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

Supporting Document 1

RISK ASSESSMENT REPORT

APPLICATION A1019
EXCLUSIVE USE OF PHYTOSTEROL ESTERS IN LOWER-FAT CHEESE PRODUCTS

Executive Summary

Plant sterols (phytosterols, phytostanols and their esters) are a group of naturally occurring steroid compounds that are structurally related to cholesterol. The usual dietary sources of plant sterols include vegetable oils and certain vegetables. Plant sterols can either be extracted from vegetable oils or from tall oil soap which is a by-product of coniferous wood pulp. Commercial plant sterol mixtures consist predominantly of the cholesterol-like compounds β-sitosterol, sitostanol, campesterol and campestanol, which are poorly soluble in lipids. To improve their solubility in food matrices with high lipid content, phytosterols and phytostanols can be esterified with food-grade long chain fatty acids derived from vegetable oils. Phytosterol and phytostanol esters are chemically stable and resistant to heat and oxidation.

Foods with added plant sterols have been in the food supply since the 1990s, particularly in Europe and the USA. In Australia and New Zealand, the addition of plant sterols is currently permitted in edible oil spreads, low fat milk and yoghurt, and low sugar/high fibre breakfast cereal. This Application seeks permission to extend this to lower fat cheese products through the addition of tall oil phytosterol esters at 1.1 g free phytosterols per slice of individually wrapped cheese or 2.2 g per 40 g mini tub of cream cheese.

The Applicant has demonstrated that tall oil phytosterol esters can be evenly dispersed through products such as lower fat cheese. The dispersibility and stability of tall oil phytosterol esters indicates that the lower fat cheese products proposed as the food vehicle should deliver a consistent amount of plant sterols per serve over the expected shelf life of these products. There is currently no specification for tall oil phytosterol esters in the Australia New Zealand Food Standards Code (the Code).

Given the comprehensive nature of previous assessments of the chemical and physical properties of plant sterols and their safety, efficacy and dietary intake, the focus of this assessment is on new information that has become available over recent years, particularly since the last FSANZ review of plant sterols in 2005.

Previous safety reviews conducted by FSANZ, other regulatory agencies, and international scientific committees using toxicity data and studies in humans have concluded that plant sterols are poorly absorbed from the gastrointestinal tract. Minor differences in the extent of absorption occur among individual sterol compounds. Irrespective of these minor differences, less than 5% of dietary plant sterols enter the circulation. By contrast, approximately 80% of structurally related cholesterol in the diet enters the circulation. The

major proportion of ingested plant sterols is excreted unchanged in the faeces. Plant sterols that are absorbed are transported to the liver, metabolised and excreted through the bile.

Based on the results of numerous short-term and sub-chronic toxicity studies showing no adverse effects associated with plant sterols administered to animals at high doses, FSANZ has previously concluded that consumption of plant sterol fortified foods raises no safety concerns and a reference health standard is not warranted. This conclusion was also reached by regulatory agencies in Europe and the USA. However in 2008, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) established an Acceptable Daily Intake (ADI) of 40 mg/kg bw, based on heart muscle degeneration in rats observed after 90 days of gavage administration. FSANZ has re-evaluated this toxicological evidence, together with other 90-day dietary feeding studies. Based on an analysis of all toxicological information, FSANZ finds no justification for establishing an ADI for plant sterols. The apparent treatment-related adverse effect is entirely explained by the background incidence of pathology reported in historical control data relevant for the strain of rats used in the experiments. Coupled with the absence of corroborating evidence from other studies in rats administered high doses of plant sterols, claims of an adverse effect due to plant sterols cannot be substantiated.

Consuming plant sterol fortified foods is associated with a reduction in serum levels of some carotenoids, particularly β-carotene, a precursor of vitamin A. However, after standardisation for reduced levels of LDL-cholesterol, only the change in serum levels of β-carotene is significant. Changes in other carotenoids are not significant. The evidence from numerous studies shows that carotenoid levels remain within a broad natural range considered to be typical of variable diets. In addition, clinical studies have demonstrated that increasing consumption of fruits and vegetables, particularly varieties rich in carotenoids, compensates for lower absorption of fat-soluble vitamins. Overall, consumption of plant sterols does not result in changes in vitamin levels that could be considered to raise any nutritional concerns.

Although poorly absorbed, the levels of plant sterols in blood do increase with consumption of these substances in the diet. Patients with a rare lipid disorder that results in hyperabsorption of dietary plant sterols develop early atherosclerosis and coronary heart disease. Some population studies have therefore investigated whether the modest increase in serum plant sterols in normal consumers is associated with a heart disease risk. A comprehensive review of the literature does not indicate any population health risk arising from consumption of plant sterol fortified foods. Even with consumption of sterol fortified foods, the levels of plant sterols in the blood remain at less than 1% of total sterols. Plant sterols are not present in sufficient amounts to be considered as an additional risk factor for cardiovascular disease under normal circumstances.

Given the lack of toxicity in animals administered extremely high doses of plant sterols, and the lack of adverse effects in human studies with plant sterol fortified foods, no new evidence has been presented that would indicate the need to change previous conclusions regarding the safety of plant sterol fortified foods. Consumption of plant sterols at the proposed levels of use does not raise any food safety concerns.

FSANZ has previously considered evidence of a cholesterol-lowering effect of various types of plant sterols and their esters in specific foods. The nature and form of the plant sterol compounds, the food matrix and method of incorporation into foods can all influence the cholesterol lowering effect to some extent, as can genetic factors, background diet and baseline cholesterol levels. For most people, it is necessary to consume between 1-3 g/day plant sterols to potentially achieve the optimal cholesterol-lowering effect. Consumption above approximately 3 g/day does not result in any additional cholesterol-lowering effect. The potential reductions in serum cholesterol are in addition to reductions due to a modified

diet or cholesterol-lowering medications such as statins. The data assessed in this Application show that tall oil phytosterol esters in a lower fat cheese matrix can deliver a similar cholesterol lowering effect to other permitted food vehicles, including other dairy foods across a range of fat contents.

Research on use of plant sterol fortified foods shows that these foods occupy a niche market. Most users are older adults, tertiary educated and have, or are at risk of, high cholesterol levels or cardiovascular disease. Users of these foods generally self-select them, have an active interest in their health (particularly cholesterol levels or cardiovascular disease) and use plant sterol products as part of a generally healthy lifestyle and diet. However there is consumption of plant sterol fortified foods for other reasons, for example because someone else in the house consumes the product.

Increased product availability does not appear to be linked to high intake of plant sterols. Users of plant sterol fortified products tend to use one product at a time, with only a small proportion consuming two or three different products per day. European consumers of plant sterol fortified foods do not consume cheese spreads containing added plant sterols on a daily basis and only eat cheese as the second or third choice after other plant sterol fortified products. Studies of consumer use of plant sterol fortified products show that many users do not consume sufficient amounts to gain a health benefit. Generally there seem to be very few consumers whose plant sterol intake would exceed 3 g/day.

Previous FSANZ estimates of intakes of plant sterols from a range of sterol fortified foods showed that mean dietary intake of plant sterols would not exceed 1.9 g per day in any population group, assuming existing consumption patterns were maintained and there was complete replacement of regular foods with their plant sterol fortified counterparts. At the 95th percentile of consumption, intake of plant sterols was estimated to be 4.8 g/day. If consumers eat the Applicant's recommended two serves of lower fat cheese, intake of plant sterols from cheese would be 2.2 g per day, which is within the range for a potential cholesterol-lowering effect. However only a small, albeit growing, proportion of consumers in Australia and New Zealand consume lower fat cheese and the average amount consumed daily would be insufficient to achieve this intake of plant sterols. Consumers would therefore need to deliberately change their eating patterns to consume more serves of lower fat cheese on a regular basis or also eat other types of foods containing plant sterols.

A 2007 survey of Australian children showed that approximately 2% of Australian children reported consuming products containing added plant sterols. This survey information enabled FSANZ for the first time to estimate intakes of plant sterols in children aged 2-16 years. Intakes were considerably lower in these children than predicted in previous FSANZ estimates of intake that assumed replacement of existing foods with foods fortified with plant sterols.

Critical uncertainties

FSANZ takes a conservative approach in assessing the health and safety risk for all novel foods to ensure consumers are adequately protected; the degree of conservatism depends on the quality of the data available and any identified data gaps. 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 safety and suitability for purpose of plant sterol fortified lower fat cheese for all population groups.

Conclusions of the risk assessment

- The properties of the tall oil phytosterol esters proposed for use by the Applicant, and the manufacturing process by which they are added to lower fat cheese, are suitable to deliver a consistent amount of phytosterols that are likely to remain stable during storage under usual conditions.
- On the basis of the available safety data, the use of plant sterols in lower fat cheese at the proposed level does not raise any food safety concerns.
- Previous assessments have concluded that a reference health standard is not warranted. There is no new toxicological evidence that would indicate the need to change previous conclusions regarding the safety of plant sterol fortified foods.
- 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. 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 lower fat cheese products containing plant sterols can potentially lower LDL blood cholesterol levels.
- If consumers adhere to the recommended size and number of serves of plant sterol fortified lower fat cheese, daily intake of plant sterols is estimated at 2.2 g, which is within the range shown to be optimal for a cholesterol-lowering effect.
- A small proportion of children are likely to consume lower fat cheese containing added tall oil phytosterol esters, however this is not considered to raise a health concern.

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1. Background

Plant sterols (phytosterols, phytostanols and their esters) are a group of naturally occurring, lipophilic steroid compounds that are structurally related to cholesterol. Phytostanols are the saturated counterparts of phytosterols and are less abundant in nature. The usual dietary sources of plant sterols include vegetable oils such as corn, sunflower and soybean oils, and certain vegetables.

When consumed in sufficient amounts, plant sterols have been shown to reduce blood cholesterol levels. Plant sterols added to foods can either be derived from vegetable oils (VO), or can be extracted from tall oil soap which is a by-product of the wood pulping process used for coniferous trees in North America and Europe, so-called tall oil (TO) phytosterols.

Regardless of their source, commercial plant sterol mixtures consist predominantly of the compounds β-sitosterol, sitostanol, campesterol and campestanol. Depending on the plant source, commercial plant sterols also contain varying amounts of minor components, such as stigmasterol and brassicasterol. To improve their solubility in some food matrices, phytosterols and phytostanols can be esterified with food-grade long chain fatty acids derived from vegetable oils.

2. Introduction

The Applicant seeks permission to use TO phytosterol esters in lower fat cheese products. Phytosterol esters derived from VO are currently permitted in edible oil spreads, low-fat milk, low-fat yoghurt and high-fibre breakfast cereal. Unesterified TO phytosterols are permitted in edible oil spreads and low-fat milk.

Previously, differences in the food technology properties and composition of VO- and TO-derived plant sterols resulted in separate assessments based on the plant source. Approvals for (i) 'phytosterol esters derived from vegetable oils'[1], and (ii) 'unesterified tall oil phytosterols'[2], as separate novel food ingredients subsequently followed in the Code.

Given the comprehensive nature of previous scientific evaluations, the primary focus of this assessment is to review aspects of the scientific evidence, and consider any new information that has become available over recent years, particularly since the most recent assessment by FSANZ in 2005.

2.1 Terminology

The following terms are used in this report:

Plant sterols	Collective term referring to free and esterified phytosterols and phytostanols, regardless of the biological source.

[1] 'Vegetable oil-derived phytosterol esters' (characterized in the schedule to Standard 1.3.4 – Identity and purity) are currently permitted in edible oil spread, low-fat milk, low-fat yoghurt, and high fibre/low sugar breakfast cereal.

[2] 'Unesterified tall oil-derived phytosterols' (characterized in the schedule to Standard 1.3.4 – Identity and purity) are currently permitted in edible oil spread and low-fat milk.

Phytosterols	Free (non-esterified) steroid alcohols occurring in plants, e.g. β-sitosterol, campesterol, stigmasterol. Dietary intake of plant sterols is expressed in terms of free phytosterols.
Phytostanols	Any of the fully saturated phytosterols e.g. sitostanol, campestanol
Phytosterol esters	Phytosterols esterified with food grade fatty acids derived from vegetable oils.
Phytostanol esters	Phytostanols esterified with food grade fatty acids derived from vegetable oils.

3. Key Risk Assessment Questions

For this application, the risk assessment questions were developed in the context of the Section 18 Objectives under the *Food Standards Australia New Zealand Act 1991*, having regard to the Ministerial Policy Guidelines for the Addition of substances other than vitamins and minerals (see Assessment Report A1019 for details).

The following key questions are addressed in the risk assessment report:

- Are the chemical properties of the plant sterol mixtures and manufacturing processes proposed by the Applicant, technologically suitable for addition to lower fat cheese?
- What new information relevant for assessing the safety of plant sterols in lower fat cheese has become available since previous FSANZ reviews of their safety?
- Are the plant sterol mixtures proposed for use capable of lowering cholesterol when added to lower fat cheese?
- What impact could the introduction of lower fat cheese fortified with TO phytosterols have on the consumption patterns of this food type in Australian and New Zealand consumers?
- Considering existing permissions for plant sterol fortified foods, what is the estimated impact on total plant sterol intakes from the addition of plant sterol fortified cheeses to the diet?

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 in this application.

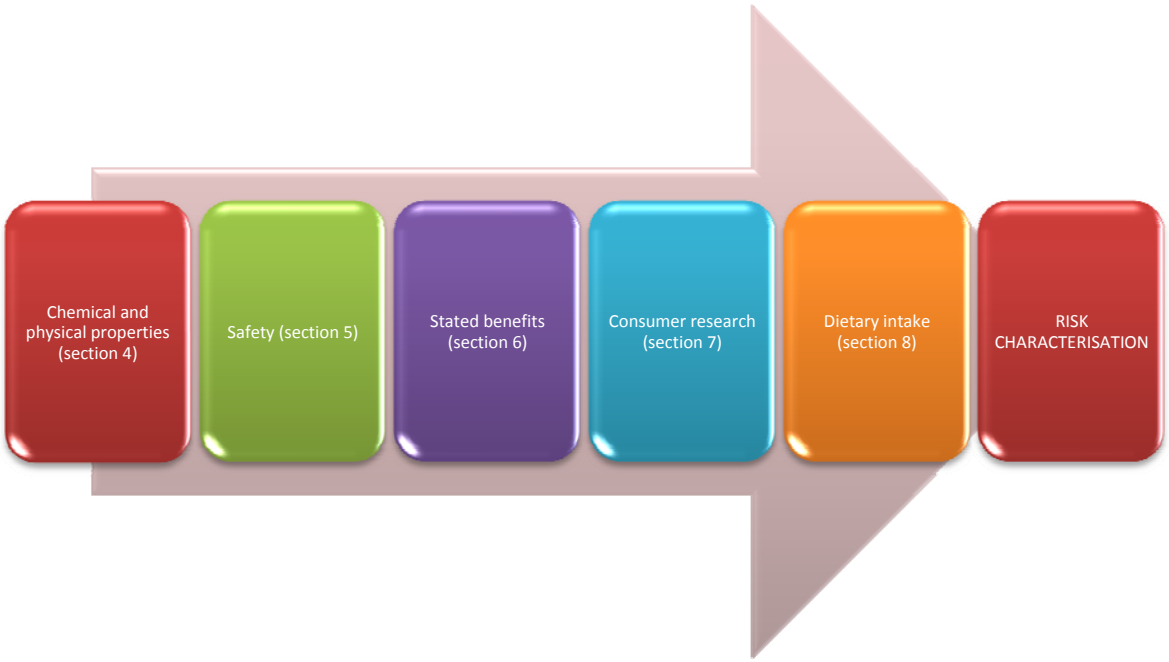

Figure 2.1: Schema for the risk assessment of plant sterols

4. Characterisation of plant sterols

4.1 Sources

Phytosterols and phytostanols and their esters are a group of steroid alcohols and esters that occur naturally in plants. Commercially, phytosterols and phytostanols in different proportions are isolated from vegetable oils, such as soybean oil, rapeseed oil, sunflower oil or corn oil, or from "tall oil", a by-product of wood pulp industry (FAO/WHO, 2008). TO phytosterols are extracted from tall oil 'soap' that is produced by alkaline treatment of tall oil woodchips and purified by distillation, extraction, crystallisation and washing, resulting in products of high purity (generally >90%) (see Table 4.1).

There have been more than 100 types of phytosterols reported in plant species but the more abundant ones are sitosterol and campesterol found in soybean oil and tall oil at varying levels (Fernandes & Cabral, 2007). Minor components, such as stigmasterol and brassicasterol, are also present in other vegetable oils (Brufau *et al.,* 2008). Phytostanols are the hydrogenated derivatives of phytosterols. They are also found in nature but in less abundance. Phytosterol blends derived from either vegetable oils or tall oil may be converted to the corresponding phytostanols by catalytic saturation. Each commercial source has its own typical composition.

Phytosterol esters also exist naturally in plant materials as esters of fatty acids. Commercially, phytosterol and phytostanol esters are produced by esterifying phytosterols and phytostanols with long chain fatty acids from vegetable oils to improve their solubility in food products.

4.2 Chemical and physical properties of plant sterols

4.2.1 Chemical structures

Phytosterols and cholesterol share the same steroid skeleton (Figure 4.1), but differ in the structure of the side-chain. Phytosterols have a role in plants similar to that of cholesterol in mammals, i.e. stabilisation of the phospholipid bilayers in cell membranes. They both have a four-ring steroid nucleus, the 3β-hydroxyl group and often a 5,6-double bond (Piironen *et al.,* 2000).

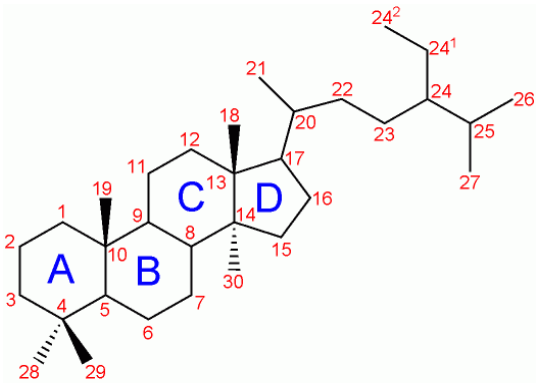

Figure 4.1: Steroid skeleton (JECFA, 2008)

Phytosterols can be divided into 3 major groups: 4-desmethyl sterols (no methyl group at position 4), 4α-methyl sterols (one methyl group) and 4,4-dimethyl sterols (two methyl groups). The 4α-methyl sterols and 4,4-dimethyl sterols are precursors of 4-desmethyl sterols (which are terminal products of the biosynthesis pathways). The 4-desmethyl sterols are the more abundant plant sterols in nature and 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. As examples of minor plant sterols, the structure of Δ^7-avenasterol as a Δ^7-sterol, ergosterol (a precursor of vitamin D) as a $\Delta^{5,7}$- sterol, gramisterol as a 4α-methyl sterol and cycloartenol as a 4,4-dimethyl sterol, are shown in Figure 4.2. Phytostanols can be produced by 5α hydrogenation of the corresponding phytosterols (e.g. sitostanol in Figure 4.2). The 3-hydroxyl group of free phytosterols/stanol may be esterified by a fatty acid from vegetable oils to form phytosterol/stanol esters, e.g. sitostanol esterified with oleic acid to form sitostanyl oleate (Figure 4.3).

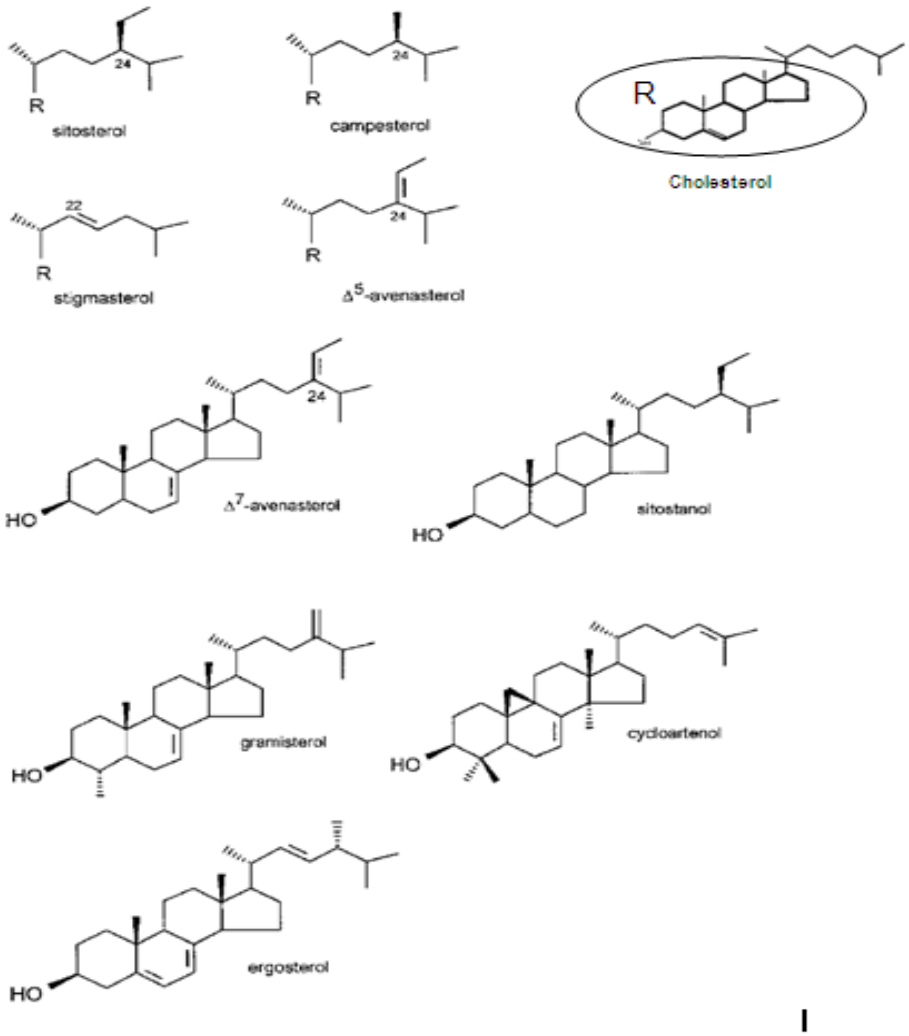

Figure 4.2: Examples of structures of major plant Δ^5-sterols with a similar sterol nucleus (R) to cholesterol but differing from cholesterol due to the additional alkyl substituents at C-24 (sitosterol, campesterol) or an alkene group (Δ^5-avenasterol) and/or a double bond at C-22 (stigmasterol). An example of a phytostanol with the bond between 5 and 6 being saturated is sitostanol. Examples of some minor components (Δ^5-avenasterol, gramisterol, cycloartenol and ergosterol) are shown (Piironen et al., 2000).

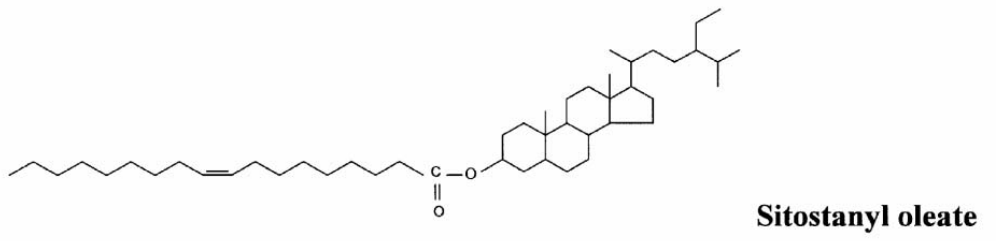

Figure 4.3: Example of a phytostanol ester

4.2.2 Physical properties

Esters of phytosterols occur as light yellow, thick liquids to waxy solids at room temperature and can be completely melted at 40 °C. In contrast, the free phytosterols are solid as inert crystalline structures at room temperatures; sitosterol, campesterol and stigmasterol have melting points of 140, 157-158 and 170 °C respectively.

4.2.3 Solubility

Phytosterols are practically insoluble in water but soluble in non-polar solvents, vegetable fats and oils. Solubility of plant sterols in water decreases as the length of their side chains increases. Thus phytosterols with 28 or 29 carbon atoms are more hydrophobic and have lower micellar solubilities in water than cholesterol with 27 carbon atoms. However, free sterols and steryl esters are soluble in non-polar solvents such as hexane.

Esterification of phytosterols with fatty acids can increase their solubility approximately ten-fold (Engel & Schubert, 2005). Therefore phytosterols and phytostanols are sometimes esterified with fatty acids derived from food grade vegetable oils. The resulting esters with improved solubility in oil allow the incorporation of phytosterols and phytostanols into various processed foods by dissolving or suspending in fat matrices (AbuMweis, 2008).

4.2.4 Stability

Plant sterols are very stable compounds and experience only limited damage during oil processing (Ferrari *et al.,* 1997). Only under specific conditions, such as high temperatures (>100 °C) in the presence of air, may some oxidation of phytosterols occur in the same way as for cholesterol (Yanishlieva-Maslrova and Marinova, 1985). Phytosterols are mono-unsaturated compounds (double bond in the B-ring) but because of steric hindrance by their ring structure, they are much more stable than mono-unsaturated fatty acids such as oleic acid. Therefore even under severe conditions, such as during deep frying, sterol oxidation products are only formed in very low concentrations (Dutta *et al.,* 1996).

As the intended application of the phytosterol esters is for addition to lower fat cheese slices and lower fat spreadable cream cheese, the storage and use of these cheese products will not expose the phytosterol esters to high temperatures for an extended time. Therefore phytosterol esters for the intended use would be stable against degradation through oxidation under likely conditions of use.

4.3 Production of tall oil phytosterols and their esters

Tall oil phytosterols are isolated from tall oil soap (a by-product of wood pulp manufacture). They are purified by distillation, extraction, crystallisation and washing, resulting in a product of high purity. Some phytosterols may be extracted as esters of fatty acids.

Phytosterol esters are produced by the sterols reacting with fatty acids derived from food grade vegetable oils. The fatty acid ester chain may be saturated, mono- or polyunsaturated depending on the source of the vegetable oil. The chain lengths of the vegetable oil fatty acids range from C14 to C18. The production process may include the use of hexane, 1-propanol, ethanol and methanol.

4.3.1 Specification of phytosterol esters

The product specification of the Application is compared with specifications from the FAO/WHO Joint Expert Committee on Food Additives (JECFA) Monograph 5, June 2008, in Table 4.1. The Applicant's specification for TO phytosterol esters meets the JECFA specification for products containing only esterified phytosterols, where a minimum of 55% phytosterol/stanols (calculated as free phytosterol/stanols after saponification of the phytosterol esters) in the total product is required.

Standard 1.3.4, Clause 2 Substances has been updated to include JECFA Monograph 5, where it can be used as a primary source for specifications.

4.4 Addition of phytosterol esters to cheese products

Incorporation of phytosterols and phytostanols into foods is difficult due to their high melting point (140 – 170˚C) and their tendency to form insoluble crystals (McClement, 2007). Phytosterols were originally added to high-fat foods (e.g. edible oil spreads) where solubilisation and dispersion of the sterols in the food matrix are relatively simple. For phytosterols to be introduced into foods that are not based on a continuous fat phase, they need to be either suspended or emulsified and stabilised in the food matrix. For this application, low solubility has been overcome by esterification of the phytosterols to polyunsaturated fatty acids, as discussed.

In this Application, the total fat level of the cheese products does not exceed 6 g/100 g before addition of phytosterol esters, and no more than 14 g/100g after the addition of phytosterol esters. In this Report, FSANZ refers to cheese <9 g fat/100g cheese as 'lower fat'. Under the Code of Practice on nutrient claims in food labels and in advertisements (CoPoNC) (National Food Authority, 1995[3]) and the Dietary Guidelines for Australian Adults (NHMRC 2003) a reduced fat product contains no more than 75% of the total fat content of the same quantity of a reference food, in this case a full fat cheese. The Applicant has provided information that an equivalent full fat cheese contains >21 g fat/100g cheese. Therefore, cheese <9 g fat/100g could be considered a reduced fat food. The New Zealand Dietary Guidelines do not define the term 'reduced fat'.

4.4.1 Production process of cheese slices containing phytosterol esters

Processed cheese slices containing phytosterol esters are prepared using the same process as low fat individually-wrapped cheese slices (IWS) as illustrated in Figure 4.4. The phytosterol esters are added to ripened cheese at the blending step where all the other dry ingredients are added. This mixture is blended until the ingredients are evenly distributed. The Applicant has validated their mixing time via sampling to ensure a homogeneous mix. The cheese is then pasteurised, formed and individually packed. According to the Applicant, the amount of phytosterol esters is controlled at point of addition and by periodic testing of phytosterols in the finished products. This process of mechanical incorporation is comparable to those described in two studies of the cholesterol lowering effects of plant sterols in cheese (Korpela *et al.,* 2006; Jauhiainen *et al.,* 2006).

