4-04
26 May 2004

DRAFT ASSESSMENT REPORT

APPLICATION A434

PHYTOSTEROL ESTERS DERIVED FROM VEGETABLE OILS IN LOW-FAT MILK & YOGHURT

DEADLINE FOR PUBLIC SUBMISSIONS to FSANZ in relation to this matter:
21 July 2004
(See 'Invitation for Public Submissions' for details)

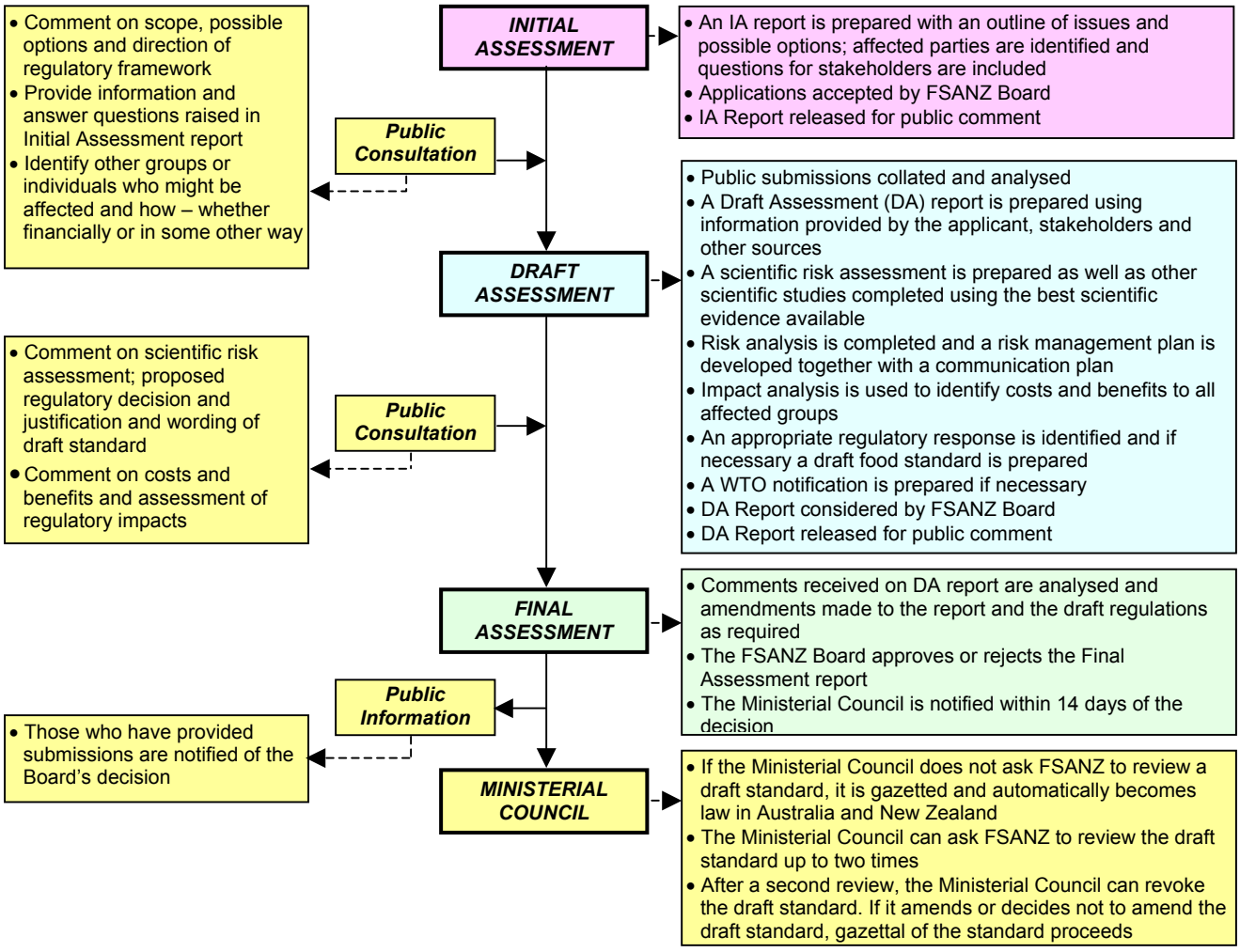
FOOD STANDARDS AUSTRALIA NEW ZEALAND (FSANZ)

FSANZ's role is to protect the health and safety of people in Australia and New Zealand through the maintenance of a safe food supply. FSANZ is a partnership between ten Governments: the Commonwealth; Australian States and Territories; and New Zealand. It is a statutory authority under Commonwealth law and is an independent, expert body.

FSANZ is responsible for developing, varying and reviewing standards and for developing codes of conduct with industry for food available in Australia and New Zealand covering labelling, composition and contaminants. In Australia, FSANZ also develops food standards for food safety, maximum residue limits, primary production and processing and a range of other functions including the coordination of national food surveillance and recall systems, conducting research and assessing policies about imported food.

The FSANZ Board approves new standards or variations to food standards in accordance with policy guidelines set by the Australia and New Zealand Food Regulation Ministerial Council (Ministerial Council) made up of Commonwealth, State and Territory and New Zealand Health Ministers as lead Ministers, with representation from other portfolios. Approved standards are then notified to the Ministerial Council. The Ministerial Council may then request that FSANZ review a proposed or existing standard. If the Ministerial Council does not request that FSANZ review the draft standard, or amends a draft standard, the standard is adopted by reference under the food laws of the Commonwealth, States, Territories and New Zealand. The Ministerial Council can, independently of a notification from FSANZ, request that FSANZ review a standard.

The process for amending the *Australia New Zealand Food Standards Code* (Food Standards Code) is prescribed in the *Food Standards Australia New Zealand Act 1991* (FSANZ Act). The diagram below represents the different stages in the process including when periods of public consultation occur. This process varies for matters that are urgent or minor in significance or complexity.



INVITATION FOR PUBLIC SUBMISSIONS

FSANZ has prepared a Draft Assessment Report of Application A434 and prepared a draft variation to the *Australia New Zealand Food Standards Code* (the Code).

FSANZ invites public comment on this Draft Assessment Report based on regulation impact principles and the draft variation to the Code for the purpose of preparing an amendment to the Code for approval by the FSANZ Board.

Written submissions are invited from interested individuals and organisations to assist FSANZ in preparing the Final Assessment for this Application. Submissions should, where possible, address the objectives of FSANZ as set out in section 10 of the *Food Standards Australia New Zealand Act 1991* (FSANZ Act). Information providing details of potential costs and benefits of the proposed change to the Code from stakeholders is highly desirable. Claims made in submissions should be supported wherever possible by referencing or including relevant studies, research findings, trials, surveys etc. Technical information should be in sufficient detail to allow independent scientific assessment.

The processes of FSANZ are open to public scrutiny, and any submissions received will ordinarily be placed on the public register of FSANZ and made available for inspection. If you wish any information contained in a submission to remain confidential to FSANZ, you should clearly identify the sensitive information and provide justification for treating it as commercial-in-confidence. Section 39 of the FSANZ Act requires FSANZ to treat in-confidence, trade secrets relating to food and any other information relating to food, the commercial value of which would be, or could reasonably be expected to be, destroyed or diminished by disclosure.

Submissions must be made in writing and should clearly be marked with the word 'Submission' and quote the correct project number and name. Submissions may be sent to one of the following addresses:

Food Standards Australia New Zealand
PO Box 7186
Canberra BC ACT 2610
AUSTRALIA
Tel (02) 6271 2222
www.foodstandards.gov.au

Food Standards Australia New Zealand
PO Box 10559
The Terrace WELLINGTON 6036
NEW ZEALAND
Tel (04) 473 9942
www.foodstandards.govt.nz

Submissions should be received by FSANZ **by 21 July 2004**.

Submissions received after this date may not be considered, unless the Project Manager has given prior agreement for an extension.

While FSANZ accepts submissions in hard copy to our offices, it is more convenient and quicker to receive submissions electronically through the FSANZ website using the Standards Development tab and then through Documents for Public Comment. Questions relating to making submissions or the application process can be directed to the Standards Management Officer at the above address or by emailing slo@foodstandards.gov.au.

Assessment reports are available for viewing and downloading from the FSANZ website. Alternatively, requests for paper copies of reports or other general inquiries can be directed to FSANZ's Information Officer at either of the above addresses or by emailing info@foodstandards.gov.au.

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Executive Summary and Statement of Reasons

Dairy Farmers has submitted an application to FSANZ seeking approval for the use of phytosterol esters derived from vegetable oils as a novel food ingredient in low-fat milk and low-fat yoghurt under Standard 1.5.1 – Novel Foods – in the *Australia New Zealand Food Standards Code*.

Standard 1.5.1 prohibits the sale of novel foods or novel food ingredients unless they are listed in the Table to clause 2 of the Standard, and comply with any special conditions of use stipulated in the Table. Approval for use requires a safety assessment to be undertaken. Current permissions to use phytosterol esters as a novel food ingredient are limited to edible oil spreads and margarines. There is currently no permission to add phytosterols to a broader range of foods.

Purpose and scope of the Application

Free phytosterols are chemically and structurally related to animal-derived cholesterol. These properties confer the ability to interfere with the mechanism of cholesterol absorption in the human intestine. When ingested in various food matrices, phytosterol esters can potentially lower low density lipoprotein (LDL) cholesterol levels in the blood. Products with added phytosterol esters are primarily targeted at consumers over 40 years. The purpose of the application is to increase the range of phytosterol-enriched foods available to the consumer.

Approval of a health claim is not a consideration in this assessment. Clinical data have been evaluated for the purposes of establishing that phytosterol esters can lower LDL cholesterol levels when added to low-fat milk and low-fat yoghurt. This ensures that there is truth in labelling, particularly where manufacturers' statements and promotional material associate plant sterols with a reduction in the absorption of cholesterol.

Risk assessment

Two new clinical studies were submitted in support of the application. As well as testing efficacy in different food matrices (breakfast cereal, fibre-increased bread, low-fat milk and low-fat yoghurt), a range of physiological/biochemical parameters were also measured. When incorporated into low-fat milk and low-fat yoghurt, phytosterol esters had a modest cholesterol lowering effect. Daily consumption rates between 2.6 g and 10.7 g phytosterol esters were well tolerated, and no adverse physical or physiological effects were detected. The results from the clinical studies are consistent with other published studies, some investigating consumption of phytosterols for periods up to 12 months.

The investigations into the nutritional effects of phytosterols on absorption of carotenoids and some fat-soluble vitamins found that β-carotene levels were the most affected, showing a reduction of approximately 25%, which to some extent was dependent on the nature of the food matrix and on cholesterol-lowering effects. However, the reduction in β-carotene levels was not associated with a reduction in retinol or vitamin A levels and was within a broad natural variation for this provitamin.

The results from the dietary exposure assessment which considered phytosterol-containing low fat milk and low fat yoghurt indicate that mean exposure to free phytosterols would be 1.6 g/day for the Australian population and 1.9 g/day for the New Zealand population.

For both countries, estimated mean dietary exposure is highest for consumers aged 40-64 years, which is a major sub-set of the target group. At the highest level of consumption (95th percentile), estimated exposure to phytosterols is between 4.2 g/day and 4.7 g/day for all population groups assessed.

When all proposed foods in Applications A433 and A434 ('healthy' breakfast cereal, plus low fat milk and yoghurt) are considered, the results of the dietary exposure assessment indicate that estimated mean dietary exposure from all foods, expressed as free phytosterols, did not exceed 1.9 g/day in any population group, and highest mean consumption levels were in the target population groups (over 40 years of age) in both Australia and New Zealand. At the 95th percentile of exposure, no population group exceeded 4.7 g free phytosterols per day, equivalent to 7.6 g phytosterol esters. The highest consumers of phytosterol esters are therefore likely to be under the upper level of consumption of 10.7 g/day used in the clinical studies. The results also suggest that the major source of dietary exposure to added phytosterols is from edible oil spreads for all population groups assessed.

The overall conclusion of the risk assessment is that low fat milk and low fat yoghurt enriched with phytosterol esters, at the levels proposed by the Applicant, are not associated with adverse effects, and can result in a cholesterol lowering effect. Adult consumers in the target population group are major consumers of the foods in question, and by maintaining their established dietary habits are likely to use the foods in amounts considered safe and appropriate to achieve a cholesterol lowering benefit.

Risk management

Phytosterol ester enriched foods can be consumed safely by the target population group and may assist in reducing LDL cholesterol levels. However, in general, children and pregnant or lactating women do not need to reduce cholesterol absorption, and products containing added phytosterols are therefore less appropriate for these groups.

Comprehensive risk management options have been considered, to encourage appropriate use by the target population group and discourage consumption by non-target groups. The recommended measures include (i) prescribing the amount of phytosterol esters that may be added to low-fat milk and low-fat yoghurt; (ii) retaining the three mandatory advisory statements currently required under Standard 1.2.3 (for edible oil spreads and margarines), and adding one additional mandatory advisory statement; (iii) imposing a restriction on the size of the package to 1 litre for milk, and 140g punnet for yoghurt; and (iv) imposing an additional condition of use prohibiting phytosterol enriched foods to be used as ingredients in other foods.

It is proposed that labelling requirements apply to all foods with added plant sterols, including the edible oil spreads and margarines.

Public consultation

Sixteen submissions were received during the first public consultation period. These were almost equally divided between those in favour of the application, and those opposed to the application. The submissions in favour of the application supported increased consumer choice and improved opportunities for product innovation.

The major issues of concern were the potential nutritional effects, the potential for adverse effects in non-target consumers, and the choice of food products, namely milk and yoghurt which are widely consumed in Australia and New Zealand. The issues raised in public submissions have been addressed in the report and, where appropriate, through the risk management strategies outlined.

Statement of Reasons

FSANZ recommends the approval of the use of phytosterol esters derived from vegetable oils in low-fat milk and low-fat yoghurt, subject to specified conditions of use, for the following reasons:

- there are no anticipated public health and safety concerns associated with the use of phytosterol esters derived from vegetable oils in low-fat milk and low-fat yoghurt when used in conjunction with the risk management measures proposed;

- there is evidence that phytosterol esters derived from vegetable oils can, following consumption, reduce levels of cholesterol in humans when incorporated into low-fat milk and low-fat yoghurt products;

- the nutrition assessment indicates that phytosterol esters derived from vegetable oils have no significant adverse nutritional effects at the proposed levels of use. The reductions in the absorption of fat-soluble nutrients (carotenoids) are within the normal variation which results from physiological and environmental factors;

- conditions of use, including an additional labelling statement, are proposed as part of a comprehensive risk management strategy to ensure appropriate use of phytosterol-containing foods by the target consumers, and to discourage use by non-target consumers;

- the proposed changes to the Code are consistent with the section 10 objectives of the FSANZ Act; and

- The Regulatory Impact Statement indicates that, for the preferred option, namely, to approve the use of phytosterol esters derived from vegetable oils as novel food ingredients in low-fat milk and low-fat yoghurt, the benefits of the proposed amendment outweigh the costs.

The proposed drafting to the *Australia New Zealand Food Standards Code* is shown at **Attachment 1** to the Draft Assessment Report.

1. Introduction

An application was received from Dairy Farmers on 15 February 2001 seeking approval for the use of phytosterol esters derived from vegetable oils as a novel food ingredient in low-fat milk and low-fat yoghurt under Standard 1.5.1 – Novel Foods – in the *Australia New Zealand Food Standards Code* (the Code). The Application was assigned to Work Group 2 and, by mutual agreement with the Applicant, was due to commence work in September 2002. Following completion of the Initial Assessment, FSANZ accepted this Application on 11 March 2003.

Phytosterol esters derived from vegetable oils can lead to a lower serum low density lipoprotein (LDL) cholesterol level when added to the diet. Phytosterol esters are currently added to lines of edible oil spreads namely Pro-activ® marketed by Unilever, and Logicol® marketed by Goodman Fielder. The Applicant seeks to extend the current permission for phytosterol esters to low-fat dairy products, in order to increase the product range available to consumers.

2. Regulatory Problem

2.1 Current Regulations

Standard 1.5.1 in the Code prohibits the sale of novel foods or novel food ingredients unless they are listed in the Table to clause 2 of the Standard and comply with any special conditions stipulated in that Table. Approval for use requires that a novel food or novel food ingredient, be subjected to a pre-market safety assessment before being offered for retail sale in Australia and New Zealand.

The current permission to use phytosterol-esters and tall oil phytosterols (TOPs) as novel food ingredients was limited to edible oil spreads and margarines primarily due to the limited safety data available and the lack of scientific evidence relating to their cholesterol-lowering effects in other food matrices. There is currently no permission to add phytosterol esters to a broader range of foods.

Dairy Farmers is seeking to extend the approval for the use of phytosterol esters to a limited number of additional food products, namely a low-fat milk and a low-fat flavoured yoghurt.

The Initial Assessment Report for this application was advertised on 19 March 2003 for public comment over eight weeks, and the application is currently at Draft Assessment.

3 Objective

The objective of this application is to establish if the food regulations should be changed to allow the use of phytosterol esters in low fat milk and low fat yoghurt. Before approval, an amendment to the Code must be agreed by the FSANZ Board, and subsequently be notified to the Australia and New Zealand Food Regulation Ministerial Council (Ministerial Council). An amendment to the Code may only be gazetted once the Ministerial Council process has been finalised.

In addressing the proposed variation to Standard 1.5.1, FSANZ is required by its legislation to meet three primary objectives that are set out in section 10 of the FSANZ Act. These are:

- the protection of public health and safety;
- the provision of adequate information relating to food to enable consumers to make informed choices; and
- the prevention of misleading or deceptive conduct.

In developing and varying standards, FSANZ must also have regard to:

- the need for standards to be based on risk analysis using the best available scientific evidence;
- the promotion of consistency between domestic and international food standards;
- the desirability of an efficient and internationally competitive food industry;
- the promotion of fair trading in food; and
- any written policy guidelines formulated by the Ministerial Council.

4. Background

Phytosterols (or plant sterols) is a collective term for cholesterol-like compounds that are naturally present at low levels in many varieties of fruits, vegetables, nuts and cereals. The most common and major plant sterols are sitosterol, campesterol and stigmasterol.

Free phytosterols occur naturally at low levels (up to 0.9%) in common vegetable oils, and are chemically and structurally related to animal-derived cholesterol. These properties confer the ability to interfere with the mechanism of cholesterol absorption in the human intestine.

Free phytosterols are extracted from vegetable oil sources, typically soybean oil. Phytosterol esters are prepared commercially by the reaction of phytosterols with fatty acid methyl esters or free fatty acids.

4.1 Previous consideration

In 1999, following consultation between the then ANZFA and Senior Food Officers in each of the Australian States and Territories and New Zealand, it was agreed that phytosterol esters derived from vegetable oils should be regarded as novel food ingredients because of a lack of history of significant consumption by the broad community, and a lack of knowledge in relation to their safety at the proposed levels of use. Based on this decision, phytosterol esters derived from vegetable oils underwent assessment as novel food ingredients and were subsequently approved for use in edible oil spreads and margarines. For phytosterol esters, the variation to the standard was gazetted on 14 June 2001.

The use of phytosterol esters derived from vegetable oils was assessed by the then ANZFA within Application A410. At the time, the Final Assessment Report (completed in May 2001) concluded that permission to use phytosterol esters should be limited to edible oil spreads and margarines at a maximum concentration of 13.7% (w/w) for the following reasons:

(a) The available data on phytosterol esters did not demonstrate any evidence of adverse effects at the levels of dietary exposure expected from their use in edible oil spreads and similar products;

(b) The available data at the time was not adequate to assess the safety of phytosterol esters at higher levels of dietary exposure that could occur from their use in a broader range of foods; and

(c) The available scientific evidence demonstrated that dietary phytosterol esters could reduce total and low density lipoprotein (LDL) cholesterol levels in blood when incorporated into an edible oil spread at 13.7% (w/w). Any marketing statements to this effect used by manufacturers on packaging or in advertising would therefore be consistent with this evidence. However, there were no data available in relation to the effectiveness of phytosterol esters in this regard when incorporated into other foods.

4.2 Related Applications

FSANZ is currently considering two other related Applications:

- A433 seeks permission to use phytosterol esters derived from vegetable oils in breakfast cereals; and
- A508 seeks permission to use tall oil phytosterols in low-fat and no-fat liquid milk products.

Dairy Farmers and the applicant for A433, Goodman Fielder, have indicated that the phytosterol-enriched foods encompassed by the two applications (breakfast cereals, breakfast cereal bars, low-fat milk and yoghurt) would be marketed to consumers in a coordinated fashion, using statements and an identifying brand name that would be consistent across the range of products. The two companies have submitted scientific information common to both applications. Due to significant similarities in terms of the safety assessment, Application A433 therefore has been considered in parallel with this Application.

4.3 Additional information requested during assessment

Since the assessment period commenced in September 2002, FSANZ has sought additional information from the Applicant on two occasions. Further information and validation of the clinical studies submitted with the application was sought on 22 October 2002. The Applicant requested additional time, and a response was received on 29 January 2003.

In order to more fully develop the risk management options, the statutory timeframe was interrupted by FSANZ on 3 September 2003, seeking details from the Applicant on the extent of consumer information that was intended for packaging, as well as labelling and proposed marketing strategies for the products. FSANZ received a written response on 12 December 2003, after the Applicant again sought additional time. Assessment of the application was re-commenced on 17 December 2003.

4.4 Use of phytosterol esters in other countries

Phytosterol ester-enriched table spreads were introduced into the food supply in the mid 1990's in Finland, and spread gradually over succeeding years into other countries including the United States (USA), Brazil, Switzerland (1999), Australia and New Zealand (2001).

Since the EU Novel Foods approval in 2000 (EC258/97), edible oil spreads containing phytosterols have become available in over 11 countries in the European Union including the Netherlands, Austria, Belgium, France, Germany, Greece, Ireland, Portugal, Spain, Sweden and the United Kingdom (UK).

A variety of foods containing plant sterols are reportedly already on the market in Europe, although their regulatory status is not clear. Examples of these include yoghurts (natural and flavoured), semi-skimmed milk, chicken meat products, sausages, mayonnaise-based salads, cereal bars, and soft cream cheeses in addition to the permitted yellow fat spreads. In 2002, the UK Food Standards Agency received an application from Unilever seeking approval for use of phytosterol-esters (equivalent to 1g of free phytosterols per serving) in milk and yoghurt type products, which is currently under consideration.

Recently in Europe (December, 2003), the Standing Committee for the Food Chain and Animal Health recommended approval for plant sterols to be added to milk type products and salad dressing/spicy sauces, in addition to the existing yellow fat spreads. The policy role of the Standing Committee was to select the categories of food that would be eligible for the addition of plant sterols, and those that would not, based on risk management views expressed by the Scientific Committee on Food. There has been a host of new applications under the EU Novel Foods Regulation for permission to use plant sterols in foods across a broad range of categories, including foods such as bakery products, soft drinks, meat products, ice cream, sweets and cereal bars.

In the USA, a number of vegetable oil sterol esters, that meet appropriate food-grade specifications and are produced by current good manufacturing practice (21 CFR section 182.1(b)), have been notified under the GRAS system. The US Food and Drug Administration (FDA) have raised no objection to a number of food products (mainly edible oil spreads) that may contain plant sterol and stanol esters in amounts up to 20%, on the basis of the GRAS notification. The FDA has not conducted an independent assessment of these compounds.

Recent phytosterol ester FDA GRAS notifications (where the FDA raised no issues) are:

- GRN 000048 (2000) - Vegetable oil phytosterols – for use in vegetable oil spreads, salad dressings, bars and yoghurt.
- GRN 000053 (2000) – Phytosterol esters – for use in vegetable oils for baking and frying, and salad dressings.
- GRN 000061 (2001) – Plant sterols/plant sterol esters – for use in vegetable oil spreads, salad dressings, health drinks, health bars and yoghurt type products.

The FDA has also issued an interim final rule (Sept 2000, 21 CFR section 101.83 [2]), which allows manufacturers of products containing added phytosterol and stanol esters to make a health claim (for reducing the risk of coronary heart disease). There are a number of specific restrictions with which the products must comply before such a health claim may be made. In the USA, a number of foods that are allowed to use this interim health claim include sterol esters in spreads and salad dressings, and stanol esters in spreads, salad dressings, snack bars and dietary supplements in soft gel form.

There is currently no permission for the use of phytosterols in foods in Canada, although some vegetable oil spreads are reportedly on the market without regulatory approval.

4.4.1 Use of plant stanols in foods

In Europe there have been a number of phytostanol ester-enriched foods (stanols esterified with fatty acids of rapeseed oil) launched on the market. Initially, the products were edible oil spreads (margarines), but this has broadened to include other foods such as fresh cheese, snack bars, salad dressing and yoghurt, and the markets include Finland, Belgium, the Netherlands, Luxembourg, UK, Ireland, Sweden, Denmark and the USA.

A yellow fat spread containing plant *stanol* esters is permitted on the market in the EU, without being subjected to review, because it was marketed in a member State before the Novel Foods Regulation came into force. A similarly-based cheese spread is also on the market in the UK from the same manufacturer who considered it as merely a variant of the yellow fat spread. However, the product was withdrawn from the market in the Netherlands on instructions from the Dutch regulatory agency that regarded it as a separate novel food requiring prior separate approval.

There is currently no permission to use a phytostanol ester novel food in Australia and New Zealand.

5. Relevant Issues

The focus of the assessment of novel foods is primarily related to the first objective identified in section 10 of the FSANZ Act, namely, *the protection of public health and safety*. However, as for all applications to change the Food Standards Code, other objectives identified in the Act must also be considered, including *the provision of adequate information relating to food to enable consumers to make informed choices*; and *the prevention of misleading or deceptive conduct*.

FSANZ has identified and addressed the relevant issues in relation to a broader use of phytosterol esters. These issues include the potential for increased dietary exposure, safety, potential nutritional effects in target and non-target consumers, truth in labelling, and the provision of appropriate consumer information, as well as some other issues raised in public submissions. The risk management measures address the issues identified in the risk assessment.

5.1 Claims concerning function or efficacy

FSANZ has previously assessed phytosterols derived from vegetable oils and TOPs primarily from a safety perspective rather than an efficacy perspective. Health claims are generally prohibited in the Code, and although there is now an abundance of published studies reporting the cholesterol-lowering effects of additional phytosterols in the human diet, approval of a health claim is not a consideration in this assessment.

However, irrespective of whether any statement on a product is considered a health claim, all statements on the label should be true and not mislead consumers. The evidence now available suggests that the nature of the food matrix in which the phytosterols are consumed is a factor in the overall cholesterol-lowering effect. As lipophilic compounds, they are suited to incorporation into lipid-rich foods such as edible oil spreads and margarines.

As these physical properties may contribute to the cholesterol lowering effects, appropriate evidence is needed to demonstrate that phytosterols have similar efficacy when added to other food-types such as dairy and cereal products. Therefore, current information on the potential physiological or functional effects of phytosterol esters when incorporated into specific food matrices is relevant only in relation to the veracity of labelling statements used by manufacturers in promoting these products.

5.2 Technical properties of phytosterol esters

A detailed report on the food technology aspects of this application is provided at **Attachment 2**.

Plant sterols are natural components of cereals, fruit, vegetables and edible vegetable oils, and as such are natural constituents of the human diet. Phytosterols have a role in plants similar to that of cholesterol in mammals, that is forming cell membrane structures. The structures of the most abundant phytosterols, β-sitosterol, campesterol and stigmasterol are very similar to cholesterol. Free phytosterols are poorly soluble in most food matrices, but their sterol esters (formed by reacting the sterols with fatty acids) are more fat soluble.

Free phytosterols are only partially extracted when edible oils (such as soybean oil) undergo normal refining. It is estimated that 2500 tonnes of vegetable oil needs to be refined to yield 1 tonne of plant sterols. This relatively low yield ratio means that phytosterols are expensive to produce.

The current applications seek to extend the range of foods with phytosterols to low fat milk and low fat yoghurt products, as well as breakfast cereals. For such products, the esters are technically preferable to the free phytosterols since they have improved solubility in fats or oils used in the manufacturing process. The production and processing methods for phytosterol-enriched products are the same as for the corresponding standard products, except for the added incorporation of the phytosterol esters before further processing. Phytosterol esters are very stable to both oxidation and heat and remain unchanged during product processing, including various heat treatments. Even under severe conditions, such as deep-frying, sterol oxidation products are only formed at parts per million (ppm) concentrations.

The technical specifications for the phytosterol esters in this Application are the same as those currently listed under Standard 1.3.4 – Identity and Purity in the Code (see Attachment 2).

5.3 Safety/efficacy of phytosterol esters

An extensive database of both animal and human studies on the safety of phytosterol esters was examined during consideration of Application A410. The animal studies indicate that free phytosterols and phytosterol esters are poorly absorbed from the gastrointestinal tract, have low toxicity, are not genotoxic, and have no effect on reproductive parameters. There was also no evidence of oestrogenic activity in both *in vitro* and *in vivo* studies. Excretion is through the bowel both as free phytosterols and as phytosterol esters. The human studies provide evidence of reduced cholesterol absorption resulting in lower plasma cholesterol levels following ingestion of up to 3.3 g/day in short term studies, and ingestion of 1.6 g/day (calculated as free phytosterols) in a 1-year study.

There was no evidence of adverse health effects in these studies, but the reduction in the levels of plasma β-carotene (a precursor for the synthesis of vitamin A) raised a potential concern regarding higher levels of phytosterol intake, particularly for groups that may be at risk of vitamin deficiency such as children or lactating women.

An updated safety assessment of phytosterol esters in other food vehicles and at higher levels of intake has been prepared, and is presented in two sections – a general safety assessment (at **Attachment 3**), and a nutritional assessment (at **Attachment 4**).

Clinical studies were conducted in mildly hypercholesterolaemic volunteers that examined the cholesterol lowering effects of phytosterol ester-enriched low-fat milk, low-fat yoghurt, fibre-increased bread and breakfast cereal, in a multi-centre trial. In the first study, the trial foods each provided 2.6 g/day phytosterol esters, and were consumed singly and sequentially for a period of three weeks each, in addition to a control period in which no phytosterol-enriched foods were consumed. As well as testing efficacy in the different food matrices, a range of physiological/biochemical parameters were also measured in the study participants (see Attachment 3).

The second study was an evaluation of the nutritional effects of phytosterol ester enriched breakfast cereal, fibre-increased bread, and margarine together providing 10.7 g/day phytosterol esters, consumed over a period of 12 weeks. The analyses specifically focussed on circulating levels of lipophilic nutrients such as the carotenoids and some fat soluble vitamins. In the second six-weeks of the trial period, participants were asked to consume at least 5 serves per day of fruits and vegetables, at least one from a list of carotenoid-rich fruits and vegetables, in conjunction with the phytosterol enriched foods (see Attachment 4).

The key findings of these recent studies include:

- each phytosterol-enriched trial food (low-fat milk, low-fat yoghurt, fibre-increased bread and breakfast cereal) providing 2.6 g/day phytosterol esters reduced serum LDL cholesterol levels (Study 1);

- milk was the most effective food vehicle, reducing serum LDL cholesterol levels by approximately 15%, whereas yoghurt reduced serum LDL cholesterol levels by approximately 8%;

- bread and breakfast cereal reduced serum LDL cholesterol levels by approximately 5% and 6% respectively;

- very low levels of phytosterols were detected in plasma with increases over controls in the range of 1-2 μg/ml;

- intakes of phytosterol esters of 10.7 g/day (Study 2) resulted in a similar reduction in serum LDL cholesterol as the lower intake level (2.6 g/day). However, if the milk phase was excluded from Study 1, the reduction in cholesterol at the higher intake level was approximately twice that of the lower intake, implying that there is a more significant cholesterol lowering effect when milk is used as the food vehicle;

- lipid-standardised plasma carotenoid levels were significantly reduced (14% - 27%), which varied with individual carotenoids throughout the trial, but were partially restored to baseline levels when target amounts of fruits and vegetables were consumed. The reduction in β-carotene levels is the most consistent finding across these studies; and

- no other significant changes were observed in other physiological/biochemical parameters tested in the studies.

A comparison between diets containing 2.6 g/day and 10.7 g/day phytosterols revealed that at higher ingestion levels, endogenous cholesterol synthesis increases almost proportionately as indicated by plasma lathosterol levels. This homeostatic synthesis may account for the plateau effect in reduction of serum cholesterol levels observed as ingestion of phytosterol esters increases. The effects observed in both of these studies are highly consistent with previous studies reported in the scientific literature.

5.3.1 Nutritional assessment

As absorption of dietary cholesterol is inhibited by ingestion of phytosterols, there is a concomitant effect on the absorption of some lipophilic micronutrients. When these nutritional effects were examined in detailed studies, reductions in α- and β- carotene, lycopene, lutein and cryptoxanthin were observed, while vitamin E and vitamin A levels remained unaffected. When the nutrient levels were adjusted to correct for lower LDL cholesterol levels, only β-carotene levels were significantly affected by ingestion of phytosterol enriched foods. However, additional fruits and vegetables (including some that were carotenoid-rich) in the diet, when co-consumed with the phytosterol-enriched foods, partially compensated for the lower bioavailability of carotenoids in the presence of phytosterols. The detailed nutrition assessment is presented in this report at **Attachment 4**.

With some variability, consumption of phytosterol-enriched foods generally results in a reduction in β-carotene levels of approximately 20-25%. This reduction does not translate into an overt nutritional deficiency as absolute levels remain within a broad natural range and there is no measurable effect on retinol or vitamin A levels. The nutritional significance of a reduction in β-carotene levels therefore cannot be directly measured or assessed. In terms of antioxidant status, other nutrients such as vitamin C and vitamin E are not affected by consumption of phytosterols and other phytochemicals present in fruits and vegetables contribute to the complexity of the diet and overall health.

5.3.2 Conclusions from the safety assessment/efficacy assessment

When incorporated into foods such as low-fat milk, low-fat yoghurt, breakfast cereal, and bread, phytosterol esters have a modest cholesterol lowering effect, with milk the most effective food vehicle, followed by yoghurt, cereal and bread in order. At daily consumption rates between 2.6 g and 10.7 g, phytosterol esters are well tolerated, with no adverse physical or physiological effects detected in clinical trials over a 12 week period. Other published studies suggest that phytosterol esters are not associated with adverse effects over periods up to 12 months.

