description of cohort	Definition of outcome	Duration of follow up (years)	N cases, control	Sitosterol level	Odds ratio (OR)	95% Confidence Interval	Adjustments and other comments
Pinedo, 2007 Examine the association between plasma levels of plant sterols and the risk of future CAD in EPIC cohort who had never suffered a myocardial infarction or stroke	EPIC-Norfolk; 25,663 men and women aged 45-79 years, resident in Norfolk, UK. Recruited 1993 and 1997	Fatal or non fatal coronary artery disease (CAD was defined as codes 410 to 414 according to the International Classification of Diseases, 9 th revision)	6	373, 758	Low <4.42 µmol/L	1	Referent	Potential confounders adjusted for in analysis: age, sex, systolic blood pressure, total cholesterol, HDL-C, body mass index (BMI), smoking (never, past, current) and diabetes Adjusted for all of the above and lathosterol to cholesterol ratio
					Med 4.42-6.23 µmol/L	0.68	0.49-0.95	
					High >6.23 µmol/L	0.79	0.56-1.13	
Assmann, 2006 Evaluate the relationship between modest sitosterol elevations seen in the general population and CHD risk in men from a large prospective population-based cohort	Prospective Cardiovascular Münster (PROCAM) study – 20,060 men and women in a large scale prospective employment based cohort Recruited 1979 -1985 Men aged 35-65 years	Myocardial infarction or sudden death - coronary event (defined as the occurrence of sudden cardiac death or a definite fatal or nonfatal myocardial infarction -on basis of ECG and/or cardiac enzyme changes).	10	159, 318	<5.25 µmol/L	1	Referent	Cases were matched on age, sex, and date of enrolment The following were higher in cases:, total and LDL cholesterol; triglycerides, systolic blood pressure and fasting blood glucose HDL levels were lower in cases than controls These differences between cases and controls were not adjusted for.
					>5.25 µmol/L	1.81	1.12-2.94 (p<0.05)	
Fassbender, 2008 Examine coronary heart disease risk associated with moderately increased plant sterol or stanol levels in a cohort of elderly subjects participating in a large community-based study.	Longitudinal Aging Study Amsterdam (LASA) - 3,107 men and women aged 55–85 years 1192 men aged 65 - 89 years participating at the Longitudinal Aging Study Amsterdam (LASA)	Coronary heart disease (measured by presence of angina pectoris and/or myocardial infarction, peripheral artery disease identified by ECG or cardiac enzyme changes)	10 or 11 years not clearly specified	279, 957	NO 8.07µmol/L	1	Referent	Corrected for cholesterol by calculating sitosterol ratio to cholesterol. Adjusted for age (males), age (females), sex, cholesterol, diabetes smoking and hypertension
					CHD 7.13 µmol/L	OR 0.78	0.62-0.98 (p=0.030)	

NO= No vascular disease; CAD=Coronary artery disease; CHD= Coronary heart disease;
*converted to µmol/L from mg/dl

5.4 Conclusion

FSANZ has undertaken a comprehensive analysis of epidemiological studies to assess whether increased serum plant sterol concentrations increase risk of cardiovascular disease. The available evidence indicates that plant sterols do not increase risk of cardiovascular disease other than in the rare group of individuals with sitosterolaemia. The previous conclusion that consumption of plant-sterol-fortified foods does not raise any safety concerns therefore remains unchanged.

5.5 Response to risk assessment questions

5.5.1 Question 1:

ARE PLANT STEROLS (CONFORMING TO THE MODIFIED JECFA SPECIFICATIONS) SAFE FOR HUMAN CONSUMPTION AT THE LEVELS OF USE CURRENTLY SPECIFIED IN THE CODE?

The evidence supporting the safety of plant sterols discussed in Sections 4 and 5 includes studies with variable preparations of phytosterols and phytostanols. No food safety concerns were identified irrespective of the proportions of individual sterol or stanol compounds used, or their source. Based on consideration of all available evidence, phytosterols, phytostanols and their esters may be considered bioequivalent.

Following a comprehensive analysis of appropriately designed epidemiological studies to assess whether increased serum plant sterol concentrations contribute to the risk of cardiovascular disease, the available evidence indicates no role of plant sterols in cardiovascular disease risk in the general population. This information confirms previous conclusions about the safety of consuming plant-sterol-fortified foods.

5.5.2 Question 2:

ARE PLANT STEROLS (CONFORMING TO THE MODIFIED JECFA SPECIFICATIONS) NUTRITIONALLY SAFE?

Reduced carotenoid uptake associated with consumption of plant sterols is not a nutritional concern in adults as serum carotenoid levels fluctuate normally according to a number of dietary factors and environmental variables. A small reduction in the absorption of carotenes with intake of plant sterols is largely explained by the reductions in serum levels of carrier LDL-cholesterol attributed to plant sterols.

However, children and pregnant or lactating women in general do not need to lower cholesterol levels and, in addition, are considered to have increased growth or physiological requirements compared with other adults and so consumption of cholesterol lowering products in these groups does not provide any benefit.

Clinical studies have shown that increasing intakes of fruits and vegetables, particularly varieties rich in β -carotene, while consuming plant-sterol-fortified foods, partially compensates for lower absorption of carotenoids.

6. FOOD TECHNOLOGY

Discussion of food technology in this part of the Risk Assessment Report precedes assessment of cholesterol-lowering effects to provide a context for consideration of these physiological effects.

6.1 Production of plant sterols

6.1.1 Production from vegetable oil

Commercially, plant sterols are isolated from vegetable oils, such as soybean oil, rapeseed (canola) oil, sunflower oil or corn oil. These vegetable oils normally undergo a series of refining steps to remove unwanted constituents and to improve their quality and shelf lives. The last step in the oil purification process is deodorisation which produces a distillate known as the “vegetable oil deodorised distillate (VOD)” (EFSA, 2007).

The VOD is the starting material for the extraction of plant sterols which are subjected to a series of distillation, filtration and crystallisation steps to remove unwanted by-products including fatty acids, di- and triglycerides, waxes, fatty acid esters and others.

Phytosterol esters are esters of fatty acids and are usually produced by esterifying phytosterol and phytosterols with long chain fatty acids from vegetable oils to improve their solubility in food products that have fat components.

6.1.2 Production from tall oil

Plant sterols can also be isolated from a by-product of the wood pulp manufacture from pine trees (*Pinus sp.*). Crude tall oil is a by-product of the wood pulping process. Phytosterols are concentrated in the residue after the crude tall oil is distilled into different fractions. This residue, called tall oil pitch, contains up to 5-15% phytosterols (JECFA 2008).

The tall oil pitch is saponified with caustic soda to hydrolyse the phytosterol esters. The mixture is then neutralised with mineral acid and the aqueous phase removed. The phytosterol fraction is recovered by distillation of the residual pitch in a number of steps. Finally, the phytosterols are purified via solvent re-crystallisation (JECFA 2008).

6.1.3 Allergenicity

Generally, the protein content of highly refined vegetable oils is expected to be very low. FSANZ notes that the European Food Safety Authority (EFSA) (2007), after considering the processing of plant sterols, including the starting materials, subsequent production process and the demonstration of low residual protein content, concluded that it is unlikely vegetable oil-derived plant sterols from soybean would trigger a severe allergic reaction in individuals who are sensitive to soybean proteins. In Australia and New Zealand however, declaration of soy and soy products is regulated by Standard 1.2.3 – Mandatory Warning and Advisory Statements and Declarations in the Code.

6.2 Specifications for plant sterols

This Application originally requested that specifications published by JECFA (June 2008) be adopted to replace specifications then existing in the Code. The JECFA specifications were adopted into the Code by a separate process in 2009 thus providing a fourth set of specifications for plant sterol preparations (as shown in Table 6.1). In submissions to this

Application, amendments to these JECFA specifications sought alteration of the proposed solvent residue limits and a minimum requirement for plant sterol content.

6.2.1 Extraction solvent residues

The process of steam stripping unreacted free fatty acids from the esterified product mixtures effectively removes all residual extraction solvents. However, formulations of unesterified plant sterols, where steam is not used, will contain low levels of particular extraction solvents. The current specifications for tall oil-derived phytosterols listed in the Code specify a maximum level of residual extraction solvents of 5000 ppm, equivalent to 0.5% (see Table 6.1). This level corresponds to the maximum limit for organic volatile impurities in the Certificate of Analysis for FCP-3P1, tall-oil derived phytosterols, used in a 90 day toxicity study in rats (Forbes Medi-Tech Study Number: 115-003). This study formed part of the safety information submitted to FSANZ in 2001, underpinning the current approvals for the use of tall oil-derived phytosterols³. The maximum level of 5000 ppm for solvents also accords with the US FDA specifications for Phytrol™ under GRAS notification number GRN 000039.

Based on updated information obtained from major manufacturers of plant sterols used in the food industry, FSANZ has determined that manufacturers are consistently able to meet a maximum level of 2000 ppm (0.2%) for residual solvents. In revising the current specifications, FSANZ is therefore proposing to lower the maximum level of solvents to 2000 ppm in accordance with the principles of Good Manufacturing Practice (GMP).