According to the Applicant, cheese contains a relatively large amount of milk proteins, which are natural emulsifiers under the influence of citrate and/or phosphate salts normally used in the making of processed cheese. Therefore no additional emulsifiers are being added in this process.

[3] National Food Authority FSANZ's predecessor

Table 4.1: Comparison of specifications of phytosterol esters provided by the Applicant with JECFA Monograph 5

Phytosterol Content(%)	Application (Tall oil phytosterol esters)	JECFA Monograph 5[1]
Free phytosterol/stanols[2] + Phytosterol/stanols (from phytosterol/stanols esters after saponification)[3]		55-95
Free phytosterol/stanols + Phytosterol/stanols esters	97 min	
Free phytosterol/stanol (for non-esterified products)[4]		95 min
Phytosterol esters	91 min	
Phytosterol/stanols after saponification of the esters[5]	59 min[6]	55 min
Free phytosterol/stanols (naturally present in esterified products)	6 max	
Steradienes	0.3 max	
Relative sterol profile (%)		
Campesterol	4 – 25	
Campestanol	0 – 14	
β-sitosterol	36 – 79	
β-sitostanol	6 – 34	
Brassicasterol		
Stigmasterol		
Δ^5-Avensterol + Δ^7-Stigmastenol		
Δ^7-Avenasterol + others		
Fatty acid methylester (%)	0.5 max	
Moisture (%) loss on drying	0.1 max	4 max
Solvents (ppm)	50 max	50 max
Residue on ignition (%)	0.1 max	0.1 max
Acidity (g KOH/kg)	0.2 max	
Heavy Metals (total, ppm)		
Iron	1.0 max	
Copper	0.5 max	
Arsenic	3 max	3 max
Lead	0.1 max	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 for phytosterols, phytostanols and their esters.
[2] Free phytosterol/stanols refer to non-esterified phytosterol/stanols.
[3] For products that are mixture of free and esterified phytosterol/stanols – the content of phytosterol/stanols, measured as free phytosterol/stanols in a native and saponified sample.
[4] For products containing only free phytosterol/stanols – on a total free phytosterol/stanol basis.
[5] For products containing only esterified phytosterols – measured as phytosterol/stanols on a saponified sample.
[6] Phytosterol/stanols measured as des-methylsterols.

FSANZ accepted the information provided by the Applicant that the blending step in this process should produce cheese that is homogeneous in composition before cheese slices are formed and the subsequent pasteurisation step is not known to cause any degradation of the phytosterol esters. Since cheese slices are individually wrapped, the quantity of TO phytosterol esters should stay constant at approximately 1.8 g per 20.5 g slice (equivalent to 1.1 g free phytosterols). The phytosterol esters should be stable to oxidative and thermal degradation through the intended storage period since this product would not be expected to be subjected to sustained high heat before consumption.

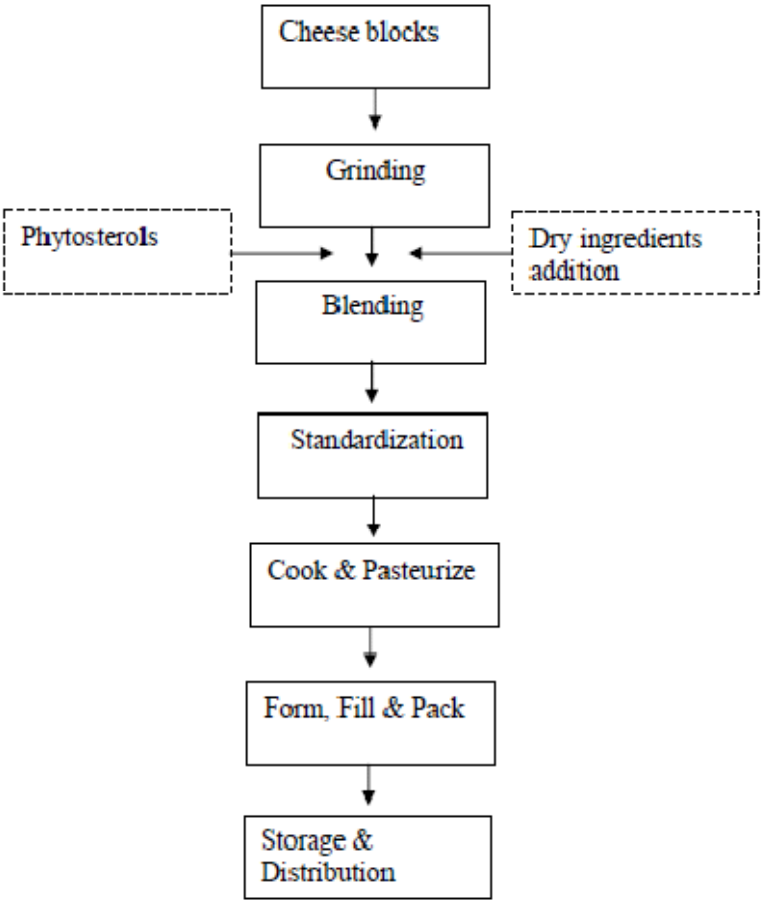

Figure 4.4: Flow chart of production process of individually wrapped cheese slices.

4.4.2 Production process of spreadable cream cheese containing phytosterol esters

Cream cheese containing phytosterol esters is prepared using the same process as for low fat cream cheese, as illustrated in Figure 4.5. The phytosterol esters are added to cheese curd at the blending step after the fermentation step, where all the other dry ingredients are added. The blending step is a vigorous process to ensure even distribution of the ingredients. The food grade gum normally added in this low fat cream cheese process, and the milk protein, stabilise the mixture and no additional emulsifier is needed. The cheese mixture is then pasteurised, filled and packed for storage and distribution. According to the Applicant, the amount of phytosterol esters is controlled at point of addition and by periodic testing of phytosterols in the finished products.

FSANZ accepts the claim by the Applicant that the blending step in this process should produce a cream cheese mix that is homogeneous in composition before filling and packing. The subsequent pasteurisation step is not known to cause any degradation of the phytosterol esters. Each 40 g mini-tub contains 2.2 g free phytosterols and can be consumed in a single serve or multi-serves. The phytosterol esters should be stable to oxidative and heat degradation through the intended storage period since this product would not be expected to be subjected to sustained high heat before consumption.

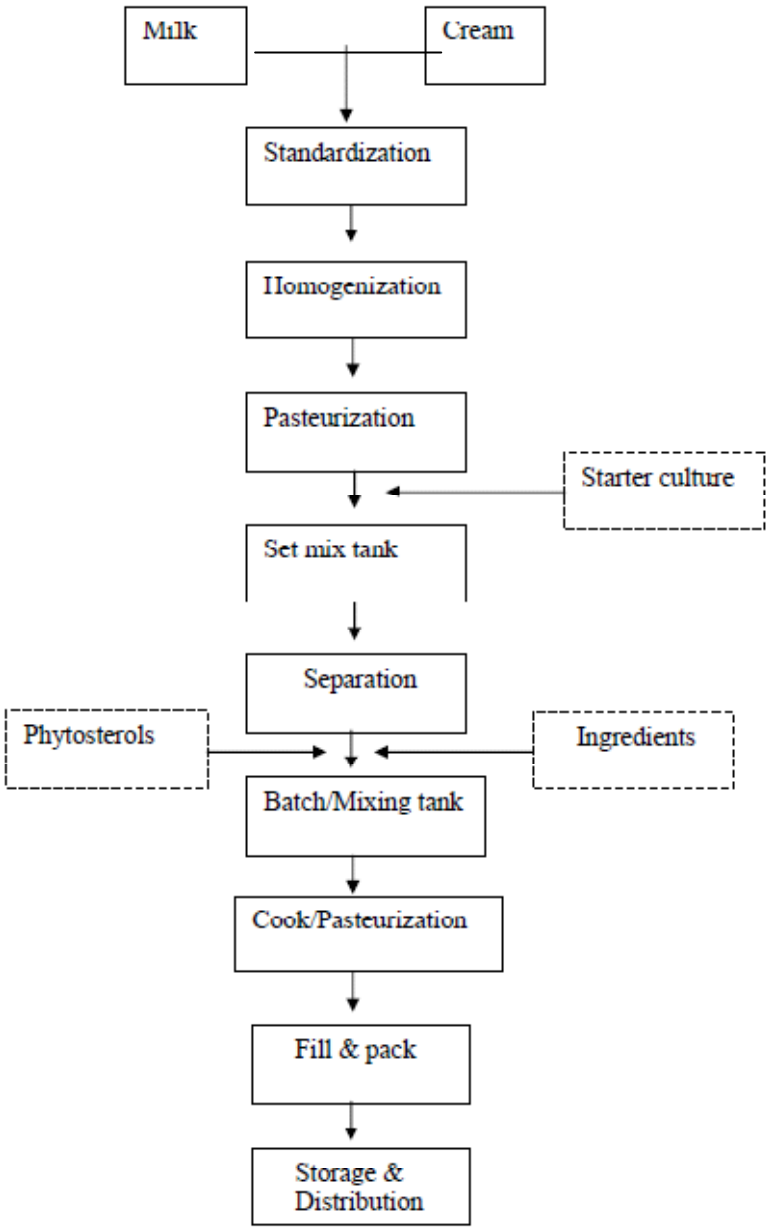

Figure 4.5: Flow chart of production process for cream cheese.

4.5 Response to risk assessment question 1

Are the chemical properties of the plant sterol mixtures and manufacturing processes proposed by the Applicant technologically suitable for addition to lower fat cheeses?

The chemical properties of the tall oil phytosterol esters proposed for use by the Applicant, and the manufacturing process by which they are added to lower fat cheese, are considered suitable to deliver a consistent amount of plant sterols that are likely to remain stable during storage under usual conditions.

5. Safety Assessment

The approach of this Assessment is to evaluate toxicological and epidemiological evidence that had not been considered in FSANZ's last safety assessment of plant sterols. It is not intended to cover all matters that would have been considered if FSANZ had not previously comprehensively reviewed the safety of plant sterols. A summary of the key safety issues and conclusions that underpin the existing permissions is presented as background information.

While TO phytosterol esters are not currently permitted in the Code, esterification of phytosterols is not a new process, and studies demonstrating equivalent nutritional and physiological properties of the various steroid compounds have been considered.

5.1 Previous FSANZ consideration

Data supporting the safety of phytosterol esters (derived from vegetable oils) and TO phytosterols (derived from coniferous trees) were assessed in 2001, in the context of separate applications to FSANZ seeking permission to add phytosterols to edible oil spreads (Applications A410 and A417).

5.1.1 Phytosterol esters in edible oil spreads

The safety assessment conclusions for Application A410 were:

1. The studies provided no evidence of adverse toxicological effects associated with consumption of phytosterol esters up to a level of 1.6 g free phytosterols/day over a one year period.

2. Studies in humans provided no evidence of any adverse health outcomes. The data indicated a potential for phytosterol esters to reduce plasma levels of carotenoids in humans.

3. The available data did not allow the assessment of any potential risk of changed serum carotenoid levels that may be associated with consumption of high levels of phytosterols.

4. The data available to address the potential long-term effects of phytosterols in humans were limited. While there were 3-4 week studies at several dose levels, the one-year human study was conducted at only one dose level. Further studies at higher dose levels would have provided more confidence in both the safety of this product and in its capacity to maintain long-term reductions in plasma cholesterol levels.

5.1.2 Tall oil phytosterols in edible oil spreads

Studies relating to the safety of TO phytosterols in animals and humans were evaluated under Application A417. The conclusions of the safety assessment were:

1. The available animal studies indicated that TO phytosterols are poorly absorbed from the gastrointestinal tract, have low toxicity, are not genotoxic and demonstrate no reproductive or developmental toxicity.

2. There was also no evidence of oestrogenic activity in the *in vitro* and *in vivo* studies evaluated.

3. The human studies indicated no clinical symptoms, apart from some minor non-significant reductions in plasma carotenoids at consumption levels of 0.9, 1.8 and 3.6 g/day without a dose-relationship.

4. The studies supported the safe consumption of TO phytosterols up to and including a level of 3.6 g/day.

5.1.3 Safety in other foods

In considering requests to broaden the permissions to low-fat milk and low-fat yoghurt (Applications A434 and A508) and breakfast cereals (Application A433), further information was needed to demonstrate both safety and efficacy when using plant sterols in other food matrices. Two clinical studies in mildly hypercholesterolaemic people were conducted in 2002, to examine the efficacy, safety and nutritional effects of phytosterols when added to low-fat milk, low-fat yoghurt, breakfast cereal (muesli type), and bread. The results of these studies have since been published (Noakes *et al.,* 2004 and Clifton *et al.,* 2005) and were evaluated in the assessment reports for Applications A433[4] and A434[5].

The following conclusions supported the approval of phytosterol esters in low-fat milk, low-fat yoghurt (Application A434), high fibre, low sugar breakfast cereal (Application A433), and the approval of TO phytosterols in low-fat milk (Application A508):

1. As reported in other studies, there was a statistically significant increase in the absorption of phytosterols, although the absolute levels in plasma remained very low. The increases in plasma sitosterol and campesterol levels were highest with consumption of phytosterol fortified milk, suggesting possible differences in the bioavailability of phytosterols with the food matrix.

2. Adjusted plasma α- and β-carotene levels decreased by up to 23%, however increased intake of fruits and vegetables partially compensated for the lowering of plasma carotenoids.

3. There were no changes in full blood count or clotting profile and no other changes in plasma biochemistry in subjects. Urine tests showed a variety of abnormalities throughout the study, but none of these changes could be statistically related to the

4 http://www.foodstandards.gov.au/standardsdevelopment/applications/applicationa433phytosterolestersinfibreinfibreincreasedbreadandcerealbars/index.cfm

5 http://www.foodstandards.gov.au/standardsdevelopment/applications/applicationa434phytosterolestersinlowfatmilkandlowfatyoghurt/index.cfm

intake of phytosterol esters in the trial foods.

4. As no treatment-related changes were detected, the results of these studies indicated that phytosterol esters may be consumed safely in amounts up to approximately 10.7 g per day, when incorporated into foods such as low-fat milk, yoghurt and breakfast cereal.

5.2 Previous international scientific evaluations

5.2.1 Scientific Committee on Food

The former Scientific Committee on Food (SCF) of the European Commission assessed commercial plant sterol mixtures on multiple occasions (SCF, 2000, 2002a, 2003a). The approach adopted for each evaluation was to consider information specific for the chemical nature of the commercial phytosterol mixture in association with a particular food matrix, on a product-by-product basis.

In its initial assessment (SCF, 2000), the Committee considered sterol mixtures of predominantly β-sitosterol, campesterol, and stigmasterol and/or their esterified forms, presented in an edible oil spread, anticipated intakes and consequences of use, and nutritional, microbiological and toxicological information. The toxicological information included data from studies on absorption, distribution, metabolism and excretion, subchronic toxicity, genotoxicity and reproductive toxicity, as well as potential oestrogenic activity, and data from human studies. The Committee considered the available data in both animals and humans to be extensive and concluded that no safety concerns were apparent.

In 2002, the potential for longer term nutritional effects with consumption of phytosterol fortified foods was the focus of further consideration. Safety studies not previously evaluated showed no adverse effects in (healthy) humans who consumed reduced-fat spread and salad dressing providing 0, 3, 6 or 9 g/day phytosterols for eight weeks. In another study in humans, no adverse effects were found after daily consumption of 1.6 g esterified plant sterols for one year. Reductions in the blood concentrations of the carotenes were observed with consumption of phytosterols, confirming that reduced absorption of certain carotenoids was a consistent nutritional finding.

A numerical upper level of total daily intake of phytosterols was not established by the Committee at this time (SCF, 2002a). However it was noted that there was a paucity of robust data available on the long-term health effects of consuming phytosterol fortified foods, particularly for periods beyond one year. Given the absence of adverse effects in shorter-term studies, the decrease in plasma β-carotene, together with any associated implications for general health, was the primary focus of any projected safety concerns expressed by the Committee.

Further applications from the food industry seeking approval to use variable mixtures of phytosterols and phytostanols (predominantly TO phytosterols) in an expanded range of food products, triggered further assessment of safety and efficacy in different food matrices (SCF, 2003b, 2003c). The Committee again considered effects on carotenoids and hormone levels in humans.

Toxicological studies in animals were also submitted. Animal data included numerous studies conducted in rats, dogs and rabbits using variable doses of phytosterols in the diet

for periods up to 22 months in rats and dogs, and up to 842 days in rabbits[6]. The animal studies showed no adverse effects associated with exposure to phytosterols through the diet, in particular, no detectable change in the gross or microscopic appearance of organs including the heart.

An early study in humans (Shipley *et al.,* 1958), not previously assessed, showed no adverse effects of phytosterols following continuous administration for periods exceeding four years. This was determined through laboratory tests of kidney and liver function, blood and urine composition, electrocardiogram and gall bladder examination. In addition, no symptoms of vascular lesions were reported, although the number of patients and the dose used in this study were not specified.

5.2.2 European Food Safety Authority

In 2005, the European Commission sought an update on the safety of phytosterols from the European Food Safety Authority (EFSA). Some data not previously evaluated by the SCF were included in the assessment. The EFSA review noted that the cholesterol-lowering effect of both the plant sterols and the plant stanols was similar.

Specifically, the Panel considered (i) the effects of the combined intake of dietary phytosterols (stanols in this case) and lipid-lowering medication, and (ii) whether elevated serum levels of plant sterols might be a risk factor for coronary heart disease. The latter concern was raised in the context of the premature development of atherosclerotic plaques and coronary heart disease in patients with sitosterolaemia, a metabolic disorder that results in abnormally high absorption of dietary phytosterols (see Section 5.6.1).

This review confirmed that, in addition to the cholesterol-lowering statins, dietary phytosterols could elicit a positive cholesterol-lowering effect due to the inhibition of cholesterol absorption. EFSA also concluded that while there was no evidence of adverse effects from absorbed phytosterols, there was a lack of knowledge on long-term exposure to higher intakes of plant sterols possibly arising from an increase in the range of phytosterol products (EFSA, 2005).

In the context of further product development by the food industry, the Data Collection and Exposure Unit of EFSA issued a report in 2008 on the pattern of usage of plant sterol fortified food and beverages by European consumers (EFSA, 2008). The findings of this report are discussed in Section 7.

5.2.3 JECFA

At its 69th meeting in June 2008, JECFA reviewed the safety information available for phytosterols. The review considered three sets of toxicity data for (i) phytosterol esters (derived mainly from soybean oil), (ii) unesterified mixtures of phytosterols and phytostanols (derived mainly from tall oils), and (iii) two types of phytostanol esters (wood (i.e. tall oil)-derived and vegetable oil-derived). Studies in both animals and humans were reviewed, including studies which examined efficacy and nutritional outcomes, as well as *in vitro* toxicity data.

On the basis of the availability of toxicological data for variable mixtures of phytosterols, the JECFA Committee considered that establishing a group ADI (Acceptable Daily Intake) for phytosterols was appropriate because of the similarities in biological effects irrespective of the precise sterol and stanol composition, or the source, of the test material. The Committee

[6] The data were derived from earlier published studies (Shipley *et al.,* 1958; Sugano *et al.,* 1977) and company-derived studies.

determined the group ADI of 0-40 mg/kg bw for the group of phytosterols, phytostanols and their esters, expressed as the sum of phytosterols and phytostanols in their free form (WHO, 2009). New specifications for the combined sterol/stanol mixture were also prepared.

In establishing the group ADI, an overall no observed adverse effect level (NOAEL) of 4200 mg/kg bw per day was determined from several key short-term studies in rats. The lowest observed adverse effect level (LOAEL) was taken to be 9000 mg/kg bw per day. A safety factor of 100 was applied to the NOAEL determined in rats to derive the ADI.

5.3 Toxicity studies

Four key sub-chronic (90 day) toxicity studies in rats using similar but not identical preparations of sterol/stanol mixtures are relevant for considering the potential hazard of phytosterols, phytostanols and their esters. Two of these studies have not been previously considered. The other two studies were evaluated by FSANZ in assessing earlier applications (A410 and A417), and summaries are presented here for comparative purposes.

Kim et al. (2002)

Test material:	Soybean derived plant sterols (sitosterol 49.4%, campesterol 27.9%, stigmasterol 18.5%, others unspecified) (Eugene Science Inc, Korea) esterified with unsaturated fatty acids from olive oil (oleic acid ≥ 70%)[7]
Test Species:	Sprague-Dawley rats obtained from Charles River Japan Inc. (Kanagawa, Japan).
Dose:	0, 1000, 3000 and 9000mg/kg bw/day administered by gavage, 90 days
GLP:	Korea Food and Drug Administration regulations
Guidelines:	Not specified

Groups consisted of 10 (low and intermediate dose groups) or 16 (control and high dose group) rats of each sex. Animals in the control group received the same volume of distilled water (9.5 ml/kg) as those on the highest dose. The animals were provided with standard rodent chow and sterilized tap water *ad libitum* for the duration of the study.

At the end of the treatment period, 10 rats/sex in each of the four groups were killed; a further six rats/sex in the control and highest dose group were killed following a four-week recovery period. The animals in the recovery groups were observed for reversibility, persistence and delayed onset of any toxic effects.

During the test period, the animals were checked daily for clinical signs, mortality and moribundity. Body weights were measured at the start and end of the study and twice per week in the intervening period. Food and water consumption were monitored for two rats in each cage at the start of treatment and then at weekly intervals. External eye examination was performed shortly before the start and conclusion of treatment.

Urine was collected for analysis from five rats per sex per group during the last week of the study. Haematology and serum biochemistry were performed on blood samples collected at necropsy. Gross findings and absolute and relative organ weights (organ-to-body weight ratios) were recorded for a large number of tissues. Histopathology was conducted on a large number of organs and tissues taken from all animals in the control and highest dose groups, and the heart and liver from other dose groups.[8]

[7] The purity of the sterol esters was ≥ 95.4%.
[8] A list of parameters typically measured in sub-chronic studies is at Appendix 1. Any significant inclusions or exclusions from this list are noted in the text.

Results

There were no clinical signs or deaths during the study that could be related to treatment. A statistically significant reduction in body weight gains in both male and female rats occurred on specific days in the highest dose group (9000 mg/kg bw/day) in the second half of the study period. It was stated that reduction in body weight gains were apparent until the end of the four-week recovery period, however the data were not shown. Minor decreases in food consumption and increases in water consumption occurred across groups over the course of the study. Ophthalmologic examinations and urinalysis did not reveal any treatment-related effects in any animals. Haematology and serum biochemistry did not reveal any significant differences between the control and treatment groups. Reported gross findings were unremarkable, and no statistically significant differences in organ weights between the control and treatment groups were observed.

Histopathological examination of the tissues revealed a significant increase in the incidence of cardiomyopathy with mononuclear cell infiltration in male rats in the highest dose group (eight of 10 animals); the lesion was reported to be more severe in that group compared with controls but the data were not shown. For all other treatment groups including the controls, cardiomyopathy of undefined severity was evident in two males per group. Apparently no recovery was observed at the end of the four-week recovery period, although no data were provided on the severity or incidence of cardiomyopathy. Cardiomyopathy was not observed among females in any group throughout treatment. There was evidence of liver inflammation in over half of the animals in the study (both males and females) including controls and all treatment groups. There were no other significant histopathological findings.

Comments

The authors concluded that the dose-dependent effects on bodyweight and bodyweight gain, recovered only at the end of the recovery phase, were related to the administration of plant sterol esters. FSANZ would agree with this conclusion on the basis that the reduced bodyweights occurred in both sexes, and were more pronounced with increasing doses of plant sterols and with the duration of treatment. This is because daily gavaging with high volumes of oil would be expected to result in significant physical and physiological stress, particularly in the rats receiving the highest dose of 9.5 ml/kg bw/day. While the effects of daily gavaging became more evident with time, similar doses of plant sterols administered to rats through their diet for the same period of time had no such effect on bodyweights or bodyweight gains (see Hepburn *et al*. *1999*). This raises the question of whether the findings in the study by Kim *et al*. can be considered relevant for risk assessment purposes.

The claim that the increased incidence of cardiomyopathy in males was treatment related is much less convincing owing to the well known high background incidence of cardiomyopathy in historical control data. While the authors concluded the LOAEL was 9000 mg/kg bw/day for males and over 9000 mg/kg bw/day for females, FSANZ concludes that the NOAEL in both sexes is 9000 mg/kg bw/day. The incidence of cardiomyopathy was 20% in the control, low and intermediate dose groups and 80% in the high dose group after 90-days of treatment; this does not indicate an unequivocal dose-response relationship. In addition, the authors reported that the finding was *not recovered* during the 4-week recovery period; however no further data were presented to report the incidence or severity of cardiomyopathy.

The incidence of cardiomyopathy in the control group and the gender-specific nature of the finding together raise the possibility of other plausible explanations. The authors stated that cardiomyopathy is generally a spontaneous lesion that occurs at some low frequency in rats and is typically of unknown aetiology. They did not appear to question the lack of correlation of their findings with other sub-chronic studies on phytosterols administered at high doses

(up to 6500 mg/kg bw/day), or consider the historical background incidence of cardiomyopathy relevant for the strain of rats used in their experiments (see section 5.3.3).

Turnbull et al. (1999)

Test materials:	Tall oil-derived phytostanol ester and vegetable oil-derived phytostanol ester (chemical composition not specified)
Test Species:	Wistar rats Crl:(WI)WU BR (Charles River Wiga GMBH, Germany)
Dose:	0, 0.2, 1, 5% total stanols w/w administered in diet, 13 weeks.
GLP:	not specified
Guidelines:	not specified

This study comprised seven groups of 20 rats per sex per group, including one control group, and six test groups. Three test groups received tall oil-derived phytostanol esters incorporated into the diet at concentrations of one of 0.2, 1 or 5%, and three test groups received vegetable oil-derived stanol esters at the same concentrations. The highest dose corresponded to dietary levels of test substances of 8-9% (in the esterified form) which the authors claimed was the maximum feasible dose without needing to fortify the diet of the animals with vitamins and minerals to ensure nutritional adequacy. Pure rapeseed oil was used to balance the energy level of the various treatment diets. Control rats received the standard rodent diet supplemented with rapeseed oil.

All animals were observed daily for clinical signs of toxicity. Body weights were recorded at the start and end of treatment, and at weekly intervals throughout the study period. Food consumption was measured per cage over successive 7-day periods and per rat (for 3 rats/sex/group) over a 1-day period during the 3-day faeces collection period. Water consumption was also measured per cage on 5 consecutive days in weeks 1, 6 and 12. Ophthalmoscopic examinations were carried out at the start and end of treatment.

On days 86-87, urine from 10 rats/sex/group was collected following food and water deprivation for a period of 16 and 24 hours respectively. Urine samples were collected for analysis (see Appendix 1) and semi quantitative observations including sediment microscopy. Fasting glucose was measured in blood collected at the end of the urine collection period. Faeces were collected from a different set of animals (3 rats/sex/group) and used to determine concentrations of neutral steroids (including sitosterol, campesterol, cholesterol and their 5α/β-saturated derivatives). Bile acids were measured in the control group and the two highest-dose groups.

Blood was collected at necropsy from 10 animals/sex/group for standard haematological and clinical chemistry measurements (see Appendix 1). Calculations were made for some additional haematological parameters. Clinical chemistry included determinations for total, HDL and non-HDL cholesterol. At the end of treatment, plasma concentrations of sterols and stanols (including sitosterol, campesterol, cholesterol, saturated derivatives, and cholesterol precursors) were measured in10 rats/sex/group. Plasma concentrations of β-carotene, vitamins A, E, D and K were measured in another 10 rats/sex/group.

A large number of tissues were harvested from all animals at terminal necropsy (see Appendix 1). Tissues were weighed and relative organ weights determined. Organs and tissues obtained from the control and high dose animals were examined microscopically. The kidneys, liver, lungs and any gross lesions were also examined in all rats of the intermediate dose group. Data were subsequently analysed by appropriate statistical techniques.

Results

There were no animal deaths or clinical signs attributable to treatment in any dose group. There were no significant differences in mean body weight between the treated groups and controls, and no differences in water consumption. Mean food intake and food conversion efficiency in male rats showed no treatment-related differences; food consumption in females tended to be higher in the higher sterol dose groups. Ophthalmoscopic eye examinations in animals of the high dose group and controls revealed no ocular changes.