The investigations into the nutritional effects of phytosterols on absorption of carotenoids and some fat-soluble vitamins found that β-carotene levels were most affected, showing a reduction of approximately 25%, which was to some small extent dependent on the nature of the food matrix and on cholesterol-lowering effects. Some evidence was provided to demonstrate that consumption of 5 serves per day of fruits and vegetables (with at least one of these rich in carotenoids), partially restores the levels of some micronutrients, particularly α-carotene, lycopene, lutein and cryptoxanthin to baseline levels. Of primary importance, the reduction in β-carotene levels is not associated with a reduction in retinol or vitamin A levels and is within a broad natural variation for this provitamin. On the basis of the currently available data, there is no evidence to support the view that a reduction in β-carotene levels of this magnitude would result in adverse nutritional effects.

Phytosterol ester enriched foods can act as convenient tools for reducing circulating LDL cholesterol levels without major changes to the diet, and can be consumed safely by the target population group.

5.4 Potential dietary exposure to phytosterol esters

A detailed dietary exposure assessment has been completed based on the availability of phytosterol ester enriched low fat milk and low fat yoghurt in addition to a baseline level of exposure from existing phytosterol-enriched edible oil spreads (at **Attachment 5).** A separate dietary exposure report has been prepared that incorporates the results of modelling for all proposed phytosterol enriched foods currently under assessment (breakfast cereals, low-fat milk and low-fat yoghurt) as well as the existing baseline exposures from edible oil spreads (at **Attachment 6**).

Since Applications A433 and A434 are being considered at the same time, the combined dietary exposure assessment was undertaken to determine the potential impact of allowing phytosterol esters to be added to all foods encompassed by the applications, as well as permitted edible oil spreads, to provide an absolute maximum exposure assessment. Both applicants propose to add phytosterol esters at a level equivalent to 0.8 g free phytosterols (1.3 g phytosterol esters) per serve. The dietary modelling was conducted for both Australian and New Zealand populations using DIAMOND, a dietary modelling computer program developed by FSANZ that uses data obtained in nutrition surveys.

5.4.1 Exposure assessment

The dietary exposure assessment took into account the existing permission under Standard 1.5.1 to add phytosterol esters to edible oil spreads and margarines (the 'baseline' scenario), but not the intrinsic level of phytosterols naturally occurring in foods. Food consumption data were derived from the 1995 Australian National Nutrition Survey (NNS) and the 1997 New Zealand NNS.

Assessments were conducted for the general Australian and New Zealand populations (2+ and 15+ years respectively), for two target populations for phytosterol products (those aged 40-64 years and 65+ years) and for two non-target populations for whom phytosterol exposure would offer no nutritional benefit, namely pregnant women and children (2-12 years). Food chemical concentration data were derived from levels proposed in both applications and from the maximum level of use permitted in edible oil spreads and margarines.

In the first report, which considered only the dairy products, intakes of phytosterol esters were estimated for mean and 95th percentile consumers for the target and non-target groups separately, and were considered in the context of a comparison between a baseline level of intake (assuming that all existing edible oil spreads and margarines contained phytosterols), and the estimated level of intake if all low-fat milk and low-fat yoghurt contained phytosterols. Dietary exposure estimates were conducted using the assumption that the pattern of consumption of foods containing phytosterols would not be different to the pattern of consumption of similar foods prior to the addition of phytosterol esters to these foods. The modelling assumptions and methodology were therefore conservative and provide an over-estimate of likely exposures to phytosterols from the specified foods.

5.4.2 Results

The results from the assessment considering baseline plus low-fat milk and low-fat yoghurt (Attachment 5) indicate that mean exposure to free phytosterols would be 1.6 g/day for the Australian population and 1.9 g/day for the New Zealand population. For both countries, estimated mean dietary exposure is highest for consumers aged 40-64 years, which is a major sub-set of the target group. The group with the highest 95th percentile exposure is the New Zealand population group 40-64 years.

The results from the combined assessment which considered all proposed foods (low fat milk and yoghurt plus healthy breakfast cereal) in addition to the baseline exposure (Attachment 6), indicate that estimated mean dietary exposure from all foods, expressed as free phytosterols, did not exceed 1.9 g/day in any population group assessed, and highest mean consumption levels were in the target population groups (over 40 years of age) in both Australia and New Zealand. At the 95th percentile of exposure, no population group assessed exceeded 4.7 g free phytosterols per day. The results also suggest that the major source of dietary exposure to added phytosterols is from edible oil spreads for all population groups assessed.

5.4.3 Discussion

These data suggest that permission to use phytosterol esters in low fat milk and low fat yoghurt would be likely to result in a level of consumption of phytosterols that has been shown in clinical studies to be safe. High consumers of all phytosterol enriched foods (existing and pending approvals) would be under the upper limit of intake (10.7 g phytosterol esters) considered in the clinical studies submitted with this application.

Notwithstanding the variation in efficacy shown to be associated with the nature of the food vehicle, consumption of up to three serves of phytosterol enriched product provides the amount of phytosterol-esters associated with reduced blood cholesterol levels. Consumption of more than three serves of phytosterol enriched foods on a daily basis provides no additional cholesterol lowering effect, but does produce a level of intake of phytosterols that is associated with other nutritional effects, namely a reduction in ß-carotene levels.

5.5 Risk assessment

5.5.1 General population

The data submitted with these applications indicate that consumption of phytosterol enriched foods providing up to approximately 10.7 g/day phytosterol-esters is safe. This conclusion is supported by other information from published studies. The effects of phytosterol esters consumption above 10.7 g/day over the long-term have not been researched, however, the available data do not raise any concerns in this regard. The potential for an adverse nutritional effect is considered to be very small, however, risk management strategies should be considered to ensure appropriate use of phytosterol esters in the target group and discourage use in the non-target groups.

5.5.2 Children

In considering the potential impact of consuming phytosterol-enriched foods by non-target consumers such as children, the recent NHMRC Dietary Guidelines for Children and Adolescents in Australia (2003) has been considered. This guideline states that, relative to their body weight, children's nutrient and energy requirements are greater than those of adults. Furthermore, in assessing the role of fat in the diet of children, the background to the guideline states: "There is some evidence that an adequate intake of cholesterol during the growth period is important for cholesterol metabolism later in life and for myelinisation of the nervous system, neurologic development in general, the formation of hormones essential for growth and sexual maturation, and the production of bile acids."[1]

At the same time, the guideline report emphasises the fact that the human body is capable of synthesising sufficient cholesterol for all its metabolic needs. Excluding genetic disorders and/or underlying pathology, hypocholesterolaemia does not occur in otherwise healthy children. In addition, the report states that increasing problems of obesity in childhood constitute a risk factor for a range of immediate and long-term health problems, among them diabetes, high cholesterol levels, hypertension, sleep apnoea, musculoskeletal problems, liver disease and potential psychological problems. It is now established also that overweight children over the age of 7 years are at greater risk of developing obesity and cardiovascular disease as adults. However, it is also noted that saturated fats in the diet contribute more to elevated blood cholesterol levels than does dietary cholesterol.

A detailed discussion of studies investigating the effects of consumption of phytosterol esters by children (with pre-existing hypercholesterolaemia) is presented in the Nutrition Safety Assessment (Attachment 4). As expected, phytosterol consumption lowered cholesterol absorption in children, with no identified adverse effects. On balance therefore the available evidence suggests that occasional or casual consumption of phytosterol-enriched foods by children does not present a risk to health. The modest reduction in cholesterol that may result from an increased intake of phytosterols is not likely to be physiologically or nutritionally significant. However, the potential reduction in some carotenoids, while not reaching nutritionally inadequate levels, may be inappropriate for an individual based on above-average nutrient needs for optimal growth and development.

[1] NHMRC Dietary Guidelines for Children and Adolescents in Australia (2003), Chapter 3.6 Limit Saturated Fat and Moderate Total Fat Intake

5.5.4 Pregnant and lactating women

Pregnant and lactating women are considered to have specific nutritional needs because of their respective physiological states, and do not need to actively lower blood cholesterol levels. Phytosterol enriched products are therefore not suitable for these women.

5.5.5 Conclusions of risk assessment

Consumption of phytosterol-enriched foods is not appropriate for children, or pregnant or lactating women since there is no necessity to lower absorption of dietary cholesterol in these groups, and they would derive no health benefit from increasing their intake of phytosterols. In contrast, for consumers over the age of 40 years, and particularly those with slightly elevated cholesterol levels, the broader availability of phytosterol enriched foods could enable them to effectively reduce LDL cholesterol, one of the known risk factors in the development of atherosclerosis and cardiovascular disease.

The results of several studies suggest daily consumption of 5 serves of fruits and vegetables, particularly those high in β-carotene, when choosing phytosterol-enriched foods, may assist in maintaining the levels of some carotenoids. The European Scientific Committee for Food (SCF) recommends that consumers be made aware of the potential β-carotene lowering effect of phytosterol-enriched products by the provision of appropriate dietary advice relating to the regular consumption of fruits and vegetables.

5.6 Risk management strategies

Based on the conclusions of the safety assessment, phytosterol ester enriched low-fat milk and low-fat yoghurt products would be considered safe and appropriate for adults over the age of 40 years who are the primary target consumer group. However, while not raising any significant safety issues, the risk assessment has concluded that habitual consumption of phytosterol enriched products is not suited to other groups such as children and pregnant or lactating women. This is consistent with previous conclusions on the potential impact of phytosterol-enriched foods on the whole consumer market in Australia and New Zealand, and with more recent international opinion. The safety assessment also found that occasional, or inadvertent, consumption of phytosterol-enriched products by non-target consumers would not raise any health concerns.

A number of strategies can be employed to achieve the goals of permitting broader choice in the range of phytosterol-enriched products available to interested consumers, whilst at the same time discouraging consumption by children and pregnant or lactating women, thus minimising the likelihood that non-target groups would become regular consumers. The risk management measures proposed in this assessment also aim to address the issue of appropriate consumption by the target group. These measures include:

- restricting permission to use phytosterol esters to defined products;
- restricting conditions of use;
- comprehensive labelling requirements, including mandatory advisory statements on packaging; and
- the provision of additional consumer information.

5.6.1 Restricting permission to use phytosterol esters

Permission to use phytosterol esters in a low-fat milk and a low-fat yoghurt represents a cautious expansion of the use of these novel food ingredients. Although milk and yoghurt are widely consumed foods, low-fat varieties are preferred by the target consumers and the dietary modelling suggests consumption rates of these products would not be excessive. Phytosterol enrichment of low-fat dairy products is also consistent with public health messages on the nature of a healthy diet.

5.6.2 Restricting conditions of use

In addition to the existing conditions of use in Standard 1.5.1 pertaining to the specifications for phytosterol esters in Standard 1.3.4 Identity and Purity, the following additional conditions of use within Standard 1.5.1 are proposed:

(i) Foods containing added plant sterols may not be used as ingredients in other foods;
(ii) The container size for low-fat milk is to be specified at a maximum of 1 litre; and
(iii) The container size for low-fat yoghurt is to be specified at a maximum of 140g.

Prohibiting the use of phytosterol enriched foods in other mixed foods will limit the ability of manufacturers to further extend the product range.

The proposed maximum container size for the low-fat milk containing phytosterol esters is 1 litre, while the proposed container size for the phytosterol ester-enriched low-fat yoghurt is a maximum of 140g. The maximum carton size for milk discourages general household use compared to 2 or 3 litre size cartons, and single serve punnets of yoghurt discourage excessive consumption while ensuring that the amounts consumed are effective.

5.6.3 Comprehensive labelling requirements

5.6.3.1 Labelling statements relating to absorption of cholesterol

The previous assessment of the use of phytosterols in edible oil spreads and margarines concluded that the available evidence from human studies indicated that total cholesterol was reduced by approximately 5% and LDL-cholesterol by 7-8% by average consumption of these products. At that time, no studies had been conducted to examine whether similar cholesterol lowering effects could be achieved when phytosterols were consumed in other food matrices. The available evidence now demonstrates that cholesterol-lowering effects can be achieved when phytosterol esters are incorporated into low-fat milk and low-fat yoghurt.

An integral part of the previous and current assessments is to consider the approach taken by manufacturers of these products in terms of advertising and promotional statements carried on retail packaging, in terms of the objective under section 10 of the FSANZ Act relating to *the prevention of misleading or deceptive conduct*. Since approval of the edible oil spreads and margarines, statements such as: ‘With plant-derived ingredients that lower cholesterol absorption’ or ‘With natural plant sterols which reduce cholesterol uptake’ are evident on existing food packaging.

The Applicant has provided copies of packaging proposed for each of the dairy products in this assessment that bear a similar statement: 'With Natural Plant Sterols to Lower Cholesterol Absorption'. This serves to demonstrate the kind of statements that manufacturers use to promote consumer identification of this range of products.

5.6.3.2 Ingredient labelling

The existing conditions of use for phytosterol esters under the standard for Novel Foods (Standard 1.5.1), include the requirement for 'phytosterol ester or plant sterol esters' to be used when declaring the ingredient in the ingredient list, as currently prescribed in Standard 1.2.4. This requirement would be maintained for any broader permission arising from this assessment.

5.6.4 Mandatory advisory statements

Existing mandatory advisory statements are:

Statements to the effect that:

1. **the product should be consumed in moderation as part of a diet low in saturated fats and high in fruit and vegetables;**
2. **the product is not recommended for infants, children and pregnant or lactating women unless under medical supervision; and**
3. **consumers on cholesterol-lowering medication should seek medical advice on the use of this product in conjunction with their medication.**

Consumption of phytosterol-enriched foods such as low-fat milk, yoghurt or breakfast cereal is less appropriate for children, or pregnant or lactating women. Potential diet-related health problems in children are better managed through improved overall dietary habits and limiting foods high in saturated fats and sugars, rather than by merely lowering cholesterol absorption. Similarly, pregnant or lactating women do not need to manipulate cholesterol levels *per se*, because of their particular physiological status. For these reasons, the current mandatory labelling statements would be maintained for any new phytosterol ester enriched products.

In addition to these statements, it is proposed to add the following mandatory advisory statement:

4. **consuming greater than 3 serves per day of products containing plant sterols provides no additional benefit.**

The previous assessment speculated that, as phytosterols exert their effect in the gut, the food matrix in which they are presented to the body is likely to be an important factor in their mode of action.

The clinical studies now available confirm that when various food vehicles are compared under controlled conditions, there is a variable outcome in terms of cholesterol-lowering effect that is apparently, to some extent, dependent on the nature of the food vehicle.

This additional statement will serve to limit excess consumption of phytosterol esters and is consistent with the data that demonstrate the safety of phytosterols for high consumers within the target group, and also ensures the integrity of the statements relating to the cholesterol lowering effects of plant sterols, where cholesterol lowering effects do not increase with increased consumption over approximately 3g phytosterol esters per day. It also achieves a consistent message for consumers across all phytosterol-containing foods and ensures an effective minimum amount is consumed.

5.6.5 Consumer information

The marketing plans for the products encompassed by the two applications using phytosterol esters equate one serving of product with 1.3g phytosterol esters (0.8 g of free phytosterols). The manufacturers have designated serving sizes for each of the proposed products. In the case of milk, one serving is designated by manufacturers as 250 ml (1 cup), one serving of yoghurt is provided in a 140 g punnet, and one serving of breakfast cereal (muesli-type) is 45-50 g. The CSIRO studies confirm that consumption of between 1-4 g of free phytosterols achieves a measurable reduction in LDL-cholesterol using these food vehicles. Thus, two to three servings of a phytosterol ester-enriched product (either milk, yoghurt, breakfast cereal or the existing table spreads) provides the optimal amounts of phytosterols associated with reduced cholesterol levels.

The applicants seeking a broader permission for phytosterol esters have provided details of symbols that would be used on all phytosterol ester-containing products, across a single brand name associated with their products. The pictorial representation clearly communicates to consumers that consumption of 3 serves per day is desirable, from a choice of cereal, yoghurt, spread or milk. The symbol also communicates appropriate serving sizes for each of these products (see diagram below).

In addition to the marketing symbol for the branded products, serving sizes pertaining to the food in question are stipulated in the nutrition information panel. These measures are considered to adequately address the issues of appropriate consumption by consumers interested in these products.

Proposed consumer information to appear on packaging for low-fat milk and low-fat yoghurt products, depicting serving sizes for all phytosterol enriched products.	Proposed consumer information to appear on breakfast cereal packaging to depict serving size and appropriate use of all phytosterol enriched products.
	

5.7 Additional risk management issues

5.7.1 Pricing of products

The applicants claim that, because of the high cost of the phytosterol esters, there will be a pricing regime on products containing these novel food ingredients that will discourage family or larger household use. This is claimed to lessen the possibility that family members other than those in the target group would have access to these products. However, although the existing phytosterol enriched edible oil spreads and margarines are more expensive than their conventional counterparts, price discounting on these products does occur. A price premium for phytosterol-enriched foods therefore cannot always be guaranteed. Price does not reflect a robust risk management tool to ensure use only by target consumers.

5.8 Issues raised in public submissions

5.8.1 Medicalisation of the food supply

The Environmental Health Unit of Queensland Health, in addition to Richard and Valerie James (NZ) and Rosemary Stanton (Aust), express concerns that a broader approval for phytosterols equates to the food supply becoming a vehicle for the delivery of a therapeutic agent not required by the whole population.

5.8.1.1 Response

Phytosterol esters derived from vegetable oils are already approved novel foods under Standard 1.5.1 in the Code. Although they occur naturally in foods such as legumes and nuts at low levels, they are regarded as novel food ingredients when used in amounts some 5-10 fold higher than normal food consumption would provide.

Several identified risk factors for major diseases such as cardiovascular disease and stroke can be correlated in varying degrees with diet. Of these, obesity, high blood cholesterol, and high blood pressure have been at the forefront of public health messages over an extended period. In Australia and New Zealand, government and non-government organisations like the National Heart Foundation as well as clinicians, nutritionists, dieticians and other health professionals have reinforced the link between dietary and lifestyle choices and improved general health.

The pursuit of a 'healthy diet' is now promoted in many countries. These messages were formulated in the early 1980's and, in the UK, were documented in a report of the National Advisory Committee on Nutrition Education (NACNE, 1983). This publication sought to establish the nature of a healthy diet in practical terms and proposed nutritional guidelines based on accumulated information. The proposed guidelines included recommendations for changes in the profile of energy and nutrient intake in the typical diet over both the short and long term. Specifically, this entailed reductions in total and saturated fat, salt and sugar intake, together with a concomitant increase in fibre intake. In Australia, similar dietary recommendations have prevailed over many years in broad nutritional health policy developed by the National Health and Medical Research Council (NHMRC), as guidelines and in food-based regulations.

In response to nutritional messages concerning the health benefits of reducing obesity in the population as a whole, and subsequent changing consumer attitudes particularly with respect to processed foods, the food industry has engaged in continuous development of new food products that reflect the changing market conditions. Consumers also have readily demonstrated the extent to which they can alter traditional eating habits in their widespread acceptance and consumption of low or reduced fat foods, even where staple foods in the New Zealand and Australian diet, such as dairy products, are targeted.

Despite the obvious market success and broad availability of fat-modified foods, they are not suitable for all consumer groups. For example, low-fat milk is not recommended for young children because of the requirement for a full complement of dietary fats necessary for their growth and development. Similarly, low or no-fat versions of many foods are not selected by many consumers, on the basis of personal choice. In general, consumers have adapted well to the co-existence of numerous product variations that cater to individual dietary requirements and food preferences. In this regard, mandatory labelling, in combination with manufacturers' information provided on packages, are significant communication tools to assist consumers to make an informed choice with respect to their food purchases.

Foods with added phytosterol esters are intended for a specific group of consumers for whom they offer a potential benefit in terms of reducing the absorption of dietary cholesterol. At the same time, these foods offer no advantages to individuals who are not primarily interested in lowering LDL cholesterol. These purchasing criteria are not significantly different from those that can be applied to other more specialised foods targeted to particular sections of the public. Restricting package sizes of phytosterol-enriched foods further reinforces the message that these foods are not for everyone.

These marketing features of phytosterol enriched products places them in a similar retail position to low fat products which do not provide benefits to all consumers and whose unsuitability to certain subgroups within the population is managed through labelling statements.

With phytosterol-enriched spreads already on the market for several years, market research has shown that consumers who are sufficiently motivated to purchase these products have demonstrated that their use of them is informed and appropriate.

5.8.2 Long term safety

Several submitters including the New Zealand Dietetic Association, Rosemary Stanton, Joanne Dellow and Queensland Health express concerns about the long term safety of phytosterols, particularly with respect to the nutritional effects, and where non-target population groups consume phytosterol enriched products.

5.8.2.1 Response

Phytosterol-esters have been assessed as safe when consumed at levels up to approximately 10 g per day. Although the presence of phytosterols in the diet affects uptake of certain fat-soluble nutrients to some extent, the most significant observed effect is a reduction in β-carotene levels (see Attachment 4). Even for this nutrient, the reduced levels are within the normal range, which is naturally broad because of individual variation due to the influence of genetic, physiological and environmental factors. For example, the bioavailability of fat-soluble nutrients can be adversely affected by a range of variables in the diet itself, including consumption of low-fat foods, the source of the nutrient (eg. whether fruit or vegetable), and whether the food has been cooked or is eaten raw.

While there are other suggested functions for carotenoids, the only generally accepted function is that of precursors of vitamin A (retinol) for β-carotene, α-carotene and β-cryptoxanthin. However, the CSIRO clinical studies indicate that retinol levels in consumers of phytosterol esters at levels up to 10.7 g/day are unaffected, remaining within the normal range.

The concerns about long-term effects generally focus on the carotenoids and especially on the potential role of antioxidant activity in health and disease. Most hypotheses on the beneficial effects of carotenoids arose from studies in animals and epidemiological studies. Although many carotenoids exert antioxidant activity under specific conditions *in vitro*, the relevance of this activity *in vivo* to the prevention of disease is as yet unknown.

Classes of carotenoids are absorbed and metabolised differently by the body and among different animal species. The diversity in structural and geometric isomers, each with differing physicochemical properties, also makes it extremely difficult to uncover a mechanism of action for a single carotenoid *in vivo* and relate it to a potential role in disease.

The risk management strategies presented in this report are intended to minimise habitual consumption by consumers not in the target group. On the basis of the safety assessment and nutrition report, occasional consumption would be considered to have no adverse effects on the health of any consumer group, including children. Furthermore, post-launch monitoring of products in Europe (conducted by industry) indicates that consumption of phytosterol-enriched foods is predominantly as intended by the manufacturers i.e. by consumers in the target age group. This information also indicates that consumption is below the expected or anticipated levels.

5.8.3 Nature of phytosterol enriched foods

Richard and Valerie James contend that phytosterols in staple consumer items such as milk and yoghurt are not appropriate because these foods are consumed by a broad section of the population including children and pregnant women. There is a claim that the type of products under assessment are therefore unsuitable because there is conflict between established food consumption patterns and the mandatory statements that are required on current phytosterol enriched products (edible oil spreads) advising against consumption by children and pregnant women.

5.8.3.1 Response

The food industry has generated a broad and varied range of dairy products over recent times, each targeted to various sectors of consumers or a niche market. Dairy foods currently available include a range of modifications to the fat content from enrichment to almost complete removal, and calcium enriched versions. Given such a varied product range, not all of the available products are suitable or appropriate for all consumers. For example, low-fat versions of milk and yoghurt are not suitable for young children unless on the advice of a health professional. At the other end of the spectrum, milks supplemented with additional cream are unsuitable for consumers on fat or calorie restricted diets, or those simply seeking to reduce their intake of saturated fats. Similar options are available within a range of probiotic yoghurt products.

There is no evidence that consumers are confused or overwhelmed by the variety of choices available within the dairy product category. Rather, industry data indicate that consumers have responded positively to the availability of different versions of milk, cheese and yoghurt and purchase according to their personal preferences and requirements. In the case of phytosterol-enriched table spreads and margarines, this is also considered to be the case.

FSANZ considers that with the requirement for prominent labelling, consumers will be able to readily identify and discriminate between a phytosterol-enriched milk or yoghurt and the conventional forms. The mandatory *advisory* statements will be required to reinforce the appropriate use of the food, as they are already with other foods such as milk, and beverages made from soy or rice up to 2.5% w/w fat, unpasteurised egg products, or kola beverages containing added caffeine. A mandatory *warning* statement is reserved for foods that represent a significant health risk to certain individuals or groups, and therefore would not be warranted on phytosterol-enriched foods.

5.8.4 Extension of use to other foods

So Natural Foods (Australia) supports permission to use phytosterol esters as ingredients in low-fat milk and low-fat yoghurt and considers that this option ought to extend to the legume-derived analogues that are consumed as alternatives to dairy products. The reasons include that as phytosterols are natural components of soy products, the addition of phytosterol esters is merely enhancing the existing nutritional profile. The company also claims that permission for use of phytosterols in certain dairy products without a corresponding permission in the non-dairy analogues would effectively discriminate against those consumers who are normally intolerant of dairy products by restricting the choices of phytosterol-containing foods.

They further claim that by expanding the available range of phytosterol-containing foods, consumers should be able to reach the required level of intake for the cholesterol-lowering benefit more readily, without altering their established eating patterns.

If consideration is not extended to the legume-derived products, it is claimed that manufacturers are likely to experience decreased market share in the novel foods arena, and potentially a reduced demand for their soy based products in particular, as well as having a reduced ability to develop products with a positive public health impact.

5.8.4.1 Response

From the data supplied to FSANZ and other literature in the public domain, it is apparent that the nature of the food matrix has some impact on the efficacy of phytosterols. The ability of phytosterol esters to reduce cholesterol absorption when present in low fat milk and low fat yoghurt is supported by the results of the CSIRO clinical studies supplied with the current application. These results ensure that statements made by manufacturers on the packaging of phytosterol-enriched products can be shown to be truthful. Although phytosterols occur naturally in certain soy products, no technical information has been provided to FSANZ on their efficacy in terms of lowering cholesterol absorption when present in higher amounts in soy foods. In addition, in order to consider extending the use of phytosterols into a separate category of foods such as soy based beverages and yoghurts, detailed information on proposed marketing strategies and likely consumption levels would also be required from the relevant manufacturers, to ensure that products were compatible with the target group of consumers.

Because of the existence of parallel permissions under Standard 1.3.2 Vitamins and Minerals for addition of certain vitamins and minerals to both dairy and legume-derived foods, it has been proposed that this assessment could be broadened to include both dairy and non-dairy categories of foods. This expansion would not be justified however as phytosterol esters are regarded as novel food ingredients in the Code, and are therefore subject to a specific regulatory process that is separate from the regulation of vitamins and minerals. An application for the use of phytosterol esters in legume-based beverages could be considered if supported by appropriate data.

5.8.5 Risks to consumers in the target age group

Richard and Valerie James raise the concern of the impact on consumers in the target group (over 40's) who have normal or low cholesterol level.

5.8.5.1 Response

It is envisaged that the phytosterol-enriched products under consideration if approved, would be easily distinguished from their non-enriched counterparts. Comprehensive labelling and a price premium are expected to act synergistically to discourage consumers who do not have a specific interest in the products. The scientific evidence indicates that consumers with normal cholesterol levels would not be exposed to additional health risks if phytosterol-enriched milk and yoghurt products were consumed.

In terms of lowering cholesterol, there are no adverse health outcomes in lowering LDL cholesterol levels in humans who do not have a pre-existing high LDL cholesterol level. In general terms, with respect to cardiovascular disease risk, any reduction in LDL-cholesterol is regarded as beneficial by the medical profession. Hypocholesterolemia only arises in certain situations where there is pre-existing liver disease, malabsorption, or genetic disorders, or through prescribed pharmacological agents. In otherwise healthy individuals, a modest reduction in LDL-cholesterol levels achievable through dietary means is generally regarded as a positive outcome.

5.8.6 Phytosterols and oestrogenic activity

Richard and Valerie James as well as the New Zealand Dietetic Association consider that phytosterols are potentially estrogenic, that is, they contribute to similar effects in the body that generally result from the activity of the female hormone oestrogen.

5.8.6.1 Response

This issue was also raised in relation to the assessment of Application A410 in 2000, when approval was subsequently given for the use of phytosterols in edible oil spreads and margarine. The response provided at that time stated that there is no experimental evidence from *in vitro* or *in vivo* studies that phytosterol esters disrupt hormonal activity. *In vitro* oestrogen binding studies indicate that phytosterols do not bind to the rat oestrogen receptor nor do they bind or activate the human oestrogen receptor. *In vivo* uterotrophic assays in immature rats did not produce any adverse clinical changes.

The European SCF has also recently reviewed this issue in some detail[2]. The Committee reports on several studies using fish, rats and the mustelid European polecat. While some studies found that, when used at high levels or when administered subcutaneously, plant sterols (especially sitosterol) might have oestrogenic activity, these findings were not consistently reported when re-investigated. The Committee concluded that additional studies, including a two-generation reproductive study in rats, provided sufficient reassurance of the absence of endocrine effects of phytosterols via the oral route.

5.8.7 Validation of efficacy

The Australian Food and Grocery Council (AFGC) contends that FSANZ is not justified in requiring that efficacy data verify with labelling statements used by manufacturers on the packaging of phytosterol-enriched products, because a health claim is not being considered in relation to phytosterols.

5.8.7.1 Response

Based on examination of current packaging for phytosterol-enriched edible oil spreads and margarines, and on information supplied with this application, manufacturers intend to differentiate foods with added phytosterols with such labelling statements as “With natural plant sterols which reduce cholesterol uptake” or “With plant-derived ingredients that lower cholesterol absorption”.

[2] 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 β-carotene. European Commission, Belgium, October 2002.

At the time of assessment of edible oil spreads, appropriate data were provided to show that phytosterol enrichment of these foods was associated with a cholesterol lowering effect. Further evidence was required to demonstrate that phytosterol esters could be similarly efficacious in lowering cholesterol absorption when they are added to other foods, such as low-fat milk and yoghurt. The availability of such evidence supports the use of the manufacturers statements concerning that food and its linkage with reduced cholesterol absorption.

These data requirements are entirely consistent with the second and third objectives set out in Section 10 of the FSANZ Act 1991. Specifically, FSANZ must ensure that *food regulatory measures provide consumers with adequate information relating to food to enable them to make informed choices* and, particularly relevant to this application, *prevent misleading or deceptive conduct*. Without the appropriate scientific evidence that phytosterol esters are efficacious in a variety of food matrices, the veracity of labelling statements could not be supported.

5.8.8 Addition of vitamins

The AFGC suggests that, in view of the potential effects on the absorption of certain fat-soluble nutrients arising from consumption of phytosterol-enriched foods, FSANZ ought to require the foods in question to contain a minimum quantity of fat-soluble vitamins either naturally occurring or by addition.

5.8.8.1 Response

A number of published studies and the clinical studies submitted with this application confirm that plant sterols can interfere with the absorption of fat-soluble nutrients such as carotenoids, at the same time as inhibiting absorption of dietary cholesterol. The nutritional effects are almost certainly related to the mechanism of action of plant sterols in the intestine, which is thought to involve the exclusion of cholesterol from micelles. The decreased absorption of nutrients dependent on dietary fat for uptake is therefore a secondary physiological effect, the consequence of reduced cholesterol absorption.

Some studies report that increased consumption of fruits and vegetables can partially compensate for the lower absorption of fat-soluble nutrients. While levels of substances such as lycopene and α-carotene are partially restored by fruit and vegetable consumption, the studies suggest that β-carotene levels do not respond to the same extent, most likely because of the greater hydrophobicity of the β-carotene molecule compared with the other carotenoids. Consequently the physico-chemical properties of β-carotene are a barrier to its uptake in a cholesterol-depleted environment. There is no available evidence to demonstrate that additional intake through fortification is likely to restore levels of β-carotene.

In addition, FSANZ has previously assessed the issue of voluntary fortification of foods with vitamins and minerals as part of Application A424 – Fortification of foods with calcium. That assessment found the eligibility of particular vitamins or minerals to be permitted for voluntary fortification in new foods would be considered only in cases where the population group intake of that vitamin or mineral is assessed as inadequate. In the case of carotene forms of vitamin A, dietary estimate for both Australia and New Zealand indicates that the estimated mean daily intake for consumers in the target age group is approximately 1.6 times the recommended dietary intake (RDI).

Of particular note, the modelling also shows that children (2 to 11 years of age) have a mean dietary intake between approximately 1.8 and 2.4 times the RDI (Australia only data). Fortification of phytosterol-enriched foods with β-carotene would therefore not be compatible with these criteria.

5.8.9 Relevant specifications for phytosterol esters

Unilever Australasia claims that the current specifications in Standard 1.3.4 for phytosterol esters derived from vegetable oils were submitted in 1999 for Application A410, and were restrictive. The company now suggests that the specifications be revised in line with those being considered for use as novel foods by other regulatory agencies, to ensure an international market for these products. Based on newer analytical methods, Unilever claims that more generic specifications would be preferable, which only include those components that have been demonstrated to affect the safety and/or efficacy of the foods.

5.8.9.1 Response

The applicants for the current phytosterol assessments have not requested a change to the technical specifications, but rather have indicated that they can comply with those that already exist. FSANZ compared the existing specifications with those suggested by Unilever, and found differences that would require further consideration. For example, it would need to be demonstrated that phytosterol esters matching the suggested generic specifications would be equivalent in terms of safety to those meeting the current specifications.

6. Regulatory Options

6.1 Option 1 – do not permit the use of phytosterol esters in low-fat milk and low-fat yoghurt

This option maintains the status quo by not permitting the use of phytosterol esters in low-fat milk and low-fat yoghurt, while retaining the current permission on the use of phytosterols in edible oil spreads and margarines only.

6.2 Option 2 – approve the use of phytosterol esters in low-fat milk and low-fat yoghurt

This option will result in an amendment to the Code to permit the addition of phytosterol esters at specified levels to a low-fat milk and a low-fat yoghurt product under Standard 1.5.1.