Solvent residues in phytosterols typically include hexane, 1-propanol, ethanol or methanol, which may be present either singly or in combination. FSANZ is advised by some manufacturers that preparations of phytosterols may contain residual levels of isopropanol and/or methyl ethyl ketone as a result of using contemporary extraction methods. While the Code currently does not specify the solvents to which the maximum level applies, FSANZ proposes to include a list of solvents in revised specifications. Residues of any of these particular solvents (hexane, isopropanol, ethanol, methanol and methyl ethyl ketone) up to a maximum level of 2000 ppm in total raise no food safety concerns and should be readily achievable by any manufacturer of plant sterols.

6.2.2 Plant sterol content

Two of the specifications currently in the Code are source specific (and hence commercial product specific), with detailed requirements for the plant sterol content, form and composition. In comparison, the JECFA specifications are more general in regard to the source and composition of plant sterol preparations with no restrictions on the form of plant sterol. The addition of an extra condition to specify the 4-des-methyl content of preparations ensures that the specifications align with preparations that have been reviewed by FSANZ (in this and previous reports) and scientifically substantiated to reduce blood cholesterol. The proposed amendment will also align the JECFA specifications with previous specifications in the Code.

6.3 Suitability of adding plant sterols to foods

Section 2.2.2 provided information on the physical properties of plant sterols. The suitability of adding plant sterols into different food types will depend on their physical properties, whether the plant sterol be fully and uniformly distributed throughout the food matrix and the stability of the plant sterol once it is incorporated into the food.

³ FSANZ previously completed the assessment of tall oil phytosterols in Application A417.

6.3.1 Incorporation challenges

a. *High melting points*

Phytosterol and phytostanol preparations have high melting points that average between 138-158 °C and they exist as solid crystalline structures at room temperatures. Incorporation of phytosterols and phytostanols into foods is difficult because their form may prevent optimal mixing into both liquid and solid food matrices and they tend to form insoluble crystals and settle out in liquid foods.

There are technologies available to overcome these problems:

- Esterify phytosterols and phytostanols with fatty acids from vegetable oils. The esterified plant sterols have much lower melting temperatures, between 26 and 40 °C. With lowered melting points, the esters can be blended more easily in semi-solid foods such as spreads and yoghurt during the processing at a temperature slightly higher than 40°C. At this temperature, the esters behave like liquid oil, for example allowing an even coating of cereal grains for breakfast cereals. The liquefied esters can also be homogenised with the help of emulsifiers in aqueous-based drinks to provide an even distribution, however, this emulsification does not necessarily guarantee stability of the emulsion over the storage (shelf life) period.
- Patented grinding of the phytosterols and phytostanols. In this process, no esterification is needed. The sterols and stanols are ground in a cryogenic (frozen below -150 °C) state to 1 – 2 µm in size and then homogenised in liquid foods to provide a homogeneous suspension of the plant sterol particles in the food. This suspension may settle over time in drinks but can be easily redistributed by shaking the container. No emulsifier is needed to be added to the product.

Table 6.1: Comparison of Specifications

Phytosterol Content (%)	JECFA Monograph 5¹	Food Chemicals Codex 6th Ed (USP 2008) (Vegetable oil phytosterol esters)	The Code (Vegetable oil phytosterol esters)⁶	The Code (Tall oil phytosterols)⁶
Free phytosterol/stanols ² + Phytosterol/stanols (from phytosterol/stanols esters after saponification) ³	55-95			
Free phytosterol/stanols + Phytosterol/stanols esters		95 min	94 min	
Free phytosterol/stanol (for non-esterified products) ⁴	95 min			97 min
Phytosterol esters		86 min		
Phytosterol/stanols after saponification of the esters ⁵	55 min			
Des-methyl-sterols		59 min		
Free phytosterols		9 max	10 max	
Steradienes			0.3 max	
Acyl-glycerides		5 max		
Sterol profile (%)				
Campesterol		10 – 40	20-29	4-25
Campestanol			0-6	0-14
β-sitosterol		30 – 65	42-55	36-79
β-sitostanol			0-2	6-34
Brassicasterol		12 max	6 max	3
Stigmasterol			12-23	
Δ ² -Avensterol		6 max	4 max	
Δ ⁷ -Stigmastenol			2 max	
Δ ⁷ -Avenasterol		7 max	2 max	
Other sterols			6 max	
Cholesterol			2 max	
<i>Trans</i> fatty acids (%)			1.0 max	
Fatty acid methylester (%)			0.5 max	
Moisture (%) loss on drying	4 max	0.1 max	0.1 max	4 max
Solvents (ppm)	50 max			5000 max
Residue on ignition (%)	0.1 max			0.1 max
Acidity (g KOH/kg)		0.2 max		
Heavy Metals (total, ppm)				2 max
Iron			1.0 max	
Copper			0.5 max	
Arsenic	3 max			0.1 max
Lead	1 max	0.1 max		0.1 max
Cadmium				0.1 max
Mercury				0.1 max
Microbiological				
Total aerobic count (CFU/g)				10,000 max
Moulds and yeasts (CFU/g)				100 max
Coliforms				Negative
E. coli				Negative
Salmonella				Negative

¹ A combined specification of phytosterols, phytostanols and their esters.

² Free phytosterols/stanols refer to non-esterified phytosterols/stanols.

³ For products that are mixture of free and esterified phytosterols/stanols –content of phytosterols/stanols measured as free phytosterols/phytostanols in a native and saponified sample.

⁴ For products containing only free phytosterols – on a total free phytosterol basis.

⁵ For products containing only esterified phytosterols – measured as phytosterols/phytostanols on a saponified sample.

⁶ Taken from Schedule to Standard 1.3.4 of the Code

b. Low solubility

Phytosterols and phytostanols have low solubility in normal food systems. Less than 0.01 g dissolves in 100 g (mL) of water and 2.5% in fat at ambient temperature.

To overcome the low solubility of phytosterols and phytostanols, phytosterol and phytostanols are esterified. The solubility of phytosterol and phytostanol esters is 10 to 20 times higher in fats and oils than their non-esterified counterparts (Engel and Schubert 2005). The esters are also soluble in non-polar solvents but not in water. The increased solubility allows the esterified phytosterols and phytostanols to be dissolved into the fat components of spreads or fat-containing food products which have their fat components already homogeneously distributed or emulsified. In addition varying the fatty acid composition of phytosterol and phytostanol esters can 'tailor' the solubility to improve incorporation into different food matrices.

c. Stability of plant sterols and products containing plant sterols

As indicated in Section 2.2.3, plant sterols are basically very stable compounds and experience only limited damage during oil processing (Ferrari et al 1997). Even under severe processing conditions such as deep-frying, plant sterols are quite stable with limited oxidation occurring. Because plant-sterol-fortified food products require no or minimal heat treatment prior to consumption, e.g. table spread, yoghurt and breakfast cereals, there will be little loss of plant sterols prior to consumption of the products.

d. Distribution stability during storage

After the plant sterol has been dispersed evenly in a solid or semi-solid matrix such as fat spreads, yoghurt or breakfast cereals, there is not usually a problem associated with redistribution of the plant sterol or the uneven consumption of the plant sterol through consumption of individual serves. Caution may need to be taken when the plant sterol is dispersed into liquid media such as milk since settling or agglomeration of the plant sterol dispersion might occur. Different technologies such as cryogenic grinding or use of emulsifiers are needed to ensure that the correct amount of the plant sterol is available in each serve.

6.3.2 Incorporation practices

This Application has requested that any appropriate plant sterol that conforms to the modified JECFA specification can be permitted to be added to any of the approved foods. The type of plant sterol used will depend on a variety of factors. Ultimately the plant sterol needs to be uniformly incorporated into the food product and to be stable during storage prior to consumption to deliver a LDL-cholesterol lowering effect.

Different technologies can be used to distribute plant sterols in different food matrices, thus overcoming some of the limitations discussed above. Table 6.2 summarises the different technologies that enable the incorporation of plant sterols into the currently permitted foods. This Table also includes comment on the suitability and stability of incorporating the different types of plant sterols in the four different food matrices. These are general statements and should not be seen as being definitive. This is because there are likely to be new technologies that allow the incorporation of plant sterols that have not been assessed by FSANZ to date. For example, patented cryogenic grinding of non-esterified phytosterols is a new technology that allows incorporation of the non-esterified phytosterol in juice without the need for emulsifiers. If the Application is successful then food manufacturers will be able to use the most appropriate plant sterol preparation suitable for their purpose, provided the

product conforms to the modified JECFA specifications and is able to be uniformly incorporated into the food (Soupas, 2006).