While no significant haematological changes were found in male rats in any groups, female rats consuming wood-derived stanols showed a significant increase in thrombocyte count, and females consuming vegetable-oil stanols showed increased percentage of neutrophils and decreased percentage of lymphocytes. The authors did not consider these findings (in the high dose group only) to be related to treatment because there was no clear dose-response relationship and there were no significant changes in the absolute numbers of these cell types.

A number of clinical chemistry parameters were significantly altered compared with concurrent control. None of the changes were considered to be toxicologically significant because the levels were within the range of historical control values and/or showed no relationship to the dose of the test substances. The plasma levels of vitamins E, D and K were decreased in males and females of the high dose groups. The levels of vitamin A in the liver showed no treatment-related changes, whereas levels of vitamins D and E were decreased in the high-dose group (measurements in females only).

Plasma levels of phytosterols and phytostanols showed multiple changes related to treatment: all sterol levels were dose-dependently decreased in the groups consuming stanol esters. Plasma concentrations of both sitostanol and campestanol were increased. Cholesterol-precursors (desmosterol and lathosterol) increased in females in the intermediate and high dose groups, however males showed decreases in both desmosterol (low and intermediate dose groups) and lathosterol (low wood-derived stanol group). The faecal analysis showed that sterols and stanols were excreted in the free (non-esterified) form.

In males in the high dose groups, the absolute weight of the kidneys was slightly decreased and, in all groups, the males showed a slight decrease in the absolute and relative liver weights which was dose-dependent. Gross examination of animals at autopsy showed no treatment-related changes. The microscopy showed no adverse effects of the test substances on the gastrointestinal tract or any of the organs and tissues examined, except for more pronounced glycogen depletion in the liver of most males and a higher incidence of uterine luminal dilatation in females in all dose groups compared to control animals.

The authors concluded that ingestion of wood- or vegetable-oil derived stanol esters at the high dose level in this study (5% free stanol) was associated with decreases in the levels of vitamins A, D, E and K in plasma and/or the liver. No toxicity or significant changes were observed at the intermediate dietary level (1% free stanol), equivalent to approximately 500 mg/kg bw/day.

Horner, S.A. (1997)[9]

Test material:	Vegetable oil-derived plant sterol esters (β-sitosterol 48.7%, campesterol 25.8%, stigmasterol 21.6%, β-sitostanol <2%), fatty acid esters from sunflower oil (Unilever ESL).
Test Species:	Wistar derived Alpk:AP_fSD rats (Zeneca Pharmaceuticals, Alderley Park),
Dose:	0, 0.1, 1, 2, 5% sterols w/w administered in diet, 90 days.
GLP:	UK GLP Compliance Programme, Department of Health 1997
Guidelines:	OECD Guideline ref. 408 (1981), USEPA, EC.

After 1 to 2 weeks acclimatisation, five groups of rats (20 each sex) were allocated to control or treatment with plant sterols in diet. The doses represented 0.1% to 5% w/w plant sterol esters. Clinical observations and body weight measurements were made prior to the feeding study and animals were observed for clinical condition and behaviour twice daily. Food consumption, body weights and detailed clinical observations were recorded weekly. Food utilisation was expressed as increase in bodyweight/100 g food consumed. Ophthalmology of all animals was performed before the study and of control and high dose groups at week 13.

At terminal necropsy all animals were assessed for measurements of haematology (listed in Appendix 1 plus erythrocyte distribution width), and clinical chemistry (listed in Appendix 1 including lactate dehydrogenase, sorbitol dehydrogenase, total bile acids, LDL and HDL, but excluding ornithine carbamyltransferase and phospholipids). Additional blood was collected for measurement of serum 5'-nucleotidase. Organ weights (listed in Appendix 1 including epididymides, but not ovaries/uterus, pituitary or thymus) and histology (listed in Appendix 1 including the oviduct, caecum, rectum, parathyroid, salivary gland, and tongue, and excluding bone, femoral bone marrow, lacrimal gland, skin and vagina) were performed. Data were analysed by appropriate statistical techniques.

Results

Three male animals from the 0.1% dosage group were sacrificed *in extremis* as a consequence of damage to the snout unrelated to the treatment regime. No deaths were associated with sterol treatment. There were no treatment related adverse effects on food consumption and body weight, nor on food utilisation. Male rats receiving sterols in diet had group mean food consumption higher than in controls but this was toxicologically insignificant. There were significant clinical observations with sterol treatment observed at week 14. There was depression of mean platelet counts in females at all sterol diet levels and in males at 1 and 2% dietary sterols. In the absence of a statistically significant depression at 5% this was considered to be incidental. Selected blood cell counts were slightly but statistically significantly increased in males at 5% sterol in diet (total white blood cell, neutrophil and lymphocyte count) and decreased at 2 and 5% (eosinophil count).

In females, sterol treatment at all levels was associated with slight but significant decreases in prothrombin time, and activated partial prothrombin time was higher in males at 2 and 5% sterol. A variety of clinical chemistry measurements were significantly altered compared with concurrent control. These included increased plasma albumin (in females at 5% sterols), increased LDL (dose related in males but significant at 2 and 5%), increased cholesterol and HDL (in males and females at 1 and 2% but not 5%), increased alkaline phosphatase (in males at 5% and females at 1, 2 and 5%), increased alanine aminotransferase (in males and females at 2 and 5% and in males at 1%), increased aspartate aminotransferase, creatine

[9] Central Toxicology Laboratory, Alderley Park, Macclesfield, England. Study KF960455, 14 October 1997. Previously assessed by FSANZ in Application A410. Study subsequently published in Food and Chemical Toxicology in 1999 (Hepburn *et al.,*1999).

kinase and lactate dehydrogenase (in females at 5%), increased phosphorus (in males at 2% but not 5%) and increased magnesium (in females at 2 and 5%).The extent of these changes, although in general statistically significant, were considered small and of little biological or toxicological importance. Organ weights, organ morphology and microscopic features were unaffected by sterol treatment in diet at 5% w/w. The lack of any histopathological findings supports the interpretation of the clinical chemistry and haematology data.

The NOAEL with daily dietary administration of plant sterols in the rat for 90 days was the highest dose tested, namely, 3911 and 4204 mg/kg bw/day in males and females respectively.

Wedig J. (2000)[10]

Test material:	Tall-oil non-esterified plant sterols (TOPs) (sitosterol 44%, sitostanol 25%, campesterol 12%, campestanol 6%).
Test Species:	Sprague-Dawley rats
Dose:	0, 1.25, 2.5, 5% administered in diet for 91 days.
GLP:	USA GLP Regulations
Guidelines:	not specified

After acclimatisation for at least seven days, four groups of rats (24/sex/group) were treated with TOPs in the diet. Control animals were given standard rat diet as provided by the manufacturer. Clinical observations were recorded twice daily, and bodyweight and food consumption were recorded weekly. A satellite group of rats (4/sex/group) were selected for measurement of phytosterol concentrations pre-dose, and at day 29, 58 and 92. Haematology, clinical chemistry, urinalysis and measurements of vitamin A, D, E, K and beta-carotene were performed prior to necroscopy. Ophthalmology of all animals was performed before the study and near termination. At the end of the study, all animals were sacrificed and a complete necroscopy performed (gross examination, organ weights and tissue sampling). Histopathology was performed on all tissues from the control and high dose groups and the caecum, colon and rectum from low and mid-dose groups were also examined. Appendix 1 lists the parameters measured.

Results

No deaths were associated with treatment. In general, there were no treatment related clinical signs, adverse effects on food consumption and body weights or bodyweight gains. There was an overall decrease in bodyweight from day 85 to 91 (not statistically significant) which was attributed to increased temperature in the animal house on day 90 (including controls). Isolated variations (increases) in absolute and relative food consumption and decreases in feed conversion efficiency were noted, however, there was no dose-response relationship.

Male rats at high-dose had significant increased feed consumption throughout the whole study. The reporting ophthalmologist concluded that there were no ocular abnormalities associated with the test material. There were no treatment related differences in haematology, clinical chemistry or urinalysis at any dose. Serum cholesterol and triglyceride levels in the treated groups were not significantly different to controls.

There were no treatment related differences between treated groups and controls in mean serum concentrations of vitamins A or E. Serum levels of vitamin D, K and beta-carotene

[10] Redfield Laboratories. Study Number 115-003, 31 August 2000. Previously assessed in Application A417.

were below the limit of quantification and therefore no levels were reported.

In general organ weights, organ morphology and microscopic features were unaffected by treatment at up to 5% in the diet. Exceptions were isolated increases in mean relative adrenal weights to bodyweights in males at 2.5 and 5% and in relative heart weights to bodyweights at 1.25, 2.5 and 5%. However, these increases were small, confined to one sex and there were no accompanying histopathological or enzyme findings. Inflammation of the caecum, colon and rectum was slightly more prevalent and severe in males at all doses, however, a dose-response relationship was not established. These observations may be attributed to possible changes in the intestinal flora, although there was a general absence of inflammatory changes in the small intestine. In conclusion, no evidence of toxicity was noted following treatment with TOPs in the diet of rats up to doses of 5%.

5.3.2 Reproductive and developmental toxicity

FSANZ has previously considered other studies in rats that have investigated whether high doses of plant sterols show any reproductive or developmental toxicity. Studies over two generations on the reproductive system in rats receiving a diet containing sterols and stanols at concentrations up to a level of 8.1% in the diet in general found no biologically significant effects on reproductive parameters. In one study, the only treatment-related effect observed was decreased pup body weights in both generations at the highest dose level (dietary concentration 5%, calculated to be equal to 8100 mg/kg bw/ day). This effect was attributed to the lack of absorption of phytosterols and the resulting reduction in caloric value of the test diet compared with controls. The NOAEL for reproductive or developmental toxicity in this study was taken to be the lower dose of 2.5% phytosterols in the diet, calculated to be equal to 4100 mg/kg bw/day.

5.3.3 Discussion

A comprehensive range of toxicity studies investigating plant sterols have shown that phytosterols, phytostanols and the esterified forms have low toxicity, are not genotoxic, and have no effect on reproductive parameters. However, there was a 90-day toxicity study in rats which did report an adverse outcome following gavage administration of a large dose of phytosterols (9000 mg/kg bw/day). This study by Kim *et al.* (2002) claimed that an increased incidence of cardiomyopathy in male rats at the highest tested dose of 9000 mg/kg bw/day was treatment related. Although the severity was only reported for the high dose group the observed incidence of cardiomyopathy was 20% in the control, 1000 and 3000 mg/kg bw/day groups and 80% at 9000 mg/kg bw/day after 90 days of treatment. There was a statement indicating that no reversal occurred during a 4-week treatment-free period for the control and high dose groups but no information was provided on the incidence or severity of cardiomyopathy in either group. Since cardiomyopathy is a very common lesion observed in many rats strains the authors apparently did not consider the possibility that the observed incidence at high dose may have arisen through chance alone. In this context the absence of information about the incidence and severity of cardiomyopathy in the control and high dose groups following the recovery period is most unfortunate.

There is an abundance of published literature reporting that cardiomyopathy is a common, spontaneous, non-neoplastic age-related lesion occurring in Sprague-Dawley, Wistar and Fischer (F344) strain rats, with males having a markedly higher incidence (Kemi *et al.,* 2000). The lesions typically involve combinations of myocardial fibre degeneration, mononuclear cell infiltration and interstitial fibrosis. Cardiomyopathy is reported to contribute to the morbidity and mortality of control rats, particularly males, in general toxicity and carcinogenicity studies to the extent that it can interfere with the interpretation of cardiotoxicity and consequently with the evaluation of the potential toxicity of test substances

(Kemi *et al.,* 2000).

A recent paper by Duffy *et al.,* 2008, studied the incidence of non-neoplastic pathology in male Sprague-Dawley rats consuming different rodent chow diets at one-year (middle age) and two-year time points. At 58 weeks of age, the predominant disease findings were liver vacuolisation and cardiomyopathy (incidence of 90% and 80% respectively) in rats fed *ad libitum*. The cardiomyopathy was moderate to severe in over half of the animals. At 114 weeks of age, cardiomyopathy was the predominant finding in 92% of male Sprague-Dawley rats. In comparison, the incidence of cardiomyopathy in vehicle control female Sprague-Dawley rats in two year chronic studies was reported to be 28.5%, and the disease was typically of minimal severity. The predominant finding in females at two years was hypertrophy of the adrenal cortex (incidence of 81%) (Brix *et al.,*2005).

Published data on the incidence of spontaneously-occurring cardiomyopathy in young Sprague-Dawley rats from Charles River Laboratories in Japan around 2001 could not be located. However, the incidence of cardiomyopathy in a range of rat strains from other sources has been published. The incidence of all spontaneously occurring lesions has been determined for control Fischer-344 rats (males and females) from 90-day (13-week) toxicity studies when treated by gavage, diet or inhalation. In control male rats, the most common lesions were nephropathy and cardiomyopathy (mean incidence 90.6% and 79.1% respectively). For gavaged animals, the background incidence of cardiomyopathy in male controls in 90-day studies was 85%. In female Sprague-Dawley and Fischer rats, the incidence of cardiomyopathy was found to be 22.8% (Dixon *et al.,* 1995). These data suggest that the pattern of development of cardiomyopathy in both Sprague-Dawley and Fischer rats is well correlated.

Table 5.1: Incidence of cardiomyopathy in control groups in 90-day Sprague-Dawley rat studies.

Study authors and year	Supplier	Test material/ compound	Mode of administration	Cardiomyopathy incidence	
				Males	females
Eason & Turck (2002)	Charles River Labs, USA	Sodium monofluoroaceatate (compound 1080)	gavage	0/10	ND
Hammond *et al.,* (2001)		DHA-rich microalgae	diet	20/20 (19 minimal 1 mild)	13/20
Hammond *et al.,* (2006a)		GMO corn	diet	11/20	7/20
Hammond *et al.,* (2006b)		GMO corn	diet	5/20	3/20
Appenzeller *et al.,* (2009)		Herbicide-resistant corn	diet	4/12	1/12
Kim *et al.,* (2002)	Charles River Labs, Japan	Phytosterols	gavage	2/10	0/10

ND=Not determined

Another source of information to establish the background incidence of cardiomyopathy in young Sprague-Dawley rats comes from a random selection of published 90-day rat studies which report the incidence of cardiomyopathy in control animals (see Table 5.1). As the data

show, the incidence in young rats is quite variable, ranging from 0 to 100%.

Kim *et al.* (2002) reported cardiomyopathy in two of ten males in the control group (20%) and 8 of ten males (80%) at the highest phytosterol dose at the conclusion of the 90-day treatment period. In comparison to the reported historical control data as well as other studies (Table 5.1) the incidence of cardiomyopathy for all groups in the Kim study was well within the expected range for untreated rats suggesting that the observed increase at 9000 mg/kg bw/day may not be treatment related. This interpretation is supported by the observation that other 90-day studies involving phytosterols in the diet show no such increase in cardiomyopathy at doses as high as 6600 mg/kg bw/day (Hepburn *et al.* 1999).

Given the uncertainties and lack of corroborative evidence implicating a causal relationship between phytosterol administration and cardiomyopathy FSANZ can see no sound scientific basis for establishing a reference health standard for phytosterols, phytostanols and their esters.

5.4 Effect of plant sterols on absorption of carotenoids and fat soluble vitamins

Consumption of plant sterols is known to lead to a reduction in concentrations of serum carotenoid (α- and β-carotenes and lycopene) levels, but not on the serum concentrations of retinol. Some studies also observed decreases in vitamin K, vitamin D and tocopherol levels. The reduction in serum concentrations of some carotenoids associated with consumption of phytosterol fortified foods was previously evaluated by FSANZ (FSANZ 2005, FSANZ 2006a). FSANZ concluded that:

- the decreases in tocopherol, vitamin K, and vitamin D levels are not significant once adjusted for decreases in lipid levels;
- as carrier LDL-cholesterol decreases by whatever means (i.e. drugs or diet), serum carotenoids are decreased;
- the levels of carotenoids in serum are known to fluctuate widely as a consequence of many dietary and environmental factors and a decrease of the range 20-25% seen in studies falls within a broad natural variation; and
- the reduction does not translate into an overt nutritional issue as absolute levels remain within the broad range in existing levels in population and there is no measurable effect on retinol levels.

Four of the studies included in the assessment of LDL cholesterol lowering effects (Section 6), published since 2006, included an investigation of the changes in serum carotenoids including ß-carotene.

All four of these studies reported no significant differences in mean serum levels or percentage changes in serum levels 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.,* 2008).

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 since have supported this conclusion (Jula *et al.,* 2002; Clifton et al., 2004; Colgan e*t al.*, 2004; Brufrau *et al.,* 2008).

5.4.1 Discussion

Additional evidence reviewed in this assessment supports FSANZ's previous consideration that there are no overt nutritional issues associated with the reductions in serum carotenoid levels associated with the use of plant sterol fortified foods. The current evidence has shown that serum concentrations of carotenoids and tocopherol remain within the normal range, and that increase in the dietary intake of carotenoids mediates the effects of plant sterols on serum carotenoids. There is no change to the previous conclusion that these reductions in serum levels do not pose a health risk to the population.

5.5 Absorption of plant sterols

A brief summary of knowledge on the absorption of plant sterols in humans is presented as background to section 5.6 of this report, which considers serum plant sterol levels and risk of cardiovascular disease. Cholesterol absorption and metabolism were previously reviewed in detail and reported in the Final Assessment Report for Application A434[11].

Cholesterol homeostasis in the body is well studied with pathways for cholesterol biosynthesis and clearance fully described (Altman *et al.,* 2004). The absorption of other sterols and their interaction with cholesterol is not yet as well defined, although the mechanism of plant sterols absorption has been investigated through studies on patients with sitosterolaemia.

Figure 5.1 provides an overview of the metabolism of cholesterol and plant sterols entering the human intestine. Current evidence suggests 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).

Research has identified several genetic factors which influence cholesterol and plant sterol absorption, explaining some of the individual variation commonly seen in plasma lipid responses (Sehayek, 2003; Sanchez-Muniz *et al.,* 2009). Absorption is due to specific transport proteins mediated through polymorphisms in genes involved in cholesterol pathways (Rudkowska *et al.,* 2008). Apo lipoprotein E (ApoE) and adenosine triphosphate-binding cassette (ABC) transporters both have important roles in the absorption and subsequent appearance of cholesterol in the circulation. The genes for ABC transporters (ABC G5/G8 genes) when expressed normally, appear to regulate transport of cholesterol and plant sterols in enterocytes and liver cells. The human ApoE gene is polymorphic but has three common alleles (Sanchez-Muniz *et al.,* 2009). People with different ApoE alleles differ in their ability to absorb cholesterol from the intestine, synthesize cholesterol and bile acids and in lipoprotein response to phytosterols (Sanchez-Muniz *et al.,* 2009).

[11] available online at www.foodstandards.gov.au/standardsdevelopment/applications/applicationa434phytosterolestersinlowfatmilkandlowfatyoghurt/index.cfm).

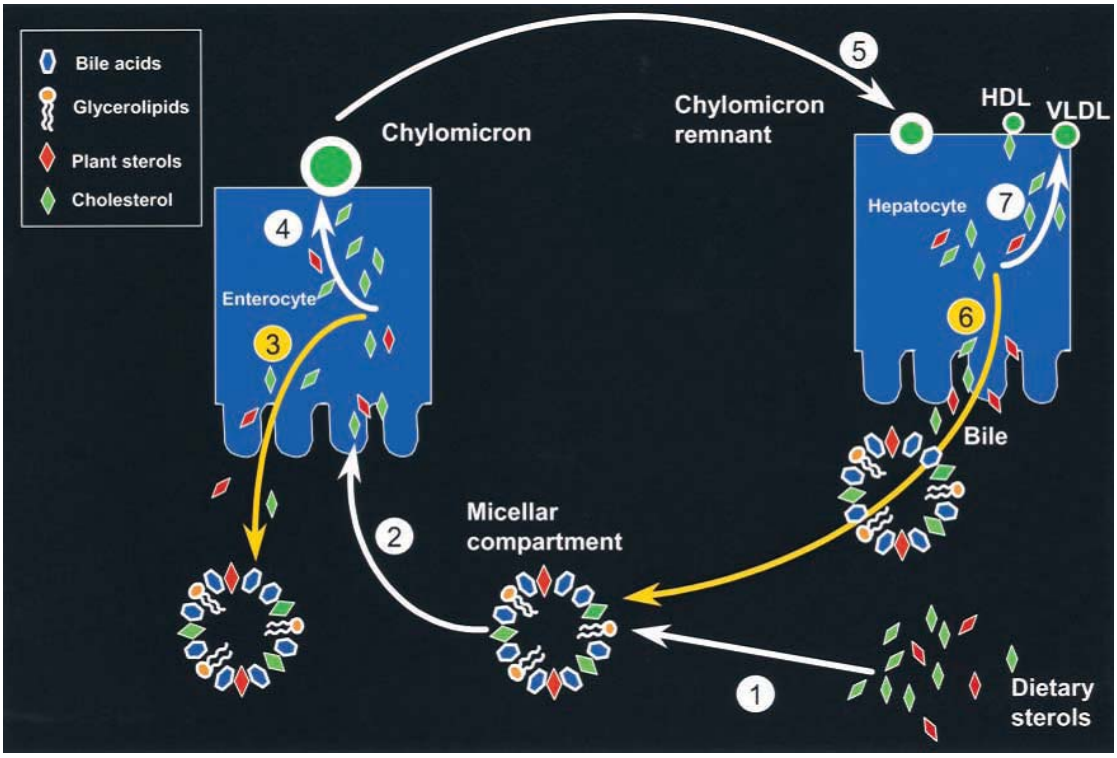

Figure 5.1. The enterohepatic metabolism of cholesterol and plant sterols.

Within the intestinal lumen, dietary cholesterol and plant sterols are transferred into the micellar compartment (1), taken up across the enterocytic brush border membrane (2), partly excreted back into the intestinal lumen (3), transported and secreted into plasma in the form of chylomicrons (4), disposed of by the liver in the form of chylomicron remnants (5), excreted through the hepatocytic canalicular membrane into the bile (6), and partly transported, packed, and secreted into plasma in the form of VLDLs and HDLs (7) (from Sehayek 2003).

Although plant sterols and cholesterol are absorbed by the same principal mechanism, 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 *et al.,* 2002; Kratz *et al.,* 2007). For example, although 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, typical serum plant sterol levels will be around 0.025 mmol/L compared to around 5 mmol/L for cholesterol (see Table 5.2). The lower net absorption of plant sterols compared to cholesterol is related to the active re-secretion of sterols back into the enteric lumen by the ABCG5 and ABCG8 transporters (Pinedo *et al.,* 2007). Findings from Kratz *et al.* (2007) showed that the ABCG5/G8 genotype was more important in determining serum phytosterol concentrations than the amount of phytosterols in the diet.

Consumption of plant sterol fortified foods will result in increases in plasma plant sterols levels. However the absolute levels remain at less than 1% of total plasma sterols, with increases generally not exceeding 0.025 mmol/L, even with high dietary intake of plant sterols (Windler *et al.,* 2009). For example in Australian clinical studies in 2002 by Clifton *et al.* (subsequently published in 2005) in mildly hypercholesterolaemic subjects consuming 6.6 g free phytosterols per day over a period of 12 weeks, plasma plant sterol levels increased from baseline by 111% for campesterol and 58% for sitosterol, to 14.9 and 12.1 µmol/L respectively. Despite the increased levels of phytosterols in plasma, the absolute amounts of phytosterols remained very low in the blood at approximately 0.02% of plasma cholesterol levels.

These changes in the levels of circulating phytosterols were similar to those reported in several other studies using phytosterol fortified foods.

Table 5.2 Typical intakes of sterols and serum sterol concentrations in healthy adults

	Average Western intake	Average serum concentration in healthy Western adults
Cholesterol	298 mg/day* (1995 NNS)	<5.5 mmol/L in populations not at increased risk of CVD
Plant sterols (from unfortified foods)	~150-360 mg/day	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) (Chan *et al*., 2006)

* Mean intake of Australians aged 19 years and above, 1995 NNS (McLennan & Podger, 1998)
**Based on review of 45 studies published from 1986-2005

5.5.1 Absorption in children

The absorption of dietary plant sterols appears similar in children and adults. This is illustrated by the findings of the larger STRIP Study (Special Turku Coronary Risk Factor Intervention Project) for children. In this study, 20 healthy, 13 month old children were randomly selected to consume a diet naturally rich in plant sterols by replacing milk fat with low erucic acid rapeseed oil over a period of several months, so that the intervention group had about twice the intake of plant sterols (132 mg/day) of the matched control group. Serum campesterol and sitosterol levels were 75% and 44% higher respectively in the intervention children compared with controls (Tammi *et al*., 2001). This effect was also seen in a study of children (aged 3-7 years) and their parents, where both children and parents were diagnosed with familial hypercholesterolaemia (Amundsen *et al*., 2004). After 26 weeks of consumption of plant sterol spreads (providing 1.6 g free phytosterols/day) there were increased serum concentrations of sitosterol and campesterol in the children, showing a similar pattern of increase as in adult consumers. The absolute increase however was considered minor and the levels of the phytosterols together remained well below 1% of total serum sterols, with cholesterol accounting for more than 99% of serum sterols.

5.6 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). 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. To examine the relationship (if any) between plant sterol levels and the risk of coronary heart disease, FSANZ has reviewed and summarised the relevant types of evidence in humans.

5.6.1 Sitosterolaemia

Sitosterolaemia (also known as phytosterolaemia) is a rare genetic (autosomal recessive) disorder[12] in lipid absorption in which affected individuals experience increased intestinal sterol absorption, decreased biliary excretion of dietary sterols and low endogenous cholesterol synthesis (Berge *et al.,* 2000). This change to sterol metabolism in sitosterolaemia results in a progressive accumulation of plant sterols in virtually every tissue except the brain (Salen *et al.,* 2004) and greatly increasing cardiovascular disease risk.

The cause of sitosterolaemia is mutations in both the ABCG5 and ABCG8 transporters in the liver and intestine (Lee *et al., 2001*). The affected genes each encode half of an ABC transporter (Chan *et al.,* 2006) (see Figure 5.1). Consequently in patients with sitosterolaemia, due to the mutation, intestinal uptake of plant sterols is increased while at the same time hepatic secretion is decreased.

In adults, the characteristic indicator of sitosterolaemia is significantly 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). The magnitude of the difference in serum plant sterol levels between otherwise normal individuals who consume phytosterol fortified foods on a daily basis, and patients with sitosterolaemia, is large.

As a result of plant sterol accumulation, individuals with sitosterolaemia are at high risk of atherosclerosis and coronary heart disease–related morbidity and mortality (Graf *et al.,* 2002; Salen *et al.,* 2004). However, the incidence of sitosterolaemia in the general population is extremely low. Affected individuals would be under medical supervision from an early age in childhood due to the early manifestation of clinical signs.

Once diagnosed, sitosterolaemia is treated by strict reduction in consumption of foods high in plant sterols. Avoidance of foods fortified with plant sterols would be essential. Close medical supervision and dietary advice from an early age means that these individuals would be advised against consuming foods fortified with plant sterols, Other treatments may also be necessary to reduce the abnormal absorption of plant sterols, for example the use of absorption inhibitors.

Although iinvestigations into the potential toxicity of plant sterols have largely demonstrated no adverse effects in either animals or humans, data from sitosterolaemia patients with increased plasma sterol levels and premature development of CVD (John *et al.,* 2007) raises 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 as to whether plant sterols are more atherogenic than cholesterol (Kratz, 2007). Although pathophysiological mechanisms underlying the associations between cholesterol homeostasis and CHD are not fully understood, the structural similarity of plant sterols to cholesterol raises the possibility that plant sterols might contribute to the development of atherosclerotic plaques in the same way as cholesterol (Silbernagel *et al.,* 2009).

5.6.2 Literature search

FSANZ undertook a literature search to identify human studies which examined the relationship between serum plant sterol levels and the risk of some forms of CVD. A total of

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

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 studies met the inclusion criteria (cohort or nested case control studies) 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 effects of this on dietary and lifestyle behavior cannot be investigated and assessed. Cross-sectional studies were excluded for this same reason.

5.6.3 Evidence from nested case-control studies

Three case-control studies nested within existing cohort studies were identified that had investigated whether levels of plasma plant sterols are related to an increase in risk of cardiovascular disease or adverse cardiac events. Study details are shown in table 5.3. All studies had 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.

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. There was a non significant inverse association with sitosterol:cholesterol ratio and CHD (after adjustment for traditional risk factors).

Assmann *et al.* (2006) investigated the risk of myocardial infarction or sudden death over 10 years 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 a known risk factor.

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)*.