6.3 Option 3 – approve the general use of phytosterol esters

This option will result in an amendment to the Code to permit the use of phytosterol esters as ingredients in foods to a maximum permitted level.

7. Impact Analysis

7.1 Affected parties

- consumers, especially target population groups such as adults over 40 years of age with health concerns about high blood cholesterol, and non-target population groups such as children and pregnant or lactating women;

- dietitians and allied health professionals providing dietary advice to consumers;

- the manufacturing and retail sectors of the food industry; and

- Government generally, where a regulatory decision may impact on trade or WTO obligations, and State, Territory and New Zealand enforcement agencies.

7.2 Impact Analysis

In the course of developing food regulatory measures suitable for adoption in Australia and New Zealand, FSANZ is required to consider the impact of all options on all sectors of the community, including consumers, the food industry and governments in both countries. The regulatory impact assessment identifies and evaluates, though is not limited to, the costs and benefits of the proposed regulation, including the likely health, economic and social impacts.

The following analysis of the costs and benefits of the identified regulatory options is based on an assessment of the information supplied by the applicant and from public submissions to FSANZ, as well as knowledge from the previous considerations relating to the use of phytosterol esters in the food supply. The relative costs and benefits may be different for sub-groups of consumers.

7.2.1 Option 1

Option 1 provides no major benefits to consumers. As permission to use phytosterol esters would apply only to edible oil spreads and margarines, there may be a limited benefit in terms of reducing the possibility of excessive intakes of phytosterol containing foods.

Option 1 represents a potential cost to adult consumers in terms of restricting the choice and availability of phytosterol-enriched food products, particularly for those who are actively seeking dietary measures to reduce the risk of cardiovascular disease, or who find the current edible oils spreads and margarines unsuitable. Similarly, there is an identifiable cost to the food industry in terms of a restriction to product range, and limiting associated marketing opportunities.

There would be no immediate impact on government if the status quo is maintained.

7.2.2 Option 2

Option 2 represents potential benefits to consumers especially in the target group in terms of access to a greater range of phytosterol-enriched food products. Dietitians and other health professionals may also benefit from a broader permission to use phytosterols in foods because of the availability of products that may be more compatible with individual needs and general ‘healthy diet’ messages to the public. The costs to consumers not in the target group is likely to be minimal, because of the mandatory labelling statements that would be required on phytosterol enriched low-fat milk and yoghurt products.

Option 2 also provides potential benefits to food manufacturers in terms of increased product range and potential greater market share. In addition, there are potential marketing opportunities for food retailers.

In terms of a potential impact on Government, Option 2 is unlikely to have any significant impact on monitoring resources, as there is already a permission for use of phytosterols in edible oil spreads and margarines.

7.2.3 Option 3

In terms of the target consumer group, option 3 would provide the greatest range of phytosterol ester-enriched products. At the same time, a broad permission would be likely to increase the possibility of over-consumption by the target group, and inappropriate consumption by non-target consumer groups, because of the likely significant expansion of products (based on information from overseas applications) and a significantly increased presence in the marketplace of products enriched with plant sterols. Furthermore, increased intake of phytosterols is not associated with an increased benefit in terms of lowering cholesterol, and therefore option 3 may be confusing or misleading for consumers, especially in relation to the amount of phytosterol enriched products required to achieve the optimal cholesterol-lowering effect. The risk management strategies proposed in this application may not be as successful where the range of available products is unrestricted, and therefore the costs would be more likely to apply to consumers in general, rather than only to non-target groups.

Option 3 provides benefits to the food industry in that it represents the least restrictive regulation of phytosterols in the food supply, allowing the greatest innovation of products (within inherent technological limitations). However, there is likely also to be costs to manufacturers associated with a broader approval in terms of increased competition in the marketplace for products that are aimed only at a defined group of consumers, albeit a group that is likely to grow in size as the population ages. Thus, by definition, the potential market is smaller than for products aimed at a more general market. In addition, as phytosterol esters are costly ingredients to produce, a broad product range directed at a limited consumer market may mean that Option 3 is likely to be less viable economically for manufacturers.

In terms of government resources, unrestricted use of phytosterol esters in foods would broaden enforcement requirements generally.

8. Consultation

8.1 Public consultation

8.1.1 Initial Assessment

FSANZ received sixteen submissions in response to the Initial Assessment Report. Seven of the submitters support approval for the use of phytosterol esters in low-fat milk and low-fat yoghurt, subject to the assessment of safety, nutrition and dietary exposure at Draft Assessment. Of those in favour, several supported Option 3, a general permission to use phytosterol esters in foods. Submitters in favour included the food industry, the Australian Dairy Corporation, food technologists, and the Dietitians Association of Australia.

Six submissions were opposed to the application, primarily because of safety or nutritional concerns. Submitters not in favour of the application included Queensland Health, nutritionists, and the New Zealand Dietetics Association.

Three submitters deferred a decision until the Draft Assessment has been completed. A summary of submissions is at **Attachment 7**. Issues raised in submissions have been addressed in section 5 of this Report.

8.1.2 Draft assessment

FSANZ is now seeking comment in relation to this Draft Assessment Report. Comments received in response to this Report will be used to assist in the development of a Final Assessment Report.

Submitters are invited to provide comments in relation to:

- the issues discussed in section 5 of this Report; and
- regulatory options, and potential impacts in relation to these regulatory options.

8.2 World Trade Organization

As members of the World Trade Organization (WTO), Australia and New Zealand are obligated to notify WTO member nations where proposed mandatory regulatory measures are inconsistent with any existing or imminent international standards and the proposed measure may have a significant effect on trade. This enables other member countries of the WTO to make comment.

Amending the Code to permit the use of phytosterol esters derived from vegetable oils as novel food ingredients in low-fat milk and low-fat yoghurt will not be notified to the WTO under either the Technical Barrier to Trade (TBT) or Sanitary and Phytosanitary Measure (SPS) agreements, as the permission is unlikely to have a significant effect on international trade, particularly since FSANZ would be expanding an existing permission. There are no relevant international standards and the potential food uses of phytosterol esters under the proposed variation are limited in terms of market size. Because the products containing phytosterol esters may be more expensive, the products are likely to be targeted at consumers looking for foods with particular attributes.

8.3 Risk communication

The risk management options identified in this report will serve to alleviate concerns from stakeholders arising from a broader permission to use phytosterol esters in foods. FSANZ is proposing additional conditions of use and labelling statements to ensure that consumers get the message that consuming 2-3 serves/day is appropriate. The current risk management options (such as mandatory labelling requirements) address the issue of consumption by non-target groups.

The Applicant has also proposed additional strategies to increase consumer awareness on the use of the products. These include the establishment of a consumer information line to assist consumers with advice on the purchase and consumption of phytosterol-containing foods; advertising to be specific for the target audience; and the distribution of educational material to health professionals.

FSANZ does not propose any additional communication requirements to those discussed above. The Executive Summary will be used for public release, pending Board approval of this Draft Assessment. In addition, the Final Assessment Report would be available on the FSANZ website and relevant stakeholders and submitters would receive a copy of the assessment.

9. Conclusion and Recommendation

FSANZ recommends the approval of the use of phytosterol esters derived from vegetable oils in low-fat milk and low-fat yoghurt, subject to specified conditions of use, for the following reasons:

- there are no anticipated public health and safety concerns associated with the use of phytosterol esters derived from vegetable oils in low-fat milk and low-fat yoghurt when used in conjunction with the risk management measures proposed;

- there is evidence that phytosterol esters derived from vegetable oils can, following consumption, reduce levels of cholesterol in humans when incorporated into low-fat milk and low-fat yoghurt products;

- the nutrition assessment indicates that phytosterol esters derived from vegetable oils have no significant adverse nutritional effects at the proposed levels of use. The reductions in the absorption of fat-soluble nutrients (carotenoids and some fat-soluble vitamins) are within the normal variation which results from physiological and environmental factors;

- conditions of use, including an additional labelling statement, are proposed as part of a comprehensive risk management strategy to ensure appropriate use of phytosterol-containing foods by the target consumers, and to discourage use by non-target consumers;

- the proposed changes to the Code are consistent with the section 10 objectives of the FSANZ Act; and

- the Regulatory Impact Statement indicates that, for the preferred option, namely, to approve the use of phytosterol esters derived from vegetable oils as novel food ingredients in low-fat milk and low-fat yoghurt, the benefits of the proposed amendment outweigh the costs.

10. Implementation and review

Following the second consultation period for this document, the Final Assessment of the Application will be completed. Following the preparation of the Final Assessment Report and consideration by the FSANZ Board, a notification will be made to the Ministerial Council and it is anticipated that this will be completed by the end of 2004. The amendments to the Code with respect to Standard 1.5.1 – Novel Foods, and other relevant Standards, would come into effect shortly thereafter upon gazettal, subject to any request from the Ministerial Council for a review.

The existing stock-in-trade provisions allow a period of 12 months for industry to comply with the new labelling requirements for all phytosterol enriched foods.

ATTACHMENTS

1. Draft variations to the *Australia New Zealand Food Standards Code*
2. Food Technology Report
3. Safety Assessment Report
4. Nutrition Assessment Report
5. Dietary Exposure Assessment – Application A434
6. Dietary Exposure Assessment – Applications A433 & A434
7. Summary of issues raised in first round public submissions

ATTACHMENT 1

Draft Variations to the *Australia New Zealand Food Standards Code*

To commence: On gazettal

[1] ***Standard 1.5.1*** *of the Australia New Zealand Food Standards Code is varied by –*

[1.1] *inserting in* column 2 *of the* Table to clause 2 *corresponding to the entry for* Phytosterol esters –

May only be added to milk in accordance with Standard 2.5.1. May only be added to yoghurt in accordance with Standard 2.5.3.

[2] ***Standard 2.5.1*** *of the Australia New Zealand Food Standards Code is varied by –*

[2.1] *inserting after the* Editorial note *to clause 4 –*

5 Phytosterol Esters

(1) Phytosterol esters may only be added to milk –

(a) that contains no more than 1.5 g/100 g of milkfat; and
(b) that is supplied in a package, the capacity of which is no more than 1 litre; and
(c) where the total phytosterol ester added is 5.2 g/litre of milk.

[3] ***Standard 2.5.3*** *of the Australia New Zealand Food Standards Code is varied by –*

[3.1] *inserting after the Editorial note to clause 3 –*

4. Phytosterol Esters

(1) Phytosterol esters may only be added to yoghurt –

(a) other than drinking yoghurt; and
(b) that contains no more than 3 g total fat per 100 g; and
(c) that is supplied in a package, the capacity of which is no more than 140 g; and
(d) where the total phytosterol ester added is 1.3 g/140g of yoghurt.

ATTACHMENT 2

FOOD TECHNOLOGY REPORT

APPLICATIONS A433 AND A434 – PHYTOSTEROL ESTERS DERIVED FROM VEGETABLE OILS

Introduction

Phytosterols (plant sterols) are natural components of cereals, fruits, vegetables and edible vegetable oils such as sunflower seed oil and, as such, are natural constituents of the human diet.

Incorporation of free sterols into edible fats/oils is not readily achieved because of their insolubility, whereas sterols esterified to fatty acids are more fat soluble. In the intestine, most sterol esters are hydrolysed to free sterols as part of the normal digestive process. Plant stanols are the hydrogenated counterparts of the plant sterols but are less abundant in nature than the corresponding plant sterols. Consequently, the normal dietary intake of plant stanols is much less than that of plant sterols [1].

These applications are an extension to a previous application (A410), which resulted in the approval for the use of phytosterol esters, sourced from vegetable oils, in edible oil spreads and margarines to a maximum amount of 13.7%. The applicants are seeking to extend the use of phytosterol esters into new food matrices, namely breakfast cereals, low-fat milk and low-fat yoghurt products. The phytosterol esters under consideration in Applications A433 and A434 are identical to those previously assessed within A410.

Structure of plant sterols and stanols

Plant sterols have a role in plants similar to that of cholesterol in mammals, e.g. forming cell membrane structures. Plant sterols fall into one of three categories: 4-desmethylsterols (no methyl groups); 4-monomethylsterols (one methyl group) and 4,4-dimethylsterols (two methyl groups). The most common plant sterols are ß-sitosterol, campesterol and stigmasterol and structurally these are very similar to cholesterol, belonging to the class of 4-desmethylsterols (Fig. 1, reference 1).

Plant stanols belong to the group of 4-desmethylsterols. Plant stanols are hydrogenation products of the respective plant sterols, e.g. campestanol/campesterol and ß-sitostanol/ ß-sitosterol (Fig. 1), and are found in nature at very low levels.

When edible oils undergo normal refining, plant sterols are partially extracted together with some tocopherols (in the process of natural vitamin E production). It is estimated that 2500 tonnes of vegetable oil needs to be refined to produce 1 tonne of plant sterols [1]. Plant stanols are obtained by hydrogenation of the plant sterols. Another source of plant sterols is tall oil, derived from the process of paper production from wood and approximately 2500 tonnes of pine is required to produce 1 tonne of plant sterols. Tall oil also contains a higher proportion of plant stanols (primarily ß-sitostanol) than do vegetable oils [1].

In nature, plant sterols can be in the free form or predominantly esterified with long chain fatty acids or with phenolic acids as in rice bran oil (ferulates) and shea butter (cinnamates). In the intestine, most sterol esters are hydrolysed to free sterols as part of the normal digestive process [1]. Details provided in the applications and from comparable products internationally indicate that 1.3 g of the phytosterol esters is equivalent to 0.8 g of free phytosterols.

Production of phytosterol esters

Phytosterols are by-products from the traditional vegetable oil refining process. The crude vegetable oil is refined to remove solvents (if used in extraction), lecithins, free fatty acids, colour, and various off-odours and off-flavours. One of these refining steps is steam distillation (deodorisation) where the resulting distillate contains the phytosterol fraction. This fraction is further refined to remove fatty acids, lecithins and other compounds by fractional distillation, ethanolysis/transesterification, distillation and crystallisation from an organic solvent. The phytosterols are further purified by recrystallisation. These processes are considered standard methods traditionally used for the production of phytosterols.

The phytosterol esters are then produced from the phytosterols using food grade vegetable oil-derived fatty acids or triglycerides and applying standard methods for esterification or transesterification commonly used in the fats and oils industry [8].

Solubility

The solubility of phytosterols in edible oil products is relevant for other food matrices. The solubility of free sterols in oil is around 2 percent, while the solubility of sterol esters in oil exceeds 20 percent. Therefore, the free plant sterols are typically esterified with fatty acids from sunflower to improve solubility.

For foods such as milk, yoghurt and cereal, the esters are preferred to free phytosterols since they have improved solubility properties in oils, analogous to their solubility in edible oil spreads. For the dairy products, low-fat milk and low-fat yoghurts, the phytosterol esters are initially solubilised in a vegetable oil base which is then dispersed and homogenised into the milk, in a similar fashion to the production of low-fat milk. For breakfast cereal bars, the esters are directly added to the mixture as an ingredient during manufacture.

Phytosterol ester-enriched products are produced using the same processes and procedures as the corresponding conventional products. The additional processing step controls the amount and quality of the phytosterol esters incorporated into the product prior to further processing (including heat treatment).

The improved solubility of phytosterol esters creates a palatable product and is associated with more uniform distribution both in the product and in the gastrointestinal tract.

Stability

The physical and chemical properties of phytosterols are similar to cholesterol, since they differ only with respect to the side chain. Phytosterols and their fatty acid esters are basically very stable compounds and experience only limited damage during processing [3].

Phytosterols and phytosterol esters are known to be stable to both oxidation and heat, and remain unchanged during product processing, including the various pasteurisation treatments used to produce milk and yoghurt type products. The applicants state for the milk and yoghurt type products these treatments are:

- standard treatment for milk products (HTST – High Temperature Short Time pasteurisation) 76°C for 15 seconds
- high temperature treatment for extended shelf life milk products (UHT - Ultra High Temperature) 143°C for 4 seconds
- batch pasteurisation for yoghurt mix, 90°C for 15 minutes.

The chemical and microbial stability of the milk and yoghurt type products with added phytosterol esters have been found to be similar to standard products[5].

Specifications

Free sterols are obtained from the vegetable oil refining process where they are recovered from the steam distillate in the deodorisation process. All commercially available vegetable oil sterols are obtained by similar methods, and the esterification process is standard throughout the industry.

The specification for phytosterol esters derived from vegetable oils is the same as that given in the earlier application A410 and which is contained in Standard 1.3.4 – Identity and Purity of the *Australia New Zealand Food Standards Code* is as follows:

Specification for phytosterol esters derived from vegetable oils

Phytosterol esters are phytosterols derived from edible vegetable oils esterified with long-chain fatty acids derived from edible vegetable oils.

Phytosterol esters + free phytosterols (%)			min.	94	
Free phytosterols (%)			max.	10	
STERADIENES (%)			**MAX.**	**0.3**	
Fatty acid methylester (%)			max.	0.5	
Iron, Fe (ppm)			max.	1.0	
Copper, Cu (ppm)			max.	0.5	
Moisture (%)			max.	0.1	
Trans fatty acids (%)			max.	1.0	
Sterol profile (%) as below:					
Cholesterol		min.	0.0	max.	2.0
Brassicasterol		min.	0.0	max.	6.0
Campesterol		min.	20.0	max.	29.0
Campestanol		min.	0.0	max.	6.0
Stigmasterol		min.	12.0	max.	23.0
β-Sitosterol		min.	42.0	max.	55.0
β-Sitostanol		min.	0.0	max.	2.5
D5-Avenasterol		min.	0.0	max.	4.0
D7-Stigmastenol	min.	0.0	max.	2.0	
D7-Avenasterol		min.	0.0	max.	2.0
Other		min.	0.0	max.	6.0

References

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

4. Hicks, K.B. and Moreau, R.A. (2001) Phytosterols and Phytostanols: Functional Food Cholesterol Busters. Food Technology, 55, 63-67.

5. Lea, L. (July 2002) Phytosterol-Esters: Use in a Range of Food Products Volume 1 of 3. Unilever UK document D02-018 (application to the UK Food Standards Agency to include phytosterol esters to 'milk' and 'yoghurt' type products).

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

7. European Commission, Scientific Committee on Food. General view 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).

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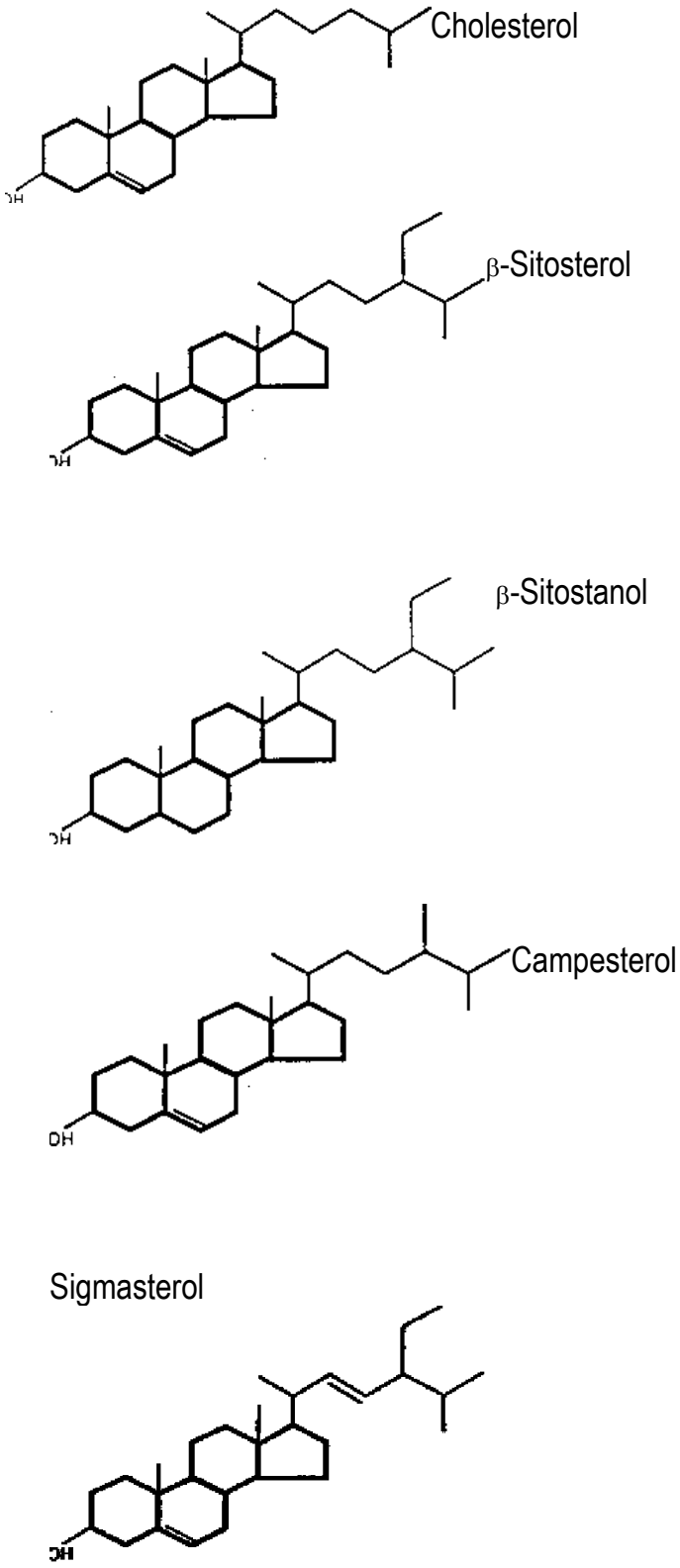

Fig. 1
Structure of cholesterol and some common phytosterols and phytostanols (taken from ref 1).

ATTACHMENT 3

SAFETY ASSESSMENT REPORT

APPLICATION A434 - PHYTOSTEROL ESTERS DERIVED FROM VEGETABLE OILS AS INGREDIENTS IN LOW-FAT MILK AND YOGHURT

1. Introduction

Dairy Farmers are seeking a variation to Standard 1.5.1 – Novel Foods - to permit the use of phytosterol esters derived from vegetable oils as novel food ingredients in low-fat milk and a low-fat yoghurt.

2. Previous safety consideration

Phytosterol esters are a novel food ingredient currently permitted for use in edible oil spreads and margarine up to a level of 13.7% (w/w). This is equivalent to 8% (w/w) free phytosterols. Phytosterols are naturally occurring plant compounds that have been reported to reduce blood cholesterol levels by inhibiting the absorption of dietary cholesterol. Phytosterols are naturally found in common vegetable oils and spreads at a level of 0.1 – 0.9% (w/w).
The permission to use phytosterol esters in table spreads and margarine was based on data and information on the safety of phytosterol esters assessed in 2001 within Application A410. The conclusions of the safety assessment report were:

1. The studies presented provide no evidence of adverse toxicological effects associated with consumption of phytosterol esters up to a level of 1.6 g free phytosterol/day over a l-year period, although the data do indicate a potential for phytosterols to reduce plasma levels of carotenoids in humans.

2. The data available do not allow the potential risk of carotenoid deficiency that may be associated with consumption of high levels of phytosterols to be assessed. Reduced carotenoid uptake is a potential risk for children and lactating women.

3. The available data indicate that plasma cholesterol levels are reduced by approximately 5% and LDL cholesterol levels by 7-8% when phytosterol esters are consumed in edible oil spreads at a level equivalent to 1.6 g free phytosterols/day over a 1-year period. There are no data available in relation to their effectiveness in this regard when present in other foods.

4. The data available to address the potential long-term effects of phytosterols in humans are limited. While there are 3-4 week studies at several dose levels, the 1-year human study has been conducted at only one dose level. Further studies at higher dose levels would provide more confidence in both the safety of this product and in its capacity to maintain long-term reductions in plasma cholesterol levels.

3. Cholesterol metabolism

Cholesterol is a natural component of animal cell membranes as well as being a precursor for the synthesis of the steroid hormones and bile acids. Both dietary cholesterol (mostly contained in egg yolks and animal fat) and that synthesized in the body (*de novo*) are transported through the circulation in lipoprotein particles, and stored in cells as cholesteryl esters. Both the synthesis and use of cholesterol is tightly regulated in order to prevent over-accumulation and abnormal deposition within the body. Deposition of cholesterol and cholesterol-rich lipoproteins in the coronary arteries is a major risk factor for disease, in particular atherosclerosis.

3.1 Biosynthesis of cholesterol

Slightly less than half of the cholesterol in the body derives from *de novo* biosynthesis. The liver accounts for approximately 10% and the intestine approximately 15% of the amount synthesized on a daily basis. Cholesterol is synthesized from acetyl-CoA, which is mainly produced in the mitochondria from fatty acid oxidation or from pyruvate and then transported to the cytoplasm. The biosynthetic pathway consists of four major steps to form squalene which then undergoes a two-step cyclisation to yield lanosterol which, in turn, is ultimately converted to cholesterol through a series of additional reactions.

3.2 Regulating cholesterol synthesis

The greatest proportion of cholesterol is used in bile acid synthesis. A relatively constant level of cholesterol in the body (150-200 mg/dL) is maintained by controlling the level of *de novo* synthesis. Normal healthy adults synthesise cholesterol at a rate of approximately 1 g/day and consume approximately 0.3 g/day. The level of cholesterol synthesis is regulated in part by the dietary intake of cholesterol and feedback inhibition of biosynthetic pathways. When dietary intake of cholesterol is high, hepatic cholesterol synthesis is decreased, and vice versa. However, the feedback compensation is incomplete, because a diet low in cholesterol and saturated fat leads to a modest decline in circulating blood cholesterol. The cellular supply of cholesterol is maintained at steady levels by several distinct mechanisms including the regulation of plasma cholesterol levels via LDL receptor-mediated uptake and HDL-mediated reverse transport.

3.3 Transport of cholesterol

Dietary cholesterol is transported from the small intestine via lymphatic ducts to the liver as chylomicrons. Cholesterol is transported in the plasma predominantly as cholesteryl esters associated with lipoproteins. Most of the cholesterol synthesized by the liver as well as any surplus dietary cholesterol in the liver is incorporated into very low density lipoprotein (VLDL) which eventually becomes low-density lipoprotein (LDL) in the circulation. LDLs provide cholesterol to the tissues to be utilized as an essential constituent in cell membranes and by gland cells to make steroid hormones. Cholesterol acquired from peripheral tissues is absorbed and transported as high-density lipoprotein (HDL).

3.4 Absorption of cholesterol

On a moderate fat intake, 95% or more of the ingested fat is absorbed. Cholesterol is readily absorbed from the small intestine if bile, fatty acids and pancreatic juice are present. Closely related sterols of plant origin are poorly absorbed. Almost all the absorbed cholesterol is incorporated into chylomicrons that enter the circulation through the lymphatics. Poorly absorbable plant sterols such as those found in soybeans reduce the absorption of cholesterol, probably by competing with cholesterol for esterification with fatty acids.

3.5 Cholesterol and bile metabolism

One of the predominant mechanisms for excretion of cholesterol is in the bile as free cholesterol, or as bile salts following conversion of excess amounts to bile acids in the liver. However, the excretion of cholesterol in the form of bile acids is insufficient to compensate for an excess of dietary intake of cholesterol. Bile acids are carried from the liver through the bile ducts to the gallbladder, where they are stored for future use. The ultimate fate of the bile acids is secretion into the intestine where they aid in the emulsification of dietary lipids by rendering fats accessible to pancreatic lipases, and facilitate the intestinal absorption of fat-soluble vitamins (vitamins A, D, E and K). Once in the gut, they undergo chemical modification and are either excreted in small amounts or reabsorbed by the gut and returned to the liver for recycling in the bile (enterohepatic circulation). The synthesis, and subsequent excretion of bile acids through the faeces represent the only significant mechanism for the elimination of excess cholesterol.

The entire bile salt pool of approximately 3.5 g recycles repeatedly via the enterohepatic circulation; it has been calculated that the entire pool recycles twice per meal and 6-8 times per day.

3.6 Fat digestion and absorption

Dietary fats are finely emulsified in the small intestine by the detergent action of the bile salts, lecithin and monoglycerides. When the concentration of bile salts in the intestine is high, as it is after contraction of the gall bladder, lipids and bile salts interact spontaneously to form micelles. Micellar formation further solubilises lipids and provides a mechanism for their transport to the enterocytes. Within the enterocytes, the lipids are rapidly esterified, maintaining a favourable concentration gradient from the intestinal lumen into the cells.

The fate of the fatty acids in enterocytes depends on their size. Fatty acids with less than 10-12 carbon atoms pass from the mucosal cells directly into the portal blood, where they are transported as free (unesterified) fatty acids. The fatty acids with more than 10-12 carbon atoms are re-esterified to triglycerides in the mucosal cells. In addition, some of the absorbed cholesterol is esterified. The triglycerides and cholesteryl esters are then coated with protein, cholesterol and phospholipids to form the chylomicrons which leave the cell and are transported to the liver.

3.7 Relation to atherosclerosis

Cholesterol is a major factor in the development of atherosclerosis which in turn predisposes to heart attack, cerebral thrombosis, and other major diseases. In individuals with elevated plasma cholesterol levels, there is an increased incidence of atherosclerosis and associated complications.

The normal range for plasma cholesterol is reportedly 120-220 mg/dL, but in men there is a clear, tight, positive correlation between the death rate from ischemic heart disease and plasma cholesterol levels above 180 mg/dL. There is now a consensus view that lowering plasma cholesterol slows the progression of atherosclerosis and may also inhibit thromboses, by reducing the risk of rupture of atherosclerotic plaques. Individuals with elevated LDL cholesterol have a higher than normal incidence of cardiovascular disease, whereas individuals with elevated HDL cholesterol have a lower incidence. In general, HDL cholesterol levels are higher in those who exercise or drink 1-2 alcoholic drinks per day, whereas they are decreased in those who smoke, are obese, or live sedentary lives.

4. Phytosterols in other foods

4.1 General safety issues

4.1.1 Effects of dietary phytosterols on blood cholesterol levels

Studies submitted:
P.M. Clifton, P.J. Nestel and D.R. Sullivan. . CSIRO Health Sciences and Nutrition (South Australia), Baker Medical Research Unit (Melbourne) and Royal Prince Alfred Hospital (Sydney), June 2002.
LDL Cholesterol Lowering with Phytosterol Ester-enriched Bread, Cereal, Milk and Yoghurt in a Multi-centre Trial (Study 1) and The Effect of Consuming Higher Dietary Intakes of Phytosterol Esters Over an Extended Period in Mildly Hypercholesterolaemic People (Study 2).
Study audits carried out by NHMRC Clinical Trials Centre, 14 November 2002.

<u>Study 1 – LDL cholesterol lowering with phytosterol ester-enriched bread, cereal, milk and yoghurt in a multi-centre trial.</u>

The objective of this study was to investigate whether consumption of certain phytosterol ester-enriched low fat foods leads to a reduction in serum cholesterol in mildly hypercholesterolaemic adults. The study was conducted at three centres, Melbourne, Adelaide and Sydney, and involved both women and men with slightly raised cholesterol levels. The subjects consumed specially prepared trial foods (bread, breakfast cereal, milk and yoghurt) containing specified amounts of phytosterols, and control foods not enriched with phytosterols, over a twelve week period. The trial foods provided the equivalent of 1.6 g per day of free phytosterols (equivalent to 2.6 g/day phytosterol esters).

Fifty-eight (35 females and 23 males) volunteer subjects were allocated to a diet in a randomized, incomplete crossover, single blind study consisting of four treatment periods of three weeks each, one of which was a control period. The subjects were selected on the basis of certain health criteria including

- Age 20-75 years
- Body Mass Index (BMI) < 31
- Total serum cholesterol >5.0 mmol/L and <7.5 mmol/L
- No lipid lowering medication
- No diabetes
- Normal thyroid status and no metabolic disorder other than hyperlipidaemia
- Not taking medications likely to affect lipid metabolism and no clinical requirement for such medication
- No history of metabolic disease
- Serum triglycerides <4.5 mmol/L

- No strong aversion and no known allergies/intolerances to the foods involved.

The study provided three active periods, in which one of the four phytosterol-enriched foods was tested per period, and one control period in which none of the four foods were enriched with phytosterol esters. During the three active periods all other test foods were supplied in their non-enriched form. An outline of the dietary regime managed by each study centre over the duration of the study is presented in Table 1.

Table 1. Arrangement of test and control diets in each study centre

	Adelaide (n = 22)	**Melbourne** (n = 18)	**Sydney** (n = 18)
Baseline (2 weeks)	Each subject's usual diet (baseline)		
Period 1 (3 weeks)	**Sterol – Yoghurt** Control – cereal, milk, bread	**Sterol – Milk** Control – cereal, bread, yoghurt	**Sterol – Yoghurt** Control – cereal, bread, milk
Period 2 (3 weeks)	**Control – All 4 foods**	**Sterol – Bread** Control – milk, cereal, yoghurt	**Sterol – Bread** Control – cereal, yoghurt, milk
Period 3 (3 weeks)	**Sterol – Cereal** Control – milk, yoghurt, bread	**Sterol – Cereal** Control – milk, bread, yoghurt	**Sterol – Cereal** Control – yoghurt, bread, milk
Period 4 (3 weeks)	**Sterol – Milk** Control – cereal, yoghurt, bread	**Control – All 4 foods**	**Control – All 4 foods**

Thus by food type, phytosterol-enriched yoghurt was tested twice (n=40), phytosterol-enriched milk was tested twice (n=40), phytosterol-enriched bread was tested twice (n=36), and phytosterol-enriched cereal was tested three times (n=58).

Food requirements

Serve sizes per day for each subject were yoghurt 200g, bread (white) 2 slices, cereal (muesli style) 45g, milk (2% fat, extended shelf life) 500 ml. The phytosterol enrichment of the test foods was such that each serve contained 1.6 g phytosterols. All subjects were requested to consume one serve of each food per day.

Food composition

Each of the control foods was analysed for relevant nutrient composition including energy, protein, fat (total, saturated, monounsaturated, polyunsaturated), carbohydrate (total and sugars), dietary fibre, calcium, sodium and potassium. The nutrient composition data presented for the test (phytosterol-enriched) foods were estimations based on calculations using the values for the control foods or other available data. The phytosterol content of the test foods was adjusted so that the equivalent of 1.6 g free phytosterols were available per serve per day.