Table 6.2: Specific food technology approaches for addition of plant sterols to food types currently approved in the Code

Food	Technical Issue	Conclusions	Technologies	Example: FSANZ Application
Edible oil spreads	suitability	Yes	Esterification of the phytosterols and phytostanols to increase solubility and lower melting points.	A410, A434
	stability	Yes		
	suitability	Yes	Free (non-esterified) phytosterols <ul style="list-style-type: none"> - Partially dissolve the phytosterols at 85°C into the fat spread mixture and recrystallise upon cooling. - Disperse the phytosterols into the water phase with stabilisers and proceed with emulsification and recrystallisation. - Melt the phytosterols at a concentration of 17% into fat and use this as a source solution to prepare the fat phase. 	A417
	stability	Yes		
Milk	suitability	Yes	Esterification – so that the phytosterol and phytostanol esters are soluble in liquid fat which is then homogenised and dispersed into the milk	A434
	stability	May be		
	suitability	Yes	Free (non-esterified) phytosterols – using company confidential technology	A508
	stability	May be		
Yoghurt	suitability	Yes	Esterification of the phytosterol and phytostanol so that they are soluble in liquid fat which is then homogenised and dispersed into the milk before fermentation	A434
	stability	Yes		
Breakfast cereals	suitability	Yes	Esterification of the phytosterol and phytostanol to make them soluble in liquid fat which is then applied to the cereal during manufacturing.	A433
	stability	May be		

Note: Most plant sterols are chemically stable unless they are subjected to high heat prior to consumption. Therefore, the stability here refers to the incorporation and uniform distribution of the plant sterol in the product.

6.4 Conclusion

Plant sterols are very stable compounds. There are very few losses due to oxidation provided the food containing the added plant sterols is not subject to very high processing temperatures and oxidising conditions. The currently permitted foods are not subjected to these extreme conditions in their manufacture or use. Phytosterol and phytostanol compounds have very low-fat solubility and high melting temperatures, making their consistent incorporation into many food types hard to achieve unless other technologies are used to overcome this limitation. One of the favoured approaches taken is to esterify phytosterols and phytostanols with long chain fatty acids from vegetable oils. These phytosterol and phytostanol esters have much lower melting points and also have greater solubility in fats and oils. These properties are used to incorporate plant sterols into fat-containing food products. The use of emulsifiers or other manufacturing techniques (e.g. grinding) may be used to assist in obtaining uniform incorporation of the plant sterol into the food.

6.5 Response to risk assessment question 3

DO THE CHEMICAL PROPERTIES OF PHYTOSTEROLS, PHYTOSTANOLS AND THEIR ESTERS (CONFORMING TO THE MODIFIED JECFA SPECIFICATIONS) MAKE THEM TECHNOLOGICALLY SUITABLE FOR ADDITION TO THE FOUR APPROVED FOODS?

Yes, phytosterols, phytostanols and their esters that conform to the modified JECFA specifications are suitable for being incorporated into the four currently approved foods in the Code. There are likely to be some technical issues around incorporating some forms of plant sterols into some foods to achieve 100% uniform distribution but some of these difficulties can be overcome using technical solutions such as fine grinding of the particles or use of emulsifiers.

7. CHOLESTEROL LOWERING EFFECT

This part of the assessment considers the blood LDL-cholesterol lowering effects of commercial preparations of phytosterols, phytostanols, either free or esterified when added to each of the four approved food matrices. This assessment is undertaken to ensure that consumption of plant sterols in advised frequency and amounts would lower LDL-cholesterol; also to ensure that consumers are not misled as to the intended effect of the fortified food. LDL-cholesterol is the outcome variable of interest in these studies because higher serum LDL-cholesterol levels increase the risk of CVD. It should be noted that the assessment considered only commercial plant sterol preparations containing >95% des-methyl sterols; studies examining other plant sterol preparations were excluded (refer to Figure A12.2 in Appendix 1).

There is a large body of literature which concludes that commercial plant sterol preparations lower LDL-cholesterol when consumed in variety of foods including spreads, vegetable oils, salad dressings, mayonnaise, milk, yoghurt, bread, juices, muffins, croissants, breakfast cereals, non-fat beverages, cereal bars, meat and chocolate. The studies differ in their study protocols, plant sterol exposures (amount consumed, food characteristics, time and frequency of plant sterols consumption) as well as their subject characteristics (baseline LDL-cholesterol, age, sex, lifestyle, background diet and co-medication with statins).

Data on changes in LDL-cholesterol levels are presented inconsistently across the literature. Some studies present results as absolute change in mmol/L either from baseline or control LDL-cholesterol levels, while others present the results as a percent change from baseline or

control. Hence both absolute and relative reductions are reported in this assessment. Several meta-analyses have reviewed the literature to quantify the LDL-cholesterol lowering effect of plant sterols consumed in a wide range of food matrices. The range of food matrices included in these reviews is broader than the edible oil spreads, lower-fat dairy, and cereal matrices of current interest to FSANZ (refer to Table 7.1). The date of publication of the earlier meta analyses (Law, 2000; Katan *et al.*, 2003; and Chen *et al.*, 2005), meant that the studies included in the meta analysis were limited to fat-based foods, mainly using esterified plant sterols. The studies included in the two more recent meta analyses include a broader range of types and forms of plant sterols in a variety of food matrices as the use of plant-sterol-fortified foods has increased. As Table 7.1 shows, the mean LDL-cholesterol reduction is slightly lower in the later two meta analyses, however the effects seen across the five meta analyses are consistent. These meta analyses indicate that plant sterols as a group lower LDL-cholesterol in a dose-dependent manner up to approximately 2 g/day. This effect tapers off at daily intakes above 2.5 g/day.

The variation reported across individual trials indicates that the effects of plant sterols on LDL-cholesterol levels are not uniform in individuals. It is now clear that several factors influence an individual's cholesterol metabolism and hence the cholesterol lowering effect of plant sterols. Polymorphisms in genes that encode key proteins in lipoprotein metabolism can explain some of the variance as can factors such as ethnicity and hormonal status (Herron *et al.*, 2006). Other factors such as background diet and baseline cholesterol concentration will also influence the magnitude of the cholesterol lowering effect; however the degree to which each factor contributes to the variations observed has yet to be determined (Naumann *et al.*, 2008; Demonty *et al.*, 2009).

7.1 Cholesterol lowering mechanism

As discussed in Section 4.2, there are minor reported differences in absorption of phytosterols and phytostanols in the gut however the cholesterol lowering mechanism is the same. Once plant sterols are within the intestinal enterocyte, they influence cellular cholesterol metabolism and decrease the cholesterol esterification rate in the intestinal epithelium (Trautwein *et al.*, 2003; Nissinen *et al.*, 2007; Brufau *et al.*, 2008). The exact molecular regulation of these mechanisms is not confirmed but absorption occurs by multiple mechanisms and is potentially a multistep process regulated by multiple genes at the enterocyte level (Sehayek, 2003; Brufau *et al.*, 2008; Calpe-Berdial *et al.*, 2009). Further detail on the cholesterol lowering mechanisms is available in the Risk Assessment Report for Application A1019.

Table 7.1: Summary of meta analyses findings

Author	Year	No. of trials included	Type and form of plant sterols included	Food matrices included	Mean plant sterol amount g/day (as free equivalents)	Mean reported change in absolute LDL-cholesterol (mmol/L) (change from control)	95% CI	Mean reported change in relative LDL-cholesterol (%) (change from control)	95% CI
Law	2000	14	STA ester STE ester STE	Edible oil spreads	2.0	-0.54	-0.46 to -0.63	-14	Not reported
Katan	2003	41	STA ester STE ester STE	Edible oil spreads; yoghurt; mayonnaise ; salad dressing	0.7-1.1 1.5-1.9 2.0-2.4 >2.5	Not reported	Not reported	-6.7 -8.5 -8.9 -11.3	-4.9 to -8.6 -7.0 to -10.1 -7.4 to -10.5 -10.2 to -12.3
Chen	2005	23	STA ester STE ester STE STA	Edible oil spreads, yoghurt; mayonnaise; salad dressing; meat; chocolate	3.4	Not reported	Not reported	-8.7	Not reported
AbuMweis	2008	59	STA ester STE ester STE STA	Edible oil spreads; yoghurt; milk; cheese; mayonnaise; salad dressing; meat; low-fat beverages	1.5-2.0 2.1-2.5	-0.29 -0.32	-0.34 to -0.24 -0.36 to -0.28	Not reported	Not reported
Demonty	2009	84	STA ester STE ester STE STA	Edible oil spreads; yoghurt; milk; cereal; cheese; cereal bars; bakery products; mayonnaise; salad dressing; vegetable oil; meat; low-fat beverages; chocolate; bread; tortilla chips	2.15	-0.34	-0.31 to -0.36	-8.8	-8.31, -9.35

STE ester = phytosterol ester preparation; STA ester = phytostanol ester preparation; STE=free phytosterol preparation; STA= free phytostanol preparation

7.2 Previous consideration of plant sterols

FSANZ has previously examined the action of several specific commercial phytosterol and phytostanol preparations and concluded that when delivered in particular foods, consumption of around 2 g/day of these substances can be effective in lowering LDL-cholesterol levels in the range of 5%-15% in normocholesterolaemic and hypercholesterolaemic people (FSANZ, 2005). Table 7.2 shows the range of preparations that conform to the modified JECFA specifications and the previous applications that supported existing permissions for phytosterols in the Code which were specific for source and form of plant sterol. As shown in Table 7.2, FSANZ has *not* previously assessed phytostanol or phytostanol ester preparations in any food. Free tall oil phytosterols have not been assessed in low-fat yoghurt or breakfast cereal and free vegetable oil phytosterols and tall oil phytosterol esters have not been assessed in any of the four foods. These are considered in this assessment.