5.6.4 Discussion

None of these three studies 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. 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 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 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. These factors have not been adjusted for in the studies relating serum plant sterol levels to coronary disease.

Chan *et al.* (2006) reviewed the literature current at that time and found no clear associations between the levels of phytosterols in serum and coronary heart disease in people with otherwise normal lipid metabolism. Apart from the issues discussed above, Chan *et al.* concluded that the variable results reported in a number of studies are a result of physiological states (particularly those associated with the metabolic syndrome), use of statin drugs, dietary intake of plant sterols and gender. The analytical methods used to measure plant sterols in serum were considered to confound investigations on a possible association between plant sterols and an increased risk of coronary heart disease because measurement techniques have improved over time allowing more certainty in analytical results.

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 vs. 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 potentially significant reduction in CVD risk associated with reductions in LDL cholesterol levels. Therefore the benefit of these foods is almost certainly far greater than any possible risk from them. Section 6 of this report investigates the cholesterol lowering action of plant sterols.

5.7 Response to risk assessment question 2

What new information relevant for assessing the safety of plant sterols in lower fat cheese products has become available since previous FSANZ reviews of safety?

Previous assessments of plant sterols have shown no food safety concerns. Based on the evaluation of the toxicity studies not previously assessed, the safety assessment has not identified any new information that would indicate the need to change previous conclusions regarding the safety of plant sterol fortified foods.

Four new studies on the effect of plant sterols on serum carotenoid concentrations reported no significant differences in mean serum levels or percentage changes compared to controls after standardisation for LDL cholesterol reductions. This new information supports the previous conclusion that these changes do not pose a health risk to the population.

For the first time, FSANZ has undertaken a comprehensive analysis of epidemiology studies to assess whether increased serum plant sterol concentrations increases risk of

cardiovascular disease. The available evidence indicates no role of plant sterols in increasing risk of cardiovascular disease other than in the rare group of individuals with sitosterolaemia. None of this information changes previous conclusions about the safety of plant sterols.

6. Cholesterol lowering action of plant sterols

The purpose of adding plant sterols to foods is to lower serum cholesterol levels. Before another category of plant sterol fortified food can be approved, FSANZ must be satisfied that addition of these substances can achieve results that are consistent with that purpose. Hence, this section of the report considers the LDL cholesterol lowering effects in adults of TO phytosterol esters in lower fat cheese products and of plant sterols in dairy foods generally.

The mechanism by which plant sterols may lower LDL-cholesterol is not fully elucidated. It was originally thought that the cholesterol-lowering action was simply a result of interference with cholesterol absorption as plant sterols displaced cholesterol from mixed micelles, thus competitively blocking cholesterol absorption from intestinal contents and resulting in reduced intestinal cholesterol absorption (Jones *et al.,* 2003). It now appears that the mechanism is more complex, although the exact molecular regulation of mechanisms is not confirmed. Evidence has shown that once plant sterols are within the intestinal enterocyte they influence the cellular cholesterol metabolism, and decrease cholesterol esterification rate in the intestinal epithelium (Trautwein *et al.,* 2003; Nissinen *et al.,* 2007; Brufau *et al.,* 2008). The cholesterol reduction is thought to be a result of the suppression of cholesterol absorption both from the diet and enterohepatic circulation combined with increased faecal excretion of cholesterol, resulting in relative cholesterol 'deficiency', thus followed by an increase in cholesterol biosynthesis and LDL receptor activity (Brufau *et al.,* 2008).

Table 5.3 Cohort and nested case control studies investigating the relationship between plasma plant sterols and risk of cardiovascular disease

Author, Year and study objective	Description of cohort	Definition of outcome	Duration of follow-up (years)	N cases, controls	Sitosterol level *	Odds Ratio (OR)	95% Conf. Interval (CI)	Adjustments and other comments
Pinedo, 2007 To examine the association between plasma levels of plant sterols and the risk of future coronary artery disease CAD in EPIC cohort who had never suffered a myocardial infarction or stroke	EPIC-Norfolk; 25,663 men and women aged between 45 and 79 years, resident in Norfolk, United Kingdom. Recruited between 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, 9th 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 the modest sitosterol elevations seen in the general population and CHD risk in men from a large prospective population-based cohort	Prospective Cardiovascular Műnster (PROCAM) study – 20,060 men and women in large-scale prospective employment-based cohort Recruited between 1979 and 1985 Men aged 35-65 years	Myocardial infarction or sudden death - coronary event was 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	The following were higher in cases: HDL levels were lower in cases than controls Matched on age, sex, date of enrolment No adjustment for x, y, z despite differences between cases and controls
					>5.25 µmol/L	1.81	1.12-2.94 (p<0.05)	

Fassbender, 2008	Longitudinal Aging Study Amsterdam (LASA) - 3,107 men and women aged 55–85 years 1192 men aged 65 - 89 years participating	Coronary heart disease (measured by presence of angina pectoris and/or myocardial infarction, peripheral artery disease	10 or 11 years -not clearly specified	279 957	NO: 8.07 µmol/L CHD: 7.13 µmol/L PAD: 6.99 µmol/L CBVD: 7.83 µmol/L	OR 0.78	0.62–0.98 (p=0.030)	Correcting for cholesterol by calculation (ie sitosterol ratio to cholesterol). age (males), age (females), sex, cholesterol, diabetes smoking and hypertension

Abbreviations used in table:

NO= No vascular disease
CAD=Coronary artery disease
CHD= Coronary heart disease
PAD=Peripheral artery disease
CBVD=Cerebrovascular disease

6.1 Previous consideration

Previous animal and human studies have found that the physical and chemical form of the plant sterols, food matrix and method of incorporation into foods can all influence the cholesterol lowering effect of the fortified food to some extent (Katan *et al.,* 2003). FSANZ has previously examined the use of several commercial plant sterol mixtures as novel foods and concluded that when delivered in specific foods these substances can be effective in lowering serum cholesterol levels (FSANZ, 2005). Low fat milk and yoghurts have previously been assessed and considered effective vehicles for some forms of plant sterols. TO phytosterol (unesterified) mixtures have been assessed as effective in lowering serum LDL cholesterol and approved for use in low fat milks by FSANZ in 2006 (Application A508).

6.2 Approach to assessment and sources of data

To assess LDL cholesterol lowering efficacy, FSANZ has considered whether consumption of TO phytosterol esters in lower fat cheese products can result in a reduction in serum LDL cholesterol levels in adults. LDL cholesterol is the outcome variable of interest in these studies because lipoprotein sub-fractions have different implications for cardiovascular disease: higher serum LDL cholesterol levels increase the risk of cardiovascular disease. LDL cholesterol has been identified as the key component in atherosclerotic plaque formation in studies examining the biological mechanism behind atherosclerosis and CHD (National Heart Foundation of Australia, The Cardiac Society of Australia and New Zealand, 2005). Also, as most of the cholesterol in plasma is transported as a component of low density lipoproteins the reduction in total cholesterol is partly influenced by simultaneous changes in LDL cholesterol. Previous evaluations have also shown that plant sterols in some food vehicles can lower LDL cholesterol but have no effect on HDL, another important sub-fraction.

The assessment includes results from trials which have investigated various commercial mixtures of phytosterols and phytostanols in food matrices similar to lower fat cheese as there are limited trials examining the LDL cholesterol lowering effect from fortified cheese products.

A large number of published studies in humans and other animals have investigated the cholesterol-lowering properties and nutritional effects of plant sterols added to foods. FSANZ has undertaken an extensive literature search and assessment of the relevant studies, using a systematic review approach (see Appendix 2 for further information).

The applicant provided eight references investigating the cholesterol lowering effects of various phytosterol or phytostanol mixtures in foods including cheese, milk, yoghurt, and breakfast cereals. Five of these studies were excluded from FSANZ's assessment as they investigated water based beverages and confectionery; food matrices which are not relevant to lower fat cheese.

A total of 163 articles were identified from the search strategy. Of these, only 38 met the inclusion criteria based on title and abstract content. After full papers were read for the 2nd selection step, 13 were excluded based on the exclusion criteria. Tables 6.1a and b list the inclusion and exclusion criteria used in study selection. For further details on the literature search refer to Appendix 2. Studies examining the effects of VO phytosterols and their esters as well as TO phytosterols, delivered in dairy products containing protein, were included and assessed as a proxy for TO phytosterol esters in lower fat cheese. As

commercial TO phytosterol mixtures commonly contain high proportions of plant stanols, studies using stanol mixtures were also included. Studies delivering phytosterols in butter and edible oil spreads were excluded because it is not known whether the absence of protein and a range of micronutrients in these foods would, or would not, independently affect LDL cholesterol levels or the action of the phytosterol mixture if not delivered in a dairy food containing protein. Use of cholesterol lowering medication and cholesterol lowering or restricted diets were included as FSANZ had previously concluded the effects of phytosterols were additive to these. Only two human studies investigating lower fat cheese as a food vehicle were identified; these two studies were also provided by the applicant.

Table 6.1a: Inclusion criteria used to screen titles and abstracts

Human study
Examined effects of TO or VO phytosterols and their esters on serum LDL cholesterol levels
Used randomised control trial (parallel or cross-over design)
Minimum intervention period of three weeks duration
Minimum participant age of 18 years
Subjects with normal or elevated cholesterol levels
Investigated dairy based foods containing protein
Administered a control diet or double blind studies that administered a placebo vehicle

Table 6.1b: Exclusion criteria used to screen titles and abstracts

Measured cholesterol absorption but not effect on serum cholesterol levels
Studies including oil based spreads or butter
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 liver and kidney disease
Results not reported for all groups or unable to be calculated for all groups
Evaluated an individual plant sterol rather than commercial mixture

6.2.1 Data analysis

The main outcome variable was the change in LDL cholesterol due to the plant sterol treatment, both as the absolute change (mmol/L) and the relative percentage change. Data on changes in LDL cholesterol levels were presented inconsistently across the various trials. Nearly half the trials only presented the difference for each trial arm from baseline levels rather than the difference between the plant sterol and control/placebo arms. Some studies presented some of their results as percent change in LDL cholesterol while others presented the results in absolute change in mmol/L. FSANZ made various calculations to obtain the absolute difference and its 95% confidence interval from the information presented, as well as the percent change allowing for control/placebo arm change for each trial arm.

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 the focus on change in LDL cholesterol in the intervention group compared to the control group. None of the trials estimated or measured the intake of naturally occurring plant sterols in habitual diets.

When the outcome variable was measured at various time points during the intervention, the value corresponding to, or closest to, the 4-week time point was taken for the comparative analysis, as three weeks of plant sterol intervention has been shown to be the point when the maximum cholesterol lowering effect is reached. Attachment 2 provides further detail on how

the information in the following tables has been derived from the data presented in the original papers.

6.3 Effects of plant sterols in cheese products on LDL cholesterol

Only two published human intervention studies investigate the effect of phytosterols added to cheese on LDL cholesterol; both are included in the efficacy assessment. Korpela *et al.* (2006) used a TO phytosterol mixture (comprised of 75% ß-sitosterol, 10% ß-sitostanol, 10% campesterol, 2% campestanol & 2% other) in fresh and hard cheese with 14 g and 10 g of fat per 100 g respectively. Jauhiainen *et al.* (2006), added a phytostanol ester mixture (comprised of sitostanol and campestanol trans-esterified with 1.4 g of rapeseed oil fatty acids) to hard cheese with 17 g fat per 100 g. These studies were both conducted in Finland, in adult volunteers with hypercholesterolaemia. The objective of both studies was to examine the effects of phytosterol fortified cheeses and yoghurt on serum levels of cholesterol, fat soluble vitamins and various plant sterols. Subjects were not permitted to use cholesterol lowering drugs or a lipid lowering dietary regime during either study.

The study by Korpela *et al.,* (2006) was a multicentre, parallel, double-blind trial. Mean baseline LDL cholesterol levels were 4.0 mmol/L and 4.1 mmol/L for the control and treatment group respectively. The sterol and control groups were further randomised into lower fat hard cheese, lower fat fresh cheese or low fat yoghurt groups, or their equivalent controls. The authors report the percent change in total and LDL cholesterol but do not report the mean difference in cholesterol levels between intervention and control groups after the intervention. The study by Jauhiainen *et al. (2006)* was a single location, randomised double blind parallel-group study. The authors measured the absolute cholesterol changes from baseline to end of intervention and compared the difference between the two groups. The mean baseline LDL cholesterol for the control group was
3.60 mmol/L and 3.58 mmol/L in the phytostanol group. Further study details are included in Table 6.2b.

Both studies found statistically significant reductions from 10-12 % in mean LDL cholesterol levels for the intervention group compared to the control group. In addition, Korpela *et al.,* (2006) found no difference in the LDL cholesterol lowering effect between the two types of cheese, or a third arm which tested the TO phytosterol mixture in yoghurt.

Both studies used cheeses that are reduced in fat compared to regular cheeses, but not as low in fat as those proposed by the Applicant (6 g/100 g before addition of plant sterols). The fat content of the plant sterol fortified cheeses in Korpela *et al.*'s study was 10 g per 100 g of fresh cheese and 14 g per 100 g hard cheese. In the study by Jauhiainen *et al*. the plant sterol fortified hard cheese had a total fat content of 17 g per 100 g. Therefore FSANZ has also investigated the efficacy of plant sterols delivered in dairy foods with lower fat contents

6.4 Effects of plant sterols in other dairy products on LDL cholesterol

6.4.1 Overview of the data

Although there is a large body of literature which concludes that plant sterols deliver a cholesterol lowering effect, studies continue to be conducted to investigate new mixtures of plant sterols, different food vehicles and the methods of incorporation of plant sterols into the food vehicles. FSANZ identified thirteen cross over or parallel designed trials with 29 intervention arms and 13 control arms which examined the LDL cholesterol lowering effects of dairy products fortified with plant sterols. Two studies included in the assessment were conducted in Australia and were included (in their unpublished form) in previous FSANZ

assessments (Clifton *et al.,* 2005; Noakes *et al.,* 2004). The other included studies have not been assessed by FSANZ previously. Many of the studies included multiple trial arms as they tested more than one food vehicle, or investigated whether the LDL cholesterol lowering effect differed when plant sterol fortified foods were consumed as snacks instead of with meals.

In total there were 1638 participants (including controls), with ages ranging from 18-75 years. The mean plant sterol dose given to participants for all trial arms was 1.9 g/day (range 1-3.2 g/day). This dose was consumed over multiple daily intakes in 11 of the 29 trial arms. Studies are available for both normocholesterolaemic and hypercholesterolaemic subjects. As with previous assessments FSANZ has included studies using subjects with both, with ten of the studies including mildly hypercholesterolaemic subjects. The studies by Li *et al.,* (2007) and Hyun *et al.,* (2005) were conducted in China and Korea respectively, while all other trials were conducted in Western populations. The mean baseline LDL cholesterol levels for all included studies ranged between 3.67 and 4.83 mmol/L.

6.4.2 Results

Table 6.2 (a & b) shows the mean LDL cholesterol levels at baseline along with the absolute and percent change in LDL cholesterol compared to placebo for different food vehicles and dose levels in all trial arms of the included studies, separated by trial design. All trials showed a post intervention decrease in LDL cholesterol levels as well as a decrease in LDL levels when compared to the control group. The average absolute decrease in LDL cholesterol was 0.31 mmol/L (95% CI:-0.36, -0.26), with an average relative decrease of 7.67%. The cross-over trials tested daily doses of plant sterols ranging from 1 to 2 g, reporting reductions in LDL cholesterol levels of 4.36 to 10.06% (Table 6a). The parallel studies tested daily doses of plant sterols ranging from 1.5 to 3.2 g. Parallel designed trials found statistically significant reductions in LDL cholesterol ranging from 2.50% to 13.80% (Table 6b).

The results presented in Table 6 a & b support previous findings that a statistically significant LDL cholesterol-lowering effect is achieved at doses around 2 g plant sterols per day, irrespective of the type of sterols consumed (FSANZ, 2006a) in cheeses and other dairy foods. These results are consistent with the findings from a recent meta-analysis (Demonty *et al.,* 2009). This meta-analysis included 84 trials and investigated the dose response curves of phytosterol and phytostanol mixtures across a variety of food matrices. Their analysis concluded that the overall pooled absolute (mmol/L) and relative (%) LDL-cholesterol reductions were 0.34 mmol/L (95% CI: -0.36, -0.31) or 8.8% (95% CI: -9.4, -8.3) for a mean daily dose of 2.15 g plant sterols (assessed with a random effects model). They also reported the cholesterol lowering effect plateaus at intakes of 3 g/day, which supports the findings of Katan *et al.*'s (2003) earlier meta-analysis of 41 trials with mainly fat-based foods like spreads, margarine, mayonnaise, or salad dressings fortified with phytosterol esters. Katan *et al.* concluded that in fat-based foods, LDL cholesterol lowering tapered off at intakes higher than 2 g/day of plant sterols, with little additional lowering at doses higher than 2.5 g/day.

Although all of the included studies have expressed an effect on LDL cholesterol of a total daily plant sterol amount per day rather than the amount per serve, Tables 6 a and b show doses of 1.6-2.0 g per day had a LDL cholesterol lowering effect which suggests that the one or two serves of cheese (20g) or one serve of cheese combined with a serve of another phytosterol fortified product will deliver a reduction in LDL cholesterol levels.

The plant sterol fortified cheese products proposed by the Applicant will have fat contents within the range of fat contents shown in these studies to be associated with lowering of LDL cholesterol levels.

6.5 Factors influencing the cholesterol lowering effect

While a statistical analysis of the impact of food matrix and other treatment characteristics on the LDL cholesterol lowering effect of plant sterols was not conducted, the relevant literature was reviewed and is summarised below.

Inter-individual variation in the LDL cholesterol lowering effect is commonly observed in the literature. As discussed in section 5.5, 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). Several other factors have been reported in the literature to influence the cholesterol lowering effect. These include characteristics of the substance, the method of incorporation into the food product as well as the nature of the food itself. While this assessment has concluded that the phytosterol mixture in lower fat cheese products can effectively deliver a cholesterol lowering effect, some variance in the cholesterol lowering effects is seen across the studies. Several individual factors that can have a small influence on the magnitude of the cholesterol lowering effect are discussed below.

6.5.1 Background diets and baseline cholesterol levels

Foods such as plant oils, nuts, seeds and legumes naturally contain plant sterols (Ostlund, 2002). There are few analytical studies measuring the plant sterol content of foods thus estimations of population intake from naturally occurring sources are limited (Ostlund, 2002). The daily dietary intake of plant sterols has been estimated to differ among populations (Clifton, 2009). Available estimations of the typical 'western' diet suggest an average intake of 150-360 mg of phytosterols per day, although vegetarian diets are likely to deliver higher intakes averaging 350-750 mg/day (Chan *et al.,* 2006). The Japanese population, for example is estimated to consume ~373 mg/day while intake for a Spanish Mediterranean diet was estimated at 276 mg/day (Jimènez-Escrig *et al.,* 2006).

The effect of dietary intakes of naturally occurring plant sterols on LDL cholesterol is the subject of ongoing research. Escuriol *et al.* (2009) investigated the cholesterol lowering effects of an increased intake of naturally occurring plant sterols. This study was a sub study of a randomized nutrition intervention trial of three diets: a low fat diet and two 'Mediterranean' diets with increased consumption of plant sterol-rich foods (vegetable oils or nuts), as protection for primary cardiovascular disease. Average phytosterol intake increased by 76 mg/day and 158 mg/day in the Mediterranean diet vegetable oil and nut groups respectively. The two intervention diets with increased amount of vegetable oils or nuts were associated with significant reductions from baseline in LDL-cholesterol (-4.2 and -6.8%, respectively). These results suggest that the small amounts of naturally occurring plant sterols in foods appear to have a small cholesterol lowering effect. Studies examining cholesterol levels and plant sterol levels in vegetarian diets have also suggested significant effects of naturally occurring plant sterols (Clifton, 2009). However Naumann *et al.* (2008) suggest that single trials are generally insufficiently powered to assess the influence of baseline characteristics.

Baseline characteristics of subjects have been suggested as sources of variation in responses to plant sterol fortified foods, with baseline cholesterol levels suggested to influence cholesterol reduction effects. Naumann *et al., (2008)* conducted a meta-analysis to observe the relationship of subject baseline characteristics and changes in serum cholesterol levels. They concluded higher baseline cholesterol levels resulted in larger absolute reductions. Demonty *et al.* (2009) also concluded that the baseline LDL cholesterol concentrations had a significant effect on absolute LDL cholesterol changes in the included studies. These findings are consistent with efficacy results in drug trials (Silbernagel *et al.,*

2009).

6.5.2 Eating occasion-frequency and timing

Variation in study results suggests that the eating occasion, timing and frequency of consumption of plant sterol fortified foods can all influence the cholesterol lowering effects. Early studies suggested that plant sterols may only be effective when consumed with fat containing foods, or that the total amount of cholesterol and fat in the diet would affect the cholesterol lowering effects. As understanding of the mechanisms of plant sterols has increased and food technology has improved, it has been determined that these factors are not as influential as first thought (Plat *et al.,* 2000; Volpe *et al.,* 2001; Maki *et al.,* 2003; Berger *et al.,* 2004).

Several of the trials included in this assessment incorporated trial arms to investigate the effects of frequency and timing of plant sterol consumption, with some directly comparing the effects of each. Seven trials used a design of one serve per day, and nine used two or more serves per day. There were mixed results regarding the influence of the frequency and timing of consumption in these studies. However the conclusions for the direct comparisons in these studies are slightly limited as most studies potentially confounded the conclusions by also changing the dose of plant sterols per day. Demonty *et al.* (2009) did however evaluate this effect in their meta-analysis and concluded that within the dose range of 1-3 g/day of plant sterols, an increase in the number of serves per day was associated with larger reductions in LDL cholesterol although the influence of dose size partly confounded the effect of frequency. They also suggested further trials should investigate the impact of frequency of intake as well as the impact of consuming the fortified food as a snack or with a meal.

6.5.3 Interactions with cholesterol-lowering medications

There are several groups of drugs used to lower serum cholesterol levels; these have different sites and modes of action, and act on different aspects of cholesterol metabolism. FSANZ previously considered the effects of plant sterols in combination with cholesterol lowering medication, concluding that plant sterols can have an additional cholesterol lowering effect in people who are using cholesterol lowering medications with no adverse effects (FSANZ, 2006a).

A number of studies have further investigated the combined effects of various plant sterol fortified foods and statins, a group of cholesterol lowering drugs (de Jong et al., 2008; Plat et al., 2009). The findings of these studies support the previous conclusion that there is a cholesterol lowering effect of plant sterol fortified foods even in those taking cholesterol lowering medication. There are no reports in the literature of adverse interactions between plant sterol fortified foods and cholesterol-lowering medications (National Heart Foundation of Australia; The Cardiac Society of Australia and New Zealand, 2005; Demonty et al., 2009). International lipid management guidelines now support the inclusion of plant sterol fortifed foods in the diet to help patients taking statins to reach cholesterol targets in preference to prescription of multiple medications (New Zealand Guidelines Group, 2003; National Heart Foundation of Australia, 2007).

Table 6.2: Change in LDL cholesterol in the intervention (given plant sterols in various dairy foods) versus control group

A: Crossover study arms ordered by daily dose amount

First Author	Number of subjects	Plant sterol form	Measured	Food vehicle	Mean baseline LDL (mmol/l)	Daily dose (g/day)	Reported absolute change mmol (int. - control)	% change (int. – control)
Volpe, 2001	30	unspecified	4 wks	low fat yoghurt	4.67	1	-0.29*	-6.2
Niittynen, 2008	15	unspecified sterols	4 wks	low fat yoghurt drink	3.90	1.0	-0.19	-4.3‡
Thomsen-A, 2004	69	VO sterols	4wks	low fat milk	4.37	1.2	-0.34*	-7.14
Thomsen –B, 2004	69	VO sterols	4 wks	low fat milk	4.37	1.6	-0.44*	-9.59
Rudkowska-A, 2008	26	TO sterols	4 wks	low fat yoghurt with meal	3.67	1.6	-0.13*	-4.2†
Rudkowska-B, 2008	26	TO sterols	4 wks	low fat yoghurt	3.72	1.6	-0.29*	-8.7†
Clifton- A, 2004	40	VO sterol esters	3 wks	milk	4.03	1.6	-0.53†	-12.4†
Clifton- B, 2004	40	VO sterol esters	3 wks	yoghurt	4.03	1.6	-0.42†	-9.8†
Noakes- A, 2005	40	VO sterol esters	3 wks	low fat yoghurt	4.48	1.8	-0.27	-6.0
Noakes- B, 2005	39	VO sterol esters	3 wks	low fat milk	4.83	2	-0.38	-7.9

† Change calculated by FSANZ
‡ Change reported
A,B,C,D = different trial arms of study
* calculated from period specific absolute data

B: Parallel study arms ordered by daily dose amount

First Author	No. of subjects (int, control)	Plant sterol form	Measured	Food vehicle	Mean baseline LDL of intervention group in mmol/L	Daily dose - free sterol equiv (g/day)	Reported absolute change mmol/L (int. - control)	% change (intervention – control)
Li – A, 2007	102 ,99	Unspecified	4 wks	Chinese milk tea	3.20	1.5	-0.15‡	-2.5
Korpela- A, 2006	22, 25	VO sterol esters	6 wks	Low fat yoghurt	4.01	1.5	**-0.31**†	***-4.69†***
Plana, 2006	43, 40	VO sterol esters	3 wks	Fermented milk	3.79	1.6	-0.48†	-12.42†
Hansel-A, 2007	95, 96	TO Sterol esters	3 wks	Low fat fermented milk	4.08	1.6	-0.21†	-9.19†
Korpela-B, 2006	33, 29	VO sterol esters	6 wks	Hard cheese	4.01	2	**-0.46**†	**-11.23**†
Korpela-C, 2006	24, 28	VO sterol esters	6 wks	Fresh cheese	4.01	2	**-0.56**†	**-13.8**†
Niittynen, 2008	12, 14	Unspecified free	8 wks	Low fat yoghurt	4.73	2	-0.28‡	-6.4
Jauhiainen, 2006	33, 34	Unspecified stanol esters	5 wks	Hard cheese	3.58	2	**-0.36**‡	**-10.07**†
Hyun, 2005	23, 28	VO Stanol esters	4 wks	Low fat yoghurt	3.07	2	-0.24	-7.84‡
Seppo-A, 2007	31,29	unspecified stanol esters	4 wks	Low fat yoghurt	3.40	2	-0.1‡	-2.9‡
Seppo-B, 2007	29, 32	unspecified stanol esters	4 wks	Yoghurt shot drink	3.40	2	-0.11‡	-3.2‡
Seppo-C, 2007	10, 9	Unspecified stanol esters	4 wks	Yoghurt shot drink	3.40	2	-0.4‡	-11.8‡
Seppo-D, 2007	32, 27	Unspecified stanol esters	4 wks	Low fat milk	3.40	2	-0.21‡	-6.2‡
Li –B, 2007	100, 99	Unspecified	4 wks	Chinese milk tea	3.10	2.3	-0.15‡	-2.5
Doornbos-B, 2006	38, 33	TO esters	4 wks	Yoghurt drink (1.5%fat)	3.90	2.8	-0.37†	-12.76‡
Mensink, 2002	20, 30	Stanol esters	4 wks	Low fat yoghurt	2.92	3	-0.4†	-10.3†
Doornbos-D, 2006	36,33	TO esters	4 wks	Yoghurt drink (1.5%fat)	4.06	2.8	-0.29†	-10.36‡
Doornbos-C, 2006	39, 33	TO esters	4 wks	Yoghurt drink (0.1% fat)	3.95	3.2	-0.21†	-8.56‡
Doornbos-A, 2006	38, 33	TO esters	4 wks	Yoghurt drink (0.1% fat)	4.07	3.2	-0.4†	-12.96‡

† Change calculated by FSANZ
‡ Change reported
A, B, C, D = different trial arms with same control group comparison
Bolded studies indicate the cheese studies

6.5.4 Long term phytosterol consumption and LDL cholesterol and total cholesterol levels

The data available to address the capacity of plant sterols to maintain long-term reductions in plasma cholesterol levels in humans using plant sterol fortified foods are limited. Miettinnen *et al.* (1995) and Hendriks *et al,* (2003) showed regular consumption of plant sterol fortified food for up to a year resulted in an LDL cholesterol lowering effect with maintained consumption of the plant sterols. More recently Jenkins *et al.* (2006) conducted a 12 month study of a dietary mix of cholesterol lowering food, of which plant sterols were a large component. This study found that the maximum cholesterol reduction was observed at week 12 and reductions were maintained to one year with continued consumption of plant sterols. An 85 week long study (de Jong *et al.,* 2008) in patients on stable statin treatment, also found that consumption of stanol fortified margarines maintained lowered LDL cholesterol levels for the period of consumption. It is important to note that this additional cholesterol lowering effect ceases once regular consumption of plant sterol fortified foods is discontinued.