The major sterol composition was beta-sitosterol, campesterol and stigmasterol, in descending order of abundance. All of the test foods were enriched with phytosterol esters, with levels expressed as the free sterol equivalent.

Monitoring and measurements

Serum lipids (total cholesterol, HDL cholesterol, triglycerides) were determined on two consecutive days at the end of each period (week 2, 5, 8, 11, 14) from venous blood samples taken after subjects fasted overnight (12 h). Plasma phytosterols were measured at the end of each period for the control, bread and milk periods in Melbourne, control and bread periods in Sydney and all periods in Adelaide. In addition, subjects were requested to complete a daily checklist of foods to monitor compliance during the periods and assess micronutrient intakes. Subjects were also provided the opportunity to report any adverse events throughout the course of the study.

Analyses

At the end of the study, all samples from each subject were analysed within the same experimental run in order to reduce reagent and instrument variation. Total cholesterol and triacylglycerols were measured using enzymatic kits and standard control sera, while plasma HDL cholesterol concentrations were measured using standard techniques. The LDL cholesterol concentration (mmol/L) in each sample was calculated from the values for total cholesterol, triacylglycerols and HDL cholesterol obtained in the laboratory analyses.

Plasma phytosterols were determined by gas chromatography and concentrations were calculated using an internal standard and reference samples of lathosterol, campesterol and sitosterol.

Results

The average age of the 58 subjects who completed the trial was 54 years. The average weight was 74 kg and there was an average gain of 0.9 kg ($p<0.01$) over the 12 weeks of the study. The food checklists indicated that dietary compliance averaged 96%, 94% and 99% respectively across the three study centres.

The effect of the phytosterol-enriched test foods on total cholesterol for each study period and centre is summarised in Table 2. The analytical results indicate that serum total cholesterol levels were lowered by phytosterol consumption in milk by 0.53 mmol/L (9.7%), and in yoghurt by 0.42 mmol/L (5.6%). Similarly, LDL cholesterol levels were lowered by phytosterol consumption in milk by 0.53 mmol/L (15.9%), and in yoghurt by 0.42 mmol/L (8.6%).

Table 2. Effect of test foods containing 1.6 g/day of phytosterols – Total cholesterol (mmol/L) at the end of each study period and at baseline (expressed as mean)

Centre	Baseline	Milk	Bread	Control	Cereal	Yoghurt
Melbourne (n=18)	6.16 (4.8-7.8)	5.90 (5.0-8.0)	6.38 (5.2-8.6)	6.50 (5.5-8.8)	6.36 (5.3-8.4)	ND
SD	± 0.83	± 0.74	± 0.81	± 0.86	± 0.80	
% change from control		-9.2	-1.8		-2.2	
Adelaide (n=22)	6.46 (4.8-8.2)	5.90 (4.7-8.0)	ND	6.60 (5.3-8.4)	6.29 (5.0-8.8)	6.23 (4.9-7.2)
SD	± 0.78	± 0.71		± 0.67	± 0.65	± 0.66
% change from control		-10.6			-4.7	-5.6
Sydney (n=18)	6.05 (5.3-7.2)	ND	5.78 (4.8-7.1)	6.14 (5.3-7.2)	6.00 (5.4-7.6)	5.80 (4.9-6.8)
SD	± 0.52		± 0.65	± 0.59	± 0.66	± 0.57
% change from control			-5.9		-2.3	-5.5
Combined	6.24	**5.90***	**6.08***	**6.43**	**6.23***	**6.04***
Total	58	40	36	58	58	40
SD	± 0.74	± 0.71	± 0.79	± 0.71	± 0.71	± 0.65
% change from control		-9.7	-3.9		-3.2	-5.6

* $p<0.05$ compared with control period
% change refers to the relevant control period in the 2 or 3 centres
ND = not done

The changes in serum lipids when phytosterol-containing cereal foods were consumed were similar, with LDL cholesterol levels reduced by 6.5% for bread and 5.4% for breakfast cereal. The changes in LDL cholesterol associated with phytosterol-enriched bread differed by a factor of two between study centres (i.e. 4.3% reduction in Melbourne vs. 9.6% reduction in Sydney), although the differences are not statistically significant. In the Adelaide and Sydney groups, there was no statistically significant difference between phytosterol-enriched bread, cereal and yoghurt in terms of lowering cholesterol.

Serum HDL cholesterol levels fell from baseline (established at the beginning of the study) to the control period by 0.05 mmol/L ($p<0.01$) which the study authors relate to the small mean weight gain (0.9 kg) observed over the duration of the study. HDL cholesterol levels rose significantly by 4.7% in the phytosterol-enriched bread period compared with control periods. When data from all study centres was combined, serum triglyceride levels did not change during the study. These results are summarised in Table 3.

Table 3. Effect of test foods containing 1.6 g/day of phytosterols – mean LDL cholesterol (mmol/L), HDL cholesterol (mmol/L) and Triglycerides (mmol/L), combined data from all centres

Combined	Baseline N=58	Milk N=40	Bread N=36	Control N=58	Cereal N=58	Yoghurt N=40
LDL cholesterol	4.03	**3.74***	**3.85***	4.27	**4.03***	**3.85***
SD	± 0.71	± 0.69	± 0.74	± 0.73	± 0.66	± 0.61
% change from control		- 15.9	- 6.5		- 5.4	- 8.6
HDL cholesterol	**1.50***	1.43	**1.50***	1.46	1.44	1.46
SD	± 0.41	± 0.32	± 0.40	± 0.37	± 0.42	± 0.41
% change from control		- 0.7	+ 4.7		+2.4	+1.2
Triglycerides	1.58	1.60	1.63	1.64	1.58	1.62
SD	± 0.73	± 0.63	± 0.87	± 0.77	± 0.85	± 0.83
% change from control		-9.7	-3.9		-3.2	-5.6

* $p<0.05$ compared with control period
% change refers to the relevant control period in the 2 or 3 centres

Absorption of phytosterols

The levels of phytosterols (campesterol and sitosterol) were measured in plasma for selected food periods in all three centers. Periods utilizing milk and bread as the active foods were selected due to their varying cholesterol-lowering effects. Measurement of plasma phytosterols indicates the availability for absorption of the phytosterols, without implying any correlation with their effects on serum lipids. Plasma lathosterol was also measured as an indicator of cholesterol synthesis.

Combining the information from all study centres, the results of these analyses show that plasma lathosterol levels did not change irrespective of the food period, whether active or control. At all centres, there were highly significant increases in the plasma levels of both campesterol (range of 24-52%) and sitosterol (range of 16-32%). Some data suggest that milk and bread apparently delivered similar amounts of phytosterols to the blood, but there was insufficient data to observe a pattern across all of the study centres. However, there was no relationship between the change in cholesterol levels and the change in levels of sitosterol and campesterol. Subjects who absorbed phytosterols well, did not appear to have a correspondingly better response to phytosterols than those who absorbed phytosterols poorly and/or had higher cholesterol synthesis (as assessed by lathosterol levels).

There are no findings reported in the study in relation to the monitoring of any adverse effects in any of the participants during the test-food periods.

Study 2 – The effect of consuming higher dietary intakes of phytosterol esters over an extended period in mildly hypercholesterolaemic people.

The objective of this study was to measure the effects on serum lipids, fat-soluble vitamins (vitamins A, D and E only), plasma phytosterols, plasma carotenoids and other physiological/biochemical parameters in humans of diets comprising a maximum of 6.6 g/day free phytosterols, when compared to a corresponding diet without phytosterol-enriched foods. The three test foods used in this study were phytosterol ester-enriched bread, breakfast cereal and table spread. The study also aimed to quantitatively investigate the effect on plasma carotenoids of additional dietary fruits and vegetables when co-consumed with foods delivering 6.6 g/day free phytosterols (equivalent to 10.7 g/day phytosterol esters).

Thirty-five mildly hypercholesterolaemic women and men were recruited into this study which was conducted over 16 weeks at two clinical research centres, one in Adelaide and one in Melbourne. As in the first study, subjects were required to comply with a specified list of health criteria. Ten subjects from the first study (Melbourne centre) continued into Study 2 after completing a control period and a 1-week gap between studies.

All subjects undertook dietary regimens in a non-randomised manner and were instructed not to consume self-purchased phytosterol-enriched products during the course of the study. The study was single blind and foods were appropriately coded. The dietary periods of the study are presented in Table 4.

Food requirements

Test and control foods were supplied by Goodman Fielder. Full compliance with the study diet was designed to contribute a total of approximately 6.6 g/day of phytosterols as follows:

- 3 x 5 g (15 g) reduced fat spread – 2.1 g/day
- 3 slices of white bread – 2.4 g/day, and
- 1 serve (60 g) of cereal (muesli) – 2.1 g/day

Table 4. Dietary regimens

Time period	Description
Weeks 1 & 2 - **Baseline** Control (2 weeks)	Usual diet plus **phytosterol-free** forms of test foods (bread, breakfast cereal and spread) at the same quantities as the next two periods.
Weeks 2-8 **Period 1** Sterol-enriched food (6 weeks)	Usual diet plus **phytosterol-enriched** bread, breakfast cereal and spread contributing 6.6 g/day phytosterols.
Weeks 9-14 **Period 2** Sterol-enriched food plus additional fruit and vegetables (6 weeks)	Usual diet plus **phytosterol-enriched** foods (as above) with **additional vegetable and/or fruit** intake.*
Weeks 15-16 **Period 3** Free living (sterol wash-out) (2 weeks)	Usual diet plus **phytosterol-free** forms of test foods at the same quantities as the previous two periods.

* Dietary advice was given to consume at least 5 serves of fruit/vegetables every day, with at least 1 serve of either pumpkin, sweet potato, carrot, tomato, apricot, broccoli, spinach (1 serve = half a cup).

In determining the food requirements for the study, the authors based the calculations on dietary intake estimates (DIAMOND program, FSANZ) from the 1995 Australian National Nutrition Survey and using the food categories of liquid milks, yoghurts, table spreads, breakfast cereals and plain breads, based on the fortification level of 0.8 g free phytosterols per serve. The mean Australian phytosterol intake from these categories was calculated to be 3.0 g/day, while the estimated intake at the 95th percentile was 6.8 g/day. For compliance reasons, the authors restricted the scope of the study to three phytosterol-enriched foods providing a daily free phytosterol intake of 6.6 g/day.

Food composition

The major nutrient composition of the three foods (bread, cereal and spread) and the phytosterol-enriched counterpart was determined either by analysis, calculation or from other sources of available data. It was noted that the control spread supplied only trace amounts of phytosterols per day (based on normal dietary intakes) that are naturally present in the oil. All test foods were enriched with phytosterol esters with the major sterols beta-sitosterol, campesterol, and stigmasterol in descending order of abundance.

Monitoring and measurements

Serum lipids (total cholesterol, HDL cholesterol, triglycerides) were determined on two consecutive days at the end of each period (week 2, 8, 14, 16). LDL cholesterol levels were calculated. Plasma carotenoids, plasma fat-soluble vitamins (A, D and E) and plasma phytosterols were measured at the end of each period (as above).

A range of physiological parameters were also measured as biochemical safety indicators of the higher daily phytosterol intake. These measurements included:

- full blood count
- routine biochemistry (electrolytes, glucose, urea and creatinine, calcium, phosphate, liver function test, clotting tests-prothrombin, partial thromboplastin test
- routine urinalysis (protein, blood, white cells, pH, bilirubin and glucose)

In addition, as in Study 1, subjects were requested to complete a daily checklist of foods to monitor compliance during the periods and assess micronutrient intakes. Weight and height of subjects were determined at entry to the study and weights were measured at each visit to the clinic. As before, subjects were also provided the opportunity to report any adverse events throughout the course of the study.

Analyses

Venous blood samples were collected from subjects that had fasted overnight (12 hrs). Total cholesterol, triacylglycerols, HDL and LDL cholesterol were all determined as previously described (see page 21).

Vitamins and carotenoids were extracted and analysed using HPLC techniques according to published methods. All samples from each subject were extracted in duplicate and analysed in one run to minimize experimental variation. Reference standards were trans α- and β-carotene, lycopene, lutein, retinal, α-tocopherol and α-tocopherol acetate.

As in the first study, plasma phytosterols were determined by gas chromatography and concentrations were calculated using an internal standard and reference samples of lathosterol, campesterol and sitosterol.

Results

All 35 subjects (23 females and 12 males) completed the 16 weeks of the study. The average age of the subjects in Adelaide was 53.3 years and there was a mean increase in weight of 0.36 kg over the duration of the study. In Melbourne, the average age was 59.7 years and weight fell by a mean 0.09 kg over the 16 weeks period.

The authors report that compliance with the dietary regimes was above 95% for all periods, except for the washout period (Period 3) at one study centre, where it fell to around 70%. Compliance with the additional fruit and vegetable intake (total of 5 per day) in Period 2 at the two study centers was 83% and 86% respectively.

There were no significant changes in total dietary fat, saturated fat, or energy between the periods in the study. Beta-carotene intake increased by 41% in Melbourne (p=0.001) and fibre intake also increased by 2 g/day (p=0.04) from period 1 to period 2. Similarly, in the Adelaide group, β-carotene intake increased by 23% (p=0.023) and fibre intake increased by 3.3 g/day (p=0.002) from period 1 to period 2.

The effects of higher daily consumption of phytosterol-enriched foods (containing 6.6 g/day in this study compared to 1.6 g/day in Study 1) on serum lipids (total cholesterol, triglycerides, HDL and LDL cholesterol) are presented in Table 5.

Table 5 Effect of diets containing 6.6 g/day phytosterols on serum lipids (mmol/L), combined data from both study centres (Mean ±SD)

	Baseline period (2 weeks)	**Period 1** (6 weeks)	**Period 2** (6 weeks)	**Period 3** (washout-2 weeks)
Total cholesterol	6.59 ± 1.01	6.04 ± 0.73*	6.03 ± 0.84*	6.42 ± 0.97
% change		**–8.3**	**–8.5**	
HDL cholesterol	1.35 ± 0.38	1.38 ± 0.38	1.40 ± 0.40	1.39 ± 0.41
Triglycerides	1.81 ± 0.96	1.71 ± 0.87	1.67 ± 0.93	1.62 ± 0.99
LDL cholesterol	4.46 ± 0.91	3.95 ± 0.80*	3.88 ± 0.70*	4.31 ± 0.91
% change		**–11.4**	**–13.0**	

* Values are significantly different from relevant baseline and washout values ($p<0.05$)

The (combined) serum lipids results in this study indicate a reduction in total cholesterol (down approximately 8%) and LDL cholesterol (down approximately 12%) following consumption of foods containing 6.6 g/day of phytosterols over a period of 12 weeks.

This level of cholesterol reduction was measured at the end of period 1, after 6 weeks of consuming phytosterol-enriched bread, cereal and table spread on a daily basis, and was maintained for the test period. At the conclusion of the study, after a 2 weeks washout period in which no phytosterol-enriched products were consumed, serum lipids were not significantly different from the baseline measurements at the beginning of the study, indicating that the cholesterol lowering effects are not maintained in the absence of the phytosterol-enriched foods.

Effects of food matrix

The magnitude of the cholesterol-lowering effect from consumption of 6.6 g/day phytosterols in enriched bread, cereal and table spread, is similar to the observed effect in Study 1 when subjects consumed 1.6 g/day phytosterols in low-fat milk. In addition, as the authors note, the magnitude of the effect is also similar to that reported in the literature in which intakes of 3.2 g/day of phytosterol or phytostanol esters were consumed in margarines (Westrate & Meijer, 1998). These results and those from other published studies suggest that there is an upper limit for efficacy in terms of the potential to lower serum cholesterol that is related to the food matrix in which the phytosterols are consumed. For example, where the food matrix is bread and cereal only, more than 1.6 g/day of phytosterols may be required to achieve a reduction in serum lipids similar to that which can be achieved when milk is used as the vehicle. However, data demonstrating that bread and cereal alone can achieve the effectiveness of low-fat milk (at 1.6 g/day) or margarine (at 3.2 g/day) are not presented here.

Plasma phytosterol levels

At both study centers, the increase in levels of campesterol and sitosterol in the blood after periods 1 and 2 when compared to baseline levels was highly significant. For campesterol, the increase was approximately 99% after period 1 and rose to approximately 110% at the end of period 2. Sitosterol levels also increased by approximately 40% at period 1 and by approximately 50% at period 2 compared to baseline levels. Plasma campesterol remained higher than baseline after two weeks of washout in both study centers, suggesting that the kinetics of the two compounds are different.

These changes in the levels of circulating phytosterols are similar to those reported in several other studies using phytosterol-enriched foods, and generally show a linear relationship to the levels of phytosterols in the diet. However, the absorption of phytosterols from the gut is still very low compared to the absorption of cholesterol.

The plasma lathosterol levels showed a significant increase (13%, $p=0.002$) after period 1 and approximately 16% increase after period 2 when compared to the starting levels, but fell significantly back to baseline in the washout period. A high lathosterol level is an index of high cholesterol synthesis and concomitantly low cholesterol absorption so that phytosterols would be expected to be less effective at lowering cholesterol absorption in subjects with both low cholesterol absorption and high cholesterol synthesis. The dynamics of the total cholesterol response therefore is dependent on numerous physical and physiological factors and subject to individual variation.

Biochemical analyses

Full routine biochemistry covering urea, creatinine, electrolytes, calcium and liver function tests were performed at all visits corresponding to the end of each period in the study. Changes in enzymes such as aspartate aminotransferase (AST) and alanine aminotransferase (ALT) can indicate liver stress and are expected to occur in a small number of subjects (about 3%) in large-scale trials using cholesterol-lowering statin drugs. A comparison was therefore considered of particular interest in this study.

Measured haematology parameters included: haemoglobin, red cell count, packed cell volume, mean cell volume, mean cell haemoglobin, mean cell haemoglobin concentration, red blood cell distribution width, platelet count, mean platelet volume, white cell count, neutrophils (%), absolute neutrophil count, lymphocytes (%), absolute lymphocyte count, monocytes (%), absolute monocyte count, eosinophils (%), absolute eosinophil count, basophils (%), absolute basophil count.

Coagulation parameters included: prothrombin time, ratio, activated kaolin partial thromboplastin time.

Biochemical parameters included: sodium, potassium, chloride, bicarbonate, glucose, urea, creatinine (blood), urate, phosphate, calcium, ionised calcium, albumin (blood), total protein (albumins and globulins), total bilirubin, gamma glutamyl transferase (GGT), alkaline phosphatase (ALP), alanine aminotransferase (ALT), L-aspartate aminotransferase (AST), lactate dehydrogenase (LD), creatine kinase (CK), magnesium, and 25-hydroxy vitamin D (the major circulating form of vitamin D).

Results

On combined haematological data from both centres (maximum number of 35 subjects), there were no changes in full blood count or clotting profile observed from baseline (after week 2) to the end of period 1 (at week 8), the end of period 2 (at week 14) and the end of period 3 (washout). The number of subjects that were outside of the normal range for any particular parameter was also recorded. These data indicate that generally a small number (1-3) of measurements were outside the expected range, but these were randomly spread across parameters and across the study periods and therefore no pattern of change was detected.

In the biochemical analyses, an increase in ALT values was noted in one subject for whom normal levels were initially recorded at baseline. The rise to double normal levels was only detected at 12 weeks, and disappeared upon withdrawal of phytosterols. In another subject, there was an increase in GGT and ALP levels, for whom abnormal levels were initially recorded at baseline. The authors note that GGT is not normally measured in drug trials, as the levels are very sensitive and are prone to change for no apparent reason. Furthermore, the change in ALT levels was considered inconsequential and did not occur at a frequency greater than expected from chance alone. There were no other changes in plasma biochemistry recorded at any period in the study.

Four subjects (out of 35) were found at baseline with vitamin D deficiency. However, no significant changes with ingestion of phytosterol-enriched foods were observed for the duration of the study.

Urine was tested with dipsticks in the 23 subjects at the Adelaide study centre. Seven subjects were found to have abnormal results at baseline. In general, there were a variety of abnormalities that appeared and disappeared throughout the study but none of these changes could be statistically related to phytosterol intake.

Plasma carotenoids and fat-soluble vitamins

Plasma carotenoids and plasma fat-soluble vitamins (A,D and E) were measured at the end of each study period at week 2, 8, 14 and 16. The combined results from both study centres for measurements of lutein, retinol, α-tocopherol, vitamin D, lycopene and α and β-carotene are presented in the Nutrition Safety Assessment at **Attachment 4**.

Conclusions from the clinical studies

The results of these studies indicate that consumption of any of the four test foods providing approximately 2.6 g/day phytosterol esters, can result in a measurable decrease in total cholesterol and LDL cholesterol in mildly hypercholesterolaemic people. The greatest cholesterol-lowering effect was measured when the test food vehicle was milk, followed by phytosterol-enriched yoghurt, with bread and cereal exhibiting the smallest effect. The results therefore suggest that phytosterol-enriched bread and breakfast cereals are less effective food vehicles for achieving a lower blood cholesterol level than phytosterol-enriched milk.

Consumption of all phytosterol-enriched foods tested in this study resulted in a statistically significant increase in the absorption of phytosterols compared to the control period, although the levels in plasma remained very low. These results are consistent with other published studies. The authors suggest that the results obtained in Adelaide, where plasma phytosterol levels increased more when milk was used as the vehicle, may indicate that milk-delivered phytosterols are more bioavailable.

As no treatment-related changes in biochemical parameters were detected, the results of the CSIRO studies indicate that phytosterol esters may be consumed safely in amounts up to approximately 10.7 g/day, when incorporated into foods such as low-fat milk, low-fat yoghurt, fibre-increased bread and breakfast cereal.

ATTACHMENT 4

NUTRITION ASSESSMENT REPORT

Application A433 Phytosterol esters in breakfast cereals
Application A434 Phytosterol esters in low-fat milk and low-fat yoghurt

1. Introduction

The aim of this review is to evaluate information on the potential nutritional effects of phytosterols in the diet arising from the proposed fortification of breakfast cereals and low-fat dairy products. This review forms part of the assessment of two applications submitted to Food Standards Australia New Zealand (FSANZ) requesting permission to add 1.3 g of phytosterol esters per serve to breakfast cereal bars, low-fat milk and low-fat yoghurt products.

Currently, only phytosterol-enriched edible oil spreads are available in both New Zealand and Australia, and are being promoted as foods that can lower cholesterol absorption.

This review considers data from recently conducted studies and other currently available information on the nutritional safety of plant sterols if consumed in a broader range of products such as the proposed low-fat milk, low-fat yoghurt and breakfast cereal. Unless otherwise stated, this report refers to phytosterol amounts in their esterified form.

2. Potential effect of phytosterols on antioxidant absorption

2.1 Sources and roles of antioxidants

Antioxidants are defined as substances that, when present at low concentrations compared with those of an oxidisable substrate, significantly prevent or delay oxidation of the substrate. This may mean that the presence of an antioxidant can inhibit or slow down a biological process involving an oxidation reaction. Dietary antioxidants may inhibit oxidative damage to proteins, lipids, carbohydrates and DNA *in vivo* which are of major interest in nutritional research.

Food-derived antioxidants, mostly from dietary plants, can exert a range of possible beneficial effects. This has been demonstrated clearly for α-tocopherol and vitamin C (Handbook of Antioxidants, Eds. E. Cadensas and L. Packer, 2002). Many hundreds of compounds present in food may act as antioxidants, in a variety of different ways depending on their particular physico-chemical properties. It is possible for an antioxidant to protect (against oxidation) in one biological or food system, but to fail to protect or even sometimes promote oxidative damage in others.

As well as some of the vitamins, a class of plant compounds known as the carotenoids (including α- and β-carotene, lycopene, β-cryptoxanthin, zeaxanthin, lutein) may function as antioxidants, although in most instances their antioxidant roles are not well-defined. Some of the carotenoids, α- and β-carotene and β-cryptoxanthin, are precursors of vitamin A.

As carotenoids are essentially hydrophobic molecules, the uptake of carotenoids in the intestinal mucosal cells is aided by the formation of bile acid micelles in the lumen of the small intestine. Plant sterols lower blood cholesterol by reducing the absorption of dietary and biliary cholesterol, and therefore might be associated with reduced absorption of some fat-soluble vitamins (such as vitamin E), and the lipophilic carotenoids (such as β-carotene). The assessment of the potential nutritional effects of phytosterol-enriched foods therefore focuses on the effects of plant sterols on the circulating levels of carotenoids and fat-soluble vitamins.

2.2 Vitamin A - Retinol

Vitamin A is a fat-soluble vitamin important for vision, immunity, growth and as an antioxidant. Vitamin A activity can be obtained from two classes of compounds – retinol and some carotenoids. The adult recommended dietary intake (RDI) of vitamin A is 750μg of retinol equivalents per day. The estimated average requirements (EAR) for vitamin A is 500μg for men and 400μg for women. Although plasma retinol concentrations are used as an indicator for vitamin A status, due to a homeostatic mechanism, they are insensitive and fall only in the later stages of deficiency. Vitamin A deficiency is common in developing countries, affecting vision with xerophthalmia and night blindness.

2.3 β-Carotene and other carotenoids

Carotenoids are the basic source of yellow, orange and red plant pigments, and are most commonly consumed as components of fruit and vegetables (Basu 2001). β-Carotene and other carotenoids are classified as either provitamin A or nonprovitamin A carotenoids. The provitamin A carotenoids (α-carotene, β-carotene and β-cryptoxanthin) can be converted into retinol. The function of these carotenoids includes antioxidant activity.

Non-provitamin A carotenoids such as lycopene, lutein and zeaxanthin have been suggested through observational studies to be inversely associated with some chronic diseases such as heart disease and cancer (Basu 2001).

2.3.1 Sources

The predominate dietary sources of carotenoids are fruits and vegetables. Sources of β-carotene include dark green leafy vegetables and yellow or orange fruits and vegetables including carrots, kale, silver beet, spinach, pumpkin/squash, sweet potato, apricots, mango and watermelon (Lister 2003).

2.3.2 Absorption

The carotenes are normally ingested in a food matrix, which is critical to their absorption. Their chemical structure, with a hydrocarbon backbone, renders them insoluble in water and they must be in the form of micelles in order to be absorbed in the intestinal tract. The presence of fat in the small intestine stimulates the secretion of bile from the gall bladder and increases the size of micelles, in turn facilitating the uptake of carotenes into the intestinal mucosa. Once in the mucosal cells, the carotenes are incorporated into chylomicrons for transport in the lymphatic system. The uptake of carotenes through the mucosal cells is via passive diffusion.

2.3.3 Bioavailability

The bioavailability of dietary carotenes depends on

i) digestion of the food matrix;
ii) formation of lipid micelles in the gastrointestinal tract;
iii) uptake of carotenoids by mucosal cells; and
iv) transport of carotenoids and their products to the lymph or portal circulation.

The source of carotenoids is also a factor in their bioavailability. Synthetic carotenoids (as dietary supplements) are absorbed far more readily than those that occur naturally in foods. Studies have indicated that up to 70% of synthetic carotenoids are absorbed compared with only 5% of naturally-occurring ingested carotenoids.

Bioavailability is optimized when dietary fat is consumed during the same period as the carotenoid. The processing and cooking of fruits and vegetables also affect bioavailability. Carotenoids are less available from raw than cooked fruits and vegetables, and processing techniques such as mechanical homogenization have also been shown to enhance the bioavailability of β-carotene (Cadenas 2002).

2.3.4 Contribution of β-carotene to Vitamin A intake

There are no known adverse health effects from consuming a diet low in carotenes provided that there is adequate retinol in the diet. The contribution from consumption of β-carotene equivalents[3] to vitamin A is about 50% in both Australia and New Zealand according to National Nutrition Surveys in both countries.

2.3.5 Seasonal variation

Fruits and vegetables are the main source of carotenoids in the diet. As might be expected from the seasonal nature of many fruits and vegetables, it has been observed that there is a concomitant seasonal variation in serum carotenoids (and thus retinol) levels in humans. This was confirmed in a study investigating seasonal variation in serum nutrient levels in 111 healthy individuals. The study reported significant differences ($p<0.05$) in serum concentrations of α-carotene, β-carotene and β-cryptoxanthin across a seasonal time scale, with both α and β-carotene levels higher in summer and β-cryptoxanthin levels higher in winter. Plasma β-carotene levels could vary naturally up to 50% between seasons (Omedilla 1994).

In addition to seasonal variation in β-carotene levels, weekly variation has also been observed in individuals. In a 12-week Australian study where consecutive blood samples were collected from 12 subjects, the intra-individual and inter-individual variation was 39 and 36 % respectively (Lux 1994). Following the initial study period, blood samples were taken monthly for the following six months showing a peak of plasma β-carotene in the months of spring.

[3] β-carotene equivalents = μg β-carotene + (0.5 μg other provitamin A carotenoids)

2.3.6 Carotenoids and chronic disease

Epidemiological studies have indicated that people with higher intakes of fruits and vegetables may have a reduced risk of heart disease, stroke or some cancers compared with those with lower intakes. With such apparent broad health benefits, research has focussed on the antioxidant components and properties of such diets. Recently, a study into the health benefits of citrus fruit[4] reported that many of the major diseases of concern in Australia and New Zealand have a dietary component. These include cardiovascular conditions such as atherosclerosis, heart disease and stroke, cancers, obesity, dental caries, asthma, periodontal disease, type-2 diabetes, osteoporosis, cataracts and many others. Reductions in the incidence of chronic disease associated with the consumption of citrus fruits for example are thought to be attributable to an array of biologically active substances in fruits including vitamin C, folic acid, carotenoids, dietary fibre, potassium, selenium and a range of other phytochemicals.

Despite the epidemiological evidence regarding the benefits of fruits and vegetables, randomised controlled trials indicate that β-carotene and vitamin E when taken as food supplements have no beneficial effects in the prevention of heart disease, and may result in a small increase in the incidence of lung cancer in the group supplemented with β-carotene (Lee 1999, Eichholzer 2001, Asplund 2002).

In general, due to the complexity of nutrients and non-nutrients in fruits and vegetables, it has not been possible to attribute the protective effects to any single nutrient or class of nutrients. Rather, and notwithstanding genetic diversity in the population, any health benefits are associated with consuming a diet that is rich in fruits and vegetables, possibly in combination with a range of other 'healthy' lifestyle choices, such as avoiding smoking and engaging in regular exercise. Nevertheless, plant compounds with antioxidant activity, such as β-carotene, are currently the focus of further scientific attention to more broadly examine potential physiological effects.

2.4 Vitamin E

There are eight naturally occurring forms of vitamin E in plants: four tocopherol and four tocotrienols. The abundance and bioavailability of each form of natural vitamin E varies considerably. Vitamin E can also be synthesised chemically. Vitamin E is a powerful antioxidant; it plays an essential role in the protection of cell membranes and plasma lipoproteins from free radical damage.

2.4.1 Sources

The major food sources of vitamin E include broccoli, dark leafy vegetables, avocado, kiwi fruit along with cold pressed vegetable oils, nuts, seeds, soy beans, wheatgerm and wholegrains (Lister 2003).

[4] The Health Benefits of Citrus Fruits, Report to Horticulture Australia Ltd Project No: CT01037, Dr. Katrine Baghurst, Consumer Science Program, CSIRO Health Sciences & Nutrition, June 2003.

2.4.2 Bioavailability

Vitamin E is a fat-soluble vitamin. Its absorption in the small intestine is enhanced by the presence of fat, causing an increase in the formation of micelles required to absorb vitamin E into the mucosal cells lining the small intestine. Once in the mucosal cells, Vitamin E is incorporated into chylomicrons and enters the circulation via the lymphatic system.

2.4.3 Deficiency

Vitamin E deficiency is rare in humans, as is toxicity (Institute of Medicine 2000). Due to the protective effect on LDL oxidation, a serum tocopherol/cholesterol ratio of 2.25μmol/mmol is thought to be the lowest satisfactory serum value for oxidative protection. The RDIs for vitamin E of 10 mg/day and 7 mg/day tocopherol equivalents for men and women respectively are based on this ratio.

3. The nutritional effects of phytosterol ingestion in the target consumer group

3.1 Recommended serum cholesterol levels

The National Heart Foundations of both Australia and New Zealand recommend that people attempt to keep their individual total serum cholesterol level below 4 mmol/L to reduce the risk of heart disease. The Australian Institute of Health and Welfare (AIHW) state that individual total blood cholesterol levels above 5.5 mmol/L are an indication of a greatly increased risk of developing heart disease and that levels above 6.5 mmol/L are considered to indicate extremely high risk.

It is suggested by the New Zealand Guidelines Group that doctors classify individuals by risk according to age, blood pressure, smoking and diabetes status. Those classified as high-risk, with a total serum cholesterol level greater than 5.5 mmol/L, be recommended for 6-12 weeks of dietary intervention, before being considered for treatment with appropriate medication: dietary intervention should be continued indefinitely[5].

Phytosterol ester-enriched foods are primarily targeted to consumers over 40 years of age with concerns about a mildly elevated blood cholesterol measurement. Due to the direct link with diet, mild hypercholesterolaemia may be adequately addressed by strategic changes to the diet such as selectively choosing low-fat versions of staple foods, by using products containing plant sterols (currently edible oil spreads and margarines), and/or by increasing relative consumption of fruits and vegetables.

3.2 Studies on the effects of phytosterol ester-enriched foods

The applicants have submitted two studies undertaken by CSIRO Health Sciences and Nutrition to investigate (a) the efficacy of phytosterol esters in a variety of food matrices (Study 1), and (b) the effects of high intakes (10.7 g/day phytosterol esters) on nutritional, blood lipid and biochemical parameters (Study 2).

[5] www.nzgg.org.nz

This nutritional assessment focuses primarily on the results and information provided by Study 2, together with some additional data from Study 1. A detailed assessment of the data provided by Study 1 is presented elsewhere in this report (see Attachment 3).

Submitted studies

Study 1 LDL Cholesterol Lowering with Phytosterol Ester-Enriched Bread, Cereal, Milk and Yoghurt in a Multi-Centre Trial. P.M. Clifton, P.J. Nestel and D.R. Sullivan, CSIRO Health Sciences & Nutrition, 2002.