Table 7.2: Summary of current permissions and assessment gaps

Food matrix	Vegetable oil phytosterol ester	Vegetable oil free phytosterol	Tall oil phytosterol ester	Tall oil free phytosterol	Phytostanol ester (any source)	Free phytostanol (any source)
Edible oil spreads	A410	-	-	A417	-	-
Low-fat milk	A433	-	-	A508	-	-
Low-fat yoghurt	A433	-	-	-	-	-
Some breakfast cereals	A434	-	-	-	-	-

- = not previously assessed by FSANZ

7.3 Approach to this assessment

The purpose of adding plant sterols to foods is to lower LDL-cholesterol levels. To approve this Application, FSANZ must be satisfied that any phytosterol, phytostanol or ester preparation that conforms to the modified JECFA specifications when consumed in any of edible oil spreads, low-fat milk, low-fat yoghurt or some breakfast cereals would lower LDL-cholesterol.

Except for vegetable oil phytosterol esters in edible oils spreads, FSANZ identified at least four plant sterol-food combinations that have not been previously assessed (refer to Table 7.2). For unassessed combinations, this assessment considers the LDL-cholesterol lowering effects in each food of:

- plant sterol source
- plant sterol type
- free or esterified form.

FSANZ undertook an extensive literature search to identify studies that directly compared the type and form of plant sterols in edible oils, milk, yoghurt and breakfast cereal (see Appendix for further information). The Applicant also provided evidence supporting the cholesterol lowering effect of various plant sterols added to fat-based foods such as edible oil spreads, and to low-fat foods such as milk and yoghurt. Over 80 randomised control trials were identified as well as several meta analyses. Tables 7.3a and 7.3b list the inclusion and exclusion criteria used in study selection.

A hierarchy of evidence was considered in assessing the suitability of certain plant sterols to lower LDL-cholesterol. Studies that directly compared the source, type and form of plant sterol in the same food and study population were considered first. Studies that examined a single plant sterol preparation, however described, in an approved food matrix were then considered. Finally, studies that examined plant sterol preparations added to similar food matrices of the same food group were considered.

Table 7.3a: Inclusion criteria used to screen titles and abstracts

Human study
 Examined effects of phytosterols, phytostanols and their esters on serum LDL-cholesterol levels
 Used randomised control trial (parallel or cross-over design)
 Minimum duration of three weeks for the intervention periods
 Minimum participant age of 18 years
 Subjects with normal or elevated cholesterol levels
 Administered a control diet or double blind studies that administered a placebo vehicle

Table 7.3b: Exclusion criteria

Measured cholesterol absorption markers but not effect on serum cholesterol levels
 Study participants were children
 Phytosterols delivered as a supplement
 Possible co-interventions included (hence independent effects of phytosterol-fortified foods could not be identified)
 Subjects with disease states including diabetes, liver and kidney disease
 Results not reported for all groups or unable to be calculated for all groups

7.4 Overview of the data

Many studies included multiple trial arms because they tested more than one food matrix, or they compared source, form or type of plant sterol. Three studies were conducted in Australia, two of which were included (in their unpublished form) in previous FSANZ assessments (Clifton *et al.*, 2004; Noakes *et al.*, 2005).

Studies are available for both normocholesterolaemic and hypercholesterolaemic subjects. Most trials did not control dietary intakes of subjects; however several used low-saturated fat, low-cholesterol diets (such as the American Heart Association diet or the USA National Cholesterol Education Program step 1 diet) as the run-in phase diet. As a result of these modified diets, some change in serum lipids would be expected; hence change in LDL-cholesterol in the intervention group compared to the control group was of interest. None of the trials estimated or measured the intake of naturally-occurring plant sterols in habitual diets.

Most of the trials were double blind however the details of allocation concealment were not well described. In addition the majority of studies did not report details of recruitment method or randomisation method. Overall, the studies had well-reported eligibility and exclusion criteria; as well as defined and reported compliance of participants.

7.5 Comparison of plant sterol source, type and form

Previous assessments suggested that the source, type, and form of plant sterols all influence the cholesterol lowering effect of the plant-sterol-fortified food (Katan *et al.*, 2003). The impact of each of these is considered in the following subsections. Table 7.4 shows the effect by source, Table 7.5 shows the effect by type; and Table 7.6 shows where studies were identified to fill the assessment gaps identified in Table 7.1.

7.5.1 Effect of plant-sterol source (vegetable oil versus tall oil) in lowering LDL cholesterol

The composition of plant-sterol preparations differs according to the source i.e. vegetable oil or tall oil (refer to Section 2.1). Such differences have been previously assumed to affect the LDL-cholesterol lowering action (Soupas, 2006). Thus the source of plant sterols was investigated as a factor determining LDL-cholesterol lowering effects.

The current plant-sterol permissions in the Code are source specific (refer to Table 7.2) because previous FSANZ assessments evaluated specific phytosterol commercial preparations derived from vegetable oil or tall oil. The new specifications proposed in this Application do not differentiate by plant sterol source because preparations derived from such sources apparently do not affect the potential LDL-cholesterol lowering capacity.

Two studies directly comparing source of phytosterol preparations and one comparing source of phytosterol preparations on the LDL-cholesterol reduction (in the same food and study population) were identified. Table 7.4 shows the direct comparison of LDL-cholesterol lowering effect by source, with all studies using a plant sterol amount in the range of 1.6 to 4.0 g/day. For each study arm there was a LDL-cholesterol reduction, consistent with the expected range of 5-15 %. The results are shown as an absolute (mmol/L) and a relative reduction (%) of LDL-cholesterol. The studies are quite small; most have not reported p value or confidence interval for the mean change. These studies reported no significant difference in the reduction of LDL-cholesterol between the vegetable oil and tall oil groups. There is no consistency in direction of the differences; thus based on the available evidence, FSANZ concludes it is not possible to identify one source as being more effective than the other in lowering LDL-cholesterol.

This conclusion is supported by other reviews. Soupas (2006) reviewed studies that compared different sources of phytosterol preparations and concluded that there was no significant difference in the LDL-cholesterol lowering effect. Recent EFSA opinions on plant sterols considered the source of the sterols (vegetable or tall oil) in terms of their cholesterol lowering effect. EFSA concluded that source is not relevant to the size of the blood LDL-cholesterol-lowering effect (EFSA, 2008a; EFSA 2008b).

Based on the evidence, FSANZ concludes that the source of plant sterols is not an important factor in the consideration of the LDL-cholesterol lowering effect.

Table 7.4: Summary of studies comparing the LDL-cholesterol lowering effect of plant sterol source in edible oil spreads

Study, author, year	Total no. of subjects	Study design	Duration	Plant sterol source and form	Mean plant sterol amount (g/day)	Absolute LDL-cholesterol change (mmol/L)	Difference in LDL change between groups	Difference reported as statistically significant?	Relative LDL-cholesterol change (%)	Difference reported as statistically significant?	Statistically significant difference between VO & TO groups?
Hallikainen & Uusitupa, 1999	55	Parallel	12 wks	TO STA ester	2.3	-0.61	-0.26	No	-8.6		No
				VO STA ester	2.1	-0.35			-13.7		
Plat & Mensink, 2000	112	Parallel	4 wks	TO STA	4.0	-0.37	0.03	No	-14.6		No
				VO STA	3.8	-0.34			-12.8		
Clifton <i>et al.</i> , 2008	151	Parallel	5 wks	TO STE	1.6	-0.38	0.07	No	-9.1		No
				VO STE	1.6	-0.45			-11.4		
			8 wks	TO STE	3.0	-0.43	0.17	No	-10.4		No
				VO STE	3.0	-0.60			-14.7		

STA ester = phytosterol ester preparation; STE=free phytosterol preparation; STA= free phytosterol preparation; VO = vegetable oil derived; TO = tall oil derived

*As calculated, adjusted for changes in control group

7.5.2 Effect of plant-sterol type (phytosterol versus phytostanol) in lowering LDL cholesterol

Early studies suggested there could be differences in the LDL-cholesterol lowering effect of phytosterols and phytosterols within food matrices, mainly as a result of absorption differences (SCF, 2002). In their meta analysis, Demonty *et al.*, (2009) established a dose response curve for plant sterols and the LDL-cholesterol lowering effect. They subsequently examined the effect of phytosterols compared with phytosterols on LDL-cholesterol reduction in a subgroup analysis of 53 phytosterol and 74 phytosterol trial arms from 84 trials. The authors concluded that the LDL-cholesterol lowering effect is similar for phytosterols and phytosterols.

EFSA has also evaluated the LDL-cholesterol lowering effects of phytosterols, phytosterols and their esters in a broad range of foods. EFSA concludes that a LDL-cholesterol lowering effect of about 10% could be achieved by daily intake of 2 g of phytosterols (EFSA, 2008b) and about 9% by a daily intake of 2-2.4 g of phytosterols (EFSA, 2008a), thus concluding phytosterols and phytosterols deliver a comparable LDL-cholesterol reduction (EFSA, 2009).