6.5.5 Considerations in children

Several studies have investigated the effects of medications (predominantly statins) and plant sterols in children and adolescents with familial hypercholesterolaemia on total and LDL cholesterol (Amundsen *et al*., 2002; Berger *et al*., 2004). A number of studies investigating the effects of phytosterol esters in children were previously considered by FSANZ. The children in these studies were either hypercholesterolaemic, or were genetically susceptible to high cholesterol levels because one or both parents were diagnosed with familial hypercholesterolaemia. These evaluations concluded that consumption of phytosterols resulted in the same physiological effects as in the target adult population – that is, a modest reduction in LDL-cholesterol levels. Further studies that have investigated the cholesterol lowering effect of plant sterols in children have supported the previous conclusions (Moruisi *et al*., 2006).

Studies examining the effects of plant sterol fortified foods in children with normal cholesterol levels are still not available.

Children have high requirements for cholesterol for normal development of the nervous system, production of bile acids, neurological development, formation of hormones for growth and sexual maturation (NHMRC, 2003). There had been concerns raised that cholesterol lowering diets and consumption of plant sterol fortified foods during peak growing years may affect growth, neurological development and nutrient adequacy (Lauer et al., 2000; Rask-Nissilä *et al*., 2000). Restricted fat and low cholesterol diets have now been studied in children and adolescents and have been shown to effectively lower LDL cholesterol in children with no adverse effects on growth or maturation compared to population growth rates (Lauer et al., 2000; McCrindle *et al*., 2007). Despite this, current international recommendations for cholesterol reduction in children have adopted a conservative approach of recommending a healthy pattern of lower fat eating combined with physical activity, rather than treatment with cholesterol lowering medication or with consumption of plant sterol fortified foods (Williams *et al*., 2002).

6.6 Discussion

Evidence shows plant sterols added to lower fat cheese and other lower fat dairy products can lower LDL cholesterol levels. The LDL cholesterol reductions seen in current studies as a result of phytosterol fortified lower fat cheese consumption at a daily intake of 1.5-2 g free

phytosterols per day, fall within previously accepted ranges by FSANZ. The fat content of the dairy foods included in these studies spanned a range that includes the fat content of the Applicant's proposed lower fat cheeses and there is no reason to believe the Applicant's products would have any different effect on serum LDL-cholesterol. FSANZ considers that TO plant sterol mixtures in lower fat cheese products can deliver a cholesterol lowering effect in adults

Previous studies of the effect of plant sterol fortified foods has shown that the cholesterol lowering effect from the sterols is in addition to reductions caused by a modified diet or medications (such as statins). These studies had concluded that the magnitude of the cholesterol lowering effect can be influenced by an individual's genetic factors, background diet and baseline cholesterol levels, however the degree to which each factor contributes to the variations observed has yet to be determined.

Elevated serum LDL cholesterol concentration is a well established risk factor for CVD, which is a leading cause of disability and death in Australia and New Zealand (AIHW, 2008, Maki *et al.,* 2003, The National Heart Foundation of Australia and the Cardiac Society of Australia and New Zealand, 2005; New Zealand Guidelines Group, 2003). The National Heart Foundation of Australia and the Cardiac Society of Australia and New Zealand (2005) recommend that individuals attempt to keep their total cholesterol below 4 mmol/L and LDL cholesterol below 2 mmol/L to reduce the risk of heart disease*.* However a cutoff of 5.5 mmol/L total serum cholesterol is used to determine populations at greater risk of heart disease (AIHW, 2008).

Based on the 1999-2000 Australian Diabetes, Obesity and Lifestyle Study, it is estimated that over six million Australian adults (aged 25 years and over) had cholesterol levels higher than 5.5 mmol/L (AIHW, 2004) equating to approximately 51% of the adult population (National Heart Foundation of Australia, 2004). The New Zealand 2006/07 Health Survey reported that one in twelve adults (8.4%) was currently taking medication for high cholesterol (Ministry of Health, 2008). The New Zealand Heart Foundation estimates that lowering LDL cholesterol by 20% reduces cardiovascular risk by about 25% over 5 years (http://www.nhf.org.nz/index.asp?PageID=2145828664).

No studies have directly examined the effects of lowering LDL cholesterol through consumption of phytosterols on CHD incidence. However there is strong evidence supporting a progressive decline in CHD risk with decreased total and LDL cholesterol (National Heart Foundation of Australia and the Cardiac Society of Australia and New Zealand, 2005; NZ Guidelines group, 2009). For example, a reduction of 1 mmol/l in LDL cholesterol is estimated to reduce risk of coronary heart disease and stroke by a third (New Zealand Guidelines Group, 2003). Primary and secondary long term prevention drug trials of statin use to reduce cholesterol have shown reductions in cardiovascular death of 23-32% with decreases in LDL-C by 28% to 35% (Jenkins et al., 2003). Katan et al., (2003) estimated that based on results from drug trials and cohort studies, lowering LDL cholesterol in adults by 10% translated to lowering CHD risk by 12% to 20% in the first 5 years, and by 20% over a lifetime.

6.7 Response to risk assessment question 3

Are the plant sterol mixtures proposed for use capable of lowering cholesterol when added to lower fat cheeses?

The proposed form of plant sterols, along with other types of plant sterols, is capable of lowering LDL-cholesterol. Cholesterol lowering effects have been demonstrated when plant sterols are added to dairy foods, including lower fat cheeses, at the levels proposed by the

Applicant.

This conclusion is supported by a recent review of the European Food Safety Authority (2009), which found reductions in LDL cholesterol of 7 to 10.5% in people consuming 1.5-2.4 g/day of plant sterols.

7. Consumers' use of sterol fortified food products

Given that plant sterol fortified food products have been available in Australia/New Zealand and in other countries for around ten or more years, FSANZ undertook a review of available evidence on how consumers use these products, particularly where more than one type of fortified product is available.

7.1 Sociodemographics of users of plant sterol foods

The use of plant sterol fortified products is not uniform across socio-demographic groups, as a range of psycho-social and demographic variables influence health-related attitudes and behaviours to food (Childs & Poryzees, 1998; Worsley & Scott, 2000; Cox & Anderson, 2004; Ikeda, 2004). Consumption of foods fortified with plant sterols by target populations[13] and non-target populations is discussed below.

A survey of Australian and New Zealand users found 50% of sterol fortified spread users were over 35 years of age. There was no significant difference in the age of users of fortified spreads and those who do not use fortified spreads. Half of fortified spread users also scored moderate to high on a scale of health consciousness (FSANZ, 2006b).

European research found that 78%-91% of households across the European Union that had purchased plant sterol fortified spreads had no children living in the household; 87%-96% of regular purchasers had no children living at home. In terms of the target population, 75-91% of purchasers were older than 45 years (Scientific Committee on Food, 2002b; Lea & Hepburn, 2006).

A survey from the UK examined consumption of sterol fortified spreads, yoghurt pots and/or yoghurt drinks (UKFSA, 2006). Of the sample of adults surveyed, 27% of males, and 30% of females had consumed plant sterol fortified products in the past 6 months. Breakdowns by age group showed that 28% of 45 year olds and over and 30% of 16-44 year olds had consumed these products in the past 6 months. Of people who consumed plant sterol fortified products in the past 6 months, age group findings showed that 21% of those aged 45 years and over had consumed these products weekly in the past 6 months, compared with 33% of those aged between 16-44 years. Consumption among the under 5 year old age group is low: approximately 0.5% of children aged under 5 years may have some consumption of plant sterols. This shows a degree of use by non-target groups, however, this research also revealed a level of confusion as to what a plant sterol fortified product was; some respondents confused them with probiotic yoghurt drinks for example.

In Ireland, 60% of plant sterol fortified food users were women. Just over 80% of users were aged 45 years and over; 19% of consumers were adults younger than 45 years; consumption by children less than 5 years was 8% (occasional use) and less than 1% (regular use). The majority of users were secondary or tertiary educated (88%); 61% had high cholesterol, whilst 36% had no medical condition (Hearty *et al.,* 2008).

[13] Assumed in this assessment to be people aged 45 years and over, with an elevated cholesterol level

In Finnish research using 1997-1998 data sets the mean age of fortified spread users was 59 years (Anttolainen *et al.,* 2001). More recent research puts the highest incidence of Finnish fortified spread use at 9% for those aged 65-74. At the next decadal cohort older (75-84), and younger (55-64), 6% of each used fortified spreads (Simojoki *et al*., 2005). Findings from Finland show that when compared with nonusers, fortified spread users were most commonly males, better educated, higher earners, employed in white-collar occupations more often, more often married and more often living in city compared with rural areas. People with the lowest educational level had the lowest intakes of phytosterols, and those with highest education level had highest intakes of phytosterols (Valsta *et al*., 2004). Almost half of Finnish users reported having cardiovascular disease, compared with a quarter of nonusers. The mean age of users with cardiovascular disease was 63 years, and 57 years for users without cardiovascular disease. Among users, there were fewer smokers and more physically-active people; however users tended to consume more alcohol than nonusers. Users were less often obese compared with non-users.

The average age of German purchasers of plant sterol fortified foods was 59 years. Overall, 78% of users were adults greater than 45 years of age; 15% of users were adults younger than 45 years; 4% children aged 5-17 and 1% of children younger than 5 years old were users of fortified products. Around half of the users were outside the target population for sterol fortified products, and almost all of the children consumed these products because the products were part of the family diet (Niemann et al., 2007). One third of the German respondents had completed university or a university of the applied sciences, one-quarter had an intermediate school-leaving certificate and almost 40% had a lower school-leaving certificate. Overall, 45% of users did not belong to the target population. These other users were either people who decided to consume these products for reasons other than an elevated cholesterol level or people who simply ate the products because they were available in the households of their families or partners. The latter group (making up 5%) includes 53 of the 54 children under the age of 18. Almost all minors were not part of the target population and most of them lived in families in which the parents were not members of the target population either.

7.2 Reasons for using plant sterol fortified foods

In the survey of Australian and New Zealand users, respondents were asked what their primary motivations were for purchasing a fortified spread. Health related concerns were the primary motivation for the largest group of fortified spread users, followed by convenience. This is an expected finding with the majority of individuals motivated by health related issues with cholesterol being the major issue. Seven percent of fortified spread users reported their primary motivation for using fortified spread as ‘someone else in my household prefers it so I use it too’ (FSANZ, 2006b).

The most common reasons for consumption among users of plant sterol fortified products in the UK were ‘to lower my/my partner’s cholesterol’ (27%) (59% of whom have been diagnosed with high cholesterol), and ‘because it’s healthy / good for you’ (27%). When examined by age group, lowering cholesterol was listed as a main reason for consuming these products by those aged 45 to 65 years and over. The main reason for product consumption by 16-44 year olds was ‘it’s good for you’ / healthy. Other reasons mentioned for purchase of fortified products included ‘because I like the taste of it’, and ‘because someone else in my family buys it’ (FSANZ, 2006b).

In Ireland, 61% reported consuming fortified foods due to diagnosis of high cholesterol, and 36% reported consuming fortified foods in absence of a medical condition. Seventeen percent of users bought the product for general health reasons (UKFSA, 2006).

Out of the German plant sterol-consuming shoppers, 87% of the product users indicated an elevated cholesterol level as the reason for consumption. Furthermore, shoppers were asked whether this statement was based on the actual measurement of the cholesterol level. Overall, 89% of the people who gave an elevated cholesterol level as the reason for consumption were based on a measurement; in 8% it was not based on a measurement and 3% didn't know whether a measurement had been taken. The elevated cholesterol level had been measured in the case of the children who were indicated in two households as the sole users of the plant sterol fortified foods. Smaller proportions of respondents indicated the wish 'to protect my health' as a reason for motivation. Others gave as reasons: 'lose weight/pay attention to my figure' and 'because it tastes good'. The consumption reason 'elevated cholesterol level' was indicated significantly more frequently by older than by younger shoppers (over 45 years of age *cf* 45 years of age and under). People with minors in their households reported 'elevated cholesterol level' as the reason for consumption less frequently than people without children in the household. Overall, 72% of the 53 minors who consumed the products during family meals, therefore live in households of shoppers who indicated other consumption reasons. The level of education had no impact on consumption motivation (Niemann et al., 2007).

7.3 Consumption of plant sterol fortified foods and intake of plant sterols

There have been limited data collected for consumption of plant sterol fortified lower fat cheeses in markets where it is available. Generally, findings (EFSA, 2008) show that:

- Users of plant sterol fortified products tend to use one product at a time, with only a small proportion of users consuming two or three plant sterol fortified products per day.
- Consumers eat cheese as the second or third choice in addition to a primary plant sterol fortified product.
- Consumers of plant sterol fortified products do not consume cheese spreads on a daily basis.
- Overall, in more mature markets where plant sterol fortified cheese is available in addition to other plant sterol fortified products, the large majority of users have a free phytosterols intake of less than 3 g/day. Instead, studies show that many users do not consume sufficient amounts of fortified products to gain a health benefit.

Data on frequency of consumption of plant sterol fortified foods were rarely collected or reported in the literature.

Table 7.1 shows the median and upper level intakes (95th percentile) for free phytosterols in various EU Member States from a variety of studies reported in the European Food Safety Authority (EFSA, 2008) review. An individual analysis of the major studies is presented below.

Table 7.1: Phytosterol intake from information provided in four studies and summarised in EFSA (2008) review

	No. of fortified foods on market	Median intake (g/day)	95th/97.5[a] percentile intake (g/day)
Post-launch	2	1.0-1.9	~2.2-3.6
Ireland	5	2.5	5.5
Germany	7	-	~3[a]
UK	3	~0.9	~3[a]

7.3.1 Edible oil spreads (Lea and Hepburn, 2006)

A post-launch market survey of edible oil spreads containing plant sterols was conducted in the Netherlands, the United Kingdom, France, Germany and Belgium (Lea and Hepburn 2006). Detailed usage information was obtained for approximately 2000 households over a 12-13 week period. Intake calculations were carried out at the household level based on the number of edible oil spread packages bought. Regular[14] purchasers had an approximate median intake of phytosterols of 1.0-1.9 g/per person/day and 2.2-3.6 g/person/day at the 95th percentile of consumption.

The majority of purchasers (75-91%) were older than 45 years. 78-91% of products were purchased by households without children. The data did not show which member of a household used the products. The authors state that the similarity of intakes between one-person and larger households indicates that the products were predominately used by one person per household.

7.3.2 Edible oil spreads and six other products (Niemann *et al.*, 2007)

Niemann *et al.* (2007) reported consumption patterns for seven products with added plant sterols in Germany, including edible oil spreads, yoghurts, milks, sliced cheese and bread. The study surveyed 1001 adult purchasers of these products and was carried out at the point of purchase in the supermarket using an interview-assisted questionnaire. The 1001 questionnaires represented 1559 consumers of these products. The study did not collect any consumption amounts; however, the authors estimated that intake of phytosterols would exceed 3 g/person/day in 22% of households. Only 5% of plant sterol containing products purchased by German consumers were of sliced cheeses. 13% of purchasers of these cheeses used them on a daily basis.

7.3.3 Edible oil spreads and yoghurts (Kemplay and Nordfjord, 2006)

Kemplay and Nordfjord (2006) examined consumption patterns of products with added plant sterols in the United Kingdom. 3906 adults were interviewed as part of an omnibus survey. 28% of respondents had consumed edible oil spreads and/or yoghurts with added plant sterols in the six months before the survey. Based on the frequency of consumption reported in this study, EFSA (2008) estimated the mean intake of phytosterols by consumers of these products to be 0.9 g/day with a corresponding 97.5th percentile intake of approximately 3 g/day.

The authors reported that 90% of consumers were adults older than 45 years. 7% of consumers were adults younger than 45 years (4% women), 2% were children aged 5-16, and 1% were children younger than 6.

7.3.4 Edible oil spreads, yoghurts, milk, cheese spreads (Hearty et al., 2008)

A study carried out in Ireland surveyed 468 consumers of plant sterol containing foods, including edible oil spreads, yoghurts, milk and cheese spreads (Hearty *et al.,* 2008). The study was carried out at the point of purchase in the supermarket using an interview-assisted questionnaire. Mean phytosterols intake was 2.5 g/day, and was 4.4 and 5.5 g/day at the 90th and 95th percentiles respectively[15].

[14] Lea and Hepburn define regular purchasers as 'those who have bought the product regularly during the period monitored'. The monitoring period was 12 or 13 weeks.

[15] Hearty *et al.,* explain the high intakes in their study as follows '…Irish people are in general high consumers of spreads and these customary dietary patterns are continued with the phytosterol enriched product...'

1% of Irish consumers purchased cheese spreads with added plant sterols with a mean daily consumption of 10 g of cheese. In this study, cheese spreads contributed approximately 0.01 g/d to total phytosterol intake. For those consumers whose intakes exceeded 3 g/day, 3% consumed cheese with added plant sterols. 81% of consumers were older than 45 years.

7.4 Consumer use where multiple sterol fortified products are available

The EFSA review (EFSA, 2008) revealed that use of plant sterol fortified-products is predominantly by one person per household, and the majority of users consume one or two products as a part of their regular diet. Table 7.2 shows the proportions of sterol fortified product users consuming single or multiple fortified foods.

Table 7.2: Multiple product consumption from information provided in four studies and summarised in EFSA (2008) review

	No. of fortified foods on market	Proportion of users eating respective no. of products/day				
		<1	1	2	3	4
Ireland	5	-	69%	27%	4%	-
Germany	7	17%	72%	9%	1%	<1%
UK	3	53%	39%	6%	2%	-

UK purchasers of plant sterol fortified foods tend to be loyal to one category of food with limited cross-purchasing evidence (AC Nielsen, 2006). In Ireland, very few respondents consumed yoghurts or cheese spreads only and none consumed fortified milk only (always in combination) (Hearty *et al.*, 2008). Of the fortified products, cheese was the only product that was not consumed daily on its own by German users; it was a second or third product for the small number of people who ate it daily (n=6) (Niemann *et al*., 2007).

EFSA (2008) also examined phytosterols intake reported in the studies summarised above. They concluded that average phytosterols intake was lower than the effective amount for the majority of consumers. An established subgroup of consumers (1-4%) had intakes of greater than 3 g of phytosterols sustained for more than a year. For these intakes to occur there would have been more than three occasions of eating a plant sterol product per day, or daily consumption of two or more products, each at the recommended level of consumption. The majority of consumers were over 45 years of age. However whole families consumed plant sterol containing products without belonging to the target group; this includes some children.

7.5 Do users understand the role/purpose of phytosterols and are they aware of advisory statements?

Data collected from enriched spread users in Australia and New Zealand (FSANZ 2006b) shows that these users have mixed understandings of the role of phytosterols and labelling information, e.g. issues such as the suitability for children, and serving size information. Forty-eight percent of enriched spread users were aware of the terms ‘plant sterol’ or ‘phytosterol’. Fifty-eight percent perceived the main benefit of phytosterols to reduce cholesterol level and 25% were not sure of the main benefit. Around 10% believed the product to generally improve health. Almost two thirds of respondents incorrectly agreed with the statement that ‘Everybody can eat plant sterol margarine’. Only a quarter of respondents correctly responded to the statement that phytosterol-fortified products are not suitable for children, as written in the advisory statement on the label of enriched margarine.

These findings suggest a low level of readership of advisory statements on phytosterol-fortified spreads.

Data from the United Kingdom (UKFSA 2006) indicated that consumption guidelines have not been optimally communicated to the majority of users. Findings show low levels of knowledge of the product and guidelines and low levels of label readership also. In terms of product knowledge, 60% correctly agreed a benefit of the product is that it can lower cholesterol. Half of respondents held the common misconception that the products help maintain a healthy digestive system, a quarter believed the products can lower blood pressure, and a fifth thought that consuming the product was more effective than other changes to lifestyle behaviours (e.g. exercising) in lowering cholesterol. Ten per cent of users also believed the products were suitable for children under 5 years of age.

Regarding label readership, half of users indicated they could recall seeing 'lowers cholesterol level' on product labels, but only 9% could remember seeing the maximum amount, and 8% the minimum amount you should eat each day to be of benefit. Only 4% of respondents reported seeing the products were not suitable for pregnant or breastfeeding women, and 4% reported reading the product is not suitable for children under 5 years old, as advised on the label.

More than half of German respondents (56%) believed that the products were suitable for all users (Niemann *et al*., 2007). Twenty-seven percent of respondents knew there was a target group for enriched products, but only 38% of this group correctly nominated children as not being a target group. This was followed by pregnant women (5%) and people on cholesterol-reducing medication (5%). Breastfeeding women were only selected in 2% of cases. Only 4% of respondents could nominate the correct reason for fruit and vegetable intake whilst consuming phytosterol-fortified products as specified on European labels.[16]

7.6 Are there any impacts of phytosterol-fortified foods on lifestyle behaviours for users?

Australian and New Zealand research (FSANZ 2006b) found no significant differences between the test and control groups in the level of exercise they carried out. In terms of diet, there was a demographic distinction with younger users of enriched spreads having a better diet than those who didn't use enriched spreads (based on daily intake of fruit and vegetables). There was no significant differences in diet between test and control groups of those 35 years and older. A minority of enriched spread users reported that their diet and exercise had improved since using phytosterol-fortified spreads. Consumption of phytosterol-fortified spread was not linked to either better or worse diet and exercise measures, though a minority of users considered they had improved diet and exercise levels.

7.7 Discussion

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 cardiovascular disease. 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.

Most purchasers of plant sterol fortified food products do not have children in the household;

[16] To prevent a reduction in plasma carotenoid levels – specified on European labels

however, there is some exposure of children to these products.

Purchasers are motivated by concern about their health, particularly cholesterol or cardiovascular disease; however, there is consumption of fortified products for other reasons such as taste, and because someone else in the household purchases the product.

Most of the evidence collected across Member States in the European Union indicates that current intakes of phytosterols are below the upper level of optimal intake of 3 g/day for cholesterol-lowering purposes.

Post-market launch monitoring in other EU Member States, where additional fortified products have been available for some time, generally suggests that increased product availability is not linked to excess consumption of plant sterols, and most users consume one or two products, and substitute fortified products for each other.

Overall, data from Australia and New Zealand, the United Kingdom, and Germany indicates that enriched spread users have mixed understandings of the role of phytosterol-fortified products and current mandatory labelling information. There seems to be low levels of label readership; misunderstanding of the role of phytosterols within respondents in the UK; and a low degree of familiarity with all of the labelling information.

Phytosterol-fortified spread users in Australia and New Zealand do not believe phytosterol-fortified spreads act as a 'magic bullet' that will absolve them of further responsibility for healthy behaviour. Users of phytosterol-fortified spreads do not exercise less or have less healthy diets than non-users.

7.8 Response to risk assessment question 4

What impact could the introduction of plant sterol fortified cheeses have on the consumption patterns of this type of food in Australian and New Zealand consumers?

While it is difficult to predict individuals' behaviour, European evidence suggests that consumers are unlikely to markedly change their eating patterns to incorporate sterol fortified foods, but are more likely to incorporate one or two different types of plant sterol fortified foods into their regular diet.

8. Potential dietary intake of plant sterols

8.1 Approach to estimating dietary intake

The general approach to assessing dietary intake of a novel food ingredient is 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 extend permissions to add plant sterols to a wider variety of 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 phytosterols 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.

FSANZ has therefore used the following approach to considering the potential dietary intake of plant sterols from lower fat cheese by:

- reviewing dietary intake estimates from previous Applications
- assessing dietary intake of phytosterols[17] from the recommended number of serves of different foods containing plant sterols, including lower fat cheese
- analysing lower fat cheese consumption data from National Nutrition Surveys and calculating dietary intake of phytosterols that could be experienced if all lower fat cheese were substituted with cheese 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.

The following data bases were used to estimate dietary intake:

- 2007 Australian National Children's Nutrition and Physical Activity Survey (NCNPAS)
- 2002 New Zealand Childrens National Nutrition Survey (NZCNS)
- 1997 New Zealand National Nutrition Survey (1997 NNS)
- 1995 Australian National Nutrition Survey (1995 NNS)
- Roy Morgan Research Single Source survey (RMRSS).

Appendix 3 provides more detail on the use of these databases in the dietary intake assessment.

8.2 Dietary intake estimates from previous applications

FSANZ's previous dietary intake estimates of phytosterol equivalents assumed:

- consumers do not change the amounts and general types of foods they eat, but that they simply substitute plant sterol-containing foods for their non-plant sterol counterparts
- all edible oil spreads, high-fibre and low-sugar breakfast cereals, and low fat milks and low fat yoghurt contained added plant sterols at the highest permitted concentration.

[17] For purposes of describing dietary intake, all plant sterols are converted to the equivalent amount of free (non-esterified) phytosterols. The intake estimates presented are not equivalent to the amounts of commercial plant sterol mixtures that are actually added to foods. For example, 1.8 g of added TO phytosterol esters is equal to 1.1 g of free phytosterols.

Assessments were conducted for the general Australian and New Zealand populations, those aged 40 years and above (assumed to be the main users of these foods), women of childbearing age (16-44 years) and children (2-12 years, Australia only). Food consumption data were derived from the 1995 Australian National Nutrition Survey (NNS, McLennan & Podger, 1997) and the 1997 New Zealand adult NNS (Ministry of Health, 1999).

Foods included in the assessment were edible oil spreads, low fat milk, breakfast cereals and low fat yoghurts. Phytosterols concentration data were derived from data supplied by applicants, indicating a concentration of 0.8 g phytosterols per serve of the food.

Estimated mean dietary intake of phytosterols did not exceed 1.9 g per day in any population group assessed. At the 95th percentile of consumption, the highest intake of phytosterols equivalents reported was estimated to be 4.8 g/day (Table 8.1).

The assessments found that 84% of Australian children aged 2-12 years consumed margarines and on this basis estimated the mean intake of phytosterols to be 1.1 g/d and 2.7 g/d at the 95th percentile of intake.

In response to a request by the Australian and New Zealand Food Regulation Ministerial Council a dietary intake assessment for the whole population was carried out assuming phytosterol concentrations of 1.0 g per serve. Under this scenario, estimated mean intakes did not exceed 2.4 g/day and 5.9 g/day at the 95th percentile of intake.

The analysis also showed that in all population groups assessed, edible oil spreads contributed more to dietary intake of added phytosterols (78-84% of intake) than other foods combined (see Table 8.2).

8.3 Dietary intake from individual foods fortified with plant sterols

8.3.1 Estimated dietary intake from the recommended number of serves of various foods

The intake of phytosterols that could be achieved in the number of serves of each food recommended by the manufacturer is listed in Table 8.3, based on the maximum permitted, or proposed, phytosterols concentration and the nominated serve sizes of the food vehicles. The amount of phytosterols 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 provide enough phytosterols to achieve the cholesterol lowering effect.

Table 8.1 Phytosterols dietary intake - summary of assessments previously reported[a]

Food vehicles included in estimates					
Edible oils spreads	✓	✓	✓	✓	✓
Breakfast cereals		✓			✓
Lower fat milk			✓	✓	✓
Lower fat yoghurt				✓	✓
Mean intake[b] g/d	1.3	≤ 1.7 [2.1]	≤ 1.9 [2.3]	≤ 1.9 [2.4]	≤ 1.9
95th percentile intake[b] g/d	3.5	≤ 4.4 [5.5]	≤ 4.8 [5.8]	≤ 4.7 [5.9]	≤ 4.7

[a] estimates of dietary intake were conducted assuming the concentration of phytosterols 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 phytosterols
[b] 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: Estimated contribution of four food vehicles to intake of plant sterols, based on the intake scenarios used in previous assessments

Food vehicle	Estimated contribution to population intake
Edible oil spreads	73-82%
Low fat milk	14-18%
Breakfast cereal (high fibre, low sugar)	3-5%
Low fat yoghurt	3-5%

Table 8.3: Dietary intake of phytosterols from the recommended number of serves of various foods

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

[a] Concentration and serving size used in previous dietary intake assessments based on information supplied by the applicants.
[b] Foods currently permitted to contain phytosterols
[c] Application to permit addition of phytosterols under consideration by FSANZ

8.3.2 Estimated dietary intake from reduced-fat cheese as a food vehicle for plant sterols

Although two serves (40 g) of plant sterol fortified lower fat cheese could deliver a phytosterols intake of 2.2 g per day, it is possible that consumers would not consume this amount every day without changing their consumption patterns, for example by eating less of another food so they could increase the amount of cheese they regularly consume. Direct replacement of unfortified lower fat cheese with plant sterol fortified lower fat cheese therefore might not deliver the recommended amount of phytosterols to all consumers. FSANZ therefore examined the consumption patterns for lower fat cheese in the Australia and New Zealand.