Study 2 The Effect of Consuming Higher Dietary Intakes of Phytosterol-esters Over an Extended Period in Mildly Hypercholesterolaemic People. P.M. Clifton, P.J. Nestel and D.R. Sullivan, CSIRO Health Sciences & Nutrition, 2002.

3.2.1 Objective and methodology

The objective of Study 2 was to measure effects on serum lipids, fat-soluble vitamins (vitamins A, D and E only), plasma carotenoids, plasma phytosterols, and other physiological/biochemical parameters in free living humans provided with specific phytosterol-fortified foods providing 6.6 g/day free phytosterols (equivalent to 10.7 g/day phytosterol esters). Three test foods were used in this study: phytosterol ester-enriched bread, breakfast cereal and table spread, as well as a matched diet with no added phytosterols (as a control). The study also aimed to investigate any nutritional effects, particularly on plasma carotenoid levels, of additional dietary fruits and vegetables when co-consumed with the test foods.

Thirty-five mildly hypercholesterolaemic (cholesterol levels 5.0 – 7.5 mmol/l) women and men were recruited into this study which was conducted over 16 weeks at two clinical research centres, one in Adelaide and one in Melbourne. All subjects undertook dietary regimens in a non-randomised manner and were instructed not to consume self-purchased phytosterol-enriched products during the course of the study. The study was single blind and foods were appropriately coded. The dietary periods of the study are presented in Table 1.

Table 1. Dietary regimen (Study 2)

Time period	Description
Weeks 1 & 2 - **Baseline** Control (2 weeks)	Usual diet plus **phytosterol-free** forms of test foods (bread, breakfast cereal and spread) at the same quantities as the next two periods.
Weeks 2-8 **Period 1** Sterol-enriched food (6 weeks)	Usual diet plus **phytosterol-enriched** bread, breakfast cereal and spread contributing 6.6 g/day free phytosterols.
Weeks 9-14 **Period 2** Sterol-enriched food plus additional fruit and vegetables (6 weeks)	Usual diet plus **phytosterol-enriched** foods (as above) with **additional vegetable and/or fruit** intake.*
Weeks 15-16 **Period 3** Free living (sterol wash-out) (2 weeks)	Usual diet plus **phytosterol-free** forms of test foods in the same quantities as the previous two periods.

* Dietary advice was given to consume at least 5 serves of fruit/vegetables every day, with at least 1 serve of either pumpkin, sweet potato, carrot, tomato, apricot, broccoli, or spinach (1 serve = half a cup).

Serum lipids (total cholesterol, HDL cholesterol, triglycerides) were determined on two consecutive days at the end of each period (weeks 2, 8, 14, 16). LDL cholesterol levels were calculated. Plasma carotenoids, plasma fat-soluble vitamins (A, D and E) and plasma phytosterols were measured at the end of each period (as above).

Carotenoid levels 'adjusted' for LDL-cholesterol

In the analysis of results, changes in carotenoids and fat-soluble vitamins have been provided as adjusted and non-adjusted levels, on the assumption that:

1. the carotenoids are transported in the circulation within low density lipoprotein (LDL) carriers, and reduced LDL-cholesterol levels will naturally result in reduced levels of these substances; and
2. due to the antioxidant role of carotenoids in protecting LDL particles against oxidation, it is generally considered appropriate to consider the magnitude of change as a ratio to LDL-cholesterol.

3.2.2 Results

Dietary compliance was monitored using food frequency questionnaires and daily records of fruit and vegetable consumption. The authors report that compliance with the dietary regimen was above 95% for all periods, except for the washout period (Period 3) at one study centre, where it fell to around 70%. Compliance with the additional fruit and vegetable intake (total of 5 per day) in Period 2 at the two study centres was 83% and 86% respectively.

There were no significant changes in total dietary fat, saturated fat, or energy between the periods in the study. Intake of β-carotene increased by 41% in Melbourne ($p=0.001$) and fibre intake also increased by 2 g/day ($p=0.04$) from period 1 to period 2. In the Adelaide group, β-carotene intake increased by 23% ($p=0.023$) and fibre intake increased by 3.3 g/day ($p=0.002$) from period 1 to period 2.

The results of the analyses of fat soluble nutrients (adjusted for total cholesterol) from Study 2, are presented in Table 2.

Table 2 Mean levels (± SD) of plasma carotenoids and fat-soluble vitamins on a diet containing 6.6 g/day phytosterols, with and without additional dietary fruit and vegetables, combined data from both study centres (n=35). The levels are adjusted for total cholesterol (μmol/L/TC mmol/L)
FV = 5 daily serves fruits and vegetables.

Period	Lutein	α-tocopherol	Lycopene	α-carotene	β-carotene
Baseline	0.077^{1} ±0.034	6.03^{1} ±0.99	0.13^{1} ±0.06	0.024^{1} ±0.025	$0.105^{1,3}$ ±0.091
Period 1 (Phytosterol)	0.067^{2} ±0.03	5.85^{1} ±0.97	$0.12^{1,2}$ ±0.06	0.020^{2} ±0.014	0.082^{2} ±0.057
% change	**-14%**	**-3%**	**-11%**	**-23%**	**-26%**
Period 2 (Phytosterol + FV)	$0.073^{1,2}$ ±0.031	5.68^{2} ±0.84	0.11^{2} ±0.05	0.023^{1} ±0.013	$0.083^{2,3}$ ±0.051
% change	**-6%**	**-6%**	**-22%**	**-5%**	**-21%**
Period 3 Washout	0.075^{1} ±0.034	6.07^{1} ±1.22	$0.12^{1,2}$ ±0.05	0.023^{1} ±0.013	0.092^{1} ±0.059
% change	**-3%**	**0%**	**-11%**	**-4%**	**-13%**

Values with different superscripts within each column are significantly different ($p<0.05$) from each other.

There was a significant fall in plasma carotenoid levels measured during the first study period ($p<0.05$), with α-carotene and β-carotene reduced by 23% and 26% respectively across the combined data for all participants. At the end of the second study period, following consumption of additional fruit and vegetables, plasma α-carotene levels had increased significantly back to baseline values. Beta-carotene levels increased again only during the washout period when all phytosterol fortified foods were removed from the diet. Plasma lutein levels decreased by 14% during period 1, and increased again during period 2 with the daily consumption of additional fruits and vegetables, to levels consistent with the baseline and washout period. Plasma α-tocopherol levels were not affected by consumption of phytosterols, with measurements lower than baseline and washout only during the high fruit and vegetable intake period. Plasma lycopene levels were decreased by 11% during the first period which extended to 22% following the period of added fruit and vegetable consumption, with some recovery during the two-week washout period. Levels of plasma vitamin D did not change significantly during any of the study periods (data not shown).

3.2.3 Additional analyses from Study 1

The results of similar nutritional and biochemical investigations were also provided from Study 1 in which the daily consumption of phytosterol esters was 2.6 g/day from 3 different phytosterol enriched foods (either bread, breakfast cereal, low-fat milk or yoghurt) tested sequentially over a period of twelve weeks. As in the previous experimental design, plasma carotenoids and two fat-soluble vitamins (A and E only) were measured.

When data from both the Melbourne and Adelaide centres were combined (Table 3), only β-carotene levels were significantly decreased (by approximately 10%) by milk providing 1.6 g/day free phytosterols (equivalent to 2.6 g/day phytosterol esters). The reduction in α-carotene levels when adjusted for cholesterol was approximately 6%, which was not statistically significant. There was no change of nutritional significance in levels of lutein, retinol, vitamin E or lycopene.

Table 3 Effect of diets containing 1.6 g/d of phytosterols on absolute and adjusted plasma carotenoids (μmol/L) and fat-soluble vitamins (μmol/L) from Melbourne and Adelaide study centres combined (n=40), milk data versus the control only. (Mean ± SD, TC = total cholesterol)

Period	Lutein	Retinol	α-tocopherol	Lycopene	α-carotene	β-carotene
Control (TC 6.56)	0.44 ±0.23	2.34 ±0.43	37.4 ±9.6	0.65 ±0.37	0.13 ±0.08	0.56 ±0.44
Milk (TC 5.90)	0.41 ±0.21	2.35 ±0.39	34.5** ±5.9	0.62 ±0.37	0.11** ±0.06	0.45** ±0.30
% change	**-6.6%**	**+0.6%**	**-7.7%**	**-4.9%**	**-16.1%**	**-19.4%**
Adjusted control	0.067 ±0.035	0.36 ±0.08	5.71 ±1.37	0.10 ±0.05	0.02 ±0.012	0.084 ±0.066
Adjusted milk	0.070 ±0.037	0.40 ±0.08	5.88 ±0.99	0.10 ±0.06	0.019 ±0.010	0.076* ±0.053
% change	**+4%**	**+33.3%**	**+2.9%**	**+4.9%**	**-5.7%**	**-9.3%**

*p<0.05, **p<0.01

The additional results from Study 1 allow a comparison of data from one type of phytosterol-enriched test food separately and therefore provides some further insights into the physiological effects of phytosterol ingestion according to the food delivery matrix. The consumption of phytosterols in both milk and bread significantly lowered adjusted β-carotene levels by 14% and 8% respectively, despite the phytosterol-enriched bread failing to show a significant reduction in cholesterol (Melbourne data). This suggests that phytosterols in milk are more effective in the gut at interfering with both cholesterol and β-carotene absorption. However, the milk data obtained from the Adelaide centre does not show the same pattern. Adjusted β-carotene levels were not significantly lower than the controls, despite a decrease in LDL-c (14.4%) and total cholesterol. The authors conclude that it is therefore not inevitable that β-carotene levels will fall in combination with a cholesterol-lowering effect.

Effects of phytosterol intake

All measures of plasma carotenoids following free phytosterol intakes of 1.6 g/day and 6.6 g/day regardless of food source are compared in Table 4. With the exception of lycopene, where the adjusted level was not significantly different (p=0.07) from the control, all measurements showed a statistically significant reduction in carotenoids at the higher level of phytosterol intake (6.6 g/day). In addition, the data indicate that the reduction in plasma carotenoids is more pronounced at higher intakes of phytosterols. There was no effect of phytosterol intake level on the change in plasma α-tocopherol level (data not shown).

Table 4 Comparison of low (1.6 g/d) and high (6.6 g/d) phytosterol intakes on plasma carotenoids (μmol/L), combined from all test periods: milk and bread in Melbourne, bread in Sydney, milk in Adelaide (Mean ± SD)

Period	Lutein Low PS intake (n=76)	Lutein High PS intake (n=35)	Lyco-pene Low PS intake (n=76)	Lyco-pene High PS intake (n=35)	α-carotene Low PS intake (n=76)	α-carotene High PS intake (n=35)	β-carotene Low PS intake (n=76)	β-carotene High PS intake (n=35)
Baseline /Control	0.43^{1} ±0.22	0.50^{1} ±0.21	0.67 ±0.35	0.87 ±0.40	0.13^{1} ±0.09	0.15^{1} ±0.15	0.53^{1} ±0.41	0.69^{1} ±0.58
Phyto-sterol	0.41 ±0.21	0.40^{2} ±0.18	0.61 ±0.36	0.71 ±0.35	0.12^{2} ±0.08	0.12^{1} ±0.08	0.47^{2} ±0.36	0.49^{2} ±0.34
% change	**-4%**	**-22%**	**-7%**	**-19%**	**-7%**	**-23%**	**-13%**	**-30%**
Adjusted control	0.068 ±0.035	0.077^{1} ±0.034	0.10 ±0.05	0.13^{1} ±0.06	0.021 ±0.014	0.024^{1} ±0.025	0.083^{1} ±0.062	0.11^{1} ±0.09
Adjusted phyto-sterol	0.070 ±0.036	0.067^{2} ±0.030	0.10^{1} ±0.05	0.12^{1} ±0.06	0.020 ±0.015	0.020^{2} ±0.014	0.078^{2} ±0.062	0.082^{2} ±0.057
% change	**+3%**	**-14%**	**-1%**	**-11%**	**-1%**	**-17%**	**-7%**	**-26%**

Values with different superscripts are significantly different ($p<0.05$) from each other.

3.2.4 Results across both studies

Ten of the subjects participated in both Study 1 and Study 2 allowing a comparison of low intakes of phytosterols (1.6 g/day in milk and bread) with higher intakes (6.6 g/day in bread, cereal and spread) on plasma carotenoid levels in the same individuals (data not shown). The authors note that although the number of subjects was small, the results indicate that the reduction in β-carotene was approximately the same at both levels of phytosterol consumption, being in the order of 20% (adjusted for cholesterol).

These results also provide some indication of the effect of the food matrix on plasma cholesterol and carotenoid measurements, observing that 6.6 g/day of phytosterols in bread, cereal and margarine did not reduce adjusted plasma β-carotene any more than 1.6 g/day of phytosterols in milk, which was highly effective in lowering serum cholesterol.

3.2.5 Discussion of results

The decreases in plasma carotenoid levels recorded in Study 2 were consistent with the decreases observed from additional biochemical analyses in Study 1, in general showing some relationship with levels of intake of phytosterols and the nature of the food matrix. Thus, while phytosterol-enriched milk showed the greatest reduction in cholesterol absorption, it also resulted in lower plasma carotenoid levels. The reduction in plasma carotenoid levels with 6.6 g/day phytosterols (in bread, cereal and spread) was not different to that reported in the literature for lower levels (1.6 – 3.2 g/day) phytosterol consumption. Even 1 g/day of phytostanols has been reported to lower lipid-standardised (adjusted) β-carotene levels by 14.4% (Mensink, 2002). In general, a comparison of the data from the different study centres highlights the great variability in plasma β-carotene levels. The authors comment that carotenoid absorption is not a well-regulated process in humans and levels can fluctuate widely according to a variety of physiological and environmental factors.

Fruit and vegetable intake

The results from Study 2, where subjects were asked in the second period to consume 5 servings per day of fruits and vegetables, suggest that increased consumption of some carotenoid-rich fruit and vegetables does not completely restore plasma levels to baseline for all of the carotenoids examined. Lutein and α-carotene levels appeared to respond positively to additional fruits and vegetables in the diet in the presence of phytosterol-enriched foods. Lutein and lycopene were reduced with higher levels of phytosterol intake (6.6 g/day) but were not affected at lower intake (1.6 g/day) levels (Tables 3 & 4).

The results also indicate that the reduction in β-carotene levels with consumption of phytosterol-enriched foods in general was not compensated by additional fruits and vegetables in the diet. The authors noted a maximum fall in β-carotene of approximately 30% (unadjusted for cholesterol) in all groups regardless of the level of phytosterol intake. However, despite this effect, after 12 weeks of consumption of phytosterol-enriched foods, plasma β-carotene levels were still at levels associated with the lowest risk of all-cause mortality in US adults, according to epidemiological studies cited in the Institute of Medicine Dietary Reference Intakes (2). Furthermore, retinol levels remained constant at all study centres irrespective of the amount of phytosterol consumption.

Study 2 did not attempt to examine the reduction in carotenoids at different time points and therefore does not provide any information on a pattern of reduction with ongoing phytosterol intakes. Nevertheless, as the effects were detectable early in the study and carotenoid levels returned almost completely to baseline in the two-week washout period when phytosterol-enriched foods were removed from the diet, it is likely that the reduction in carotenoid absorption had stabilised, along with the reduction in cholesterol absorption, due to the physiological linkage. Data with respect to carotenoid levels after long-term use (years rather than months) of phytosterol-enriched foods has not been presented.

3.2.6 Nutritional issues

The results from both CSIRO studies provide evidence that the effects of phytosterol ester consumption up to 10.7 g/day have no significant impact on the general nutritional status of adults over the medium-term. The data also suggest that this level of consumption may be safe over longer periods of time.

Comparisons between Study 1 and Study 2 suggest that a higher intake of phytosterols has a greater potential to compromise levels of certain carotenoids, most significantly β-carotene, without any concomitant benefit in terms of a reduction in LDL-cholesterol. The nature of the food matrix in which the phytosterols are presented is a factor in the cholesterol-lowering effects and therefore also in the secondary nutritional effects.

However, there is no evidence in the literature that the observed reduction in β-carotene, with consumption of phytosterol-enriched foods, will result in adverse health outcomes. Epidemiological studies show unequivocally that fruit and vegetable consumption is inversely associated with cardiovascular disease and some cancers (eg gastric cancer), but to date it has not been possible to elucidate the role of individual plant components with any certainty. Clinical intervention trials using β-carotene supplements in the diet either had no benefit or caused harm, leading to speculation that a host of other compounds (or a synergistic mix) in fruits and vegetables contribute to the beneficial effects, or that an increased intake of β-carotene may merely be a marker of a 'healthy' lifestyle which in itself has been associated with a lower risk of some chronic diseases.

Increasing the intake of fruit and vegetables when consuming phytosterol-enriched foods resulted in a modest improvement in the levels of some carotenoids and therefore validates the use of advisory statements on the packaging of these products. In addition, additional consumption of fruits and vegetables is consistent with other public health messages in relation to the prevention of a range of common diseases with a dietary component.

The authors claim that the cholesterol lowering effect of phytosterol-enriched spreads can conservatively be translated to an estimated reduction of 15-20% in the risk of developing cardiovascular disease. Epidemiological studies suggest that a similar reduction in the risk of heart disease can apply to high consumers of fruits and vegetables (at the 90^{th} percentile) compared to low consumers (at the 10^{th} percentile). For the same reduction in cardiovascular disease risk, the authors claim that use of phytosterol-enriched products represents a smaller dietary change for consumers when compared to the magnitude of the dietary changes required to convert from a low to a high consumer of fruits and vegetables.

In assessing the overall risk that can be attributed to a reduction in plasma β-carotene levels resulting from consumption of phytosterol-enriched foods, the authors cite European studies (Westrate and Meijer, 1998 & Hendriks *et al*, 1999) that claim α- and β-carotene levels measured in the Dutch population are 20% lower that the baseline levels in the submitted CSIRO studies. In addition, the plasma lycopene levels are reported to vary between 26-60% of Australian mean levels. A broad natural variation therefore already exists in different geographical populations, and significant fluctuations in carotenoid levels may also arise from adherence to a low-fat diet, seasonal variation and a variety of other environmental variables.

One environmental variable is in the nature of the diet itself. A short-term study measuring the effects of fibre and fibre sources on plasma carotenoids (Nutrition Epidemiology Group, Nuffield Institute for Health, UK – 2001) reported that both plasma α- and β-carotene are negatively affected by the consumption of cereal and cereal products. In a free-living population consuming their usual diet, fibre from cereals had a negative effect particularly on α- and β-carotene (8.4% and 6.6% reduction in plasma levels respectively for a doubling of fibre intake). These results are consistent with other reports indicating that high intakes of dietary fibre impair the bioavailability of carotenoids[6].

3.3 Published studies

Table 5 summarises results from the CSIRO studies and other studies published in the scientific literature investigating the nutritional effects of phytosterol-enriched foods. Taken together, these studies provide evidence that consumption of phytosterol esters up to 3 g/day by mildly hypercholesterolemic adults would have no significant nutritional effects on fat-soluble vitamin or carotenoid status. Although most studies do show a reduction in plasma β-carotene and α-carotene levels, only some have shown the reduction to be statistically significant (CSIRO 2002, Gylling 1999, Davidson 2001, Mensink 2002, Hendriks 2003, Raeini-Sarjaz 2002).

Two studies have investigated the effects of phytosterol intakes higher than 3 g/day, however the majority of studies are not long-term. In addition, because of differences in experimental design and in some cases the absence of specific dietary information, the majority of results show effects of dietary phytosterols only in terms of the cholesterol:β-carotene ratio, and do not record changes in any other fat-soluble nutrients.

3.3.1 Studies with higher intakes of phytosterols

Davidson *et al* (2001) studied three test groups of 23 subjects each, who consumed 0, 3, 6, or 9 g/day of phytosterol esters in reduced fat spreads for eight weeks. Blood concentrations of measured fat-soluble vitamins (vitamins A, D and E) remained within normal reference ranges. There was no statistical difference in serum vitamin response for these nutrients in those subjects who consumed 9 g/day phytosterols compared with the two groups consuming 3 g/day and 6 g/day respectively.

Pair wise comparisons of β-carotene levels after the intervention period indicated significant differences between the 9 g/day group compared to the control and the 3 g/day group ($p<0.05$). Only the control group and 9 g/day group also differed significantly with respect to serum α-carotene levels. The authors concluded that consumption of phytosterols at a level of 9 g/day was safe and well tolerated.

It should be noted that the reduced-fat spread and salad dressing used as phytosterol-ester delivery vehicles in this study did not produce the expected magnitude of reduction in LDL-cholesterol levels. Despite this, reductions in levels of fat-soluble vitamins and serum carotenoids were recorded. In addition, all groups receiving phytosterols showed a relatively small increase in corresponding serum phytosterol levels indicating that the significance of the results from a nutritional perspective may be limited.

[6] Reidl, J. *et al*. Some dietary fibers reduce the absorption of carotenoids in women. J. Nutr. 129, 2170-6, 1999.

3.3.2 Research with controlled diets

Although there are two published studies investigating the nutritional effects of phytosterol consumption in the context of a controlled diet, only one provides information that is relevant to this assessment.

Raeini-Sarjaz *et al*. (2002) reported no effect of consumption of esterified plant sterols (or stanols) on serum fat-soluble vitamins or carotenoid concentrations when consumed in conjunction with a diet adequate in fruit and vegetables, compared to baseline diets. The study involved 15 hypercholesterolemic males administered a daily amount of 1.92 g/70 kg body weight of plant sterol esters in a metabolic kitchen setting in the context of a diet formulated to meet the Canadian Recommended Nutrient Intakes. Measurements for serum retinol, α- and γ-tocopherol, vitamins D and K, lycopene, lutein, α- and β-cryptoxanthin, and α- and γ-carotene were conducted. The authors concluded from their results that moderate consumption of plant sterol and stanol esters would not be expected to affect fat-soluble vitamin and carotenoid concentrations in conjunction with a healthy diet.

3.3.3 Fruit and vegetable consumption

A study by Noakes *et al.* (2002) specifically examined whether consuming daily amounts of foods high in carotenoids prevents a reduction in plasma carotenoid concentrations in subjects who consume plant sterol (or stanol) esters. Forty-six hypercholesterolemic subjects completed a three way, double blind, crossover comparison in which 25 g/day of one of the following 3 spreads were consumed for 3 weeks: control (placebo/sterol free), sterol–ester (2.3 g/day plant sterol esters) or stanol-ester (2.5 g/day plant stanol esters). During the study period, subjects were advised to eat five or more servings per day of fruits and/or vegetables, of which at least one serving was to be carrots, sweet potatoes, pumpkin, tomatoes, apricots, spinach or broccoli.

As expected, there was a reduction in total cholesterol with consumption of sterol esters (-6.1%) and stanol esters (-7.3%), compared with the control spread. The decrease in the LDL-cholesterol concentration was 7.7% with consumption of sterol ester-enriched spread and 9.5% with consumption of stanol ester-enriched spread. There were no significant changes in HDL-cholesterol or triacylglycerol concentrations.

Consumption of the different spreads did not significantly change the concentrations of retinol and lutein, which the authors note is consistent with their transport by retinol binding protein, and HDL (40%) respectively. Similarly, α-tocopherol concentrations were not significantly different among the spread periods or between the spread periods and baseline period. After standardising for lipids, there were no significant differences in plasma carotenoid concentrations between the experimental groups and the control. However, before lipid adjustment, both the sterol and stanol periods significantly lowered the β-carotene concentration by 9% compared to the control period, but not compared with the baseline period. When the 1-week baseline and control periods were analysed separately, the levels of lutein, α-carotene and β-carotene increased by 11%, 29% and 13% respectively, demonstrating the effects of increasing dietary intake of the specified fruits and vegetables, in the absence of plant sterols. Interestingly, the concentration of plasma lycopene did not change significantly during the study.

The authors concluded that daily consumption of an average of one extra daily serving of high-carotenoid fruit or vegetables, compensates plasma concentrations of α- and β-carotene and maintained concentrations of lipid-standardised plasma carotenoids in subjects consuming sterol or stanol-enriched spreads. The conclusions of this study suggest that compliance with dietary advice to consume specified fruits and vegetables, in conjunction with phytosterol-enriched foods, is likely to compensate for a decrease in carotenoid levels.

3.3.4 Long-term studies

There are few long-term studies investigating the nutritional effects of phytosterol consumption. Gylling (1999) investigated the effects on carotenoids and fat-soluble vitamins of ingestion of 2-3 g/day phytosterols over 12 months. Serum cholesterol and vitamin concentrations were measured at 0 and 12 months. The levels for serum α-tocopherol, α-carotene, β-carotene and cholesterol were all significantly lower in experimental subjects compared with controls after 12 months. However, when levels were adjusted for LDL concentration, β-carotene was the only nutrient significantly lower than the controls.

A one-year study by Hendriks *et al*. (2003) involved 185 volunteers randomised into either a control or experimental group who consumed 1.6 g/day of phytosterol esters in a margarine-type spread. Carotenoids were measured at both 26 and 52 weeks and compared to baseline and to the control group. In absolute terms, serum β-carotene levels were reduced by 22% at 26 weeks and by 25% at 52 weeks in the experimental group compared to baseline. Serum α-carotene levels were reduced by 11% at 26 weeks and by 15% at 52 weeks in the experimental group compared to baseline. When the results were corrected for LDL concentration, only α-carotene was reduced in the experimental group who consumed the phytosterol-fortified spread.

The study reported no change in LDL and cholesterol concentration (a plateau effect) in the second half of the study period between 26-52 weeks, and the researchers concluded that the nutritional effects had reached a plateau by the mid-time point.

3.3.5 Studies in hypercholesterolaemic children

Five studies investigated the effects of phytosterol esters in children (Gylling 1995, Tammi 2000, 2001 & 2002, Amundsen 2002), however only two of these investigated nutritional parameters. All children in these studies were either hypercholesterolaemic, or were genetically susceptible to high cholesterol levels.

One study of 38 children (each of whom had a parent with hypercholesterolemia) who were supplemented with 1.6 g/day phytosterol esters, showed significant decreases in serum concentrations of β-carotene and lycopene, with the difference in β-carotene disappearing after statistical adjustment for cholesterol. Twenty-one of the 38 children took either fish oil, or vitamin A, D or E supplements (Amundsen 2002).

Another study that measured the serum antioxidant levels of 72 six-year old children consuming 1.5 g/day plant stanols over a three-month intervention period showed that serum β–carotene and β-carotene/LDL concentration was significantly lowered as a result of treatment. α-Carotene and lycopene were not measured (Tammi 2000).

The results of these studies confirm that consumption of phytosterols can result in a reduction in carotenoid levels in all consumers irrespective of age, where there is a concomitant reduction in cholesterol absorption.

3.3.6 Normocholesterolemic children

Studies examining the effects of phytosterol-enriched foods in children with normal cholesterol levels are not available. This is because the primary research interest in the cholesterol-lowering effects apply to adult consumers with slightly raised cholesterol levels that are not high enough to require therapeutic intervention, but are above recommended levels for reducing risk factors associated with the development of cardiovascular disease. Given the target consumer group, it is unlikely that data in children other than with genetic/familial hypercholesterolaemia will become available.

3.3.7 Older adults

The NHMRC Dietary Guidelines for Older Australians (1999) and other papers (for example, Heseker 1994) suggest that older adults (over 65 years) generally have changing nutrient requirements because of age-related changes in body composition and physiological function. The changing nutrient requirements could include a higher dietary requirement for carotenoids (eg β-carotene), and vitamins C and E due to increased oxidative stress. At the same time, due to a general decline in physical activity and subsequent energy intake, and reductions in the bioavailability of certain nutrients with increasing age, it is recognised that meeting any increased nutritional requirements depends on varying factors affecting diet, eating habits and lifestyle.

Despite these variables, according to data from the Australian National Nutrition Survey (1995-96), the mean nutrient intakes for both males and females in the over 65 age-group of vitamin A as retinol equivalents is almost double the RDI for males and approximately 1.5 times the RDI for females. The New Zealand National Nutrition Survey (1996-97) also indicated the average intake of vitamin A as retinol equivalents was approximately 1.5 times the RDI for men and women over 65 years of age. These data indicate that in terms of retinol equivalents, the current levels of intake by elderly consumers in Australia and New Zealand are generally well above daily requirements.

While there are no studies currently available that specifically examine the nutritional effects of phytosterol-enriched foods in older-age consumers, the NHMRC guidelines stress the importance of variety in the diet in order to provide a more complete profile of nutrients and non-nutrients. This recognises the importance of whole foods, particularly fruits and vegetables, as beneficial in reducing the risk of developing chronic diet-associated diseases. Health benefits to be derived from a diet rich in fruits and vegetables are likely to be attributable to the synergistic effects of a complex mix of phytochemicals including carotenoids, flavonoids and isoflavonoids, polyphenols, isothiocyanates, indoles, sulphoraphane, monoterpenes, xanthin, and non-digestible polysaccharides.

Given this information, the significance of a reduced level of one carotenoid, β-carotene (a pro-vitamin), with consumption of phytosterol-enriched foods should be considered in the context of the significant increase in the incidence of peripheral vascular disease, cerebrovascular disease and arteriosclerosis in the older adult population, and the measurable health benefits provided by a lower blood cholesterol level in this age group.

In the context of a changing physiology, older consumers may need to adapt dietary habits and eating patterns to compensate for a variety of changing nutrient requirements, in order to maintain optimal health. The dietary advice to consume greater amounts of fruits and vegetables when consuming phytosterol-enriched foods is therefore consistent with broad public health messages to this population group.

3.3.8 Pregnant and lactating women

Currently there is no research specifically investigating the nutritional effects of consumption of phytosterol-enriched foods by pregnant and lactating women. On the contrary, pregnant and lactating women have been excluded as subjects on nutritional grounds. The Scientific Committee on Food (SCF, 2003) considers that use of phytosterol-enriched foods by pregnant and lactating women is inappropriate because of the resultant lowered absorption of both dietary cholesterol and β-carotene, and the lack of information on whether this would have an adverse nutritional impact on women with increased physiological load.

Currently in Australia and New Zealand, phytosterol-enriched edible oil spreads and margarines are required to carry a mandatory advisory statement to ensure that pregnant and lactating women do not consume these products. This cautionary approach is therefore consistent with the views expressed by other independent scientific committees.

3.3.9 Phytosterolaemia

Sitosterolaemia is a rare genetic (autosomal recessive) disorder in which affected individuals hyper-absorb and retain both cholesterol and other (plant, fish) sterols. The effects of this genetic condition are tendon and tuber xanthomas, arthralgias and arthritis, accelerated atherosclerosis and premature coronary artery disease (SCF 2003). The potential impact of phytosterol-enriched foods on patients with this disorder is discussed in more detail in the safety assessment at Attachment 3.

3.3.10 Phytosterols as antioxidants

The oxidation of biological molecules is known to be associated with the development of numerous disorders and pathological events such as atherosclerosis, cancer and various age-dependent processes. Chemical compounds and substances such as vitamin E that suppress oxidation have therefore become a focus of study over recent times to explore more fully their potential *in-vivo* antioxidant properties. As well as vitamins and other nutrients, plant substances such as polyphenols (rich in red wine and tea) act to protect biological molecules and tissues from oxidative damage, thereby contributing to the antioxidant pool in the body.

A recent paper (Yoshida and Niki, 2003) explored the antioxidant properties of plant sterols (campesterol, β-sitosterol, stigmasterol) and reported that phytosterols themselves can act as an antioxidant *in-vitro*, a modest radical scavenger in solution, and physically as a stabiliser in liposomal membranes. The possible antioxidant role of phytosterols *in-vivo* remains as a future subject for study.

3.4 Summary of nutritional effects of phytosterol esters

Plant sterols (phytosterol-esters in this assessment) have been shown in a large number of studies to lower the absorption of dietary and biliary cholesterol thereby decreasing the levels of LDL-cholesterol in the circulation. As cholesterol absorption is reduced, there is a concomitant effect on the absorption of some lipophilic nutrients. When these secondary physiological effects were examined in further studies, reductions in α- and β- carotene, lycopene, lutein and cryptoxanthin were observed, while the levels of vitamins A, D and E remained unaffected. Additional carotenoid-rich fruits and vegetables in the diet, when co-consumed with the phytosterol-enriched foods, partially compensated for the lower bioavailability of carotenoids in the presence of phytosterols.

With some variability, consumption of phytosterol-enriched foods generally results in a reduction in β-carotene levels of approximately 20-25%. This reduction does not translate into an overt nutritional deficiency as absolute levels remain within a broad natural range and there is no measurable effect on retinol or vitamin A levels. The nutritional significance of a reduction in β-carotene levels therefore cannot be directly measured or assessed. In terms of antioxidant status, other nutrients such as vitamin C and vitamin E are not affected by consumption of phytosterols and other phytochemicals present in fruits and vegetables contribute to the complexity of the diet and overall health.

In light of the nutritional effects, consumption of phytosterol-enriched foods is not appropriate for children, or pregnant or lactating women on the general assumption that there is no direct necessity to lower absorption of dietary cholesterol in these groups. Given their requirements for optimal nutrition, these population groups would therefore derive no particular immediate health benefit from increasing their intake of phytosterols. In contrast, consumers over the age of 40 years, and particularly those with slightly elevated cholesterol levels, can make simple dietary changes that may effectively reduce one of the known risk factors in the development of atherosclerosis and cardiovascular disease.

The data submitted with these applications indicate that consumption of phytosterol-enriched foods providing up to approximately 10.7 g/day phytosterol-esters is safe from a nutritional perspective. Furthermore, other information from published studies suggests that intake of phytosterol esters at these higher levels (up to approximately 9 g/day) is not associated with adverse effects arising from a reduction in some carotenoids. The effects of phytosterol ester consumption above 10.7 g/day on nutritional parameters, or over the long-term, have not been extensively researched, and there is therefore a lack of detailed information in this area. As there is no additional cholesterol lowering effect with increased phytosterol ester intake above approximately 4 g/day, there is no additional benefit in consuming unlimited amounts of phytosterol-enriched foods.