The following subsections assess the evidence for a LDL-cholesterol lowering effect from previously unassessed types of plant sterols when consumed in each approved food matrix. Overall the identified randomised control trials had small samples. All the studies used a daily intake of plant sterols in the range of 1.6 to 3.2 g/day; each intervention group reported a LDL-cholesterol reduction, consistent with the expected range of 5-15 %. There were no clear differences between the groups in LDL-cholesterol reductions.

The literature search (refer to Appendix 12.1 for details) limited direct comparisons of type to studies of edible oil spreads, low-fat yoghurt, and breakfast cereal. For edible oil spreads, studies allow direct comparison of the two types of preparations. For other low fat milk, and low fat yoghurt, studies of single plant sterol preparations provided the evidence. Studies that examined plant sterol preparations added to similar food matrices of the same food group were also considered (refer to Table 7.5).

7.5.2.1 Edible oil spreads

Phytosterols and their esters are currently permitted in edible oil spreads; however FSANZ has not assessed phytosterol preparations in this food. Edible oil spreads were the first plant-sterol-fortified foods available to consumers. The LDL-cholesterol lowering effect of both phytosterols and phytosterols in edible oil spreads is well established with numerous published trials consistently showing LDL-cholesterol reductions. Table 7.4 shows the results of studies that directly compare phytosterols and phytosterols in edible oil spreads. Some studies found a greater reduction with phytosterols, whereas others found a greater reduction with phytosterols. The only significant difference in LDL-cholesterol reduction between groups was reported by Jones *et al.* (2000). The available evidence comparing phytosterols and phytosterols in edible oil spreads concludes that there is no clear difference between LDL-cholesterol reductions for phytosterols and phytosterols, thus it is not possible to identify one type as being more effective than the other.

The evidence base for edible oil spreads is extensive and consistent such that the addition of any preparation of phytosterols, phytosterols or their esters to this food delivers a LDL-cholesterol lowering effect of the expected magnitude.

Table 7.5: Summary of studies comparing the LDL-cholesterol lowering effect of phytosterol versus phytostanol preparations in certain food matrices

Study	Total no. of subjects	Study design	Food matrix	Type and form of plant sterol preparation	Baseline LDL	Mean plant sterol amount g/day [^]	Absolute LDL change (mmol/L)*	Difference in LDL change between groups (STA to STE)	Reported significant difference between groups?#	Relative LDL (%) change*
Hallikainen <i>et al.</i> , 2006	34	Cross over	Edible oil spread	STE ester	4.43	2.06	-0.45	+0.09	No	-12.3
				STA ester	4.43	2.02	-0.54			-9.3
Jones <i>et al.</i> , 2000	15	Cross over	Edible oil spread	STE	4.29	1.84	-0.56	-0.29	Yes	-13.2
				STA	4.35	1.84	-0.27			-6.4
Noakes <i>et al.</i> , 2002 Study 1	46	Cross over	Edible oil spread	STE	4.38	2.3	-0.33	+0.08	No	-7.5
				STA	4.38	2.5	-0.41			-9.4
Vanstone <i>et al.</i> , 2002.	15	Cross over	Edible oil spread	STE	4.0	1.8	-0.41	+0.01	No	-11.3.
				STA	4.11	1.8	-0.42			-13.8
Noakes <i>et al.</i> , 2005	40	Cross over	Low-fat yoghurt	STE	4.48	1.8	-0.27	-0.04	Not reported	-7.9
				STA	4.48	1.7	-0.23			-6.0
Nestel <i>et al.</i> , 2001	22	Crossover	Edible oil spread, cereal & bread	STE ester	Not specified	2.40	-0.45	-0.15	No	-13.6
	22			STA	Not specified	2.40	-0.30			-8.3
	22			STA	Not specified	2.40	-0.30			-8.3
De Jong <i>et al.</i> , 2008a	15	Parallel	Edible oil spread	STE	3.57	2.5	-0.29	+0.15	No	-8.2
	15			STA	3.44	2.5	-0.44			-12.8
De Jong <i>et al.</i> , 2008b	18	Parallel	Edible oil spread	STE ester	3.14	2.5	-0.28	+0.14	Not reported	-8.3
	19			STA ester	3.4	2.5	-0.42			-12.4

STE ester= phytosterol ester preparation; STA ester= phytostanol ester preparation; STE= phytosterol preparation; STA=phytostanol preparation

[^]as free equivalents

*Compared to change in control group

Papers contained a verbal report as to whether the difference between groups was significant but none reported p.

7.5.2.2 Low-fat milks

Phytosterols and their esters are currently permitted in low-fat milks; however FSANZ has not assessed phytostanols and their esters in this food.

No studies were identified that directly compare phytosterols and phytostanols in low-fat milks. Also, no studies were identified that investigate (free) phytostanols in low-fat milks, however Seppo *et al.*, (2007) examined intakes of 2.3 g/day of phytostanol esters in low-fat milk by 59 adults and found a LDL-cholesterol reduction of 6.2%, which is consistent with the expected cholesterol lowering effect.

Table 7.6 shows studies that were identified for previously unassessed type of plant sterol in one of the dairy food matrices. Studies of lower fat cheeses were included in this assessment as the food matrix is similar to low-fat milk and low-fat yoghurt. These studies consistently report LDL-cholesterol reductions in the range of 5 – 15%, as previously accepted by FSANZ. A recent FSANZ assessment of lower-fat cheese and other dairy foods concluded that a LDL-cholesterol lowering effect is achieved at doses around 2 g plant sterols per day, irrespective of type of plant sterol in cheeses and other dairy foods (FSANZ, 2009).

Based on the available data for phytosterols, phytostanols and their esters in the dairy food matrices that show consistent LDL-cholesterol reductions, FSANZ concludes that any preparation of phytosterols, phytostanols or their esters in low-fat milks deliver LDL-cholesterol lowering effects.

7.5.2.3 Low-fat yoghurt

Phytosterol esters are currently permitted in low-fat yoghurts, however (free) phytosterols, phytostanols and their esters have not previously been assessed by FSANZ. As shown in Table 7.5, Noakes *et al.*, (2005) directly compared phytosterols and phytostanols in low-fat yoghurt and concluded that there was no difference in the LDL-cholesterol lowering effect of either type. In addition to this direct evidence, several studies examining the LDL-cholesterol lowering effects of low-fat yoghurt and low-fat-yoghurt beverages containing phytosterols (n=5), phytostanols (n=1) and phytostanol esters (n=6) were identified (refer to Table 7.6). The studies listed in Table 7.6 consistently report LDL-cholesterol reductions ranging from 2.9% to 12.6% with plant sterol intakes in the range of 1 - 3 g/day (Volpe *et al.*, 2001; Mensink *et al.*, 2002; Hyun *et al.*, 2005; Pineda *et al.*, 2005; Salo and Wester, 2005; Plana *et al.*, 2008; Hansel *et al.*, 2007; Niittynen *et al.*, 2008; Rudkowska *et al.*, 2008). As discussed above, the range of data available for phytosterols, phytostanols and their esters in the dairy food matrices shows similar LDL-cholesterol reductions to those of currently approved phytosterol esters.

Table 7.6: Summary of identified studies that examine currently unapproved plant sterol preparations in different approved food matrices

Food Matrix		Phytosterols	Plant sterol amount (g/day)	Mean relative (%) LDL cholesterol change*	Phytosterols	Plant sterol amount (g/day)	Mean relative (%) LDL cholesterol change*	Phytosterols	Plant sterol amount (g/day)	Mean relative (%) LDL cholesterol change*
DAIRY	Low-fat milk	Previously approved in Code	-	-	-	-	-	Seppo <i>et al.</i> , 2007 [^]	2	-6.2
	Low-fat yoghurt	Volpe <i>et al.</i> , 2001	1	-6.2,	Noakes <i>et al.</i> , 2005 [^]	1.7	-5.0	Mensink <i>et al.</i> , 2002	3	-10.3
		Rudkowska <i>et al.</i> , 2008 [^]	1.6	4.2, -8.7				Hyun <i>et al.</i> , 2005;	2	-7.84
			1.6					Pineda <i>et al.</i> , 2005	1.2	-8.5
	Low-fat yoghurt beverages	Plana <i>et al.</i> , 2008	1.6	-12.42	--			Seppo <i>et al.</i> , 2007 [^]	2	-2.9,
		Hansel <i>et al.</i> , 2007	1.6	-9.19				Salo and Wester, 2005;	2	-3.2,
	Cheese	-	-	-				Jauhiainen <i>et al.</i> , 2006	2	-11.8
										2
CEREAL PRODUCTS	Breakfast cereal, bread and edible oil spread	-	-	-	Nestel <i>et al.</i> , 2001 [#]	2.4	-8.3	-	-	-

*Compared to change in control group

[^] Seppo *et al.*, and Noakes *et al.* included more than one dose or tested different plant sterol exposures (i.e. with or without meal)

[#]Nestel *et al.* also shown in Table 7.5

Based on the evidence directly comparing phytosterols and phytostanols as well as studies of single phytosterol, phytostanol and phytostanol ester preparations in low-fat yoghurt, low-fat-yoghurt beverages and other dairy food matrices, FSANZ concludes that preparations of phytosterols, phytostanols or their esters in low-fat yoghurt can deliver a LDL-cholesterol lowering effect.