In the 1995 NNS, approximately 6% of Australian respondents were consumers of low and lower fat cheeses and reported consuming a mean of 32 g/day of these products, somewhat less than the recommended 2 serves (40 g) (Table 8.4) per day. Lower fat cheeses consumed included cheddar, edam, feta, gouda, mozzarella and Swiss style and cottage cheeses that were low or reduced in fat as well as lower fat processed cheeses and cheese spreads. Only a small number of products had a fat content as low as proposed by the Applicant. It should be noted that the product range proposed by the Applicant is restricted to processed and cream cheese which may be consumed less frequently.

Among respondents aged 40 years and above, 4-7% of men and 8-9% of women were consumers of these foods. The mean amount of lower fat cheeses consumed in this group was 24-37 g/day, equivalent to 1-2 serves of 20 g each, and therefore delivering at or below the target 2.2 g of phytosterols if substituted with a fortified product. The highest mean amounts of lower fat cheeses were consumed by 19-24 year old males, followed by 8-11 year old males. The highest proportion of consumers of lower fat cheeses were women over the age of 18, in particular women aged 25-44 and 45-64 years. However, the amounts of cheese consumed by these women were below 40 g.

In New Zealand, 2.2% of consumers reported consuming lower fat cheeses[18]. Mean consumption was 31 g and 74 g at the 90th percentile. This is very similar to the mean amount of lower fat cheese reported for the 1995 NNS in Australia.

Considering children separately, approximately 5% of respondents in the 1995 NNS and 14% of respondents in the 2007 NCNPAS aged 2-16 years reported consuming lower fat cheese (Tables 8.4, 8.5). While it appears that the proportion of Australian children consuming lower fat cheese might have increased since 1995, these differences may reflect methodological differences between the two surveys.

The mean and median consumption of lower fat cheese of children aged 2-16 years was 31 and 21 g respectively, equivalent to 1-1.5 serves per day or 1.2-1.7 g phytosterols, if substituted with a fortified product. The 95th percentile of consumption was 93 g (3-4.5 serves, or 5.1 g phytosterols). Consumption was highest in the 9-13 years age group and lowest in the 2-3 year age group (Table 8.5).

In New Zealand, 1.1% of children in the NZNCS reported eating lower fat cheese. Mean consumption was 30 g/day and 89 g/day at the 90th percentile. This is very similar to the amounts of lower fat cheeses reported for Australian children.

[18] Lower fat cheeses in the 1997 NNS are cheeses that contain less than 20% fat. The New Zealand NNS uses a different coding for cheeses which makes direct comparison between the databases difficult.

Other than the NCNPAS data, no recent data on the consumption of lower fat cheese is currently available to FSANZ. Data from the Roy Morgan Single Source Survey shows that the proportion of adults consuming cheese over a seven day period in Australia and New Zealand has remained steady at close to 70% from 2001-2008. However, there has been a steady upward trend in consumption of no fat/low fat milks by Australian aged 14 years and above between 2001 and 2008. A similar trend can be observed in the New Zealand population. This trend towards lower fat dairy is further supported by retail data which shows that lower fat and low fat milk sales are now approximately 31% of total milk sales by volume (Dairy Australia, 2010).

It is therefore probable that the increase in the number of Australian children consuming lower fat cheese reflects a similar trend in the Australian adult and New Zealand population as a whole.

Table 8.4: Food summary statistics for consumption of lower fat cheese reported in the 1995 Australian NNS

Gender	Age	Proportion of consumers (%)	Mean amount of cheese eaten (g/day)	Phytosterols* (g/day)
Male	2-3	3.5	14	0.8
	4-7	5.0	28	1.5
	8-11	3.9	47	2.6
	12-15	4.3	32	1.8
	16-18	4.7	26	1.4
	19-24	2.7	54	3.0
	25-44	4.2	44	2.4
	45-64	5.5	37	2.0
	65+	6.9	31	1.7
Female	2-3	5.2	25	1.4
	4-7	5.2	22	1.2
	8-11	4.5	27	1.5
	12-15	5.6	32	1.8
	16-18	5.0	30	1.7
	19-24	7.1	28	1.5
	25-44	8.1	34	1.9
	45-64	8.6	32	1.8
	65+	8.0	24	1.3
All	2-16	4.5	29	1.6
All	All ages	6.2	32	1.8

*Assuming all lower fat cheese consumed contains phytosterols

Table 8.5: Consumption of low and lower fat cheese and intake of phytosterols in Australian children (2007 NCNPAS) *

Age group	Consumers (n)	Mean bw (kg)	Consumption of Cheese (g/day)			Phytosterols** (g/day)		
			mean	median	95th centile	mean	median	95th centile
2-3	185	16	14	11	31	0.8	0.6	1.7
4-8	179	25	38	28	82	2.1	1.5	4.5
9-13	167	45	43	30	88	2.4	1.7	4.8
14-16	161	66	33	27	63	1.8	1.5	3.5
2-16	692	38	31	21	93	1.7	1.2	5.1

* Data are two day averages, weighted to reflect national population distribution

**Assuming all lower fat cheese consumed contains phytosterols

8.4 Children's intake of phytosterols from current fortified products

In the 2007 Australian NCNPAS (Commonwealth of Australia, 2008), Australian brand and product names were recorded for some major food group categories, including edible oils spreads and milk. A comprehensive food list and brand name database enabled interviewers to identify the brand during the interview, reducing the possibility of errors associated with the subsequent food coding. For the first time, this has enabled FSANZ to estimate intakes of phytosterols in children based on consumption data for foods containing added plant sterols.

The 2002 NZCNS has some limited information on brands; however, information collected on brands fortified with plant sterols was limited to a few brands and did not include all edible oil spreads with plant sterols. Eight children (or 0.2%) reported consuming the brands of edible oil spreads with plant sterols that were recorded in the survey. These data show that New Zealand children do eat these products but are insufficient to estimate dietary intakes.

Among Australian children participating in the NCNPAS, 2.2% consumed foods fortified with plant sterols, predominantly edible oil spreads (Table 8.6). 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 NNS. The majority of children consuming edible oil spreads are not consumers of spreads containing plant sterols and any dietary intake assessment based on the assumption that plant sterol containing edible oil spreads fully substitute for ordinary edible oil spreads does not reflect current consumption patterns of Australian children.

The age of children that consumed these products covered the full age range of the Survey (2 to 16 years). Most of these children ate products containing plant sterols on only one day of the two days for which consumption data were collected. Only 12% of children that consumed these foods did so on each of the two days of the survey (non-consecutive days).

The weighted mean consumption of plant sterol containing margarines averaged over two days for those 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 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 phytosterols intakes were highest for 14-18 year olds, but 90th percentile intakes were highest for 9-13 year olds (Table 8.6).

Table 8.6 Intake of phytosterols from plant sterol containing foods consumed by age groups, consumers only (2007 NCNPAS)*

Age group	Consumers (n)	Phytosterols (g/day)		
		mean	median	90th centile[a]
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

* Values are means of two days and are weighted to reflect national population distribution
[a] Numbers too low to calculate 95th percentiles

8.5 Discussion

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. Consequently, consumption patterns for these fortified foods may diverge from the consumption patterns of the equivalent non-fortified foods that are reflected in national nutrition surveys.

Dietary intake estimates from previous applications showed that mean intakes were not expected to exceed 1.9 g/d and 95th percentile intakes not to exceed 4.8 g/d. These estimates assumed that consumers do not change the amounts and types of food they eat but substitute plant sterol fortified foods for their non-plant sterol counterparts. However, data from the 2007 NCNPAS suggests that the majority of consumers of edible oil spreads are not consumers of edible oil spreads fortified with plant sterols.

When the potential consumption of lower fat cheese is considered it is clear that, when consumed following the label recommendations, intake of plant sterols would fall within the optimal range for achieving the stated purpose of these products. However the proportion of the population consuming lower fat cheese in Australia and New Zealand is relatively low, based on 1995 and 1997 NNS data. Similarly, the mean amounts of cheese consumed are below the manufacturer's recommended number of serves. Current phytosterols intake in children (0.4 g/day) is well below that predicted the last time FSANZ estimated dietary intake of phytosterols (1.1 g/day).

However, these estimates assume that all lower fat cheese currently consumed is replaced with lower fat cheese fortified with plant sterols and that the amounts eaten would remain constant. More realistically, some replacement of regular fat cheeses may also occur and the use of prepacked serves is likely to influence consumption amounts. Further, low fat dairy products continue to grow in popularity and their consumption by adults is likely to have increased since 1995.

European consumer research has shown that some consumers of plant sterol fortified foods fall outside the products' target market (see Section 7). This is supported by the findings from the 2007 NCNPAS indicate that Australian children consumed plant sterol containing products (edible oil spreads and lower fat milk), including children under the age of five years.

In conclusion, it is unlikely that any dietary intake assessment based on the assumption that plant sterol fortified foods substitute for ordinary foods would reflect current consumption patterns. If target consumers adhere to the recommended size and number of serves of plant sterol fortified lower fat cheese, intake of plant sterols would be 2.2 g of free phytosterols. There is at least some incidental intake of plant sterols by children but the number of children that reported consuming plant sterol containing products is less than 3%.

8.6 Response to risk assessment question 5

Considering existing permissions for plant sterol fortified foods, what is the estimated impact on total plant sterol intakes from the addition of plant sterol fortified cheeses to the diet?

An examination of consumption patterns for lower fat cheeses in Australia and New Zealand suggests that plant sterol fortified lower fat cheeses are unlikely to be major contributors to plant sterol intake because they are not consumed by the majority of the population in either country and are typically consumed at less than two serves per day by those who do eat lower fat cheeses.

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 safety and suitability for purpose of plant sterol fortified lower fat cheese for all population groups. However, whilst there are good data for adults on the effects of consuming plant sterol fortified foods, there have been limited data generated for specific population subgroups such as children and pregnant women. Based on knowledge of the mechanisms of phytosterol action, the now extensive experience of use of plant sterol fortified foods in the general population and an absence of effects in pregnant animals and their offspring, there is no basis for postulating a risk to these population subgroups. Nonetheless, FSANZ has taken a conservative approach in assessing the effects of plant sterols in the food supply that is protective of consumers.

Overall, FSANZ considers that there is now less uncertainty about the data supporting the safety of plant sterols in foods than there was when last assessed in 2005. The nature of plant sterols together with their chemical and physical properties are well described in the scientific literature. Detailed toxicity studies in animals, predominantly rats, are available. In addition, there is a wealth of published studies examining the safety, efficacy and nutritional effects in humans following consumption of plant sterol-fortified foods. Plant sterols have a history of safe use in the food supply of more than ten years.

FSANZ considers that there is little uncertainty about the efficacy of plant sterols in lower fat cheeses in lowering cholesterol. There are over 80 randomised controlled trials assessing the cholesterol lowering effect of plant sterols added to foods across a range of matrices, including dairy foods of widely varying fat contents. There are two published human intervention studies that investigate the effect of plant sterols when added to cheese in amounts comparable to those proposed by the applicant and consumed daily in the suggested quantity.

There is some uncertainty about the intakes of plant sterols that will be achieved in practice from consumption of lower fat cheeses because of the difficulty in predicting actual consumer behaviour once these products become available. European post-launch post-marketing surveys suggest they will not be major contributors to intake of plant sterols unless consumers change their eating patterns, which seems unlikely based on the European evidence.

10. Risk Characterisation

On the basis of the available food technological, safety and dietary intake data, the use of plant sterols in lower fat cheese at the proposed level does not represent a food safety concern.

Previous investigations into the potential toxicity of plant sterols have indicated no adverse effects in either animals or humans. Based on an evaluation of new information relating to the toxicity of plant sterols and a comprehensive analysis of epidemiology studies, including an assessment of whether increased consumption increases risk of cardiovascular disease, there is no new evidence that would indicate the need to change previous conclusions regarding the safety of plant sterol fortified foods.

While the safety of plant sterols has been clearly demonstrated and there is no evidence to suggest that children and pregnant women respond differently to plant sterols than other population groups, there remain few clinical studies of plant sterols conducted in children and none in pregnant women. In addition, there is unlikely to be a purpose for them to consume plant sterol fortified foods.

The stated purpose for addition of plant sterols to lower fat cheese, to lower serum cholesterol levels, is supported by the available evidence for the level of addition proposed by the Applicant. A daily intake of 2.2 g TO plant sterols can be achieved from two serves of sterol fortified lower fat cheese. This intake of phytosterols is consistent with amounts used in studies that demonstrated reductions in serum LDL-cholesterol levels.

The uncertainty associated with this risk assessment is in estimating plant sterol intakes. An examination of consumption patterns for lower fat cheeses in Australia and New Zealand, taken together with the findings of European consumer research in this area, suggests that plant sterol fortified lower fat cheese is unlikely to be a major contributor to plant sterol intake because it is not consumed by the majority of the population in either country and is typically consumed at less than two serves per day by those who do eat lower fat cheese. Consumers would need to deliberately alter their food consumption patterns to increase consumption of these foods, or consume them with other sterol fortified foods, before intakes would reach the amounts recommended as optimal for cholesterol lowering purposes. However, although there is uncertainty in the estimation of potential intakes, it is highly unlikely that consumers could achieve plant sterol intakes in excess of the intake levels shown to present no safety concerns.

11. References

AbuMweis, S.S. and Jones, P.J.H. (2008). Cholesterol-lowering effect of plant sterols. *Current Atherosclerosis Reports* 10:467-472

AC Nielsen (2006). "Homescan" consumer monitor of purchase of fast moving consumer goods 2006. UK: AC Nielsen.

Altman, S.W., Davis, H.R., Zhu, L. Yao, X., Hoos, L.M., Tetzloff, G., Iyer, SP., Maguire, M., Golovko, A., Zeng, M., Wang, L., Murgolo, N., Craziano, MP. (2004. Niemann-Pick C1 Like 1 Protein is critical for intestinal cholesterol absorption, *Science 303 (5661):1201-1204.*

Amundsen, A.L., Ose, L., Nenseter, MS and Ntanios FY (2002). Plant sterol ester–enriched spread lowers plasma total and LDL cholesterol in children with familial hypercholesterolemia, *American Journal of Clinical Nutrition*, 76:338–44.

Amundsen, A.L., Ntanios, F., van der Put, N. and Ose, L. (2004). Long-term compliance and changes in plasma lipids, plant sterols and carotenoids in children and parents with FH consuming plant sterol ester-enriched spread. *European Journal of Clinical Nutrition,* 58: 1612-1620.

Anttolainen, M., Luoto, R., Uutela, A., Boice, J.D., Blot, W.J., McLaughlin, J.K. & Puska, P. (2001). Characteristics of users and nonusers of plant stanol ester margarine in Finland: An approach to study functional foods. *Journal of the American Dietetic Association, 101*(11): 1365-1368.

Appenzeller, L.M. *et al.* (2009). Subchronic feeding study of grain from herbicide-tolerant maize DP-09140-6 in Sprague-Dawley rats. *Food Chem Toxicol*. 47: 2269-2280

Assmann, G., Cullen, P., Erbey, J., Ramey, D.R., Kannenberg, F. and Schulte, H. (2006). Plasma sitosterol elevations are associated with an increased incidence of coronary events in men: Results of a nested case-control analysis of the Prospective Cardiovascular Munster (PROCAM) study. *Nutrition, Metabolism and Cardiovascular Diseases*, 16:13-21.

Australia New Zealand Food Authority (ANZFA) (1995). Code of Practice on Nutrient Claims in food labels and in advertisements, Canberra, Australia.

Australian Institute of Health and Welfare (AIHW) (2004). Heart, stroke and vascular diseases – Australian facts 2004. *Cardiovascular disease series no. 22*, AIHW Cat. No. CVD 27. Canberra: AIHW

Australian Institute of Health and Welfare (AIHW) (2008). Health care expenditure on cardiovascular diseases 2004–05. *Cardiovascular disease series no. 30*. Cat. no. CVD 43. Canberra: AIHW.

Berge,K.E., Hui Tian,H., Graf,G.A., Yu,L., Grishin, N.V., Schultz, J., Kwiterovich, P., Shan,B., Barnes,R., Hobbs, H.H. (2000). Accumulation of Dietary cholesterol in Sitosterolemia caused by mutations in adjacent ABC Transporters, *Science,* 290: 1771- 1775.

Berger, A., Jones, P.J. and Autret, B.C. (2004). Plant sterols: factors affecting their efficacy and safety as functional food ingredients. *Lipids in Health and Disease*, 3(5) available online at http://www.lipidworld.com/content/3/1/5.

Bohn, T., Tian, O., Chitchumroonchokchal, C., Failla, M.L., Schwartz, S.J., Cotter, R. and Waksman, J.A. (2007). Supplementation of test meals with fat-free phytosterol products can reduce cholesterol micellarization during simulated digestion and cholesterol accumulation by caco-2 cells. *Journal of Agricultural and Food Chemistry,* 55:267-272.

Bradford 2006, unpublished data

Brix, A.E., Nyska, A., Haseman, J.K., Sells, D.M., Jokinen, M.P. and Walker, N.J. (2005). Incidences of Selected Lesions in Control Female Harlan Sprague-Dawley Rats from Two-Year Studies Performed by the National Toxicology Program. *Toxicologic Pathology* 33:477-483.

Brufau, G., Canela, M.A. and Rafecas, M. (2008). Phytosterols: physiologic and metabolic aspects related to cholesterol-lowering properties. *Nutrition Research*, 28(4):217-225.

Calpe-Berdiel, L., Escolà-Gil, J.C., Blanco-Vaca, F. (2009). New insights into the molecular actions of plant sterols and stanols in cholesterol metabolism, Atherosclerosis, 203:18-31.

Chan,YM., Varady, K.A., Lin, Y., Trautwein,E., Mensink, R.P. and Jones, P.J.H. (2006). Plasma concentrations of plant sterols: physiology and relationship with coronary heart disease, *Nutrition Reviews*, 64(9):385-402.

Charest, A., Desroches, S., Vanstone, C.A., Jones, P.J. and Lamarche, B. (2004). Unesterified Plant sterols and stanols do not affect LDL electrophoretic characteristics in hypercholesteremic subjects. *Journal of Nutrition* 134:592-595.

Childs, N.M. & Poryzees, G.H. (1998). Foods that help prevent disease: consumer attitudes and public policy implications. *British Food Journal, 100*(9), 419-426.

Clifton , PM., Noakes, M., Sullivan, D., Erichsen, N., Ross, D., Annison, G., Fassoulakis, A., Cehun, M. and Nestel, P. (2004). Cholesterol-lowering effects of plant sterol esters differ in milk, yoghurt, bread and cereal, *European Journal of Clinical Nutrition*, 58: 503–509.

Clifton, P.M. (2009). Lowering cholesterol: A review on the role of plant sterols, *Australian Family Physician*, 38(4):218-221.

Colebank, S. (31/3/03). The Rising Stock of Sterols & Stanols. www.naturalproductsinsider.com/articles/341FunctionalFood.html

Colgan, H.A., Floyd, S., Noone, E.J., Gibney, M.J., Roche, H.M. (2004). Increased intake of fruit and vegetables and a low-fat plant sterol-enriched spread consumption: effects on plasma lipoprotein and carotenoid metabolism, *Journal of Human Nutrition and Dietetics*, 17(6): 561-569.

Commonwealth of Australia (2000) *Getting it Right. How to use the data from the 1995 National Nutrition Survey*. http://www.health.gov.au/internet/main/publishing.nsf/Content/B9B915EAFC32F7CDCA256F19000409BD/$File/gettingitright.pdf

Commonwealth of Australia 2008a. *2007 Australian National Children's Nutrition and Physical Activity Survey. Main Findings*. http://www.health.gov.au/internet/main/publishing.nsf/Content/phd-nutrition-childrens-survey

Commonwealth of Australia 2008b. *User Guide. 2007Australian National Children's Nutrition and Physical Activity Survey.* http://www.health.gov.au/internet/main/publishing.nsf/Content/AC3F256C715674D5CA2574D60000237D/$File/user-guide-v2.pdf

Cox, D.N. & Anderson, A.S. (2004). Food Choice. In *Public health nutrition*. M.J. Gibney, B.M. Margetts, J.M. Kearney & L. Arab. Oxford, Blackwell Science Ltd: 144-166.

Dairy Australia (2010) Packaged Milk Sales Volume 09/10 by Type - National Summary. http://www.dairyaustralia.com.au/Our-Dairy-Industry/Industry-Statistics/Latest-Statistics.aspx

De Jong, A., Plat, J., Lutjohann, D. and Mensink, R.P. (2008). Effects of long-term plant sterol or stanol ester consumption on lipid and lipoprotein metabolism in subjects on statin treatment, *British Journal of Nutrition*, 100: 937–941

Demonty, I., Ras, R.T., van der Knaap,H.C.M., Duchateau, G.S.M.J.E., Meijer, L., Zock, P.L., Geleijnse, J.L. and Trautwein, E.A. (2009). Continuous dose-response relationship of the LDL-cholesterol–lowering effect of phytosterol intake, *Journal of Nutrition*, 139: 271–284.

Dixon, D., Heider, K. and Elwell, M.R. (1995). Incidence of Non Neoplastic Lesions in Historical Control Male and Female Fischer-344 Rats from 90-Day Toxicity Studies. *Toxicologic Pathology* 23(3): 338-349.

Doornbos, A., Meynen, E., Duchateau, G., van der Knaap, H. and Trautwein, E. (2006). Intake occasion affects the serum cholesterol lowering of a plant sterol-enriched single dose yoghurt drink in mildly hypercholesterolaemic subjects. *European Journal of Clinical Nutrition* 60:325-333.

Dunstan, D., Zimmet, P., Welborn, T., Sicree, R., Armstrong, T., Atkins, R., Cameron, A., Shaw, J., Chadban J., on behalf of the AusDiab Steering Committee (2001). *Diabesity & Associated Disorders in Australia – 2000 The Accelerating Epidemic*, The Australian Diabetes, Obesity and Lifestyle Study (AusDiab), International Diabetes Institute, Melbourne.

Dutta, P.C., Przybylski, R., Appelqvist, L.A. and Eskin, N.A., (1996). Formation and analysis of oxidised sterols in frying fat. *Deep frying*, 12-150.

Eason, C.T. and Turck, P. (2002). A 90-day toxicological evaluation of Compound 1080 (sodium monofluoroacetate) in Sprague-Dawley rats. *Toxicol. Sci.* 69: 439-447.

Engel, R. and Schubert, H. (2005). Formulation of phytosterols in emulsions for increased dose response in functional foods. *Innovative Food science & Emerging Technologies*, 6, 233-237.

Escurriol V., Montserrat Cofa´n Merce` Serra, Bullo, M., Basora, J.,Salas-Salvado, J., Corella, D., Zazpe, I., Martınez-Gonzalez, M.A., Ruiz-Gutierrez, V., Estruch, R., Ros, E. (2009). Serum sterol responses to increasing plant sterol intake from natural foods in the Mediterranean diet, *European Journal of Nutrition*, published online ahead of 3 may 2009 , DOI 10.1007/s00394-009-0024-z

European Food Safety Authority (EFSA) (2005). Opinion of the Scientific Panel on Dietetic Products, Nutrition and Allergies on a request from the Commission related to two scientific publications concerning aspects of serum levels of phytosterols. *The EFSA Journal* (2005) 211, 1-6.

European Food Safety Authority (EFSA) (2008). Consumption of Food and Beverages with

Added Plant Sterols in the European Union. A Report from the Data Collection and Exposure Unite in Response to a Request from the European Commission. *The EFSA Journal,* 133, 1-21.

European Food Safety Authority (EFSA) (2009). Scientific opinion. Plant stanols and plant sterols and blood LDL-cholesterol. Opinion of the Panel on Dietetic Products, Nutrition and Allergies. *The EFSA Journal,* 1175, 1-9.

FAO/WHO (2008). *Joint FAO/WHO Expert Meeting on Food Additives, Summary and Conclusions*, Rome, Italy 2008.

Fassbender, K., Lűtjohan, D., Dik, M.G., Bremmer, M., Kőnig, J., Walter, S., Liu, Y., Letémbre, M., von Bergmann, K., Jonker, C. (2008). Moderately elevated plant sterol levels are associated with reduced cardiovascular risk—The LASA study, *Atherosclerosis,* 196: 283–288

Ferrari R.A., Esteves W., Mukherjee K.D. and Schulte, E. (1997). Alterations of sterols and steryl esters in vegetable oils during industrial refining. *Journal of Agricultural and Food Chemistry*, 45, 4753-4757.

Fernandes, P. And Cabral, J.M.S. (2007). Phytosterols: applications and recovery methods, *Bioresource Technology*, 98, 2335–2350

Food and Drug Administration (2000). Food and Drug Administration 21 CFR Part 101, Food Labeling: Health Claims; 'Plant Sterol/Stanol Esters and Coronary Heart Disease'. *Interim Final Rule*, September 8 2000.

Food Standards Australia New Zealand (FSANZ) (2005). First Review Report Application A433 - Phytosterol esters derived from vegetable oils in breakfast cereals; Application A434 - Phytosterol esters derived from vegetable oils in low-fat milk & yoghurt; Application A508 - Phytosterols derived from tall oils as ingredients in low-fat milk, available at http://www.foodstandards.gov.au/standardsdevelopment/applications/applicationa508phytosterolsderivedfromtalloils/index.cfm

Food Standards Australia New Zealand (FSANZ) (2006a). Second Review Report Application A433 - Phytosterol esters derived from vegetable oils in breakfast cereals; Application A434 - Phytosterol esters derived from vegetable oils in low-fat milk & yoghurt; Application A508 - Phytosterols derived from tall oils as ingredients in low-fat milk, available at http://www.foodstandards.gov.au/standardsdevelopment/applications/applicationa508phytosterolsderivedfromtalloils/index.cfm

Food Standards Australia New Zealand (FSANZ) (2006b). *Exploring consumer perceptions and use of phytosterol-enriched spreads*. Prepared by TNS Social Research for Food Standards Australia New Zealand: Canberra.

Fransen, H.P., de Jong, N., Wolfs, M., Verhagen, H., Verschuren, M., Lutjohann, D., von Bergmann, K., Plat, J. and Mensink, R.P. (2007). Customary use of plant sterol and plant stanol enriched margarine Is associated with changes in serum plant sterol and stanol concentrations in humans. *Journal of Nutrition,* 137: 1301-1306.

Graf, G.A., Li, W.P, Gerard, R.D., Gelissen, I., White, A., Cohen, J.C. Hobbs, H.H. (2002). Coexpression of ATP-binding cassette proteins ABCG5 and ABCG8 permits their transport to the apical surface. *J. Clin. Invest.* 110(5): 659-669

Hammond, B.G. *et al* (2001). Safety assessment of DHA-rich microalgae from *Schizochytrium* sp. 1: Subchronic rat feeding study. *Regulatory Toxicol. Pharmacol*. 33: 192-194

Hammond, B.G. *et al* (2006a). Results of a 90-day safety assurance study with rats fed grain from corn rootworm-protected corn. *Food Chem Toxicol*. 44: 147-160

Hammond, B.G. *et al* (2006b). Reults of a 90-day safety assurance study with rats fed grain from corn borer-protected corn. *Food Chem Toxicol*. 44: 1092-1099

Hansel, B., Nicolle, C., Lalanne, F., Tondu, F., Lassel, T., Donazzolo, Y., Ferrieres, J., Krempf, M., Schlienger, J., Verges, B., Chapman, M., Bruckert, E. and Autret, B.C. (2007). Effect of low-fat, fermented milk enriched with plant sterols on serum lipid profile and oxidative stress in moderate hypercholesterolemia. *American Journal of Clinical Nutrition*, 86:790-796.

Hearty, A., Duffy, E., Joyce, J., O'Connor, C., Gibney, M.J. (2008). Phytosterol enriched products on the Irish market: examination of intake and consumption patterns. *Public Health Nutrition,* 12:51-8.

Hendriks, H.F.J., Brink, E.J., Meijer, G.W., Princen, H.M.G. and Ntanios, F.Y. (2003) Safety of long-term consumption of plant sterol esters-enriched spread. *European Journal of Clinical Nutrition*, 57, 681 – 692.

Hepburn, P.A., Horner, S.A. and Smith, M. (1999). Safety evaluation of phytosterol esters. Part 2. Subchronic 90-day oral toxicity study on phytosterol esters – A novel functional food. *Food and Chemical Toxicology*, 37:521-532.