The results of several studies suggest daily consumption of 5 serves of fruits and vegetables, particularly those high in β-carotene, when choosing phytosterol-enriched foods, may assist in maintaining the levels of some carotenoids. The European SCF recommends that consumers be made aware of the potential β-carotene lowering effect of phytosterol-enriched products by the provision of appropriate dietary advice relating to the regular consumption of fruits and vegetables.

4. International reviews on the nutritional aspects of phytosterols in foods

4.1 European assessment

Foods containing added phytosterols have been available in Europe since the mid 1990's. As part of the process of assessment, the Scientific Committee on Food (SCF) of the European Commission has considered various safety aspects of phytosterol esters and has produced several opinion reports (2000a, 2002a, 2002b, 2003) reviewing in particular the nutritional effects of phytosterols, and the long-term effects of elevated levels of phytosterols from multiple dietary sources.

Previously, the Committee concluded that yellow fat spreads containing up to 8% of free phytosterols are safe for human consumption. It was noted that ingestion of approximately 20g of phytosterol-enriched spread per day for one year reduced β-carotene concentration by 20%. The Committee considered that although this reduction was within the normal range and within normal seasonal variation, it may become of greater nutritional relevance for individuals with a sub-optimal vitamin A status.

On the basis of results from several different trials using plant sterols or stanols, decreases in blood carotenoids plateau at consumption levels of 2.2 g/day (Plat et al. 2000). Apart from the carotenoid lowering effect, the Committee found that no other nutritionally relevant changes were evident when considering the results of several randomised trials of plant sterol or stanol margarines in humans, some of which lasted for one year.

The SCF considers that the greatest nutritional effect of phytosterol esters appears to be upon β-carotene, with only minimal effects on fat-soluble vitamins and other carotenoids. Based on the general acceptance that consumption of up to 10 mg/day of β-carotene from carotenoid-rich fruits and vegetables confers non-specific health benefits, the Committee has recommended the consumption of carotenoid rich fruit and vegetables to counterbalance the expected reduction of blood β-carotene arising from long-term consumption of phytosterol enriched foods.

The Committee concluded that, due to the lack of evidence of benefits from phytosterols at higher levels of intake, consumption of free phytosterols exceeding a range of 1-3 g/day (equivalent to 1.6 – 4.8 g/day phytosterol esters) was inadvisable. They also considered that with an ever-increasing number of potential foods as candidates for phytosterol enrichment, additional measures may be required to manage potentially excessive intakes (SCF 2003).

4.2 Review by the Mayo Clinic

In 2003 the Mayo clinic published a paper summarizing the deliberations of 32 experts on the safety of sterols and stanols (Katan 2003). The paper was a meta-analysis of 41 trials aimed at determining the safety of phytosterol intake at a level of 2 g of free stanols or sterols per day in relation to heart disease. The authors suggest that reduction of LDL cholesterol levels by 10% could be expected to reduce the incidence of ischaemic heart disease by between 12 and 20 % over 5 years.

The meta-analysis of 18 trials investigating the effects of sterol and stanols intake on plasma concentrations of fat-soluble vitamins showed statistically significant reductions in α-carotene (9%), β-carotene (28%) and lycopene (7%). On statistical correction for total cholesterol, only the decrease in β-carotene remained significant. The authors considered that the decrease in β-carotene could be prevented by the addition of "adequate" fruit and vegetables to the diet.

This review noted that plasma β-carotene levels are affected by a variety of dietary factors. Olestra and wheat bran have been shown to significantly decrease β-carotene levels, as have some lipid lowering drugs (probucol and cholestyramine). Therefore, based on currently available information, there is no evidence that decreased levels of β-carotene are associated with increased health risks.

TABLE 5 Studies of the Effect of Phytosterol Consumption on Plasma Fat-soluble Vitamins and Carotenoids: Details of the studies

Authors	Number of Subjects	Cholesterol status at baseline	Dietary intake	Smokers/non smokers	Mean Age	Weight at baseline	Fruit and vegetable intake
CSIRO, 2002	Adelaide 13 women 10 men	Combined centres: TC 6.59 ±1.01 mmol/l HDL 1.35±0.38 mmol/l LDL 4.46±0.91 mmol/l	8281 kJ/day Fat 24% TE SAFA 10.3% TE	Not discussed	53.3 yrs	BMI 27.9	phase 1 not discussed, phase 2-83% compliance with 5 serves / day
	Melbourne 10 men 2 women	Combined centres: TC 6.59 ±1.01 mmol/l HDL 1.35±0.38 mmol/l LDL 4.46±0.91 mmol/l	6853 kJ/day Fat 33% TE SAFA 12.5% TE	Not discussed	59.7 yrs	BMI 27.6	phase 1 not discussed, phase 2- 86% compliance with 5 serves /day
Gylling, 1999	102 active subjects 49 controls	TC >5.58 mmol/l	Fat 85g/day SAFA 34g MUFA 32g PUFA 15g	Not discussed – not in exclusion criteria	50±1 yrs	BMI 26	not discussed
Davidson 2001	0 g/day n=21 3.0 g/day n=21 6.0 g/day n = 19 9.0 g/day n=23	mildly hypercholesterolemic	TE 2019 Kcal/day Fat 33% TE SAFA 11%TE MUFA 12.6% TE PUFA 6.4%TE	74 smokers 10 non-smokers	46 yrs	79 kg	not discussed
Nestel P 2001	22 subjects 4 men 18 women	mildly hypercholesterolemic TC >5.5 mmol/l	Fat 34%TE SAFA 11.5%TE	non smokers	60±9 yrs (34-70 yrs)	BMI 24±1 (18.3-26.9)	not discussed
Raeini-Sarjaz 2002	15 men	hypercholesterolemic TC 6-10 mmol/l	All food prepared in metabolic unit. Fat 35% TE SAFA 15% MUFA 10% PUFA 10%	not discussed	37-64 yrs	not discussed	not discussed

Mensink 2002	30 subjects 30 controls 16 men 44 women	TC 5.14±0.78 mmol/l men TC 5.12±0.80 mmol/l women	Energy/day S 9.5 MJ, C 11.3 MJ Fat % TE S 29.1%, C 31.7% SAFA S 10.8%, C 11.4% MUFA S 10.9%, C 12.2% PUFA S 5.3%, C 5.9%	7 smokers	36±14 yrs	BMI 23.3±2.7	not discussed
Westrate 1998	95 subjects	TC 5.35±1.06 mmol/l	FAT 41% TE SAFA 15.5% MUFA 14% PUFA 10%	not discussed	45±12.8 yrs	24.2±2.16	not discussed
Hendricks 1999	100 subjects 42 men 58 women	TC 5.10±0.97 mmol/l (2.71-7.42 mmol/l)	Fat 33% TE SAFA 13.5% MUFA 11.6% PUFA 6.0%	not discussed	37±10 yrs	22.8 ± 2.5 (17.7-28.6)	not discussed
Hallikainen 2000	22 subjects 14 women 8 men	TC 6.87±1.28 mmol/l	Standardised background diet designed for 8 different levels of energy requirement Fat 34% TE SAFA <12% MUFA 14% PUFA 8%	not discussed	50±11 yrs	26 ± 3.4	no information on fruit and vegetable intake
Hendricks 2003	190 subjects Experimental 44 men, 45 women Controls 46 men, 50 women	TC 5.9 ± 0.98 mmol/l		6 smokers control 11 smokers exp.	48±8 yrs	24.9 ± 3.2	no information

ns No significant difference
TC Total Cholesterol

TABLE 6 Studies of the Effect of Phytosterol Consumption on Plasma Fat-soluble Vitamins and Carotenoids: Results

Study	Level of intake	Food source	Length of study	Cholesterol	α-Tocopherol	α -Carotene	β -Carotene	Retinol	Lutein	Lycopene
CSIRO	6.6 g/day	bread, breakfast cereal, table spread	total 12 weeks 2 phases-6 weeks each 2nd phase with extra F&V		P1 –10% p<0.05 P2 - 13% p<0.05	ns	cf baseline P1 -28% p<0.05 P2 –28% p<0.05	ns	P1 –23% p<0.05 P2 –15% p<0.05	P1 –18% p<0.05 P2 –30% p<0.05
					P1 –15% p<0.05 P2 –7% p<0.05	ns	ns	ns	P1 -15% p<0.05 P2 –7% p<0.05	P1 -18% p<0.05 P2 –23% p<0.05
			Adelaide and Melbourne combined	TC –8.5% LDL –13% (p<0.05)	ns	P1 –31% p<0.05 P2 ns p<0.05	P1 –30% p<0.05 P2 –26% p<0.05	ns	ns	ns
Gylling 1999	3 g/day for 6 months, then either 2 or 3 g/day for 6 mths	Margarine	52 weeks	TC -9% (P<0.001)	-10±1% (P<0.05) Proportion to cholesterol unchanged	ns	sig ↓ (p<0.05)	ns	-	-
Davidson 2001	0 g/day 3 g/day 6 g/day 9 g/day	Reduced fat spread and salad dressing	8 week treatment period	ns between groups	ns	ns	ns	ns	ns	ns
				ns between groups	ns	ns	ns	ns	ns	ns
				Total:HDL -9.6±14.9% p<0.008	ns	Significantly lower than 0 and 3g/day p<0.004	Significantly lower than 0 and 3g/day p<0.001	ns	ns	ns
Nestel P 2001	2.4 g/day	breads and breakfast cereal	12 weeks	LDL –13.6% P<0.001	ns	ns	ns	ns	-	ns

Raeini-Sarjaz 2002	sterols 1.92 g/70kg bw/day	margarine (controlled diet)	3 weeks		ns	increased P<0.01	ns	ns	ns	ns
Mensink 2002	3 g/day	low fat yoghurt 450 ml/day	4 week double blind, placebo controlled	control vs. ex groups: TC –8.7% (P<0.001) LDL –13.7% (P<0.001)	ns	not measured	β-C:LDL -12.9 ±21.2% cf. control P=0.038	ns	ns	-
Westrate 1998	1.5-3.3 g/day	Margarine sterols - soybean sheanut rice-bran sitostanol ester 5.0 mg/kg carotene	3.5x 4 weeks	control vs. ex groups: TC – 8-13%	-	α and β-carotene combined decreased from av 220µg/l in first period to 168µg/l in fourth.		-	-	↓ from period 1 – 4 85µg/l to 63µg/l
Hendricks 1999	0.83, 1.61, 3.24 g/day	Margarine	3.5x 4 weeks	Sig ↓ TC in all phases HDL ↓ after 1.61 &3.2g cf baseline	-6% 1.61g -8% 3.2g α-toc/TC ns	Combined α and β-carotene concentrations -11% w/ 0.83g/day -19% w/ 3.24g/day		-	-	lyco/TC ns
Hallikainen 2000	5x4 weeks in the order: 2.4, 2, 1.6, 0, 0.8 g/day	Rapeseed oil Margarine 25g/day	20 weeks	1.6,2.4&3.2g TC sig lowered LDL sig lowered	ns	ns	ns	-	-	-
Hendricks 2003	1.6 g/day	Margarine 5.7 mg/kg carotenoids	1 year	TC ↓4% LDL ↓ 6%	ns	sig ↓ cf with controls after 26 and 52 weeks but no sig diff for /TC	sig ↓ cf with controls after 52 weeks	ns	sig ↓ cf with controls after 26 and 52 weeks	ns

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ATTACHMENT 5

DIETARY EXPOSURE ASSESSMENT REPORT

Application A434 – Phytosterol esters derived from vegetable oils as a novel food ingredient in low fat milks and low fat yoghurts.

Summary

The application under assessment seeks permission to use phytosterol esters derived from vegetable oils as a novel food ingredient, under Standard 1.5.1 – Novel Foods, in low fat milk (fat content < 1.5%) and low fat yoghurt (fat content < 1.5%).

A dietary exposure assessment was undertaken to determine the impact of allowing phytosterol esters to be added to the above foods. The assessment took into account the existing permission under Standard 1.5.1 to add phytosterol esters to edible oil spreads (the 'baseline' scenario) as well as the proposed addition of phytosterol esters to low fat milks and low fat yoghurts (the 'low fat milks and low fat yoghurts only' scenario) and a combination of theses products (the 'low fat milks and low fat yoghurts plus baseline' scenario). In each scenario, the modeling was based on the addition of phytosterol esters at the level of 0.8 g free phytosterols per serve. The analysis also assumed that all foods considered (edible oil spreads, low fat milks and low fat yoghurts) contained added phytosterol esters. Intrinsic levels of phytosterols in foods were not taken into consideration.

Modeling was conducted assuming that consumers do not change the amounts and general types of foods that they eat, simply substituting phytosterol containing edible oil spreads, low fat milks or low fat yoghurts for their non-phytosterol counterparts. Food consumption data from the most recent Australian and New Zealand National Nutrition Surveys (NNSs) were used – the 1995 Australian NNS of those aged 2 years and above, and the 1997 New Zealand NNS of those aged 15 years and above were used. Exposure was estimated for the target populations (40-64 years and 65 years and above), and for the general population, as well as for two specific non-target groups – children aged 2-12 years (Australia only) and women of childbearing age (16-44 years), as a proxy for pregnant and lactating women.

Assuming that consumers maintain their existing eating patterns, and simply substituting phytosterol containing spreads, low fat milks, and low fat yoghurts for their non-phytosterol counterparts, the estimated mean dietary exposure (expressed as free phytosterols) did not exceed 1.9 grams per day (g/day) in any population group under any of the scenarios considered in this assessment. At the 95th percentile of exposure, no population group exceeded 4.7 g free phytosterols per day for any of the scenarios. The analysis shows that, for the target population group (40 years and above) in particular, edible oil spreads contribute more to dietary exposure to added free phytosterols (78-83% of exposure) than low fat milks and low fat yoghurts, according to the available data on food consumption patterns.

1. Introduction

1.1 Information supplied by the applicant

The applicant is seeking approval to use phytosterol esters in foods at levels formulated to provide between approximately 2 g/day and 3 g/day of free phytosterols specifically to consumers in the target group (through 2-3 serves of products). Target consumers are adults over the age of 40 years with concerns about their blood cholesterol level. However, casual consumption by other non-target population groups, including children, must also be considered.

The applicant did not provide new data relating to dietary exposures to phytosterol esters in Australia or New Zealand. Instead, the applicant referred to a previous assessment (Application A410) which concluded that an 'average' consumer would not exceed the level of intake of phytosterols shown to be safe by the available data at that time, and that the 95th percentile exposures[7] would marginally exceed this level.

However, the dietary exposure assessment provided by the applicants was not sufficiently detailed to draw conclusions on projected exposure to phytosterols among specific population subgroups of particular interest. FSANZ has therefore conducted a dietary exposure assessment to estimate potential exposure to phytosterols if these are permitted in low-fat milks and low-fat yoghurts.

1.2 Existing phytosterol enriched products

There are a small number of edible oil spreads currently on the market that contain phytosterols. These products carry statements to the effect that plant sterols assist in lowering cholesterol absorption. Permissions for the use of phytosterol esters at no more than 137 g/kg of food (equivalent to 1.37 g per 10 g serve of spread) is under Standards 1.5.1 and 2.4.2 of the Code.

1.3 Natural presence of phytosterols

Major sources of naturally occurring phytosterols are vegetable fats and oils, nuts and seeds (Food Standards Agency, 2002). Reported average intakes of phytosterols from unfortified foods vary in the range of 160 to 500 mg per day (Thurnham, 1999). These levels are therefore 5–10 fold lower than would result from addition of phytosterols to foods.

1.4 Post launch monitoring in Europe

The Unilever Company conducted post launch monitoring in Europe of the use of yellow fat spreads containing added phytosterol esters, following approval to add the esters by the European Commission. For regular users of phytosterol enriched spreads, median household consumption of spread was between 15 g/day and 18 g/day, which represents slightly less than 2 x 10 g serves per day. Surveys suggested that these amounts represent consumption by a single person in the household and are lower than predicted at the time of approval of the products, when consumption of the spreads was predicted to be 20–30 g per person per day. The ninety-fifth percentile consumption did not exceed 45g of spread (4.5 serves) per day.

[7] The exposure level that is equalled or exceeded by only 5% of the population.

Of particular note, the survey information indicated that the majority of households where these spreads were used did not include children and between 87% and 91% of regular purchasers of these spreads had no children living at home (Scientific Committee on Food, 2002).

2. Dietary modelling

The dietary exposure assessment was conducted using dietary modelling techniques that combine food consumption data with food chemical concentration data to estimate the exposure to the food chemical from the diet. The dietary exposure assessment was conducted using FSANZ's dietary modelling computer program, DIAMOND.

Dietary exposure = food chemical concentration x food consumption

The exposure was estimated by combining usual patterns of food consumption, as derived from national nutrition survey (NNS) data, with proposed levels of use of phytosterol esters in foods.

2.1 Dietary Survey Data

DIAMOND contains dietary survey data for both Australia and New Zealand: the 1995 NNS from Australia that surveyed 13 858 people aged 2 years and above, and the 1997 New Zealand NNS that surveyed 4 636 people aged 15 years and above. Both of the NNSs used a 24-hour food recall methodology.

The dietary exposure assessment was conducted for both Australian and New Zealand populations. For the Australian population, the following groups were included in the exposure assessment: the whole population aged 2 years and above; the target groups of people aged 40 – 64 years and those aged 65 years and above; and specific non target groups of special interest including children aged 2–12 years and females of child bearing age, aged 16–44 years. For the New Zealand population, the sub-groups included: the whole population aged 15 years and above; the target groups of people aged 40 – 64 years and 65 years and above; and non target group of females of child bearing age, aged 16–44 years. No New Zealand survey data are available for children aged 2-12 years in a form suitable for modelling data.

The target group for phytosterol-containing products is identified as people aged 40 years and above because it is this age group who are likely to have increasing concerns about their general health and who are likely to be interested in reducing a slightly elevated blood cholesterol level through dietary means. People aged 65 years and above were assessed separately because of the potential for some people in this target group to experience inadequate diets or reduced nutrient bioavailability.

Children generally can experience higher dietary exposures due to their smaller body weight, and higher consumption of food per kilogram of body weight compared to adults. An exposure assessment was therefore also conducted on younger non-target age groups because of a possibility that children could consume these products on a casual basis if available in the household.

However, the Unilever post-launch monitoring of households in Europe using phytosterol-enriched products suggests this is not particularly likely. In addition, to estimate the exposure of pregnant and lactating women to phytosterols from enriched products, exposure was estimated in a proxy group, women of childbearing age (16-44 years).

2.2 Additional Food Consumption Data or Other Relevant Data

No further information was required or identified for the purpose of refining the dietary exposure estimates for this application.

2.3 Concentration levels and serving sizes

The levels of free phytosterols in low fat milks and low fat yoghurts used in the exposure assessment were derived from the application. Levels of free phytosterols per serve were converted to concentrations in mg/kg to enable them to be entered into DIAMOND. Serve sizes are based on average product serve sizes from food packages - including 1 serve of edible oil spreads (10 g), 1 glass of milk (250 mL) and 1 small punnet of low fat yoghurt (140 g). The foods and proposed levels of use are summarised below in Table 1.

Table 1: Proposed levels of use of free phytosterols in foods

Food Code	Food Name	Serve size (g)	Proposed level of free phytosterol per serve (g/serve)	Concentration Level used in modelling (mg/kg)
1.1.1.2	Low fat milks (<1.5% fat), unflavoured	250	0.8	3 200
1.2.2.2	Low fat flavoured yoghurts (<1.5% fat content), including low fat fruit yoghurts	140	0.8	5 714
2.2	Edible oil spreads including reduced fat spreads	10	0.8	80 000

In estimating dietary exposure using DIAMOND, the whole category for each food was assumed to contain phytosterol as neither NNS has specific consumption data for phytosterol containing foods since such foods were not available at the time of the surveys.

2.4 Estimating risk

Estimated dietary exposures are normally compared to a reference health standard in order to determine any potential risk to health of the population or sub-groups. As novel food ingredients, free phytosterols do not have an established reference health standard and therefore estimated exposures were simply reported in gram amounts per day.

Intakes of free phytosterols up to 4.2 g/day have been associated with reductions in LDL cholesterol and have been used in recent clinical trials to study safety and efficacy in different food matrices.

2.5 How were the estimated dietary exposures calculated?

The DIAMOND program allows free phytosterols concentrations to be assigned to food groups. All foods in each of these groups included in this assessment were assigned the concentration of free phytosterols shown in Table 1. Estimated dietary exposures were calculated for the following three scenarios:

- edible oil spreads including margarines (baseline scenario);
- low fat unflavoured milks and low fat flavoured and fruit yoghurts (low fat milks and low fat yoghurts scenario); and
- edible oil spreads, low fat milks and low fat yoghurts combined (low fat milks and low fat yoghurts + baseline scenario).

An individual's exposure to free phytosterols was calculated using their individual food records from the dietary survey. The DIAMOND program multiplies the specified concentration of free phytosterols by the amount, if any, of edible oil spread, low fat milk or low fat yoghurt that an individual consumed in order to estimate the exposure to each of these foods. Once this has been completed for the foods specified to contain phytosterols, the total amount of free phytosterols consumed from all foods is summed for each individual. Population statistics (mean and high percentile exposures) are then derived from the ranked exposures of individuals who consumed added phytosterols.

The consumer populations differ in each of the three scenarios assessed. Consumers who choose to eat edible oil spreads do not necessarily also choose low fat milks and low fat yoghurts. In the baseline plus low fat milks and low fat yoghurts scenario, the consumer population includes those who consume only edible oil spreads, those who consume only low fat milks, and those who consume only low fat yoghurts as well as those who consume any combination of these foods. Therefore mean consumer exposure in the baseline + low fat milks and low fat yoghurts scenario does not represent the result of simply summing mean consumer exposure from the baseline scenario and from the low fat milks and low fat yoghurts scenario, as the consumer population is not exactly the same.

Percentage contributions of each food group to total estimated exposures are calculated by summing the exposures for a food group from each individual in the population group who consumed from that food group, and dividing by the sum of the exposures of all individuals from all food groups containing free phytosterols, and multiplying by 100.

Food consumption amounts for each individual take into account where each food in a classification code is consumed alone and where it was used as an ingredient in other foods prepared by the consumer (for example, margarine used in cooking).

2.6 Assumptions in the dietary modelling

Assumptions made in the dietary modelling include:

1. food consumption amounts are those reported in the NNSs, as it is assumed people will not change eating habits but simply substitute one product type for another;
2. where a permission is given to a food group classification, all foods in that group contain phytosterols at the concentration specified in Table 1; and

3. there is no contribution to phytosterols exposure through the use of complementary medicines (Australia) or dietary supplements (New Zealand).

The second assumption leads to a conservative estimate of dietary exposure to phytosterols, as it is highly unlikely that all foods within a group, such as all available brands of margarine, would contain added phytosterols.

2.7 Limitations of the dietary modelling

A limitation of estimating dietary exposure using 24-hour recall data is that it may not be an accurate reflection of typical exposure over a lifetime. Hence, estimated dietary exposure for high consumers is likely to be an overestimate.

While the results of national nutrition surveys can be used to describe the usual intake of groups of people, they cannot be used to describe the usual intake of an individual (Rutishauser, 2000). In particular, they cannot be used to predict how consumers will change their eating patterns as a result of an external influence such as the availability of a new type of food.

3. Results

3.1 Estimated dietary exposures to phytosterols assuming food selection patterns do not alter

The estimated dietary exposures to free phytosterols for the different food groups for the mean and 95th percentile, consumers only, are shown below in Figures 1 and 2 respectively (baseline scenario, 'low fat milks and low fat yoghurts only' scenario and 'low fat milks and low fat yoghurts plus baseline' scenario) for Australia and Figures 3 and 4 (baseline scenario, 'low fat milks and low fat yoghurts' scenario and 'low fat milks and low fat yoghurts plus baseline' scenario) for New Zealand mean and 95th percentile exposure. Numerical data are also provided for New Zealand and Australia in Table 2 (edible oil spreads), Table 3 (low fat milks and low fat yoghurts) and Table 4 (edible oil spreads, low fat milks and low fat yoghurts). Results for consumers only (eaters of foods containing free phytosterols) are presented rather than data from the whole survey population because the purpose of the risk assessment is to consider the potential impact of phytosterol addition to a variety of foods on people who report eating these foods. All values reported are expressed as free phytosterols and are reported in grams/day.

Figure 1: Estimated mean dietary exposure to free phytosterols for different population groups and scenarios for Australia

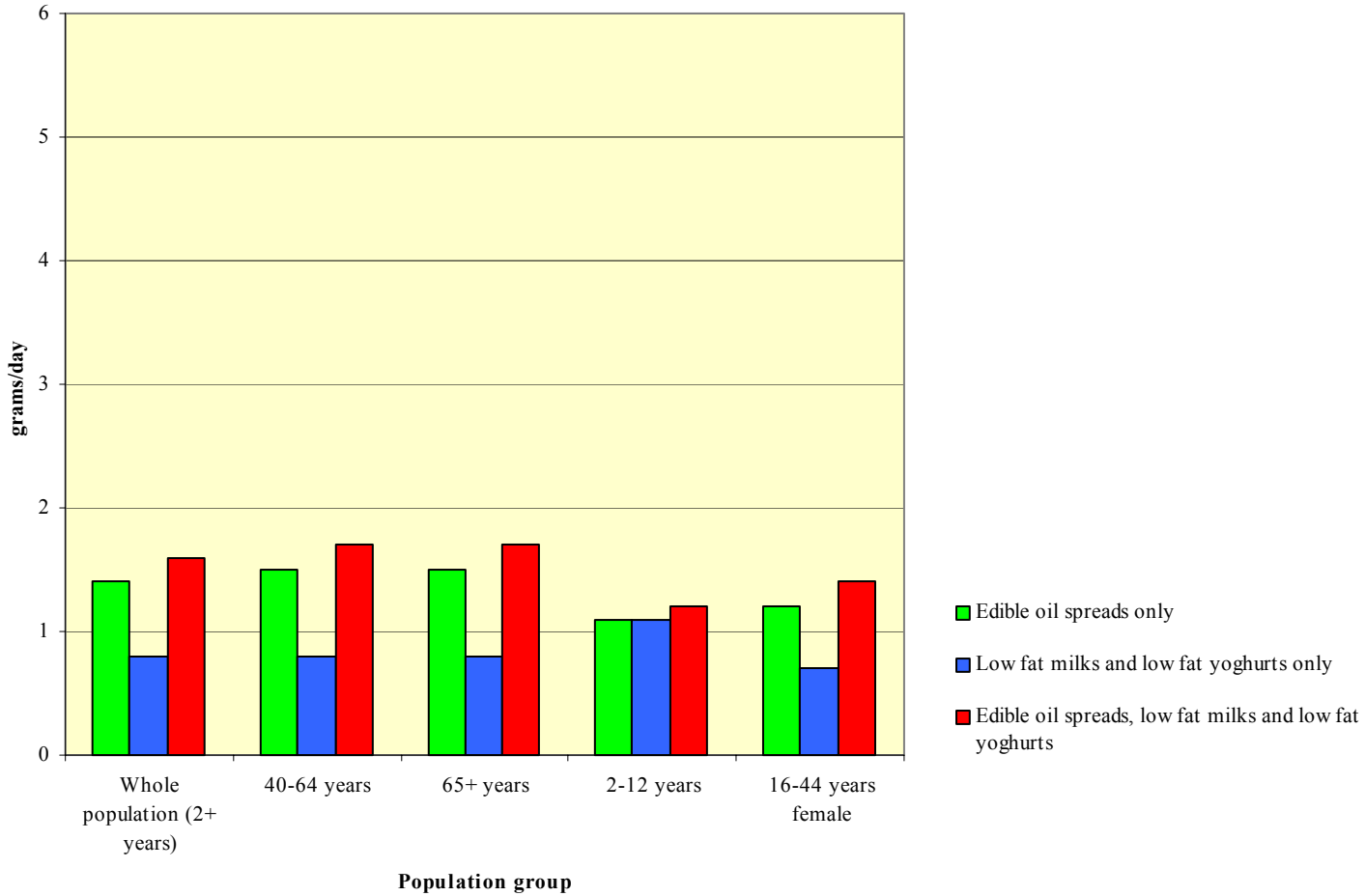


Figure 2: Estimated 95th percentile dietary exposure to free phytosterols for different population groups and scenarios for Australia

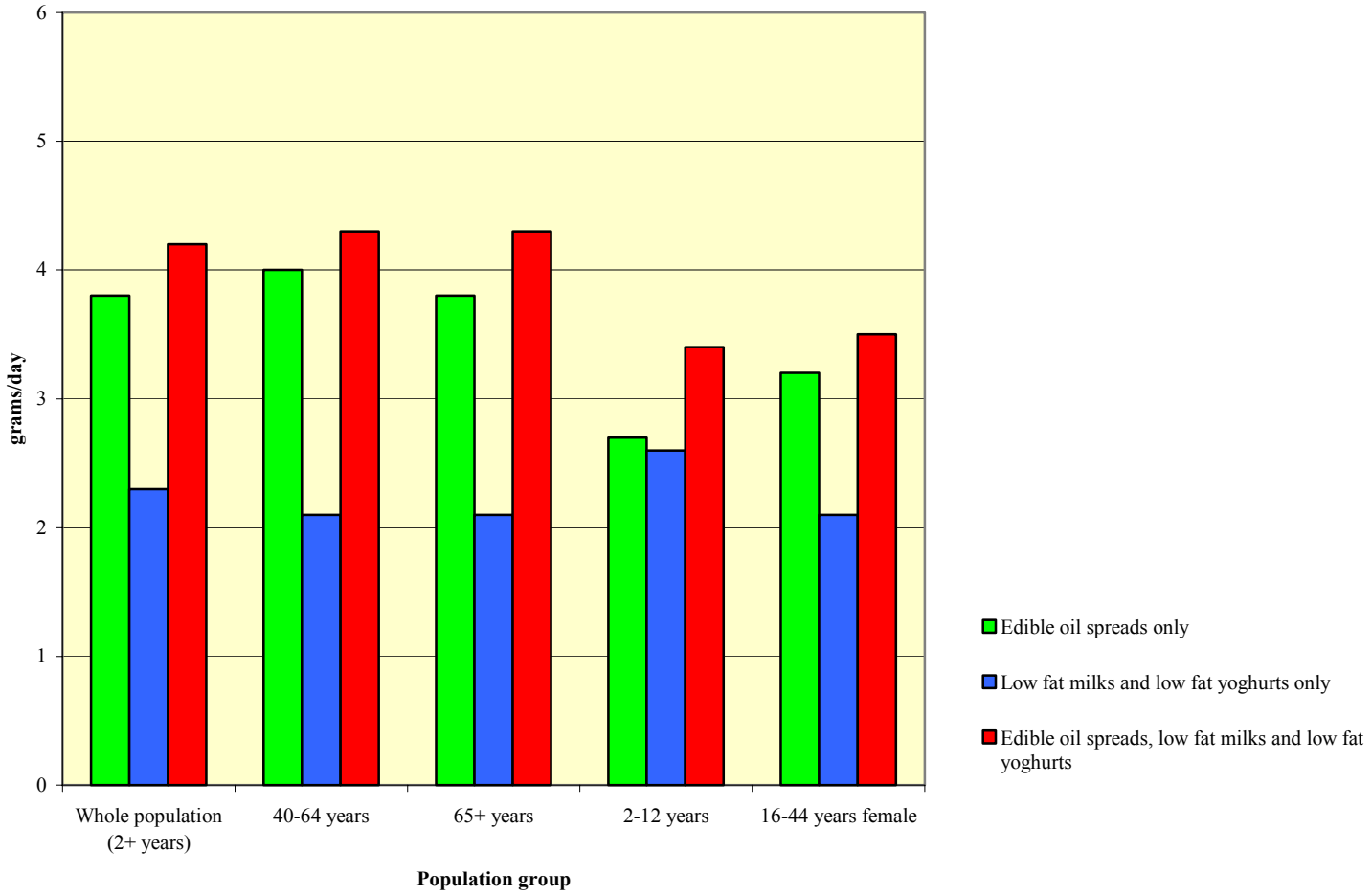


Figure 3: Estimated mean dietary exposure to free phytosterols for different population groups and scenarios for New Zealand

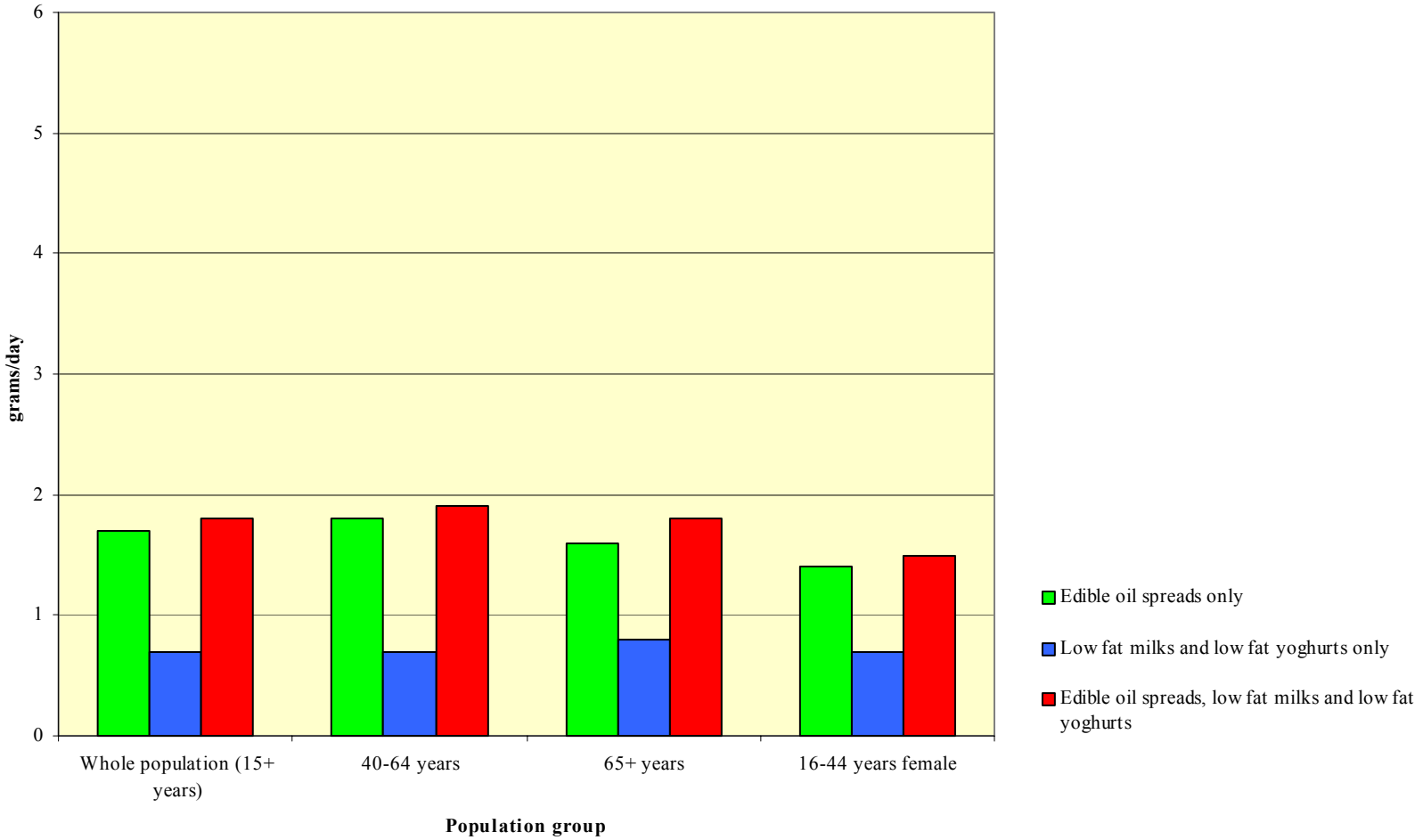


Figure 4: Estimated 95th percentile dietary exposure to free phytosterols for different population groups and scenarios for New Zealand