7.5.2.4 Breakfast cereals

FSANZ has previously approved the addition of phytosterol esters to some breakfast cereals based on LDL-cholesterol lowering effects within the expected magnitude. The evidence base for plant sterols in many low-fat food matrices (excluding dairy foods) is limited; breakfast cereal is one of these. There are no comparisons or direct evidence supporting the LDL-cholesterol lowering effects of phytosterols and phytostanols and phytostanol esters in breakfast cereal or other similar cereal products (refer to Table 7.6). Many studies which have examined phytosterols, phytostanols and phytostanol esters in breakfast cereal have also examined substances such as beta-glucan which are considered potential co-interventions (hence independent effects of phytosterol-fortified foods could not be identified).

As shown in Tables 7.5 and 7.6, Nestel *et al.*, (2001) directly compared phytosterol esters and phytostanols when added to breakfast cereal, bread and edible oil spread consumed together (in the same meal), reporting cholesterol reductions of 13.6% and 8.3% respectively. However, because the plant-sterol-fortified cereal effects were not measured separately from other plant-sterol-fortified foods eaten concurrently, this mean reduction may not reflect the effect of the cereal.

Based on the available data FSANZ concludes that preparations of phytostanol esters can deliver LDL-cholesterol lowering effects. There are no comparisons or direct evidence supporting the LDL-cholesterol lowering effects of phytosterols and phytostanols in breakfast cereal or other cereal products.

7.5.3 Effect of plant-sterol form (esterified versus free) in lowering LDL cholesterol

Free phytosterols and phytostanols were originally difficult to incorporate into foods (Plat, 2000). Esterification of plant sterols with fatty acids enhances their solubility and their dispersion in the intestine, thereby promoting their efficacy (Katan *et al.*, 2003). Thus, possible differences in LDL-cholesterol lowering effects of free and esterified plant sterols have also been studied on the basis of potential differences in absorption. However, studies have shown that following ingestion, esterified phytosterols and phytostanols are hydrolysed to their corresponding free phytostanols by an enzyme called pancreatic cholesterol esterase in the same manner as dietary cholesterol esters (Miettinen *et al.*, 2000; SCF, 2000). This suggests that the absorption differences of free and esterified plant sterols will not result in significant differences in the LDL-cholesterol lowering mechanism.

Demonty *et al.*, (2009) evaluated the effect of plant sterol esterification on an LDL-cholesterol dose response curve. The studies in the analysis covered a variety of plant-sterol-fortified foods. The authors concluded that the effect of free and esterified forms of plant sterols did not differ, thus properly formulated free phytosterols and phytostanols were considered to be as effective as phytosterol and phytostanol esters.

AbuMweiss *et al.*, (2006) directly compared free and esterified phytosterols in edible oil spreads. Although they reported minor (not statistically significant) reductions in LDL-cholesterol for all the treatment groups (as compared to control), the study found no difference between the free and esterified form. Single studies that compared free and

esterified forms of plant sterols in low-fat dairy foods were identified which showed comparable LDL-cholesterol reductions.

No direct comparisons of free and esterified forms were identified in breakfast cereal or similar cereal products. Several studies were identified for phytosterols and phytostanol esters in these foods, however little evidence was available on the LDL-cholesterol lowering effects of free forms in breakfast cereal and cereal products. Thus there is some uncertainty about the LDL-cholesterol lowering effect of free plant sterols in breakfast cereal. Plant sterols cholesterol-lowering effect is determined by their availability for absorption, thus the formulation into the food matrix plant sterols is important for solubility (Engel & Schubert, 2005; Abumweis *et al.*, 2008). As discussed in Section 2 and Section 6 of this report, the physical and chemical properties of plant sterols can result in technical incorporation difficulties in some low-fat food matrices. However, it is anticipated that new processes and methods could be applied to address such incorporation difficulties. Demonty *et al.*'s., (2009) analysis concluded that free and esterified plant sterols did not differ in the maximal LDL-cholesterol reduction, supporting earlier conclusions that proper formulation of plant sterols results in LDL-cholesterol lowering effects.

Despite the evidence gaps in the literature identified in Table 7.6 for each type of plant sterol in each food matrix, the evidence for the effect of free or esterified form suggests that form will not determine the LDL-cholesterol lowering effect providing plant sterols are adequately incorporated into the food.

The range of available data for free and esterified plant sterols shows similar LDL-cholesterol reductions to currently approved plant sterols for edible oil spreads, low-fat milk and low-fat yoghurt. There is some uncertainty around free form plant sterols in breakfast cereal, as no direct efficacy evidence was identified, however evidence suggests free plant sterols will deliver a cholesterol lowering effect if they are soluble, once incorporated into the food matrix.

7.6 Conclusion

FSANZ concludes that the source of plant sterols is not an important factor in the consideration of the LDL-cholesterol lowering effect. The totality of evidence suggests that when consumed in edible oil spreads, low-fat milk and low-fat yoghurt, phytosterols, phytostanols and their esters can deliver cholesterol lowering effects in the same magnitude as those of previously approved plant sterols. The limited direct evidence for the free form of plant sterols in breakfast cereal and cereal products results in some uncertainty, however evidence suggests they will deliver a LDL-cholesterol lowering effect if suitably dispersed in the food matrix.

7.7 Response to risk assessment question 4

DO PLANT STEROLS (CONFORMING TO THE MODIFIED JECFA SPECIFICATIONS) LOWER BLOOD LDL-CHOLESTEROL WHEN CONSUMED IN EACH OF THE FOUR APPROVED FOODS?

FSANZ concludes that plant sterols that conform to the modified JECFA specifications do lower LDL-cholesterol when consumed in the four currently approved food matrices, providing that they are suitably dispersed in the food matrix.

8. DIETARY INTAKE AND CONSUMPTION PATTERNS

8.1 Previous assessments

Previous risk assessments carried out by FSANZ have estimated the dietary intake of phytosterols in Australia and New Zealand (FSANZ, 2002; FSANZ, 2004a; FSANZ, 2002b) and reviewed the literature on purchasing behaviours and consumption patterns (FSANZ, 2009). Market share and product substitution issues have previously been considered by FSANZ as part of the impact analysis in a number of assessment reports (FSANZ, 2009).

8.2 Dietary intake

8.2.1 Approach to estimating dietary intake

Before Application A1019, the general approach to assessing dietary intake of a novel food ingredient was to predict intake based on food consumption amounts reported in national nutrition surveys, assuming:

- all foods that could contain the novel food do indeed contain it; and/or
- only a proportion of these foods contain the novel food ingredient, with the proportion determined based on predicted market share for the novel food.

Neither of these approaches is entirely appropriate for carrying out a dietary intake assessment of applications seeking to add plant sterols to foods. The first approach will provide an overly protective estimate of intake for the whole population because, in practice, only a subset of foods permitted to contain plant sterols will actually contain them and only some people will choose these products. The second approach may indicate long-term intake of plant sterols across the population as a whole, taking into account consumers and non-consumers of plant sterols. However, market share estimates are unlikely to reflect consumption patterns among those individuals who are regular consumers of foods fortified with plant sterols. Neither approach estimates intake in those consumers who deliberately alter their eating habits to include the manufacturers recommended number of serves of foods fortified with plant sterols.

In Application A1019 (FSANZ, 2009), FSANZ therefore used the following approach to consider the potential dietary intake of plant sterols:

- reviewing dietary intake estimates from previous applications
- assessing the dietary intake of phytosterols from the recommended number of serves of different foods containing plant sterols
- analysing consumption data from National Nutrition Surveys and calculating dietary intake of phytosterols that could be experienced if conventional products were substituted with food fortified with plant sterols
- assessing dietary intakes of phytosterols by children based on the findings of the 2007 Australian National Children's Nutrition & Physical Activity Survey.

Appendix 3 of the Risk Assessment Report for Application A1019 discusses the use of these databases in the dietary intake assessment in detail.

8.2.2 Estimating dietary intake based on specific plant sterols

The estimates of plant sterol dietary intake undertaken by FSANZ and those published in the literature are based on their plant sterol equivalents, i.e. irrespective of the source of the plant sterols; their intake has been expressed as free phytosterols. For purposes of

describing dietary intake, all plant sterols are converted to the equivalent amount of free phytosterols. The intake estimates presented are not equivalent to the amounts of commercial plant sterol preparations that are actually added to foods. For example, 1.8 g of added phytosterol esters is approximately equal to 1.1 g of free phytosterols. Consequently, broadening the specification and the associated permissions to include a wider variety of plant sterol preparations to the same foods does not change estimates of dietary intake. Were target consumers to adhere to the recommended size and number of serves of plant-sterol-fortified food, intake of plant sterols would be equivalent to 2.2 g of phytosterol, irrespective of the source or particular preparation of plant sterols used in the products.