Herron,K.L., McGrane, M.M., Waters, D., Lofgren, I.E., Clark, R.M., Ordovas,J.M., and Fernandez, M.L. (2006). The ABCG5 polymorphism contributes to individual responses to dietary cholesterol and carotenoids in eggs, *Journal of Nutrition*, 136: 1161–1165.

Hicks, K.B. and Moreau, R.A. (2001). Phytosterols and phytostanols: functional food cholesterol busters. *Food Technology*, 55, 63-67.

Hovenkamp E., Lourbakos E., Duchateau G. S. M. J. E., Tareilus E. W., Trautwein, E. A. (2007). Preferential efflux of phytosterols over cholesterol from macrophages, *Lipids* 42:1125–1132.

Hyun, Y.J., Kim, O.Y., Kang, J.B., Lee, J.H., Jang, T.Y., Liponkoskie, L., Saloe, P. (2005). Plant stanol esters in low-fat yogurt reduces total and low-density lipoprotein cholesterol and low-density lipoprotein oxidation in normocholesterolemic and mildly hypercholesterolemic subjects, *Nutrition Research*, 25: 743–753

Ikeda, J. P. (2004). Culture, food, and nutrition in increasingly culturally diverse societies. In *A sociology of food and nutrition. The social appetite*. J. Germov & L. Williams. Melbourne, Oxford University Press: 288-313.

Jauhiainen, T., Salo, P., Niittynen, L., Poussa, T .and Korpela, R. (2006). Effects of low-fat hard cheese enriched with plant stanol esters on serum lipids and apolipoprotein B in mildly hypercholesterolaemic subjects, *European Journal of Clinical Nutrition*. 60, 1253–1257.

Jenkins, D.J.A., Kendall, C.W.C., Faulkner, D.A., Nguyen, T., Kemp, T., Marchie, A., Wong, J.M.W., de Souza, R., Emam, A., Vidgen, E., Trautwein, E.A., Lapsley, K.G., Holmes, C., Josse, R.G., Leiter, L.A., Connelly, P.W. and Singer, W. (2006). Assessment of the longer-

term effects of a dietary portfolio of cholesterol-lowering foods in hypercholesteraemia. *American Journal of Clinical Nutrition*, 83:582-591.

Jenkins, D.J.A., Kendall, C.W.C., Marchie, A., Faulkner, D.A., Wong, J.M.W., de Souza, J.M., Emam, A., Parker, T.L., Vidgen, E., Lapsley, K.G., Trautwein, E.A., Josse, R.G., Leiter, L.A. and Connelly, P.W. (2003). Effects of a dietary portfolio of cholesterol-lowering foods vs lovastatin on serum lipids and C-reactive protein. *Journal of the American Medical Association,* 290(4):501-510.

Jimnez-Escrig, A., Santos-Hidalgo, A.B. and Saura-Calixto, F. (2006) Common sources and estimated intake of plant sterols in the Spanish diet. *Journal of Agricultural and Food Chemistry*, 54 (9), 3462-3471.

John, S., Sorokin, A.V. and Thompson, P.D. (2007). Phytosterols and vascular disease. *Current Opinions in Lipidology,*18:35-40.

Jones, P.J.H., Vanstone, C.A., Mahmoud, R. and St-Onge, M.P. (2003). Phytosterols in low- and nonfat beverages as part of a controlled diet fail to lower plasma lipid levels. *Journal of Lipid Research,* 44(9):1713-1719.

Jula, A., Marniemi, J., Huupponen, R., Virtanen, A., Rastas, M., Rőnnemaa, T. (2002). Effects of diet and simvastatin on serum lipids, insulin, and antioxidants in hypercholesterolemic men, A randomized controlled trial, *JAMA, 287:598-605.*

Katan, M.B., Grundy, S.M., Jones, P., Law, M., Miettinen, T. and Paoletti, R. (2003). Efficacy and safety of plant stanols and sterols in the management of blood cholesterol levels. *Mayo Clinical Proceedings,* 78, 965–978.

Kemi, M., Keenan, K.P., McCoy, C., Hoe, C-M., Soper, K.A., Ballam, G.C. and Van Zwieten, M.J. (2000). The Relative Protective Effects of Moderate Dietary Restriction versus Dietary Modification on Spontaneous Cardiomyopathy in Male Sprague-Dawley Rats. *Toxicologic Pathology* 28(2):285-296.

Kemplay, G. and Nordfjord, H. (2006). *Consumer research on the consumption of phytosterols*. TNS, Wembley Point, London, UK.

Kim, J-C., Kang, B-H., Shin, C-C., Kim, Y-B., Lee, H-S., Kim, C-Y., Han, J., Kim, K-S., Chung, D-W. and Chung, M-K. (2002). Subchronic toxicity of plant sterol esters administered by gavage to Sprague-Dawley rats. *Food and Chemical Toxicology*, 40:1569-1580.

Korpela, R., Tuomilehto, J., Hogstrom, P., Seppo, L., Piironen, V., Toivo, J., Lamberg-Allardt, C., Karkkainen, M., Outila, T., Sundvall, J., Vilkkila, S. and Tikkanen, M.J. (2006). Safety aspects and cholesterol-lowering efficacy of low fat diary products containing plant sterols. *European Journal of Clinical Nutrition,* 60:633-642.

Kratz, M., Kannenberg, F., Gramenz, E., Berning, B., Trautwein, E., Assmann, G. and Rust, S. (2007). Similar serum plant sterol responses of human subjects heterozygous for a mutation causing sitosterolemia and controls to diets enriched in plant sterols or stanols *European Journal of Clinical Nutrition,*61, 896–905.

Lauer, R.M., Obarzanek, E., Hunsberger, S.A., Van Horn, L., Hartmuller, V.W., Barton, B.A., Stevens, V.J., Kwiterovich, P.O., Franklin, F.A., Kimm, S, Lasser N.L., Simons-Morton, D.G (2000). Efficacy and safety of lowering dietary intake of total fat, saturated fat, and cholesterol in children with elevated LDL cholesterol: the Dietary Intervention Study in Children, *American Journal of Clinical Nutrition,* 72(supp), 1332S-1342S.

Lea, L.J. and Hepburn, P.A. (2006). Safety evaluation of phytosterol esters. Part 9: Results of a European post-launch monitoring programme. *Food Chemical Toxicology*, 44, 1213-22.

Lee, M-H., Lu, K. and Patel, S.B. (2001). Genetic basis of sitosterolaemia, *Current Opinions in Lipidology,* 12(2): 141-149.

Li, N.Y., Li, K., Qi, Z., Demonty, I., Gordon, M., Francis, L., Molhuizen, H.O.F. and Neal, B.C. (2008). Plant sterol-enriched milk tea decreases blood cholesterol concentrations in Chinese adults: a randomised control trial. *British Journal of Nutrition* 98:978-983.

Maki, K.C., Shinnock, F., Seeley, M.A., Veith, P.E., Quinn, L.C., Hallissey, P.J., Temer, A. and Davidson, M.H. (2003). Food products containing free tall oil based phytosterols and oat β-glucan lower serum total and LDL cholesterol in hypercholesterolemic adults, *Journal of Nutrition*, 133: 808-813.

McClements, D.J., Decker, E.A. and Weiss,J. (2007). Emulsion-based delivery systems for lipophilic bioactive components. *Journal of Food Science*, 72(8): R109-124.

McLennan, W. and Podger, A. (1998b). *National Nutrition Survey. Nutrient Intakes and Physical Measurements. Australia. 1995*. (ABS Catalogue number 4805.0), Commonwealth of Australia, Canberra.

McCrindle, B.W., Urbina, E.M; Dennison, B.A., Jacobson, M.S, Steinberger, J., Rocchini, A.P., Hayman, L.L., Daniels, S.R (2007) .Drug therapy of high-risk lipid abnormalities in children and adolescents, A scientific statement from the American Heart Association Atherosclerosis, Hypertension, and Obesity in Youth Committee, Council of Cardiovascular Disease in the Young, With the Council on Cardiovascular Nursing, *Circulation*, 115:1948-1967

Mensink, R.P., Ebbing, S., Lindhout, M., Plat, J., van Heugten, M.M.A.(2002). Effects of plant stanol esters supplied in low-fat yoghurt on serum lipids and lipoproteins, non-cholesterol sterols and fat soluble antioxidant concentrations, *Atherosclerosis*, 160: 205–213

Miettinen, T., Puska, P., Gylling, H., Anhanen, H.,And Vartiainen, E (1995). Reduction of serum cholesterol with sitostanol-ester margarine in a mildly hypercholesterolemic population, *The New England Journal of Medicine,* 1308.

Ministry of Health, 1999. NZ Food: NZ People. Key results of the 1997 National Nutrition Survey, retrieved from http://www.moh.govt.nz/moh.nsf/ea6005dc347e7bd44c2566a40079ae6f/8f1dbeb1e0e1c70c4c2567d80009b770/$FILE/nns.pdf

Ministry of Health (2003). *NZ Food NZ Children. Key results of the 2002 National Children's Nutrition Survey.* Ministry of Health, Wellington

Ministry of Health. (2008). *A Portrait of Health: Key results of the 2006/07 New Zealand Health Survey*. Wellington: Ministry of Health

Moruisi, K.G., Oosthuizen, W, Opperman, A.M. (2006). Phytosterols/stanols lower cholesterol concentrations in familial hypercholesterolemic subjects: a systematic review with meta-analysis, *Journal of the American College of Nutrition,* 25(1):41-8.

National Food Authority (1995) *Code of Practice Nutrient claims in food labels and in advertisements (CoPoNC),* Australian Government Publishing Service, Canberra.

National Health and Medical Research Council (NHMRC) (2003). *Dietary Guidelines for Children and Adolescents in Austra*lia. Canberra: NHMRC http://www.nhmrc.gov.au/publications/synopses/_files/n33.pdf. Accessed 20 August 2009

National Heart Foundation of Australia, (2004). *Heart, Stroke and Vascular Diseases. Australian Facts 2004*, available online at http://www.heartfoundation.org.au/SiteCollectionDocuments/Stats%20aihw%20hsvd.pdf

National Heart Foundation of Australia, (2007). *Summary of evidence on phytosterol/stanol enriched foods*, available online at http://www.heartfoundation.org.au/Professional_Information/Lifestyle_Risk/Nutrition/Pages/default.aspx

National Heart Foundation of Australia and the Cardiac Society of Australia and New Zealand (2005). Position Statement on Lipid Management—2005, *Heart Lung and Circulation*, 14:275–291.

National Health and Medical Research Council (NHMRC) (2003). *Food for Health*, Dietary Guidelines for Children and Adolescents in Australia incorporating the Infant Feeding Guidelines for Health Workers, Commonwealth of Australia.

Naumann, E., Plat, J., Kester, A.D.M. and Mensink, R.P. (2008) The Baseline Serum Lipoprotein Profile Is Related to plant stanol induced changes in serum lipoprotein cholesterol and triacylglycerol concentrations. *Journal of the American College of Nutrition*, 27(1), 117–126.

New Zealand Guidelines Group (2003). *The Assessment and Management of Cardiovascular Risk. Evidence-based Best Practice Guideline*, http://www.nzgg.org.nz/guidelines/0035/CVD_Risk_Full.pdf

New Zealand Guidelines Group (2009). *New Zealand Cardiovascular Guidelines Handbook: A summary resource for primary care practitioners*. 2nd ed. Wellington: New Zealand Guidelines Group. http://www.nzgg.org.nz/guidelines/0154/090202_CVD_web_pdf_Final.pdf

New Zealand Heart Foundation (2008). *Managing your Cholesterol level* information page available at http://www.nhf.org.nz/index.asp?PageID=2145860167

Niemann, B., Sommerfeld C., Hembeck A., Bergmann, C. (2007). *Lebensmittel mit Pflanzensterinzusatz in der Wahrnehmung der Verbraucher*. BfR Wissenschaft, BfR Hausdruckerei Dahlem, Berlin, Germany.

Niemann, B., Sommerfeld, C., Hembeck, A. & Bergmann, C. (2007). *Plant sterol enriched foods as perceived by consumers.* Federal Institute for Risk Assessment, pp 1-62. CfR-Wissenschaft: Berlin.

Nissinen M., Vuoristo, M., Gylling, H., Miettinen, T.A. (2007). Respective hydrolysis and esterification of esterified and free plant stanols occur rapidly in human intestine after their duodenal infusion in triacyl- or diacylglycerol, *Lipids*, 42:603-612.

Niittynen, L. H., Jauhiainen, T. A., Poussa, T.A. and Korpela, R. (2008). Effects of yoghurt enriched with free plant sterols on the levels of serum lipids and plant sterols in moderately hypercholesterolaemic subjects on a high-fat diet, *,International Journal of Food Sciences and Nutrition*,59(5): ,357 — 367

Noakes, M., Clifton, P., Ntanios, F., Shrapnel, W., Record, I., McInerney J. (2002). An increase in dietary carotenoids when consuming plant sterols or stanols is effective in maintaining plasma carotenoid concentrations, *American Journal of Clinical Nutrition*, 75, 79-86.

Noakes, M., Clifton, P., Doornbos, A.M.E., Trautwein, E.A. (2005). Plant sterol ester-enriched milk and yoghurt effectively reduce serum cholesterol in modestly hypercholesterolemic subjects, *European Journal of Nutrition*, 44: 214–222

Ostlund R.E. Jnr, McGill J.B., Zeng, C.M., Covey, D.F., Stearns, J., Stenson W.F., Spilburg, C.A. (2002). Gastrointestinal absorption and plasma kinetics of soy Delta(5)-phytosterols and phytostanols in humans. *American Journal of Physiology. Endocrinology and Metabolism,*. 282:E911-916.

Ostlund, R.E., Jr. (2004). Phytosterols and cholesterol metabolism. *Current Opinion in Lipidology,* 15(1):37-41.

Ostlund, R.E., Jr. (2007). Phytosterols, Cholesterol absorption and healthy diets. Lipids 42:41-45.

Patel, S.B., Honda, A., Salen, G.. (1998). Sitosterolemia: exclusion of genes involved in reduced cholesterol biosynthesis. *Journal of Lipid Research*, 39: 1055-1061.

Pinedo, S., Vissers, M.N., von Bergmann, K., Elharchaoui, K., Lutjohann, D., Luben, R., Wareham, N.J., Kastelein, J.J.P., Khaw, K-T. and Boekholdt, S.M. (2007). Plasma levels of plant sterols and the risk of coronary artery disease: the prospective EPIC-Norfolk Population Study. *Journal of Lipid Research*, 48: 139-144.

Piironen,V., Lindsay, D.G., Miettinen, T.A., Toivo, J. and Lampi AM.(2000) Plant sterols: biosynthesis, biological function and their importance to human nutrition, *Journal of the Science of Food and Agriculture*, 80:939-966.

Plana, N., Nicolle, C., Ferre, R., Camps, J., Cos, R., Villoria, J., Masana, L. on behalf of the DANACOL group (2008). Plant sterol-enriched fermented milk enhances the attainment of LDL-cholesterol goal in hypercholesterolemic subjects, *European Journal of Nutrition*, 47:32–39.

Plat, J., Kerckhoffs AJM., Mensink, RP. (2000). Therapeutic potential of plant sterols and stanols, *Current Opinions in Lipidology*, 11:571-576.

Plat,J.,Brufau,G., Dallinga-Thie, G.M., Dasselaar, M., and Mensink, R.P. (2009). A plant stanol yogurt drink alone or combined with a low-dose statin lowers serum triacylglycerol and non-hDL cholesterol in metabolic syndrome patients, *Journal of Nutrition*, 139 (6): 1143.

Leena Rask-Nissilä, L., Jokinen, E., Terho, P., Tammi, A., Lapinleimu, H., Rönnemaa, T., Viikari, J., Seppänen, R., Korhonen, T., Tuominen, J., Välimäki, I., Simell, O. (2000) Neurological Development of 5-Year-Old children receiving a low–saturated fat, low-cholesterol diet since infancy a Randomized Controlled Trial. JAMA, August 23/30, 2000—284 (8): 993-1000.

Reid P., Walker R., Wilson B. (1999). *NZ Food: NZ People. Key results of the 1997 National Nutrition Survey*
http://www.moh.govt.nz/moh.nsf/49ba80c00757b8804c256673001d47d0/8f1dbeb1e0e1c70c4c2567d80009b770/$FILE/nns.pdf

Rudkowska, I., AbuMweis, S.S., Nicolle, C., Jones, P.J.H. (2008). Association between non-responsiveness to plant sterol intervention and polymorphisms in cholesterol metabolism genes: a case-control study, *Applied Physiology, Nutrition and Metabolism*, 33: 728-734.

Russell D., Parnell W., Wilson N., Faed J., Ferguson E., Herbison P., Horwath C., Nye T., Salen G, Tint GS, Shefer S, Shore V, Nguyen L. (1992). Increased sitosterol absorption is offset by rapid elimination to prevent accumulation in heterozygotes with sitosterolemia. *Arteriosclerosis and Thrombrosis,* 12(5):563-8.

Salen, G., von Bergmann, K., Lütjohann, D., Kwiterovich, P., Kane, J., Patel, S.B., Musliner, T.P. Stein, B., Musser and the Multicenter Sitosterolemia Study Group (2004) Ezetimibe Effectively Reduces Plasma Plant Sterols in Patients With Sitosterolemia, *Circulation*;109;966-971.

Sanchez-Muniz, F.J., Maki, K.C., Schaefer, E.J., 5 and Ordovas, J.M. (2009). Serum lipid and antioxidant responses in hypercholesterolemic men and women receiving plant sterol esters vary by apolipoprotein E genotype, *Journal of Nutrition*, 139: 13–19

Sanders, D.J., Minter H.J., Howes, D., Hepburn P.A. (2000). The safety evaluation of phytosterol esters Part 6: The comparative absorption and tissue distribution of phytosterols in the rat. *Food Chemical Toxicology,* 38: 485-491.

Scientific Committee on Food (SCF) (2000). *Opinion on a request for the safety assessment of the use of phytosterol esters in yellow fat spreads*. Opinion adopted by the Scientific Committee on Food on 6 April 2000, Brussels, European Commission.

Scientific Committee on Food (2002a). *General view of the Scientific Committee on Food on the long-term effects of the intake of elevated levels of phytosterols from multiple dietary sources, with particular attention to the effects on ß-carotene*. *SCF/CS/NF/DOS/20 ADD 1 Final* (3 October 2002), Brussels, European Commission.
2
Scientific Committee on Food (SCF) (2002b). *Opinion of the Scientific Committee on Food on a report on Post Launch Monitoring of yellow fat spreads with added phytosterol esters*. *Document SCF/CS/NF/DOS/21 ADD 2 Final*. Brussels, European Commission.

Scientific Committee on Food (SCF) (2003a). *Opinion of the Scientific Committee on Food on Applications for Approval of a Variety of Plant Sterol-Enriched Foods*. *SCF/CS/NF/DOS/15 ADD 2 Final* (13 March 2003), Brussels, European Commission.

Scientific Committee on Food (2003b). *Opinion of the Scientific Committee on Food on an application from ADM for approval of plant sterol-enriched foods*. *SCF/CS/NF/DOS/23 ADD2 Final* (7 April 2003), Brussels, European Commission.

Scientific Committee on Food (2003c). *Opinion of the Scientific Committee on Food on an application from MultiBene for approval of plant-sterol enriched foods*. SCF/CS/NF/DOS/24 ADD 2 Final.), Brussels, European Commission.

Sehayek, E (2003). Genetic regulation of cholesterol absorption and plasma plant sterol levels: commonalities and differences, *Journal of Lipid Research*, vol. 44: 2030-2038.

Seppo, L., Jauhiainen., J., Nevala, R., Poussa, T., Korpela, R. (2007). Plant stanol esters in low-fat milk products lower serum total and LDL cholesterol, *European Journal of Nutrition*, 46:111–117.

Shipley, R.E, Peeiffer, R.R., Marsh, M.M., Anderson, R.C., (1958) .Sitosterol Feeding:

Chronic Animal and Clinical Toxicology and Tissue Analysis *Circ. Res.* 6: 373-382

Silbernagel, G., Fauler, G., Renner, W., Landl, E.M., Hoffman, M.M., Winkelmann, B.R., Boehm, B.O. and Marz. W. (2009). The relationships of cholesterol metabolism and plasma plant sterols with the severity of coronary artery disease. *Journal of Lipid Research,* 50: 334-341.

Simojoki, M., Luoto, R., Uutela, A., Rita, H., Boice, J.D., McLaughlin, J.K. & Puska, P. (2005). Use of plant stanol ester margarine among persons with and without cardiovascular disease: Early phases of the adoption of a functional food in Finland. *Nutrition Journal, 4*(20).

Sudhop, Gottwald, von Bergmann (2002). Serum plant sterols as a potential risk factor for coronary heart disease, *Metabolism*, 51:1519-1521.

Tammi, A., Ronnemaa, T., Valsta, L., Seppanen, R., Rask-Nissila, L., Miettenen, T.A., Gylling, H., Viikari, J., Anttolainen, M. and Simell, O. (2001). Dietary plant sterols alter the serum plant sterol concentration but not the cholesterol precursor sterol concentrations in young children (The STRIP Study). *Journal of Nutrition,* 131: 1942-1945.

Thomsen, A.B., Hansen, H.B., Christiansen, C., Green, H. and Berger, A. (2004). Effect of free plant sterols in low-fat milk on serum lipid profile in hypercholesterolemic subjects. *European Journal of Clinical Nutrition,* 58:860-870.

Tobias, M. (2001). *Burden of Disease and injury: New Zealand* 1997-2011. Wellington: Ministry of Health.

Trautwein, E.A., Duchateau, G.S.M.J.E., Lin, Y., Mel'nikov, S.M., Molhuizen, H.O.F., Ntanios, F.Y. (2003). Proposed mechanisms of cholesterol-lowering action of plant sterols, *European Journal of Lipid Science and Technology,* 105, 171–185.

Turnbull, D., Whittaker, M.H., Frankos, V.H. and Jonker, D. (1999). 13-Week Oral Toxicity Study with Stanol Esters in Rats. *Reg. Toxicol. Pharmacol*. 29: 216-226.

United Kingdom Food Standards Agency (UKFSA). (2006). *Consumer research on the consumption of phytosterols.* Prepared by TNS for COI and the Food Standards Agency: Wembley Point, London.

Valsta, L.M., Lemstrom, A., Ovaskainen, M.-L. Lampi, A.-M. Toivo, J., Korhonen, T. & Piironen, V. (2004). Estimation of plant sterol and cholesterol intake in Finland: quality of new values and their effect on intake. *British Journal of Nutrition, 92*, 671-678.

Vanstone, C.A., Jones, P.H. (2004). Limitations of plasma plant sterols as indicators of cholesterol absorption, *American Journal of Clinical Nutrition*, 79(2): 340-341.

Volpe, R., Niittynen, L., Korpela, R., Sirtori, C., Bucci, A., Fraone, N. and Pazzucconi, F. (2001). Effects of yoghurt enriched with plant sterols on serum lipids in patients with moderate hypercholesterolaemia. *British Journal of Nutrition*, 86:233-239.

Weingärtner, O., D. Lütjohann, S. Ji, N. Weisshoff, F. List, T. Sudhop, K. von Bergmann, K. Gertz, J. König, H. J. Schäfers, (2008). Vascular effects of diet supplementation with plant sterols, *Journal of the American College of Cardiology*, 51: 1553–1561

Wilund KR.; Yu, L., Xu, F., Vega, G.L., Grundy, S.M., Cohen, J.C., Hobb, H. (2004). No Association between plasma Levels of Plant Sterols and Atherosclerosis in Mice and Men *Arterioscler Thromb Vasc Biol.*,24(12):2326-32.

Williams CL., Hayman, L.L., Daniels, S.R., Robinson, T.N., Steinberger, J., Paridon, S., Bazzarre, T. (2002). Cardiovascular health in childhood: A statement for health professionals from the committee on cardiovascular disease in the young, American Heart Association, *Circulation*, 106, 143-160.

Williams, C.L., Bollella, M.C., Strobino, B.A., Boccia L., Campanaro, L. (1999). Plant Stanol ester and bran fiber in childhood: effects on lipids, stool weight and stool frequency in preschool children, *Journal of the American College of Nutrition*, 18(6), 572–581.

Windler, E., Zyriax, B.C., Kuipers, F., Linseisen, J., Boeing, H. (2009). Association of plasma phytosterol concentrations with incident coronary heart disease Data from the CORA study, a case-control study of coronary artery disease in women, *Atherosclerosis*, 203(1):284-90.

World Health Organisation (WHO) (2009). Evaluation of certain food additives.69th report of the Joint FAO/WHO Expert Committee on Food Additives. *WHO Technical Report Series 952*

Worsley, A. & Scott, V. (2000). Consumers' concerns about food and health in Australia and New Zealand. *Asia Pacific Journal of Clinical Nutrition, 9*(1), 24-32.

Yanishlieva-Maslarova, N.V. and Marinova, E.M. (1985). Autoxidation of sitosterol in lipid systems of different unsaturation degree. *Journal of the American Oil Chemists Society*, 62, 622.