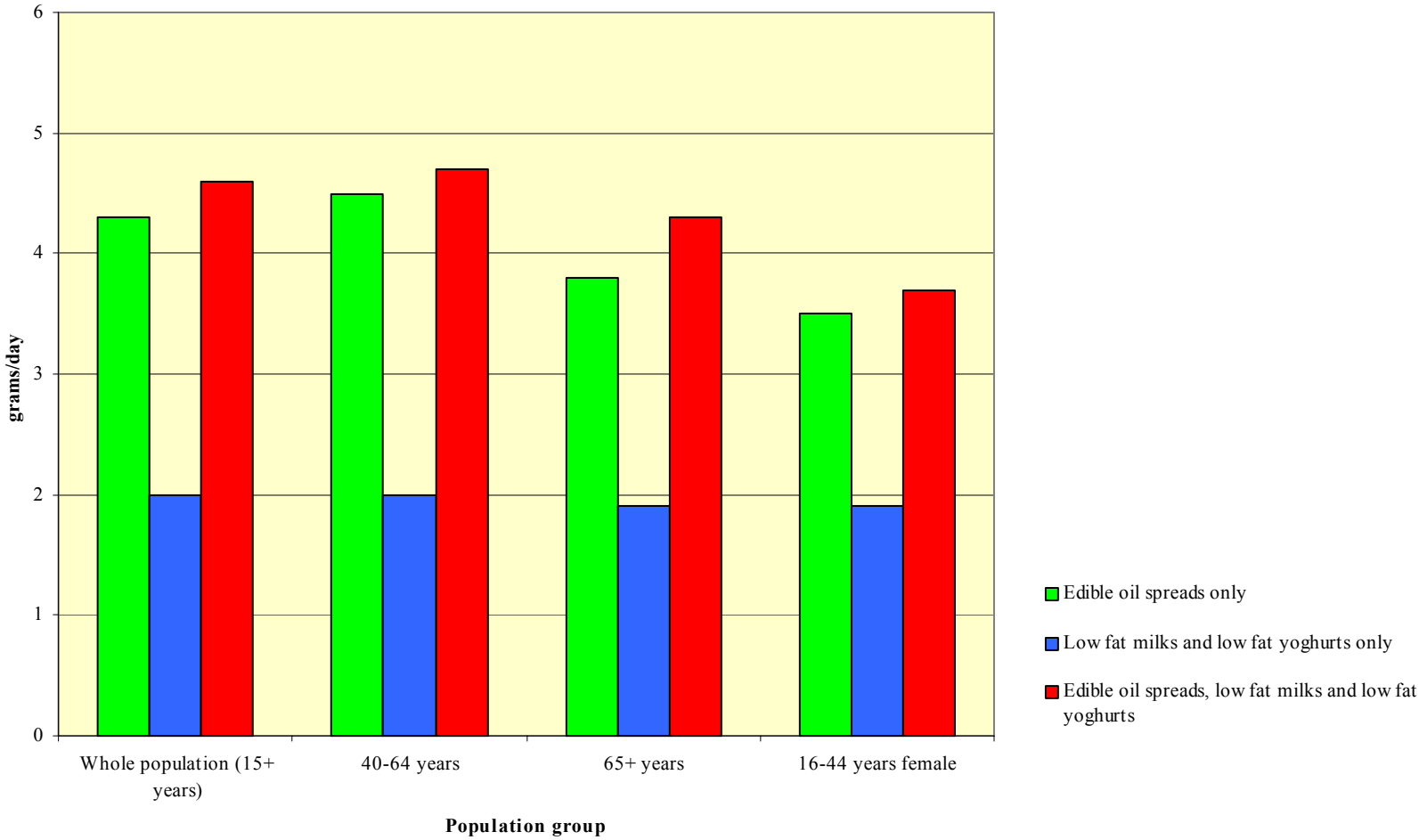


Table 2: Estimated dietary exposure to free phytosterols from edible oil spreads, for different population groups for Australia and New Zealand

Country	Population group	Number of consumers of phytosterols	Consumers as a % of total respondents[#]	Mean consumer exposure g/day	95th percentile consumer exposure g/day
Australia	Whole population (2 years+)	11 002	79	1.4	3.8
	40-64 years	3 372	78	1.5	4.0
	65+ years	1 557	79	1.5	3.8
	2-12 years	1 754	84	1.1	2.7
	16-44 years female	2 428	76	1.2	3.2
New Zealand	Whole population (15 years+)	3 093	67	1.7	4.3
	40-64 years	1 184	69	1.8	4.5
	65+ years	606	74	1.6	3.8
	16-44 years female	946	63	1.4	3.5

Total number of respondents for Australia: whole population (2 years and above) = 13 858, 2-12 years = 2 079, 40-64 years = 4 318, 65+ years = 1 960, 16-44 years female = 3 178; New Zealand: whole population (15 years and above) = 4 636, 40-64 years = 1 725, 65+ years = 817, 16-44 years female = 1 509;

(1) Estimated baseline dietary exposure to free phytosterols from edible oil spreads

Estimated mean dietary exposure to free phytosterols among consumers of edible oil spreads does not exceed 1.8 g/day (see Table 2 above), equivalent to slightly more than 2 serves of phytosterol-containing spreads per day. The highest mean exposure in both countries is for those aged 40-64 years (a sub-set of the target group for these products), and also the whole population (aged 15+ years) for New Zealand and those aged 65+ years for Australia. High consumers (95th percentile) of spreads have estimated dietary exposures to phytosterols up to 4.5 g/day, equivalent to 5½ serves of phytosterol-containing spreads per day. Again the group with the highest 95th percentile exposure is the 40-64 year age group for both countries, and also the whole population (aged 15+ years) for New Zealand.

These data show that the mean consumers of edible oil spreads in all population groups consume more than one serve of edible oil spreads per day, with the maximum mean consumer intake being 1.8 g (or 2.25 serves) per day for New Zealanders aged 40-64 years.

Table 3: Estimated dietary exposure to free phytosterols from low fat milks and low fat yoghurts, for different population groups for Australia and New Zealand

Country	Population group	Number of consumers of phytosterols	Consumers as a % of total respondents[#]	Mean consumer exposure g/day	95th percentile consumer exposure g/day
Australia	Whole population (2 years+)	4 510	33	0.8	2.3
	40-64 years	1 785	41	0.8	2.1
	65+ years	727	37	0.8	2.1
	2-12 years	348	17	1.1	2.6
	16-44 years female	1 166	37	0.7	2.1
New Zealand	Whole population (15 years+)	1 632	35	0.7	2.0
	40-64 years	689	40	0.7	2.0
	65+ years	341	42	0.8	1.9
	16-44 years female	469	31	0.7	1.9

Total number of respondents for Australia: whole population (2 years and above) = 13 858, 2-12 years = 2 079, 40-64 years = 4 318, 65+ years = 1 960, 16-44 years female = 3 178; New Zealand: whole population (15 years and above) = 4 636, 40-64 years = 1 725, 65+ years = 817, 16-44 years female = 1 509;

(2) Estimated ('low fat milks and low fat yoghurts') scenario dietary exposure to free phytosterols from low fat milks and low fat yoghurts

Estimated mean dietary exposure to free phytosterols among consumers of low fat milks and low fat yoghurts does not exceed 1.1 g/day (see Table 3 above), reflecting the smaller number of serves consumed per day of these foods than of edible oil spreads except in the 2-12 years age group. The highest mean exposure is for Australians aged 2-12 years. High consumers (95th percentile) of free phytosterols from low fat milks and low fat yoghurts have estimated dietary exposures up to 2.6 g/day, equivalent to more than 3 serves of low fat milk and low fat yoghurt per day.

These data show that the mean Australian consumers of low fat milks and low fat yoghurts from all population groups assessed consume equal to about one serve per day of phytosterol enriched low-fat milk/low-fat yoghurt with the highest mean consumer intake being 1.3 serves of low-fat milk/low-fat yoghurt per day for Australian children aged 2-12 years. For the New Zealand population, the highest mean consumer of low-fat milk/low-fat yoghurt has an estimated exposure to these products of 1.0 serve per day.

Table 4: Estimated dietary exposure to free phytosterols from edible oil spreads, low fat milks and low fat yoghurts, for different population groups for Australia and New Zealand

Country	Population group	Number of consumers of phytosterols	Consumers as a % of total respondents[#]	Mean consumer exposure g/day	95th percentile consumer exposure g/day
Australia	Whole population (2 years+)	11 941	86	1.6	4.2
	40-64 years	3 757	87	1.7	4.3
	65+ years	1 691	86	1.7	4.3
	2-12 years	1 809	87	1.2	3.4
	16-44 years female	2 704	85	1.4	3.5
New Zealand	Whole population (15 years+)	3 570	77	1.8	4.6
	40-64 years	1 376	80	1.9	4.7
	65+ years	681	83	1.8	4.3
	16-44 years female	1 114	74	1.5	3.7

Total number of respondents for Australia: whole population (2 years and above) = 13 858, 2-12 years = 2 079, 40-64 years = 4 318, 65+ years = 1 960, 16-44 years female = 3 178; New Zealand: whole population (15 years and above) = 4 636, 40-64 years = 1 725, 65+ years = 817, 16-44 years female = 1 509;

(3) Estimated ('low fat milks and low fat yoghurts plus baseline') scenario dietary exposure to free phytosterols from edible oil spreads, low fat milks and low fat yoghurts

When free phytosterols are added to low fat milks and low fat yoghurts as well as edible oil spreads, estimated mean exposure to free phytosterols increases slightly from 1.4 g to 1.6 g/day for the Australian population, and from 1.8 g/day to 1.9 g/day for the New Zealand population (see Table 4 above). Estimated mean dietary exposure does not exceed 1.9 g/day for any population group and is highest for those aged 40-64 years in New Zealand. High consumers of free phytosterols (95th percentile) from edible oil spreads, margarines and low fat milks and low fat yoghurts have estimated dietary exposures of between 4.3 g/day to 4.7 g/day for all population groups assessed. Again, the group with the highest 95th percentile exposure is the New Zealand population group 40-64 years.

Discussion

At first inspection, it may appear surprising that the addition of phytosterols to low fat milk and low fat yoghurt, as well as to edible oil spreads, indicates only a slight increase in predicted mean intakes of phytosterols compared to baseline exposure; an increase of 0.2 g/day for all Australians and New Zealanders.

These findings reflect both the greater number of serves of edible oil spreads consumed on average and the much larger number of consumers of edible oil spreads than of low fat milks or low fat yoghurts in the low fat milks and low fat yoghurts + baseline scenario. The proportion of Australians and New Zealanders who consume low fat milks and low fat yoghurts (33% and 35%, respectively) is substantially lower than the proportion who consume edible oil spreads (79% and 67%, respectively). As noted earlier, the DIAMOND program derives results from each individual's food consumption patterns.

Major contributing foods to total estimated dietary exposures

The relative contributions of edible oil spreads, low fat milks and low fat yoghurts to estimated exposures to free phytosterols are displayed in Figures 5 and 6. More detailed results are presented in Appendix 1.

Foods may be high contributors to phytosterol exposure when they have a high concentration of free phytosterols, when they are consumed in large quantities and/or are consumed by a large proportion of the survey population.

Edible oil spreads are more important contributors to dietary exposure to free phytosterols than are low fat milks and low fat yoghurts, on a population basis, assuming that eating patterns recorded in 1995 have been maintained.

Figure 5: Percent contribution of each food group to free phytosterols dietary exposure for Australia

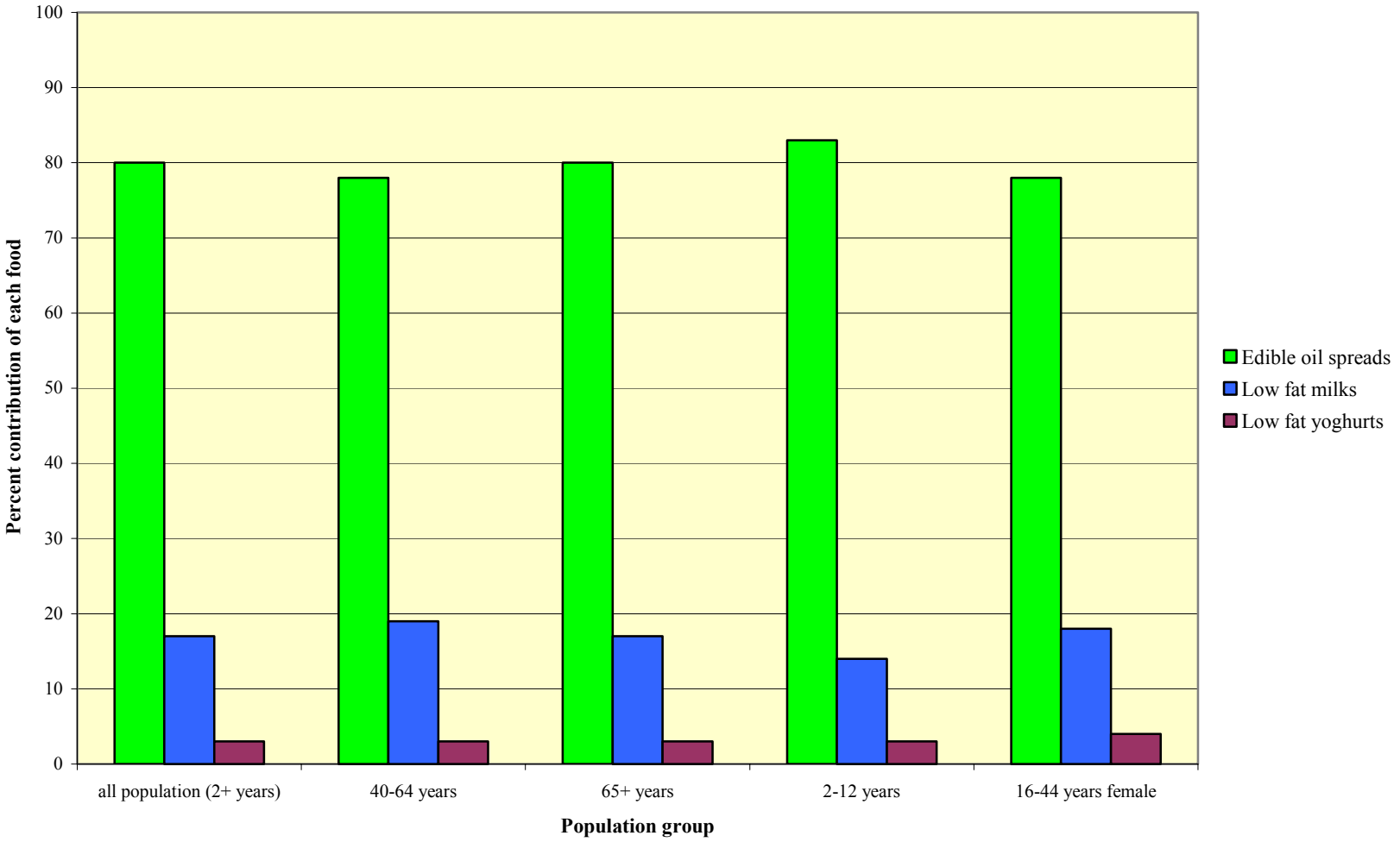
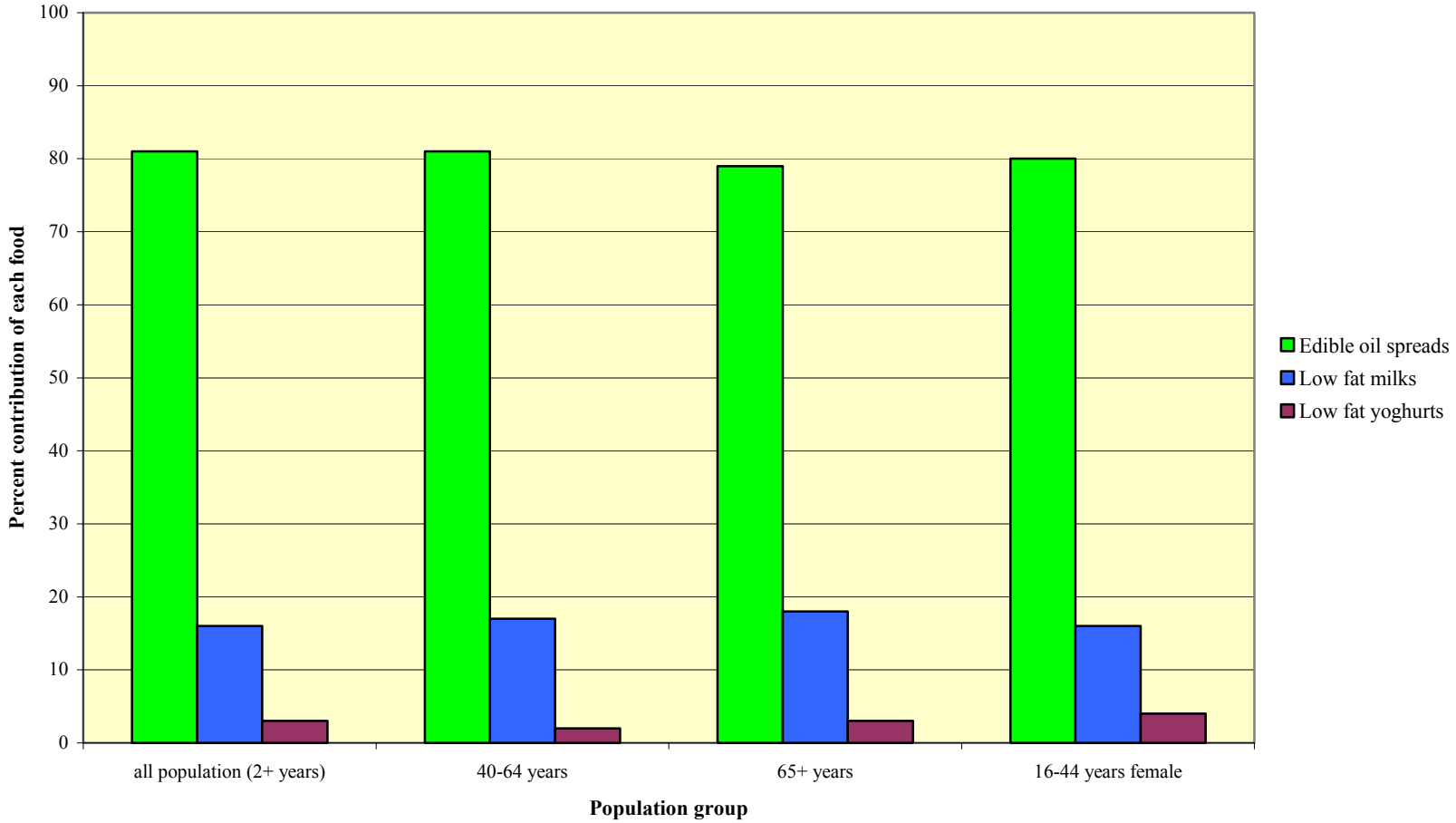


Figure 6: Percent contribution of each food group to free phytosterols dietary exposure for New Zealand



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Appendix 1

Major contributors to free phytosterols from edible oil spreads, low fat milks and low fat yoghurts for Australia and New Zealand.

Table A1.1: Major contributing foods to total free phytosterols dietary exposure for Australia and New Zealand, for different population groups

Country	Population group	Major contributing foods and percent of projected total free phytosterols intake
Australia	Whole population (2+ years)	Edible oils and margarines (80%) Low and milks (17%) Low fat yoghurts (3%)
	40- 64 years	Edible oils and margarines (78%) Low and milks (19%) Low fat yoghurts (3%)
	65+ years	Edible oils and margarines (80%) Low and milks (17%) Low fat yoghurts (3%)
	2 - 12 years	Edible oils and margarines (83%) Low and milks (14%) Low fat yoghurts (3%)
	16 – 44 years female	Edible oils and margarines (78%) Low and milks (18%) Low fat yoghurts (4%)
New Zealand	Whole population (15+ years)	Edible oils and margarines (81%) Low and milks (16%) Low fat yoghurts (3%)
	40- 64 years	Edible oils and margarines (81%) Low and milks (17%) Low fat yoghurts (2%)
	65+ years	Edible oils and margarines (79%) Low and milks (18%) Low fat yoghurts (3%)
	16-44 years female	Edible oils and margarines (80%) Low and milks (16%) Low fat yoghurts (4%)

ATTACHMENT 6

COMBINED DIETARY EXPOSURE ASSESSMENT REPORT

Applications A433 & A434 – Phytosterol esters derived from vegetable oils as a novel food ingredient in breakfast cereals, low fat milk and low fat yoghurt.

Summary

FSANZ is currently considering two separate applications seeking permission to use phytosterol esters derived from vegetable oils as a novel food ingredient under Standard 1.5.1, in 'healthy' (i.e. high-fibre, low-sugar) breakfast cereals (A433) and in low fat milk and low fat yoghurt (A434). This dietary exposure assessment was undertaken to determine the combined impact of allowing phytosterol esters to be added to all of the above foods, and incorporates the results of the dietary exposure assessment at **Attachment 5** to this report. The results therefore are an indication of likely exposures to phytosterol esters should both applications be approved.

This report provides a detailed dietary assessment of baseline plus all proposed foods (edible oil spreads, healthy breakfast cereals, low fat milk and low fat yoghurts). Assessments were conducted for the general Australian and New Zealand populations (2 years and above and 15 years and above, respectively), for two target populations for phytosterol products (those aged 40-64 years and 65 years and above) and for two non-target populations - 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) and the 1997 New Zealand NNS. Food chemical concentration data were derived from levels proposed in both applications and from the maximum level of use already permitted in edible oil spreads and margarines in the Code.

Estimated mean dietary exposure from all foods, expressed as free phytosterols, did not exceed 1.9 grams per day (g/day) in any population group assessed. At the 95th percentile of exposure, no population group exceeded 4.7 g free phytosterols per day. The major source of dietary exposure to added phytosterols was edible oil spreads for all population groups assessed. It should be noted that the modelling approach was conservative as it assumed all requested foods contained phytosterols at the maximum concentration, and therefore dietary exposure is overestimated.

1. Introduction

This dietary exposure assessment combines the results of separate dietary exposure estimates for each of the applications A433 and A434 (at Attachment 5 to each Draft Assessment Report), to provide an estimate of overall phytosterol ester intake, if all foods under assessment contained phytosterols (a worst-case scenario).

2. Methodology

The dietary exposure assessment took into account the existing permission under Standard 1.5.1 which allows the addition of phytosterol esters to reduced fat edible oil spreads and margarine, but not the intrinsic levels of phytosterols in foods. Both applicants propose to add phytosterol esters in amounts equivalent to 0.8 g free phytosterols per serve (1.3 g phytosterol esters). All values reported are expressed as free phytosterols.

As indicated in the individual dietary exposure assessment for each application, there are a number of assumptions and limitations in the dietary modelling process and therefore each dietary exposure assessment should be used only as a guide for risk management decisions regarding food regulation.

3. Concentration levels and serving sizes

The levels of free phytosterols in healthy breakfast cereals used in the exposure assessment were derived from Application A433, while the levels of use for low fat milk and low fat yoghurt were derived from Application A434. Levels of free phytosterols per serve were converted to concentrations in mg/kg to enable them to be entered into DIAMOND. Serve sizes are based on average product serve sizes from food packages - including 1 serve of edible oil spreads (10 g), 1 serve of healthy breakfast cereal (45 g), 1 glass of milk (250 mL) and 1 small punnet of low fat yoghurt (140 g). The foods and proposed levels of use are summarised below in Table 1.

Table 1: Proposed levels of use of free phytosterols in foods

DIAMOND Food Code	Food Name	Serve size (g)	Proposed level of free phytosterol per serve (g/serve)	Concentration Level used in modelling (mg/kg)
1.1.1.2	Low fat milks (<1.5% fat), unflavoured	250	0.8	3 200
1.2.2.2	Low fat flavoured yoghurts (<1.5% fat content), including low fat fruit yoghurts	140	0.8	5 714
2.2	Edible oil spreads including reduced fat spreads	10	0.8	80 000
6.3.4	Healthy Breakfast cereals	45	0.8	17 778

4. Results

Estimated dietary exposures to added phytosterols

The estimated exposures to phytosterols from all foods encompassed by A433 and A434 combined were calculated and are presented in Figure 1 (means) and Figure 2 (95th percentile) for Australia, and Figure 3 (means) and Figure 4 (95th percentile) for New Zealand. For comparative purposes the following are presented:

- estimated exposure baseline (edible oil spreads only);
- estimated exposure A433 (edible oil spreads plus healthy breakfast cereals);
- estimated exposure A434 (edible oil spreads plus low fat milk and low fat yoghurt); and
- estimated exposure all foods combined

A complete set of numerical results are provided for Australia and New Zealand in Appendix 1, Table A1.1 (all proposed foods). Results for consumers only (eaters of foods containing phytosterols) are presented, rather than results based on all respondents. This is because the purpose of the risk assessment is to consider the potential impact of phytosterol addition to a variety of foods on people who report eating these foods.

Estimated mean exposure to phytosterols among consumers of any phytosterol containing food does not exceed 1.9 g/day (see Appendix 1). The highest mean exposure is for those aged 40-64 years in New Zealand, which is one of the target groups for phytosterol-containing products. High consumers (95th percentile) of the specified foods have estimated dietary exposures to phytosterol esters up to 4.7 g/day. Again, the group with the highest 95th percentile exposure is the 40-64 year age group in the New Zealand population.

Figure 1: Estimated mean dietary exposure to free phytosterols for different population groups and scenarios for Australia

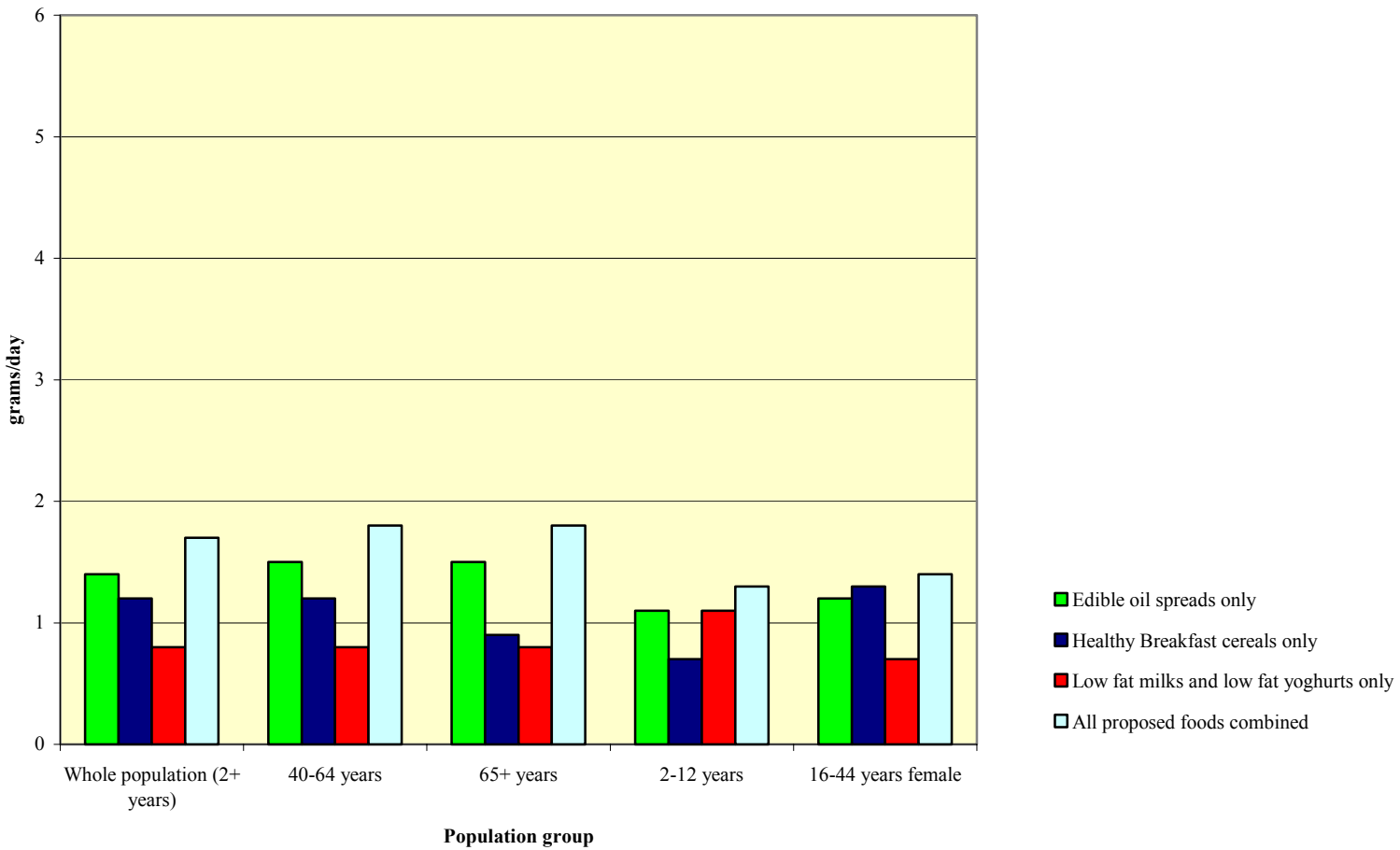


Figure 2: Estimated 95th percentile dietary exposure to free phytosterols for different population groups and scenarios for Australia

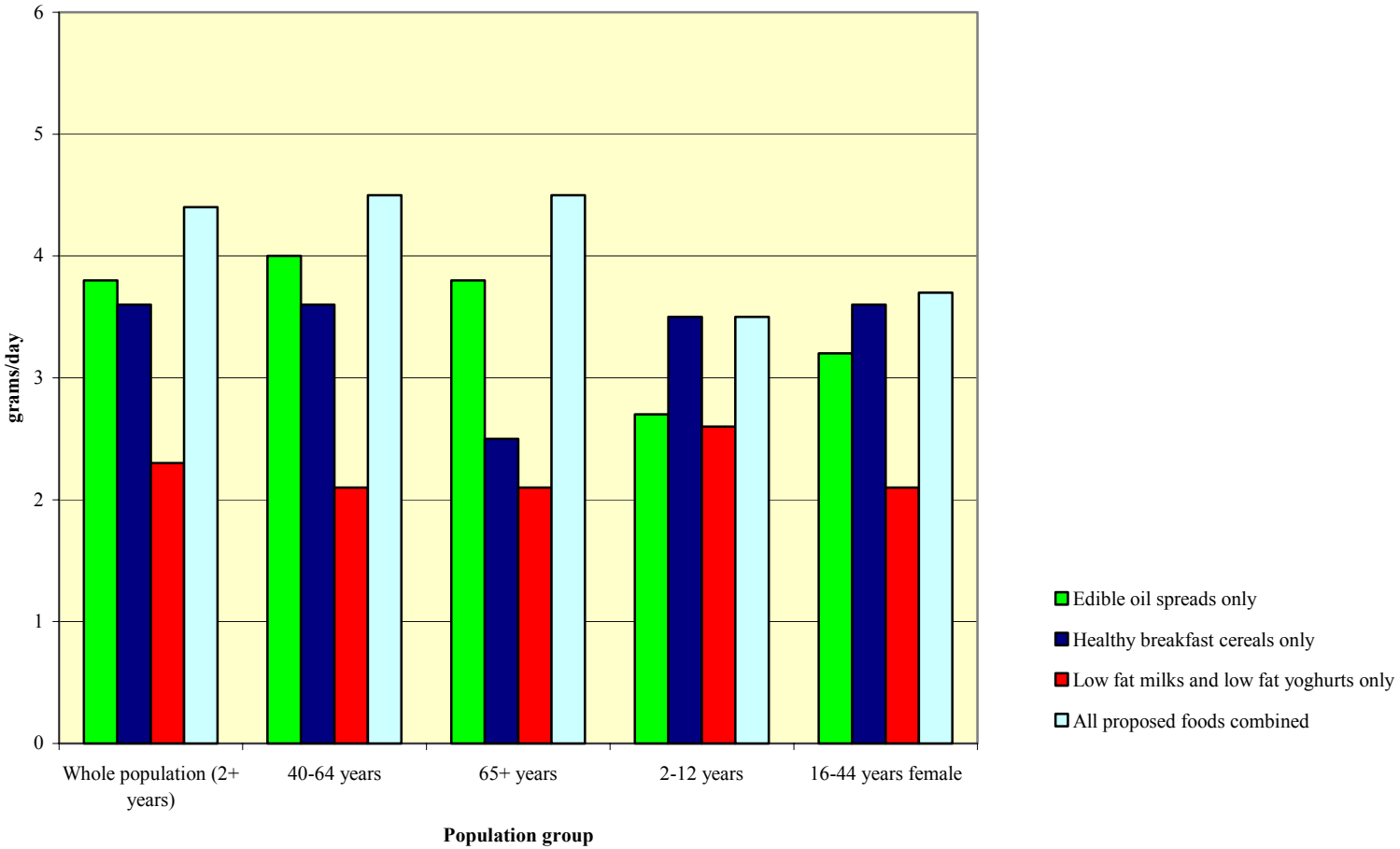


Figure 3: Estimated mean dietary exposure to free phytosterols for different population groups and scenarios for New Zealand