8.2.3 Dietary intake estimates from previous assessments

Dietary intake estimates for the whole population based on the assumption that all foods that could contain plant sterols do indeed contain it, showed that mean and 95th percentile intakes based on consumption amounts for non-fortified products were not expected to exceed 1.9 g/day and 4.8 g/day phytosterol respectively (Table 8.1). It should be noted that the demographics and consumption patterns of those consumers that intentionally purchase fortified foods are likely to diverge from the consumption patterns and demographics of those consumers that reported consuming the equivalent non-fortified foods in national nutrition surveys. Any dietary intake assessment that estimates intakes of fortified products on the basis of the consumption patterns and demographics of consumers of non-fortified equivalents therefore includes a number of uncertainties. This conclusion is supported by the findings reported in European post-launch marketing surveys that showed that overall plant sterol equivalent intake was low for the majority of consumers and less than the amount required for achieving the promised benefit (refer to A1019; FSANZ, 2009).

Plant sterols are added with the intention of providing a specific health benefit and consumers are encouraged to actively select a specified number of serves of foods containing plant sterols with the intention of realising this benefit. The intake of phytosterols that can be achieved in the number of serves of each food recommended by the manufacturer is listed in Table 8.2. This is based on addition of plant sterols to the permitted maximum equivalent amount of phytosterols permitted or proposed and the nominated serve sizes of the food. The amount of plant sterols added to each food by the manufacturer is such that consumption of two-three serves of a single food or a mixture of different foods would be equivalent to an amount of phytosterols that achieves a cholesterol lowering effect.

Plant-sterol-fortified products are targeted at consumers with elevated blood cholesterol, typically above 40 years of age. Incidental consumers are any consumers who inadvertently consumed plant sterol containing products. While products fortified with plant sterols are mainly consumed by adults, there is at least some incidental intake of plant sterols by children. Among Australian children participating in the 2007 Australian National Children's Nutrition and Physical Activity Survey (NCNPAS), 2.2% consumed foods fortified with plant sterols; predominantly edible oil spreads (Table 8.3). In contrast, 78% of children reported eating any type of edible oil spread. Similarly, 84% of 2-12 year olds reported eating edible oil spreads in the 1995 National Nutrition Survey. The majority of children consuming edible oil spreads were not consumers of spreads containing plant sterols.

The weighted mean consumption of plant sterol containing margarines averaged over two days for those children reporting consumption was 5 g/day. In comparison, the mean consumption averaged for all edible oil spreads was estimated to be 7-8 g/day. The equivalent mean intake of phytosterols for consumers based on their maximum permitted levels in edible oil spreads and low-fat milks and averaged over two days of consumption ranged from 0.4 to 2.5 g/day. Mean plant sterol equivalent intakes were highest for 14-18 year olds, but 90th percentile intakes were highest for 9-13 year olds (Table 8.3).

8.3 Purchasing behaviours and consumption patterns

As was outlined in the Risk Assessment Report for Application A1019, plant-sterol-fortified food products appear to occupy a niche market. Most users of these products are older adults, tertiary educated and have (or are at risk of) high serum cholesterol levels or CVD. Users of fortified products generally self-select have an active interest in their health, and use plant-sterol-fortified foods as part of a generally healthy lifestyle and diet. Users are predominantly motivated by concern about their health, particularly cholesterol or CVD.

International evidence suggests that in markets where a variety of plant-sterol-fortified products (i.e. a number of foods fortified with a diverse range of plant sterol preparations) have been available for some time (Netherlands, the United Kingdom, France, Germany, and Belgium), increased product availability is not linked to increased or excess consumption of plant sterols. This is because most users consume one or two products, and substitute fortified products for each other. Most of the evidence collected across Member States in the European Union indicates that current intakes of plant sterols are below 3 g/day (EFSA, 2008c; SCF, 2002).

Overall, the evidence suggests that in a mature plant sterol market, consumers substitute between plant-sterol-fortified products and that there is a tendency for suboptimal intakes of plant sterols. It is unlikely that this behaviour pattern would change based on the type of plant sterols that may be added to these products.

Table 8.1 Plant sterol^a dietary intake - summary of assessments previously reported^b

Foods included in estimates:	Edible oils spreads	✓	✓	✓	✓	✓
	Breakfast cereals		✓			✓
	Low-fat milk			✓	✓	✓
	Low-fat yoghurt				✓	✓
Estimated Intakes ^c (g/day):	Mean	1.3	≤ 1.7 [2.1]	≤ 1.9 [2.3]	≤ 1.9 [2.4]	≤ 1.9
	95 th percentile	3.5	≤ 4.4 [5.5]	≤ 4.8 [5.8]	≤ 4.7 [5.9]	≤ 4.7

^a equivalent amount of non-esterified plant sterols
^b estimates of dietary intake were conducted assuming the concentration of plant sterols was 0.8 g/serve, except for reduced and low-fat milk in Application A508 which was assumed to contain 0.9 g/serve. During the review of applications, concentration in the foods assessed was assumed to be 1.0 g/serve plant sterols
^c Less than or equal to the value presented for consumers across all of the population groups assessed in Australia and New Zealand. Values in square brackets show mean and 95th percentile intakes calculated in the Review of the applications

Table 8.2: Dietary intake of phytosterols^a from the recommended number of serves of various foods

Food	Number of serves ^b (total weight of serves in g)	Phytosterol intake (g/day)
Edible oil spread ^c	3 (30)	2.5
Breakfast cereal ^c	3 (135)	2.7
Low-fat milk ^c	3 (750)	2.9
Low-fat yoghurt ^c	3 (440)	2.1
Lower-fat cheese ^d	2 (40)	2.2

^a equivalent amount of unesterified phytosterols

^b Concentration and serving size used in previous dietary intake

assessments based on applicant's information. ^c Foods currently permitted to contain phytosterols

^d Application to permit addition of phytosterols under consideration by FSANZ

Table 8.3 Intake of phytosterols^a from plant-sterol-containing foods consumed by age groups, consumers only (2007 NCNPAS)^b

Age group	Consumers (n)	Phytosterols (g/day)		
		mean	median	90 th centile ^c
2-3	27	0.2	0.1	0.5
4-8	22	0.4	0.4	0.9
9-13	30	0.7	0.4	2.4
14-16	23	0.4	0.3	1.1
2-16	102 (2.2%)	0.4	0.3	1.4

^a equivalent amount of unesterified phytosterols ^b Values are means of two days and are weighted to reflect national population distribution ^c Numbers too low to calculate 95th percentiles

8.4 Understanding of the products

Empirical studies on labelling of plant sterol products suggest there are low levels of label readership within users in the UK, and Australia and New Zealand (UKFSA, 2006; FSANZ, 2006b). Misunderstanding of the role of plant sterols within respondents in the UK, a high proportion of German users from outside of the specific targeted group (e.g. over 45 years of age, elevated cholesterol level), and a low degree of familiarity with the labelling information suggest that labelling requirements mandated on products containing plant sterols, alone, has not been an effective way of communicating information about the appropriate use of these products.

In Australia and New Zealand, fortified-spread users have mixed understandings of the role of plant-sterol-fortified products and current mandatory labelling information (FSANZ, 2006b). However, users do not believe plant-sterol-fortified spreads act as a 'magic bullet' that will absolve them of further responsibility for healthy behaviour. Data around diet and exercise highlight that those who consume plant-sterol-fortified spreads do not have significantly less healthy diets and exercise levels than those who do not use plant-sterol-fortified spreads (FSANZ, 2006b).

These findings, combined with evidence that in more mature international plant sterol markets consumers substitute between plant-sterol-fortified products, suggests it is unlikely that consumers would pay attention to any detailed information on the type of plant sterols provided on the label of plant-sterol-fortified foods.

8.5 Market share

The European Union has the world's most mature market for foods with added plant sterols. EFSA (2008) estimated the apparent consumption of plant sterols in 2007 at 7600 tonnes and the market share of foods containing plant sterols in any of the ten categories available in Europe to be close to 10%. The total European plant sterol market has been estimated to be worth US\$184.6 million in 2005, and is predicted to reach US\$395.2 million in 2012 (Frost & Sullivan, 2006). Currently the three major players in the European market (Cognis, ADM and Raisio) combined dominate the market, accounting for about 80% of the market share in terms of revenue (Frost & Sullivan, 2006).

Over the 2004-06 period, edible oil spreads with added plant sterols had about 2% of the German edible spreads market (Anon., 2006a). In the UK, plant-sterol-fortified products comprised 2-12% of the total spreads market, up to 15% of the yoghurt and yoghurt drink market and up to 6% of the total milk market (Anon., 2005, quoted by EFSA 2008). In Finland it was estimated from a consumer survey that 4.5% of the study group used phytosterol ester margarines (Simojoki *et al.*, 2005).

In summary, the market share for plant-sterol-fortified products in a very mature market using a wide variety of plant sterol preparations in a wider variety of foods than in Australia and New Zealand is likely to be less than 10%.