Appendix 1 Laboratory Investigation Parameters for toxicity studies

LABORATORY INVESTIGATION PARAMETERS

Haematology	**Clinical Chemistry**	**Urinalysis**
Activated partial thromboplastin time (APTT) Erythrocyte count (RBC) Haematocrit (Hct) Haemoglobin (Hb) Leucocyte count (WBC) Leucocyte differential Mean corpuscular haemoglobin (MCH) Mean corpuscular haemoglobin concentration (MCHC) Mean corpuscular volume (MCV) Platelet count Prothrombin time	Albumin Albumin/globulin ratio Alkaline phosphatase (AP) Alanine aminotransferase (ALT, GPT) Aspartate aminotransferase (AST, GOT) Bilirubin (total) Calcium Chloride Cholesterol Creatinine Creatine kinase (CK) Globulin Glucose Magnesium Ornithine carbamyltransferase Phospholipids Phosphorus (inorganic) Potassium Protein (total) Sodium Triglycerides Urea	Bile Bilirubin Glucose Ketones Nitrite Occult blood pH Protein Sediment Specific gravity Urobilinogin Volume

Organs Weighed	**Tissues Examined Microscopically**	
adrenals brain heart kidneys liver ovaries pituitary spleen testes thymus thyroid uterus	adrenals aorta bone (sternum) bone marrow (femur, sternum) brain (3 levels) epididymes eyes with optic nerve heart intestine (small) intestine (large) kidneys lacrimal gland liver lungs and bronchi lymph nodes mammary gland oesophagus ovaries	pancreas pituitary peripheral nerve (sciatic) prostate seminal vesicle skeletal muscle skin spinal cord spleen stomach testes thymus thyroid trachea urinary bladder uterus vagina tissues with gross lesions

Appendix 2: Literature search details, calculations and statistical analysis: efficacy assessment

Figure A2.1: Literature search details for potential risk of plant sterol and CVD studies

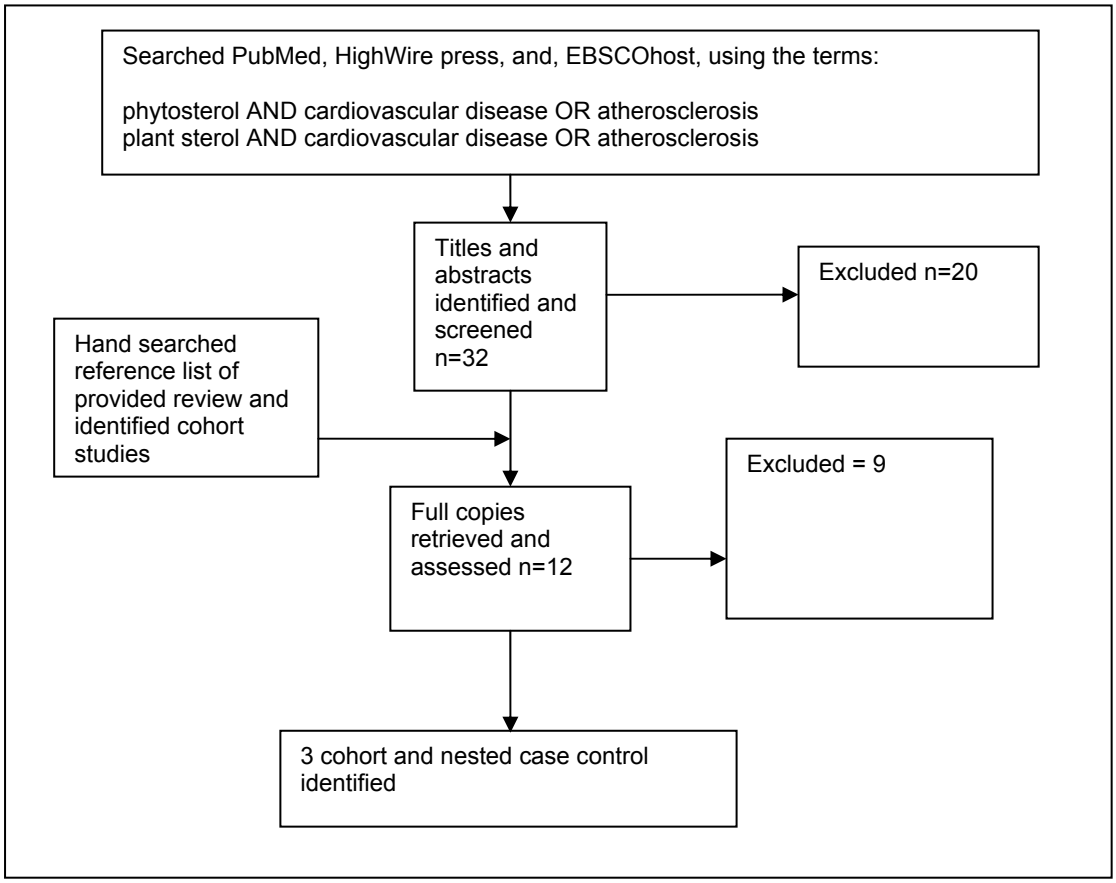

Figure A2.2: Literature search details for efficacy in lowering LDL-cholesterol of phytosterols and dairy products

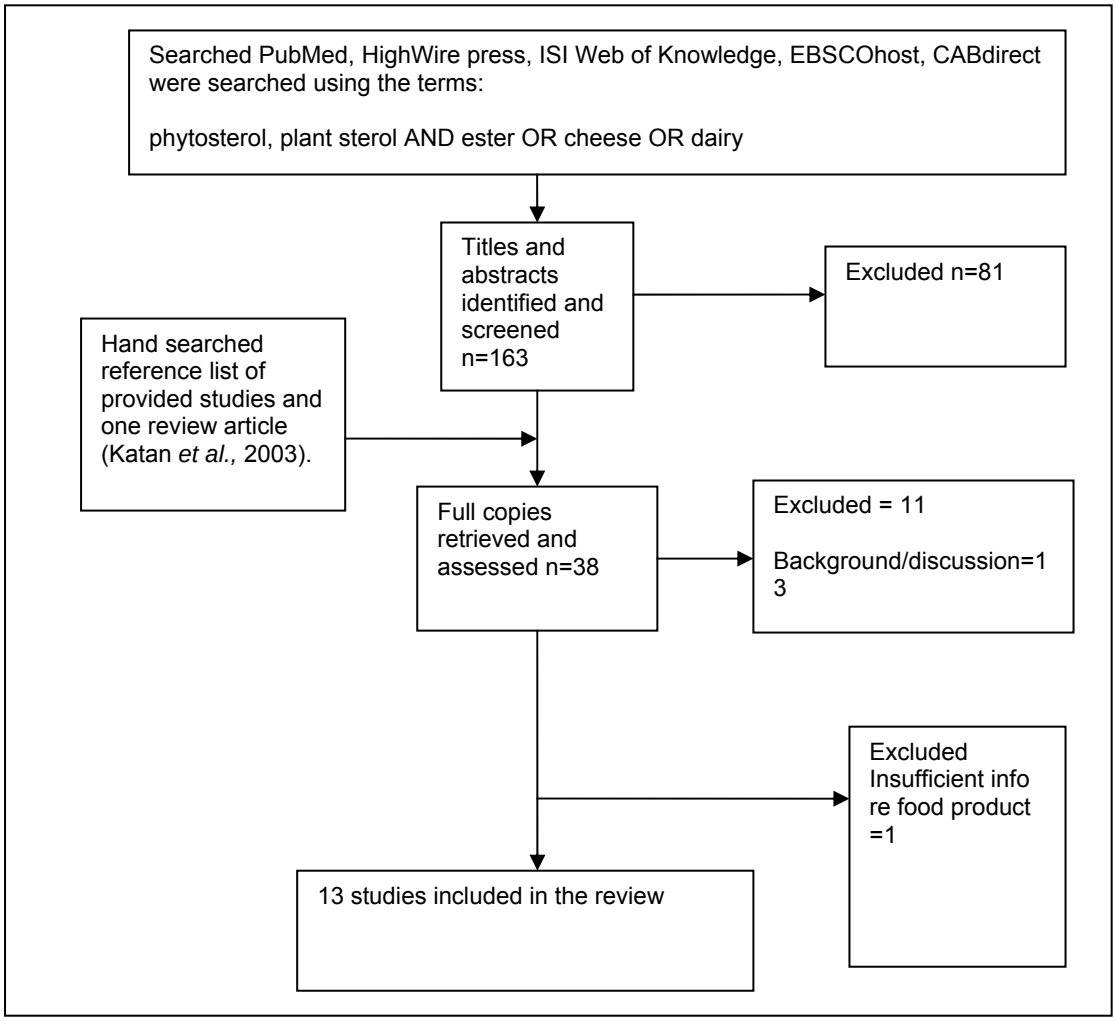

Table A2.1 Cross over study characteristics for studies included in the cholesterol lowering efficacy assessment

Subject characteristics						Study Details					
Study	**N reported (M/F)**	**N included in calculation**	**Mean BMI (kg/m^2)**	**Physical State**	**Mean age (y)**	**Food type**	**Study Length (weeks)**	**Placebo per day**	**Sterol or stanol**	**Composition of plant sterol mixture**	**Intervention amount (g/d)**
Volpe, 2001	30	30	Not reported	Hyper-cholesterolaemic	Not reported	Low fat low lactose yoghurt drink	18	100 ml	STE	soybean derived 37-55% ß-sitosterol, 20-30%campesterol and 15-25% stigmasterol	1 g
Niittynen, 2008	15	15	26	Moderate hyper-cholesterolaemic (TC 5.4-7.5 mmol)	41	Low fat yoghurt	14	One serve low fat strawberry yoghurt drink	STE	37-55% β-sitosterol, 20-30% campesterol and 15-25% stigmasterol	1 g
Thomsen, 2004	81	69 (18 m/51 f)	25.9	Hyper-cholesterolemic (TC 5.6 and 8.4 mmol)	60	Low fat milk	12	500 ml low fat (1.2%) UHT milk	STE	Not specified- mainly derived from soybeans	Low- 1.2 g High 1.6 g
Rudkowska, 2008	30	26	26.4	Hyper-cholesterolaemic	59.6	yoghurt	20	Low fat yoghurt (amount unspecified)	STE	free sterol tall oil derived 75% ß-sitosterol and 8.4% campesterol	Low fat yoghurt with 1.6 g
Clifton, 2004		58	26.2	Mildly hyper-cholesterolemic (TC 5.0-7.5 mmol)	54	Yoghurt, bread, cereal & low fat milk	14	200g yoghurt; 2 slices white bread; 45 g muesli; 500 ml milk	STE	Soy derived phytosterol ester: 50% sitosterol, 20% stigmasterol and 20% campesterol. Esterified with soy fatty acids.	1.6
Noakes, A 2005	40	39 (21/18)	25.9	Moderately hyper-cholesterolemic (TC 5.0-7.5 mmol)	51.5	300 ml low fat milk plus spread	**9**	300ml/d low fat milk	STE	Veg oil derived 47% sitosterol, 27% campesterol, 16%stigmasterol	2 g consumed either alone or with spread
Noakes, B 2005	42	40 (17/23)	26.5	Moderately hyper-cholesterolemic (TC 5.0-7.5 mmol)	60.4	Low fat yoghurt.	12	2 x 150 g low fat yoghurt	STE & STA	Not specified	1.8 g STE 1.7 g STA

Table A2.2 Details of reporting from cross over studies included in the cholesterol lowering efficacy assessment

Author, year	Study aim & design	Study locations	Reported inclusion & exclusion criteria	Washout period	Sample size justified	Were groups similar at baseline or differences dealt with?	Reported compliance?	Reported details of A. Recruitment method B. Randomisation method C. Allocation concealment
Volpe, 2001	8 wk run in on American Heart Association diet. Random gp assignment. 2 x 4 week intervention period	Rome and Milan, Italy	Inclusion criteria not reported; exclusion reported.	2 week	No	Not reported	Yes	A. no B. no C. no
Niittynen, 2008	2 x 4 week treatment periods in random order	Finland	Yes; yes	2 week	No	Not reported	Yes, 98%	A. no B. no C. no
Thomsen 2004	3 arms, 4x 3 wk intervention periods. Random assignment to treatment sequence.	Copenhagen, Denmark.	12 wks;	No washout – 4 wk control period	Yes	Yes (sex, age, BMI, BP and baseline lipid profile)	Reported 88%	A. yes B. no C. yes
Rudkowska, 2008	Aim to examine differences of consumption as snack or with meal. single blind randomised 3 x 30 day phases with controlled diet	Quebec, Cananda	Yes; yes	Yes 4 weeks	not reported	Only reports for TC	not reported	A. yes B. no C. no
Clifton, 2004	Compare the effects of different enriched foods on serum lipids, plasma PS levels and carotenoids Multicentre (3) randomised incomplete crossover single blind trial. 4 x 3 wk intervention periods	SA, NSW & VIC Australia	yes; not reported;	No	Yes	Yes	Yes (98%)	A. yes B. yes C. no.

Author, year	Study aim & design	Study locations	Reported inclusion & exclusion criteria	Washout period	Sample size justified	Were groups similar at baseline or differences dealt with?	Reported compliance?	Reported details of A. Recruitment method B. Randomisation method C. Allocation concealment
Noakes, 2005	Study 1) assess effects on serum lipids and serum carotenoids from enriched milk and enriched spread Study 2) compare cholesterol lowering of STE yoghurt vs STA yoghurt Study 1: single blind, 4 x 3 wk intervention periods study 2: yoghurt crossover study 3 x 3 wk interventions. 150g yoghurt twice a day	Adelaide, Australia	Yes; yes	na/2 wk run with usual diet	Study 1) No Study 2) No	Study 1) Yes Study 2) Yes	Study 1) Yes Study 2) Yes	Study 1 A. yes B. no C. single blind partial randomisation, Study 2 A. yes B. no C. Double blind fully randomized

Table A2.3 Details of reporting from parallel studies included in the cholesterol lowering efficacy assessment

Study	N reported Int gp, Cont. gp	N included in calculations Int. Gp, Cont. gp	Mean BMI (kg/m^2)	Baseline cholesterol classification	Mean age (y)	Plant sterol fortified food type	Study Length (weeks)	Placebo	Sterol or stanol	Composition of plant sterol mixture	Intervention plant sterol amount (g/d)
Li 2007	309	301	26	Mixture of normo- and hyper-cholesterolemic	44	Chinese milk tea	6	Milk tea containing a total of 2.3g of plant sterol each day administered in two divided doses of 1.15g	STE	not specified	Low 1.5 g High 2.3 g
Korpela, 2006	Int gp 82 Control gp 82	82 82	Int gp 27.1 Control gp 26.8	Moderately hyper-cholesterolemic (TC 5.0 - 8.5 mmol/l)	Int gp 57.6 Contl gp 57.0	Hard cheese (10% fat), fresh cheese (14 %fat), yoghurt (3% fat)	9	low fat flavoured- 1.65 g (ii) low fat hard cheese- 2g (iii) low fat fresh cheese- 2 g	STE & STA	Tall oil derived free sterols in cyrstalline form 75% ßsitosterol, 10% ßsitostanol, 10%campesterol, 2% campestanol & 2% other.	2 g
Plana, 2006	84	83	Int gp 26.5, Cont gp 26.9	Hyper-cholesterolemic	Int gp 51.8, Cont gp 50.9;	fermented milk drinks (1.2 g fat)	6	100 ml low fat (1.2 g) fermented milk	STE	unspecified source ester: 80% β-sitosterol, 10% campesterol, remaining 10% β-sitostanol, campestanol, stigmasterol and brassicasterol	1.6 g free equivalents
Hansel, 2007	Int gp 95 Cont gp 99	95 99	Int gp 23.6 Cont gp	Hyper-cholesterolemic	Int gp 48.4 cont gp 49.5	low fat fermented milk	10	125 g low fat (0.7 g fat) fermented milk.	STE	Tall oil derived 75% % β-sitosterol, 8.4% campesterol esterified with rapeseed oil.	1.6 free equivalents
Niittynen, 2008	33 int gp 34 cont gp	33 *34*	26	Moderate hypercholesterolemia	M- 54 F- 40	low fat yoghurt	8	100g low fat (2 g fat) yoghurt	STE	unspecified source 37-55% β-sitosterol, 20-30% campesterol and 15-25% stigmasterol	2 g

Study	N reported Int gp, Cont. gp	N included in calculations Int. Gp, Cont. gp	Mean BMI (kg/m^2)	Baseline cholesterol classification	Mean age (y)	Plant sterol fortified food type	Study Length (weeks)	Placebo	Sterol or stanol	Composition of plant sterol mixture	Intervention plant sterol amount (g/d)
Jauhiannen 2006	67 total; 34 33			mildly hypercholesterolaemia	43.3	Hard cheese	8	Hard Cheese (8.5g fat)	STA	sitostanol and campestanol	2 g
Hyun, 2005	28 23	28 23	Int 22.5 Cont gp 22.8	Normocholesterolemic LDL 4.5 – 6.5 mmol	Int 28.5 Cont 28.9	yogurt	4	Yoghurt (<1 g fat)	STA	soy derived 69% sitostanol, 28% campestanol, 2 % campesterol and 1 % sitosterol. esterified with rape seed oil fatty acids	2 g free equivalents
Seppo, 2007	199 total	Int 1: 31 29 Int 2: 29, 32 Int 3: 10, 9 Int 4: 32, 27	I 24.9 Cont 25.5	mild or moderate hypercholesterolemia.	Int 46.7 Cont 46.4	Yoghurt, Yoghurt shots and milk	5	150 ml Yoghurt (0.9 g fat) 100 ml Yoghurt shots (1.4 g fat) 500 ml Milk (1.3 g fat)	STA	sitostanol and campestanol (amount not specified), transesterified with rapeseed oil fatty acids	2 g
Mensink, 2002	60 total: 16, 44	experimental group (four men and 16 women	23.3	mild or moderate hypercholesterolemia.	36		6	3 x 150 g low fat yoghurt (0.7 % fat)	STA	71% sitostanol ester and 29% campestanol ester	3 g
Doornbos 2006	Int 1: 38 Int 2: 28 Int 3: 39 Int 4:36 Cont: 33	Int 1: 38 Int 2: 28 Int 3: 39 Int 4: 36 Cont: 33	All 25.2	Normocholesterolemic	All 56.8	Two types of low fat (2.2% fat & 3.3% fat) yoghurt drink	8	Yoghurt drink (1.5 g fat)	STE	sitosterol78.7%, sitostanol 8.9%, campesterol 7.7%, stigmasterol 1.1%, campestanol,1.0	2.8 g, 3.2 g

Abbreviations used in tables:
Int gp= intervention group; Cont gp = control group; STE= phytosterol; STA = Phytostanol; PS = Plant sterols; M = males; F = females

Table A2.4 Parallel study characteristics for studies included in the cholesterol lowering efficacy assessment

Author, year	Study location	Study aim & design	reporting of inclusion & exclusion criteria	Reported details of A. Recruitment method B. Randomisation method C. Allocation concealment	Were groups similar at baseline or differences dealt with?	Reported compliance?	Sample size justified? (power calculation
Li, 2007	Beijing, China	To define the effects on blood lipid concentrations of a novel food product and two PS amounts compared to placebo in healthy Chinese adults. 10 day run in, randomised into 3 parallel groups: high, low and placebo for 5 wk intervention.	Yes; yes	A. yes B. yes C. yes	yes; na	yes	yes
Korpela, 2006	Finland	To examine effects on cholesterol and serum plant sterol and fat-soluble vitamin levels in hypercholesterolaemic subjects. Multicentre trial- subjects randomly assigned to groups. 3-week run-in, 6 weeks intervention	Yes; yes	A. no B. no C. no	Yes	yes; na	not reported
Plana, 2006	fermented milk drinks	Multicentre (7) 4 wk single blind run in – Mediterranean diet placebo controlled parallel clinical study	Yes; yes	A. no B. stratified C. not reported for intervention period.	yes; na	reported at 80%	Yes

Author, year	Study location	Study aim & design	reporting of inclusion & exclusion criteria	Reported details of A. Recruitment method B. Randomisation method C. Allocation concealment	Were groups similar at baseline or differences dealt with?	Reported compliance?	Sample size justified? (power calculation
Hansel, 2007	France	To examine the effect on plasma lipid profile, circulating PS and quantify influence of PS on oxidative stress & inflammation. Multicentre (5) study, with 4 wk run in, randomly assigned to gp for 6 wks.	Yes; yes	A. yes B.no C. no	yes; na	yes defined and reported	Yes
Niittynen, 2008	Finland	To examine the effect of a on serum lipid and plant sterol values in moderately hypercholesterolemic subjects. Study 1- 14 week placebo controlled cross over trial, 2 wk run in-4 wk intervention-2 wk washout-4 wk treatment. Study 2- Two arm randomly assigned 8 wks-	Study 1 No; yes Study 2 No; yes	For both studies: A. no B. no C. no	Study 1 Not reported Study 2 yes	Study 1 Yes Study 2 yes	For both studies: no
Jauhiannen, 2006	Finland	To investigate effect of low fat hard cheese w stanol ester on serum chol levels in mildly hypercholesterolaemic subjects as part of normal diet	yes; yes	A. no B. no C. no	Yes	Yes	Yes
Hyun, 2005	South Korea	Examine effects on lipid profile and LDL oxidation in young Koreans. 2 wk run in, 4 wk intervention period	N= 51; yes	Yes; yes; not reported.	No	Yes	No

Author, year	Study location	Study aim & design	reporting of inclusion & exclusion criteria	Reported details of A. Recruitment method B. Randomisation method C. Allocation concealment	Were groups similar at baseline or differences dealt with?	Reported compliance?	Sample size justified? (power calculation
Seppo, 2007	Finland	consisted of four sub-studies of three low-fat milk products: (1) yoghurt (n = 60), (2) a yoghurt single-shot drink, Study I (n = 61), (3) a yoghurt single-shot drink, Study II (n = 19), and (4) milk (n = 59) St 1 St2 St 3 St 4	Yes same for all	For all A. No B. No C. No	Yes	No	Not for each study-used meta analysis to improve power
Mensink, 2002	Netherlands	consume daily three cups (150 ml) of placebo yoghurt for a period of 3 weeks. Cups of yoghurt were either consumed with each meal,	Yes	A. no B. no C. yes	Yes	Yes	Yes
Doornbos, 2006	Netherlands	Demonstrate the effect of two different fat levels of PS ester single dose yoghurt drink on plasma lipid and lipoprotein cholesterol concentrations in mildly hypercholesterolaemic subjects.	Yes	A. yes B. No C. yes,	Yes	Yes	Yes

Abbreviations used in tables:
Int gp= intervention group, Cont gp = control group, STE= phytosterol, STA = Phytostanol, PS = Plant sterols

Table A2.5 Summary of data reported and calculated from studies included in efficacy assessment

Study	Absolute change (int. - control)	95% CI for absolute change	% change (intervention – control)	95% CI for % change	Convert to mmol/L from mg/dL
Korpela, 2006	X	X	X	X	_
Thomsen, 2004	X	X	-	-	_
Plana, 2008	X	X	X	X	-
Niittynen, 2008	-	-	X	X	-
Doornbos 2006.	-	X	X	X	-
Noakes, 2005	-	-	-	X	-
Volpe, 2001.	X	X	X	X	-
Hansel, 2007.	X	X	X	X	X
Li, 2007.	-	-	-	-	-
Rudkowska, 2008.	X	X	X	X	-
Clifton, 2004	-	-	-	-	-
Seppo, 2007	X	X	-	-	-
Mensink, 2002	X	X	X	X	-
Hyun, 2005	X	X	X	X	X
Jauhiainen, 2006.	X	X	-	-	-

X = value was calculated by FSANZ using data reported

- = value was reported

Appendix 3 Dietary intake assessment methodology

A3.1 Chemical concentration and food consumption data

There are two major data sources that are required in order to conduct dietary intake assessments – food chemical concentration data and food consumption data.

The food chemical concentration data used in this intake assessment are the maximum proposed levels of use of plant sterol in each food matrix expressed as phytosterol equivalents.

The use of appropriate food consumption data in dietary intake assessments is obviously extremely important. There are many methods that can be used to collect food consumption data with the type of food chemical and the purpose of the assessment determining the most appropriate source of data. The data used in this assessment included the Australia and New Zealand National Nutrition surveys, Roy Morgan Single source and post-launch marketing surveys.

A3.1.1 2007 Australian Children's Nutrition & Physical Activity Survey (NCNPAS) in dietary modelling

The 2007 NCNPAS collected data on nutrition and physical activity for 4,487 children aged 2-16 years across Australia. The survey was conducted over a seven month time period, from February to August 2007.

In contrast to other national nutrition surveys used to date by FSANZ (the 1995 Australian and 1997 and 2002 New Zealand surveys), in the 2007 NCNPAS each respondent completed two 24-hour recalls on non-consecutive days. The availability of two days of food consumption data provides a more realistic estimate of long term consumption of infrequently consumed foods, because it takes account of those who may eat a food on one day of the survey but not on the other. Using one 24-hour recall may capture an unusual eating occasion for an individual that does not describe how they normally eat.

Australian brand and product names were recorded for some major food group categories, including edible oils spreads and milk. A comprehensive food list and brand name database enabled interviewers to identify the brand during the interview, reducing the possibility of errors associated with the subsequent food coding. In this assessment, intake of physterols was estimated from each consumer's average intake from foods containing plant sterols across Day 1 and Day 2. For most consumers of foods containing plant sterols, consumption of these foods only occurred on one of these two days.

The results of the 2007 NCNPAS were weighted to represent the overall population of Australian children because stratified sampling with non-proportional samples was used.

DIAMOND, the FSANZ computer program used for dietary intake estimates, first calculates each individual's total intake of phytosterols using their actual food consumption data. The individual's unweighted exposure is then multiplied by their sampling weight and divided by sum of sampling weights for all respondents in the survey sample, as follows:

$$\textit{Individual weighted intake} = \frac{\left(y_i \times sample\,weight \times n\right)}{sum\,of\ consumer\,weights}$$

Where y_i = consumption or intake for each consumer i, and n= number of consumers.

From these individual weighted intakes, the weighted mean population intake is generated. Weighting is applied after all other calculations, including calculation of two-day adjustments to nutrient intake, are undertaken. The derivation of the appropriate weights for non-proportionate sampling is described in the user guide to the 2007 NCNPAS (Commonwealth of Australia, 2008b).

A3.1.2 2002 New Zealand Children's Nutrition Survey (CNS)

The 2002 CNS was a cross sectional population survey using a computer assisted, multiple pass 24-hour diet recall on a randomly selected sample of 3275 New Zealand children aged 5 to 14 years from 172 schools throughout New Zealand. Information was obtained on food and nutrient intake, frequently eaten foods, eating patterns and physical activity patterns, among other measures (Ministry of Health, 2003). Sufficient Maori and Pacific children were included so that ethnic-specific analyses could be undertaken. To achieve this, Maori and Pacific people children were over-sampled.

In this assessment the CNS was used to estimate consumption of lower fat cheese by New Zealand children. The CNS has some very limited information on brands. Therefore, intake of phytosterols was not estimated from each consumer's average intake.

A3.1.3 1997 New Zealand National Nutrition Survey (1997 NNS)

The 1997 NNS questioned 4,636 adults aged 15 years and above (Ministry of Health, 1999). There was over-sampling of Maori and Pacific people, ensuring over 700 Maori and over 300 Pacific participants. This was done in order to obtain robust estimates of dietary intake and nutritional status for these ethnic groups. A similar 24-hour recall methodology to the Australian 1995 NNS was used with 15% of respondents reporting a second 24-hour recall, along with an FFQ for all respondents. These data are used unweighted in DIAMOND.

In previous assessments, the 1997 NNS was used to estimate intakes of plant sterols for adults from reported consumption of similar foods without plant sterols. These findings are summarised in the main report. In addition, the 1997 NNS was used to estimate consumption of lower fat cheeses in New Zealand adults.

A3.1.4 1995 Australia National Nutrition Survey (1995 NNS)

The 1995 NNS provides comprehensive information on dietary patterns of a sample of 13,858 Australians aged from 2 years and above (McLennan and Podger, 1998a). It is the most recent NNS for Australians aged above 16 years. The survey used a 24-hour recall method for all respondents, with 10% of respondents also completing a second 24-hour recall on a second, non-consecutive day. Food frequency data are available for a subset of the national sample (respondents aged 12 years and above) as are responses to a series of short dietary questions about food habits. These data are used unweighted in DIAMOND.

In previous assessments, the 1995 NNS was used to estimate intakes of plant sterols for adults and children from reported consumption of similar foods without plant sterols. These findings are summarised in the main report. In addition, the 1995 NNS was used to estimate consumption of lower fat cheeses by gender and age for the Australian population.

A3.1.5 Roy Morgan Research Single Source (RMRSS)

Since 2006, FSANZ has subscribed to the Roy Morgan Research Single Source (RMRSS) databases and has data available for the period 2001-2008. The survey is carried out annually using representative samples of Australian and New Zealand populations based on the electoral rolls. Respondents are interviewed face-to-face with follow-up self-completion surveys also carried out on the adult population sample. The RMRSS is a survey of more than 25,000 Australians and 12,000 New Zealanders aged 14 years and above per year, and is periodically revised and updated to ensure that the information collected is truly representative of the market. The Single Source is unique in that all questions are asked of each respondent building an accurate individual profile of consumers across a large base survey sample.

In this assessment the RMSS is used to describe trends in lower fat dairy product and cheese consumption in Australia and New Zealand.

A.3.1.6 Post-launch marketing surveys

There are four major surveys that provide information on the intake of plant sterols in Europe:

1. Hearty, A., Duffy, E., Joyce, J., O'Connor, C., Gibney, M.J. 2008 Phytosterol enriched products on the Irish market: examination of intake and consumption patterns. *Public health Nutrition* 12, 51-8.

2. Kemplay, G. and Nordfjord, H. 2006. Consumer research on the consumption of phytosterols. TNS, Wembley Point, London, UK.

3. Lea, L.J. and Hepburn, P.A. 2006. Safety evaluation of phytosterol esters. Part 9: Results of a European post-launch monitoring programme. *Food Chemical Toxicology* 44, 1213-22

4. Niemann, B., Sommerfeld C., Hembeck A., Bergmann, C. 2007. Lebensmittel mit Pflanzensterinzusatz in der Wahrnehmung der Verbraucher. BfR Wissenschaft, BfR Hausdruckerei Dahlem, Berlin, Germany.

In addition, EFSA (2008) have summarised these studies and recalculated the data provided to estimate overall EU intakes, expressed as free phytosterols. These studies form an important part of the evidence base on plant sterol intake which have been considered in the dietary intake assessment.

A3.2 Data analysis and extraction

A.3.2.1 DIAMOND

The DIAMOND program is a custom made, stand alone program mostly used for FSANZ's core work of developing/modifying food regulations. It is also used for many other purposes, for example to conduct the dietary exposure assessments for the Australian Total Diet Survey. DIAMOND stands for **DI**et**A**ry **M**odelling **O**f **N**utritional **D**ata.

DIAMOND has recently been enhanced to include the results of the 2007 Australian National Children's Nutrition and Physical Activity Survey (the 2007 NCNPAS, Commonwealth of Australia, 2008a) and the 2002 New Zealand Children's Nutrition Survey.

Each individual's intake of phytosterols was calculated using his or her individual food consumption records from the NCNPAS, as set out below. The DIAMOND program multiplies the specified phytosterol equivalents concentration of the food by the amount of that food that an individual consumed to estimate the intake of phytosterols from each food for each individual. For any individual in an NNS, their intake of phytosterol equivalents is calculated as:

$$\text{Phytosterols}_{\text{mg/day}} = \sum_{\text{all foods eaten}} (\text{Consumption amount}_{\text{kg/day}} \times \text{phytosterol concentration}_{\text{mg/kg}})$$

Once this has been completed for all of the foods containing plant sterols, the total intake of phytosterol equivalents from all foods is summed for each individual.

A.3.2.2 ASTEROID

ASTEROID is a tabulation package developed by Roy Morgan Research extract and analyse data from Roy Morgan databases. In this assessment, ASTEROID was used to tabulate frequency of consumption of dairy products over the 2001-2008 time periods.