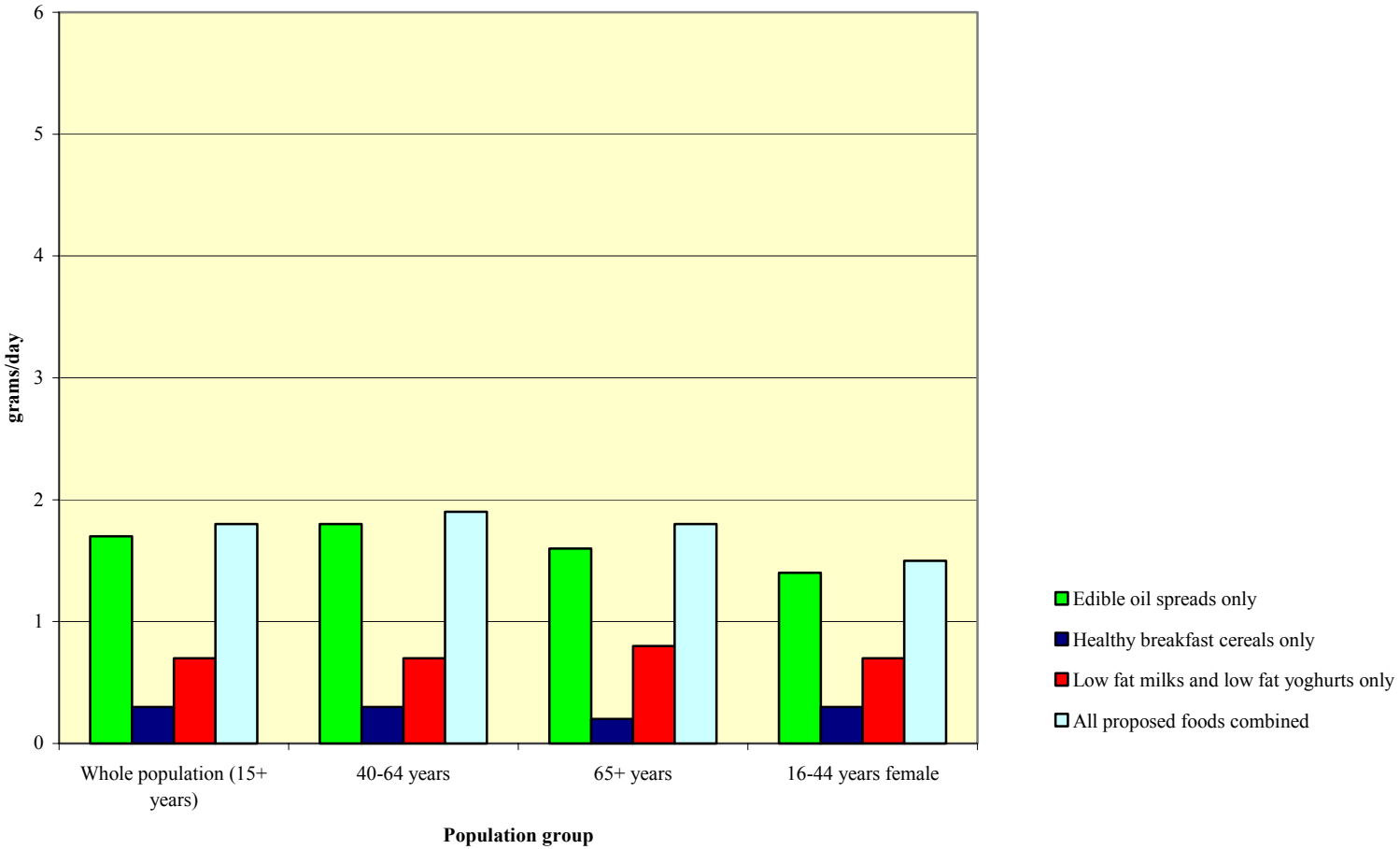
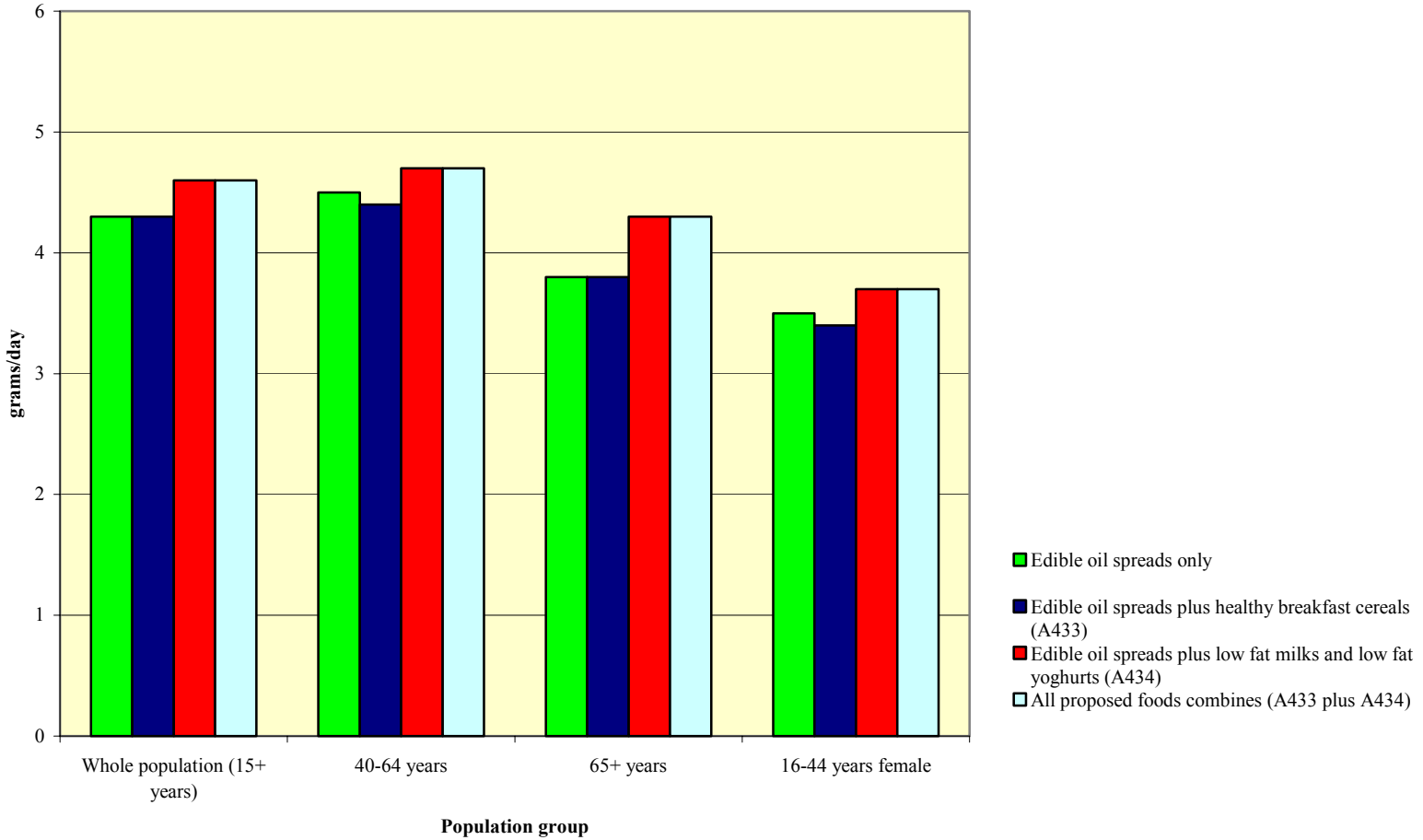


Figure 4: Estimated 95th percentile dietary exposure to free phytosterols for different population groups and scenarios for New Zealand



Major contributing foods to total estimated dietary exposures

The relative contributions of each phytosterol containing food to combined dietary exposures to free phytosterols are displayed in Figure 5 for Australia, and Figure 6 for New Zealand. More detailed results are presented in Appendix 2.

Foods may be high contributors to phytosterol exposure when they have a high concentration of free phytosterols, when they are consumed in large quantities and/or are consumed by a large proportion of the survey population.

When all foods are combined in the exposure assessments, edible oil spreads are the major contributors to dietary exposure to free phytosterols, for every population group assessed.

Figure 5: Percent contribution of each food group to free phytosterols dietary exposure for Australia

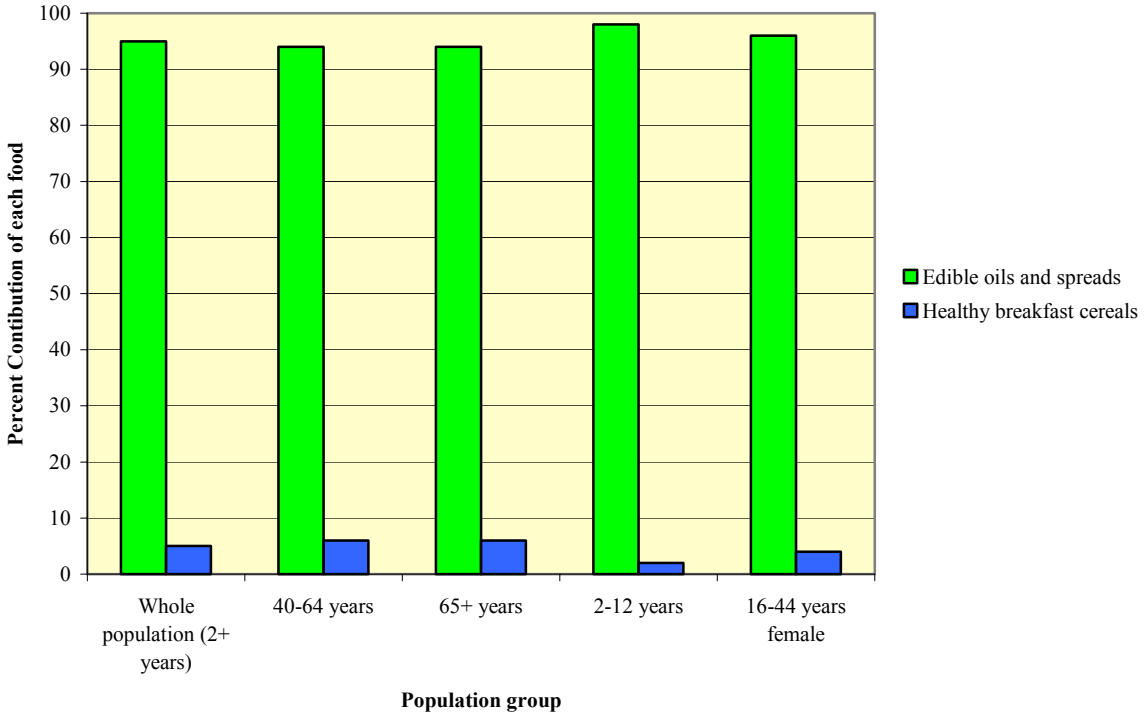
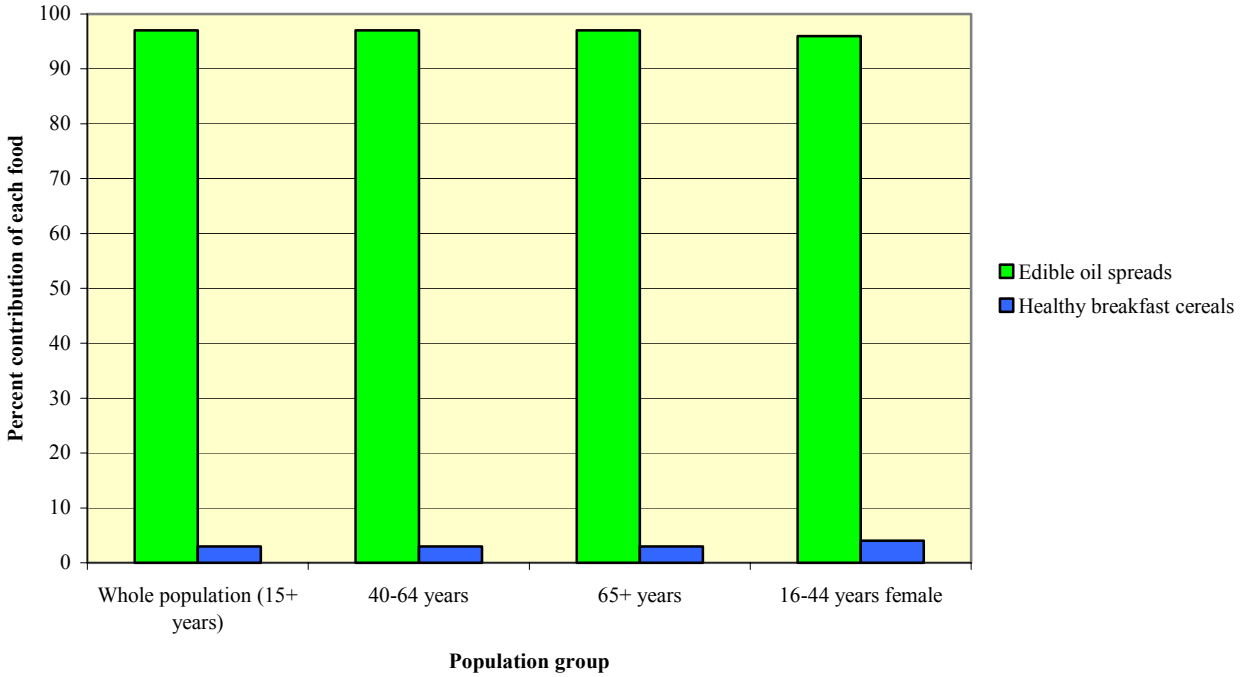


Figure 6: Percent contribution of each food group to free phytosterols dietary exposure for New Zealand



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Appendix 1

Estimated exposures to free phytosterols from all foods combined for Australia and New Zealand

A1.1: Estimated dietary exposures to free phytosterols from all foods combined (edible oil spreads, healthy breakfast cereals, low fat milk and low fat yoghurts), for different population groups for Australia and New Zealand

Country	Population group	Number of consumers of phytosterols	Consumers as a % of total respondents[#]	Mean consumer exposure grams/day	95th percentile consumer exposure grams/day
Australia	Whole population (2 years+)	12 016	87	1.3	4.4
	40-64 years	3 787	88	1.4	4.5
	65+ years	1 709	87	1.5	4.5
	2-12 years	1 814	87	1.3	3.5
	16-44 years female	2 720	86	1.4	3.7
New Zealand	Whole population (15 years+)	3 686	84	1.8	4.6
	40-64 years	1 420	82	1.9	4.7
	65+ years	705	86	1.8	4.3
	16-44 years female	1 148	76	1.5	3.8

Total number of respondents for Australia: whole population (2 years and above) = 13 858, 2-12 years = 2 079, 40-64 years = 4 318, 65+ years = 1 960, 16-44 years female = 3 178; New Zealand: whole population (15 years and above) = 4 636, 40-64 years = 1 725, 65+ years = 817, 16-44 years female = 1 509;

Appendix 2

Major contributors to free phytosterols from all proposed foods for Australia and New Zealand

Table A2.1: Major contributing foods to total free phytosterols dietary exposure for Australia and New Zealand, for different population groups

Country	Population group	Major contributing foods and percent of total free phytosterols exposures
Australia	Whole population (2+ years)	Edible oil spreads (77%) Low fat milk (16%) Healthy breakfast cereal (4%) Low fat yoghurt (3%)
	40- 64 years	Edible oil spreads (73%) Low fat milk (18%) Healthy breakfast cereal (5%) Low fat yoghurt (4%)
	65+ years	Edible oil spreads (76%) Low fat milk (16%) Healthy breakfast cereal (5%) Low fat yoghurt (3%)
	2 - 12 years	Edible oil spreads (82%) Low fat milk (14%) Low fat yoghurt (3%) Healthy breakfast cereal (2%)
	16 – 44 years female	Edible oil spreads (75%) Low fat milk (18%) Low fat yoghurt (4%) Healthy breakfast cereal (3%)
New Zealand	Whole population (15+ years)	Edible oil spreads (78%) Low fat milk (15%) Low fat yoghurt (4%) Healthy breakfast cereal (3%)
	40- 64 years	Edible oil spreads (78%) Low fat milk (16%) Low fat yoghurt (3%) Healthy breakfast cereal (3%)
	65+ years	Edible oil spreads (76%) Low fat milk (17%) Low fat yoghurt (4%) Healthy breakfast cereal (3%)
	16-44 years female	Edible oil spreads (76%) Low fat milk (16%) Low fat yoghurt (5%) Healthy breakfast cereal (3%)

ATTACHMENT 7

SUMMARY OF PUBLIC SUBMISSIONS

THE FIRST PUBLIC CONSULTATION PERIOD FOR THIS APPLICATION WAS CONDUCTED BETWEEN 19 MARCH 2003 AND 30 APRIL 2003. THE FOLLOWING SUBMISSIONS WERE RECEIVED:

1. Australian Dairy Corporation

- Supports Option 2 – approval of phytosterol esters in low-fat milk and low-fat yoghurt, with careful consideration as to appropriate labelling of these products to provide advice to consumers.
- Does not support Option 3 – a general approval for the use of phytosterol esters in foods because of concerns that a broad approval could lead to over-consumption and use of foods that are incompatible with the Australian Dietary Guidelines.
- Cites the UK Advisory Committee on Novel Foods and Processes (ACNFP) judgement that increasing the product range to include milk and yoghurt-type products would not lead to over-consumption of phytosterols.
- States that research shows that phytostanols in yoghurt can lower LDL cholesterol as effectively as enriched margarines.
- Considers that enrichment of specific foods with either phytostanols or phytosterol esters would benefit public health by:

  – improving access to phytosterol enriched foods;
  – improving the clarity of dietary advice to patients with risk of heart disease;
  – allowing consumers of phytosterol enriched milk and yoghurt to experience all of the benefits of these foods in terms of other nutrients that may be lacking;
  – consuming additional low-fat milk and yoghurt in conjunction with a low-fat diet rich in fruits and vegetables can also benefit other coronary heart disease risk factors such as high blood pressure and plasma homocysteine levels.

2. Joanne Dellow – University of Otago

- Considers that phytosterol enrichment of low-fat dairy products such as milk and yoghurt should not be approved at this time because of insufficient evidence of efficacy and safety at higher consumption levels, despite strong evidence that phytosterols are effective in lowering cholesterol when present in edible oil spreads.
- Provides detailed analysis of phytosterols in terms of their safety, efficacy in various food matrices, possible mechanisms of action, and dietary or nutritional effects.
- Questions whether low-fat dairy products are appropriate foods for older adult males. Considers that it is important that the proposed products are already part of the target population's habitual diet because both dietary and lifestyle modifications are difficult to implement and long-term adherence is difficult to maintain.
- Considers that the published studies so far do not unequivocally demonstrate no adverse effects in humans and that the long-term effects of phytosterols are generally unknown.
- Cites studies that show that intake of phytosterols of 1.6g per day adversely affects beta-carotene levels, and therefore the proposed level of use of 2.4g per day from three servings of phytosterol enriched products will have deleterious nutritional effects.

3. SoNatural Foods Australia

- Supports Option 2, to approve the use of phytosterol esters in low-fat milk and yoghurt
- Considers that there should be additional permissions for use of phytosterol esters in non-dairy beverages derived from legumes that are consumed as alternatives to dairy products because:

    - it is unfair to discriminate against those consumers who are intolerant to dairy products;
    - soybeans naturally contain phytosterols and the addition of the esters to soy-derived dairy alternatives will enhance the inherent health benefits;
    - soy foods are recommended by the American Heart Association for people with elevated total and LDL-cholesterol, and the addition of phytosterols to soy products will further enhance their cholesterol lowering ability. Consumers will therefore not need to change or increase their consumption of enriched foods to meet dietary recommendations;
    - in expanding the current application to include legume-derived alternatives, a greater variety of cholesterol-lowering foods will be available, allowing consumers to more easily reach the required intake; and
    - as phytosterols are found naturally in soy, they are likely to be as effective in lowering cholesterol as they are when present in other foods such as beverages, margarines, ground beef and baked goods.

- There are benefits to industry and to consumers by broadening the permission to include analogues derived from legumes. The benefits to industry include increased domestic market opportunities, increased sales and profitability, increased opportunity to impact on public health, and increased opportunity to provide information to consumers about healthy eating and lifestyle.
- There are costs to industry by not allowing the use of phytosterol esters in the corresponding non-dairy beverages by way of decreased market share in the novel foods arena, and a reduced ability to develop products which positively impact on public health.
- The benefits of adding phytosterol esters to low-fat milk and low-fat yoghurts, and their legume-derived alternatives, significantly outweigh the potential costs to business and to consumers.
- If the application is expanded to include analogues derived from legumes, industry would support a responsible approach to the marketing and labelling of the phytosterol enriched products to foster appropriate consumer usage.

4. Australian Food and Grocery Council (AFGC)

- Supports Option 3 – a broad permission for the use of phytosterol esters in food, but also supports Option 2 – permission to use phytosterol esters in low-fat milk and low-fat yoghurt as the next best option. Considers that this application could at least be extended to cover all yoghurts and all milks.
- Disputes FSANZ's authority to require efficacy data in relation to labelling as the Novel Foods Standard (Standard 1.5.1) refers only to the safety of the food, and there is already sufficient prohibitions on misleading or deceptive labelling in State, Territory and New Zealand Food Acts, the Trade Practices Act, and State, Territory and New Zealand Fair Trading Acts.
- States that if a reduction in absorption of fat-soluble vitamins is demonstrated, FSANZ can require phytosterol-enriched foods to contain a minimum quantity of the vitamins either naturally-occurring or by fortification. This disadvantage should not be the basis of a rejection of the application, as the ability to reduce cholesterol offers significant health advantages and reduced absorption of fat-soluble vitamins can be overcome.
- Considers that the over-use scenario by target consumers is unlikely partly because of the cost disincentive.

- States that habitual consumption by non-target groups is also unlikely and of minimal health risk.
- Agrees that appropriate labelling is essential, consistent with the existing requirements for phytosterol enriched table spreads and margarines.
- FSANZ cited health risk factors such as high blood cholesterol as part of the economic argument in favour of mandatory nutrition labelling. Approval of cholesterol lowering foods would therefore be consistent with this position.
- Supports FSANZ in proposing that maximum permitted levels and specifications for identity and purity may be required.
- Supports the same quantity of phytosterols being required in a serve of each of the foods to which phytosterols are added,
- A broad permission would be advantageous to obviate the necessity for further applications of a similar nature. At the very least, consideration should apply to all breads, breakfast cereals and breakfast cereal bars.

5. New Zealand Dietetic Association

- Opposes the application because there is neither sufficient nor substantial evidence yet available regarding the safety of higher intakes of phytosterols and prolonged exposure to phytosterols in fortified foods.
- Considers that the current permission for phytosterol-enriched table spreads enables controlled use of the products, and allows defined quantities to be recommended by health professionals to targeted people.
- Considers that general use of phytosterol-enriched foods by non-target groups e.g. the young, is premature since the scientific evidence is unclear on potential effects on carotenoids, lycopene, vitamin E, oestrogenic activity, and other biological activities.
- States that the lack of long term and large scale safety studies and the potential for widespread use of the products would preclude any recommendation of use by target consumers.
- increasing the range of foods will lead to an increase in the daily intake of phytosterols to say > 3g/day, with a paucity of supporting safety studies.
- Existing evidence shows reductions in plasma antioxidants, particularly lipophilic β-carotene, α-carotene, lycopene and other carotenoids (e.g. lutein, zeaxanthin) with higher intakes of phytosterols. Consumers cannot be relied upon to increase their consumption of fruit and vegetable to offset this decrease in absorption.
- Expresses particular concern for vulnerable groups such as children, adolescents and the elderly because the foods in question are favoured by these groups in large quantities and it is possible that, if available to the entire household, the foods will not be consumed exclusively by one target family member. In addition, consumer familiarity with the phytosterol-enriched foods may erode compliance with the dietary recommendations on the labels.
- Considers that the high cost of purchase will not work as a limiting factor.

6. Rosemary Stanton (Aust)

- Opposes the application on the basis of concerns about the nutritional issues for both target and non-target consumers including:

    - the possibility of side effects, particularly a reduction in levels of fat-soluble vitamins, α-tocopherol, β-carotene, lycopene.
    - a paucity of studies looking at the longer term nutritional effects from higher consumption of phytosterol- enriched foods.
    - reported undesirable reduction in HDL cholesterol
    - potential for unknown effects on other carotenoids and fat-soluble compounds such as the family of tocopherols and tocotrienols that may be important nutrients.

- Considers that dietary advice on the labels of phytosterol-enriched foods recommending consumption of fruits and vegetables will be ineffectual, as similar dietary advice has not been heeded by the Australian public.
- States that food manufacturers are unlikely to provide information to consumers that explains why additional fruits and vegetables are desirable.
- Potential effects on children ought to be investigated because of the nature of the foods in question i.e. fibre-increased bread, breakfast cereal bars, low-fat milk and yoghurt, that are favoured foods of the young.
- Considers that there are other effective measures for reducing cholesterol, namely reducing consumption of saturated fat and/or losing excess weight which have other beneficial health effects on the risks of developing diabetes or hypertension. The availability of a range of phytosterol-enriched foods may encourage a reliance only on these foods as a means of reducing cholesterol.
- Medicalisation of the food supply is not the best method of dealing with health problems, as it can be misinterpreted by consumers.
- Considers that the consumption levels of foods containing phytosterols will be difficult to control and there is no evidence that higher consumption is needed to achieve the desired effect. To minimise the possibility of over consumption, messages on food labels would need to be carefully worded and it is doubtful that this would be as effective as limiting the availability of phytosterol-enriched food products.
- Proposes that it would be more appropriate to market phytosterols as supplements.

7. Sanitarium Health Food Company (Aust)

- Opposes the application because of a current lack of adequate information regarding the safety of higher intakes of phytosterols from fortified foods.
- States that studies are required on the effects of phytosterol esters on a wide range of antioxidants in the blood, including β-carotene and other carotenoids like lutein and zeaxanthin.
- Considers that consumers may choose phytosterol-enriched products without increasing their consumption of carotenoid-rich foods such as fruits and vegetables, possibly because of a lack of advice about dietary effects.
- Doubts that consumers will comply with dietary advisory statements on the label of a phytosterol-enriched product.
- Advises that there could be an impact from a broader availability of phytosterol-enriched foods on people with the rare condition sitosterolaemia.

8. New Zealand Food Safety Authority

- Considers that the assessments of both applications are especially important in terms of the potential impacts of phytosterol-enriched products on non-target consumer groups such as children.
- Labelling information for consumers is important and should advise that consumption in excess of the recommended number of servings will not provide additional cholesterol-lowering benefit.
- The addition of phytosterol-esters to bread and cereals poses regulatory problems because the composition of these products in not prescribed in the Code. Linking permission to these product types would require product definitions in the Code.
- Reinforce the importance of manufacturers being aware of the permitted claims about the benefits of phytosterols, and staying within the provisions of the Code and the New Zealand Medicines Regulations.

9. Dietitians Association of Australia

- Considers that there are potential benefits for consumers and dietitians if a broader range of plant sterol containing foods is available.
- Expresses concerns about the possible higher intakes of plant sterols by non-target groups particularly pregnant/lactating women, elderly people, healthy and obese children and adolescents.
- A safety assessment using relevant studies must be completed.
- Nutrient criteria for any food under consideration should be applied before products qualify for the addition of plant sterols.
- Supports mandatory advisory statements on labels (but none specified).
- An informed decision on the merits of the application can only be done when more information is available.

10. Unilever Australia

- Supports Option 2, to approve phytosterol esters in fibre-increased bread and breakfast cereal bars, and endorses the comments of the AFGC.
- FSANZ should consider applications for like foods currently being assessed in the international arena in terms of the proposed levels of free phytosterols per serving of food. In these applications (A433 and A434), the applicants are seeking permission to use 0.8 g free sterols per serve of requested foods. The current Unilever application to the EU seeks approval to use 1 g of free sterols per serving of dairy food (milk and yoghurt). Information derived from Post Launch Monitoring of yellow fat spreads containing phytosterol esters has demonstrated that even regular users do not use the recommended quantities of spread, and this is confirmed also by data for Australian and New Zealand consumers. Therefore, permission to use 1 g of free phytosterols per serving of milk and yoghurt would enable consumers to reach the target amount of 2-3 g per day in 2-3 servings, rather than requiring a minimum of three servings.
- A common and easily understood labelling protocol ought to apply to all plant sterol containing foods to facilitate proper use by consumers. For example, the European Commission are in the process of preparing a directive on a common labelling format for all products containing phytosterols/esters/stanols. In addition to the mandatory advisory statements already required on products for sale in Australia/New Zealand, statements to this effect are suggested:

    - the product is for people who want to lower their blood cholesterol levels;
    - a declaration of the amount of phytosterols in the food eg. *one (package) of this food contains (x) g of plant sterols/stanols*; and
    - a recommendation on a suitable number of servings per day eg. *it is recommended to consume no more than 3 g sterol each day for the best cholesterol lowering effect.*

- The specifications for the phytosterol esters derived from vegetable oils in the Code are now not generic enough to apply to the phytosterols produced from the current range of available plant sources.
- Submits draft specifications under consideration by Food Chemicals Codex, and questions the need for minimum limits for the typical sterol distribution.

11. Richard James (NZ)

- Opposed to both Applications A433 and A434
- Considers that the advisory statements currently required on the table spreads and margarines are “warning” consumers that these foods should not be eaten by infants, children, pregnant or lactating women, and that the foods proposed in the applications are most likely to be consumed by children.

- Provides information on the dangers to children from diets that reduce their cholesterol.
- considers that phytosterols are estrogenic and raised this issue during the previous assessment of phytosterols in the food supply under Application A410. Considers that plant estrogens are already too prevalent in the diet with widespread adverse public health outcomes.
- Expresses concerns with the medicalisation of the food supply to benefit a particular section of the population, which will inevitably lead to consumption by people who do not need to lower their cholesterol.
- Lowering cholesterol levels below a normal level can have deleterious effects on health.
- If approved, the products should have mandatory warning statements because it is not known what cumulative effects may occur, or what the long-term effects of continual consumption may be.

12. Valerie James (NZ)

- opposed to both Applications A433 and A434
- provided material claiming that phytosterols can act as endocrine disrupters in fish, and exhibit a classic estrogenic effect in animals.
- phytosterol-containing products should carry conspicuous warnings to the effect that these products are unsuitable for pregnant women, infants and children.
- suggests that phytosterols have a 'drug-like' effect and consumers could actually be taking part in a large, uncontrolled human experiment.
- proposes that the benefits from lowering cholesterol are uncertain, and that there is almost no evidence that dietary cholesterol induces hypercholesterolaemia.
- suggests an association between a low serum cholesterol and the risk of lung cancer may be through lowering beta-carotene levels. Natural forms of carotenes are more beneficial than supplements.
- the application fails to show that any benefits outweigh the potential risks
- are associated with lung cancer and phytosterols serum cholesterol with lung cancer

13. Nestlé Australia

- Supports the general approval of the use of phytosterol-esters as ingredients in foods, including low-fat milk and low-fat yoghurt.
- Considers that use of phytosterol esters in foods can assist in the reduction of diet-related conditions such as high blood cholesterol, which is a known risk factor for disease.
- Approval of this application has the potential to reduce public health costs incurred by government.

14. Environmental Health Unit, Queensland Health

- Strongly opposed to this application, on the basis of expert dietary advice.
- Express a range of concerns including:

    - the long term effects and safety of consumption of phytosterols is unknown;
    - the inappropriate use of food as a medicine;
    - the potential for inequity in the marketplace, where the foods containing phytosterols are more costly than the traditional counterpart and are therefore less affordable to lower socio-economic groups who already carry a greater chronic disease burden;
    - consumption of phytosterols is reported to lower serum alpha-tocopherol and carotenoids;
    - phytosterol-esters reported to have an oestrogenic activity in animals, with unknown relevance in humans;

- phytosterol enriched foods present particular health risks to individuals with sitosterolaemia and who are homozygous for the condition. The risks to heterozygous individuals are not known; and
- the safety and use of these products by non-target groups (children, pregnant women, people with normal or low serum cholesterol) needs to be fully investigated.

- Suggests that the results from one clinical study are unlikely to provide sufficient information to alter previous conclusions about the lack of available data to assess the safety of phytosterols in a broad range of foods. The study would need to have used a large number of participants, and other similar studies would also be required using overseas populations.
- A statement to consume 2-3 servings per day of a particular food to ensure adequate consumption of the ingredient implies a therapeutic dose, which is inappropriate.
- All consumers may be affected, not just those with health concerns about high serum cholesterol.
- A wider range of foods containing added phytosterols may lead to excess consumption of phytosterols.
- There could be significant impact on Government educational resources and capacity if either of the options to approve the use of phytosterol-esters is successful, because in general, consumers do not understand what phytosterol-esters are and the role they may play in healthy eating and chronic disease management (specifically cardiovascular disease).

15. Food Technology Association of Victoria Inc

- supports option 2 i.e. approval of the use of phytosterol-esters as ingredients in low-fat milk and low-fat yoghurt.

16. Australian Quarantine and Inspection Service (AFFA)

- AQIS will defer comment until the Draft Assessment Report is completed and released for public comment.