8.6 Product substitution

There are a number of possible ways in which consumers integrate a new fortified food product into their diets, ranging from complete substitution of an existing food through to complete addition of a new food without any changes to intake of other foods. There is limited consumer research on this specific aspect of plant-sterol-fortified food consumption. However the research suggests that consumers substitute between like food products, e.g. fortified milk replacing normal milk in the case of a new consumer, or fortified milk replacing another type of fortified product in the case of an existing consumer. In the context of this Application, the 'new products' would be similar products but with different plant sterol ingredients. Given the limited levels of label readership, and the like for like substitution that occurs within the plant sterol product range, it is considered unlikely that consumers would differentiate products on the basis of type of plant sterol.

8.7 Pricing signals

It is possible that the wider availability and permission to use a wider range of plant sterol preparations could result in a greater number of brands entering the Australia New Zealand market place. This may result from imported products that previously could not get market access as well as from locally produced products. Existing plant-sterol-fortified products are more expensive than their conventional counterparts. Price discounting on these products does already occur, but there is no evidence from consumer research and dietary intake assessments that this leads to increased intakes by consumers who inadvertently consumed plant-sterol-fortified products. In particular, it is considered unlikely that the potential availability of less costly plant sterol preparations would lead to any substantial reduction in

the price premium for plant-sterol-fortified foods, or translate into increase purchasing or consumption of these products.

Plant-sterol-fortified products command a market premium. Depending on the product, prices may be up to five times higher than their non-fortified equivalent. According to EFSA (2008), phytostanol esters are more expensive to produce than phytosterol esters because they require the additional step of hydrogenation. This is probably the reason that the market shares for phytosterol ester and phytostanol-ester-containing products in the European Union is approximately two thirds and one third respectively (Anon., 2006b). Products containing phytosterols or phytostanols (rather than their esters) have less prominence in the European market. In contrast, products that are fortified with preparations rich in phytostanol esters are not available in the Australia and New Zealand marketplace. Making these more highly priced preparations available to local manufacturers is unlikely to result in cheaper end products.

8.8 Conclusion

The estimates of plant sterol dietary intake undertaken by FSANZ and those published in the literature are based on their plant sterol equivalents. Consequently, broadening the specification and the associated permissions to include a wider variety of plant sterol preparations in the same foods does not change existing estimates of dietary intake. It is unlikely that behaviour pattern would change based on the type of plant sterols that may be added to these products or that consumers would pay attention to any detailed information on the type of plant sterols provided on labels.

The evidence suggests that consumers substitute between plant-sterol-fortified products in a mature plant sterol market. An increase in the total number of products is unlikely to result in a substantial increase in the share of these products in individual food product market segments. It is also unlikely that the potential availability of less costly plant sterol preparations would lead to any substantial reduction in the price premium for plant-sterol-fortified foods, which in turn would translate into increase purchasing or consumption of these products. The market share for plant-sterol-fortified products in a very mature market using a wide variety of plant sterol preparations in wide variety of foods is likely to be no more than 10%.

8.9 Response to risk assessment questions 5 and 6

8.9.1 Question 5:

DOES DIETARY INTAKE, UNDERSTANDING OF THE PRODUCT OR PURCHASING BEHAVIOUR DIFFER ACCORDING TO THE TYPE AND FORM OF PLANT STEROLS?

Broadening the specification and the associated permissions to include a wider variety of plant sterol preparations in already approved foods does not change existing estimates of dietary intake. It is highly unlikely that the form of plant sterols added to an existing food vehicle could substantially change purchasing behaviour or product understanding.

8.9.2 Question 6:

WOULD A PERMISSION TO ADD PLANT STEROLS (CONFORMING TO THE MODIFIED JECFA SPECIFICATIONS) TO APPROVED FOODS BE LIKELY TO:

- A) *INCREASE THE NUMBER OF BRANDS AVAILABLE IN THE MARKET?*
- B) *RESULT IN FLOW-ON CHANGES IN CONSUMPTION PATTERNS?*

It is possible that the wider availability and permission to use a wider range of plant sterol preparations could result in a greater number of brands entering the Australia New Zealand market place. It is unlikely that this would lead to any substantial reduction in the price premium for plant-sterol-fortified foods, or translate into increase purchasing or consumption of these products.

9. UNCERTAINTIES IN THE RISK ASSESSMENT

The available data for plant sterols are considered to be sufficient to provide a high level of confidence in the conclusions of this report in regard to the safety and suitability for purpose of plant sterols that conform to the JECFA specifications and when consumed in the four approved foods by all population groups. One uncertainty identified in the assessment is whether free phytosterols and phytosterols added to breakfast cereals could deliver a LDL-cholesterol lowering effect if such plant sterols could not be uniformly distributed throughout the food matrix.

10. CONCLUSION

The evidence supports the safety of plant sterols at present levels of consumption irrespective of the proportion of the individual phytosterol or phytosterol components used or their source. New investigation of the effects on serum sterols of plant sterol consumption indicated no increased risk of CVD other than in the rare group of individuals with sitosterolaemia, a severe disease of lipid metabolism. FSANZ concludes that phytosterols, phytosterols and their esters are bioequivalent in terms of their food safety properties.

All compositional variants of plant sterols that conform to the modified JECFA specifications are suitable for incorporation into the four foods approved in the Code. There are likely to be some technical issues around incorporating free forms of plant sterols into some foods to achieve 100% uniform distribution but there is a range of technical solutions to this issue.

Plant sterols under consideration in this Application can lower blood cholesterol when added to the four approved foods and consumed in appropriate quantities.

It is possible that the wider availability and permission to use a wider range of plant sterol preparations could result in a greater number of brands entering the Australia and New Zealand market place. Such a change in the market is unlikely to reduce the current price premium for these products or modify purchasing behaviour or product understanding. Therefore, existing estimates of dietary intake are not expected to change.

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12. APPENDIX

12.1 Details of literature search

Figure A12.1: Literature search details for potential risk of plant sterol and CVD studies

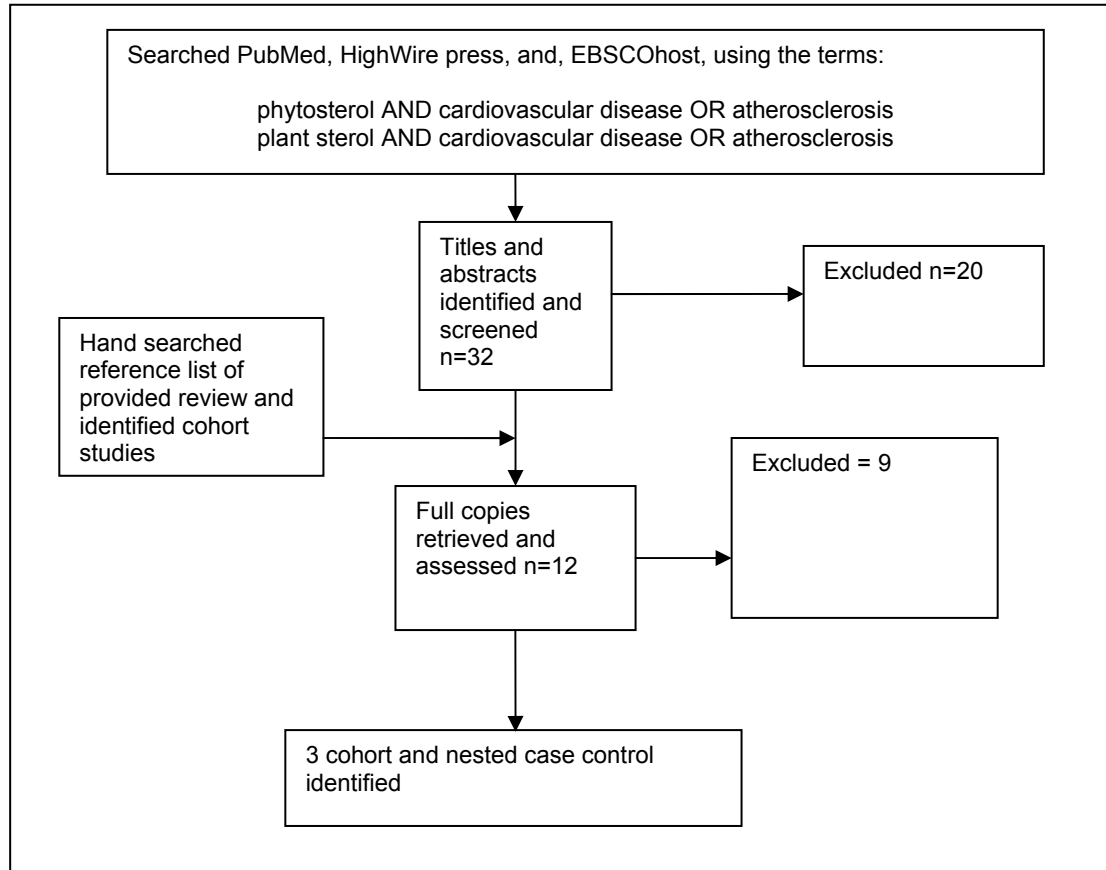


Figure A12.2: Literature search details for LDL - cholesterol lowering effect

