4-04
26 May 2004

DRAFT ASSESSMENT REPORT

APPLICATION A508

PHYTOSTEROLS DERIVED FROM TALL OILS AS INGREDIENTS IN LOW FAT MILK

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

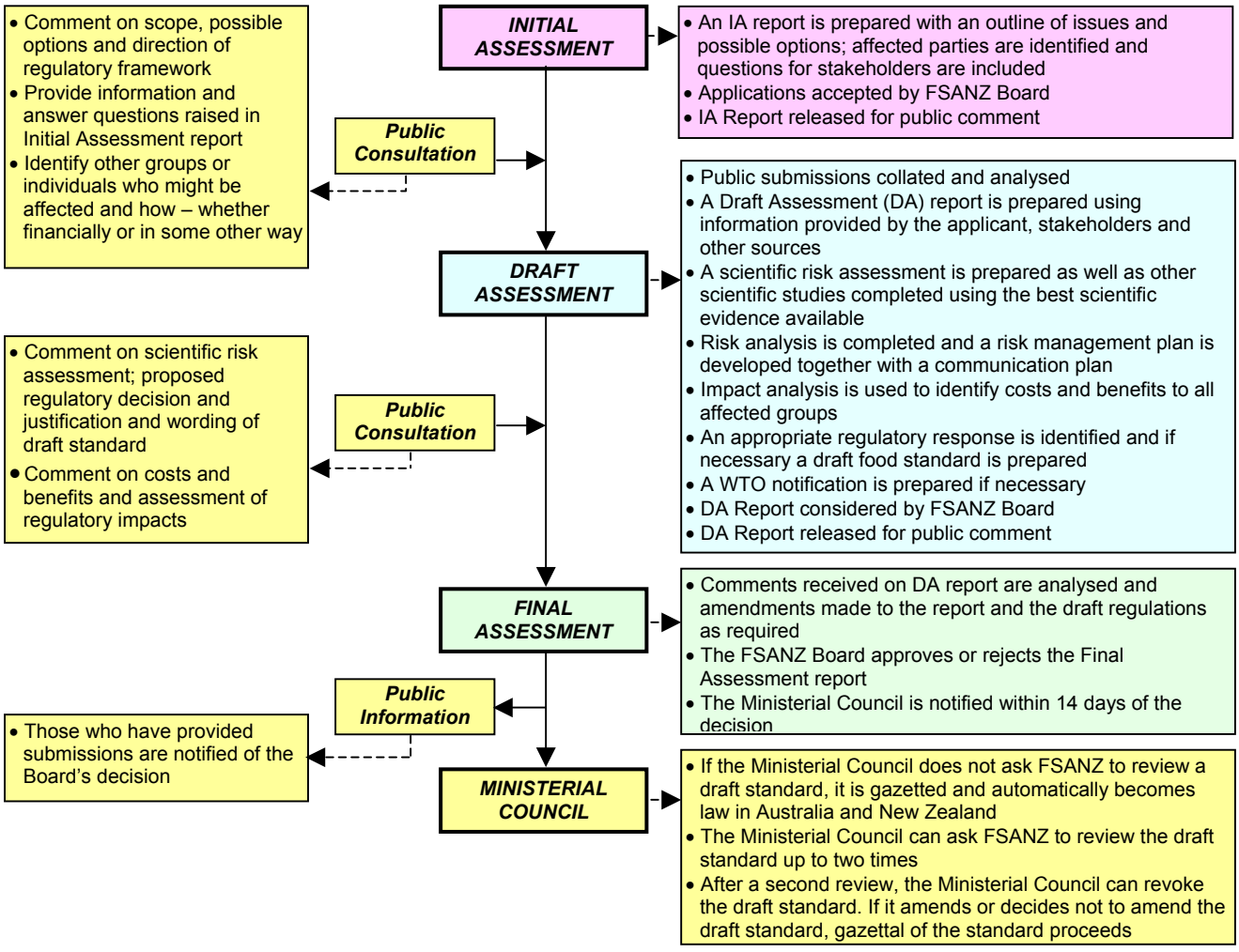
FOOD STANDARDS AUSTRALIA NEW ZEALAND (FSANZ)

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* (the 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 A508; 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 Draft Assessment for this Application. Submissions should, where possible, address the objectives of FSANZ as set out in section 10 of the 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 7 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 Liaison 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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Purpose and scope of the Application

Parmalat Australia Ltd has submitted an application to FSANZ seeking approval for the use of tall oil phytosterols[1] (TOPs) as a novel food ingredient in low-fat milk products under Standard 1.5.1 – Novel Foods – in the *Australia New Zealand Food Standards Code* (the Code). Standard 1.5.1 requires that novel foods undergo a safety assessment before being permitted in the food supply. If approved, the novel food is listed in the Table to the Standard and must comply with any special conditions of use also listed in the Table.

TOPs are added to foods with the intended purpose of lowering cholesterol absorption in humans. The Applicant has submitted efficacy studies including the data and results from clinical studies involving mildly hypercholesterolaemic individuals in a variety of food matrixes, including milk. The available human studies do provide information in relation to the effectiveness of TOPs incorporated into food products to reduce cholesterol absorption. However, there is no specific evaluation of any health claim being considered as part of this Application. Irrespective of whether any statement is considered a health claim, all statements on the label should be true and not mislead consumers.

Technical properties of TOPs

Tall oil phytosterols as well as phytosterols derived from edible vegetable products are comprised of varying ratios of the same four primary phytosterol substances sitosterol, sitostanol, campesterol and campestanol, with varying amounts of minor components such as stigmasterol and brassicasterol. The physiological activity of phytosterol products is due to the presence of these compounds. However, TOPs do not necessarily need to be esterified to improve their solubility as the Applicant has indicated that they can be incorporated into low-fat milks.

Risk assessment

The data support the safety of TOPs in both the target and non-target population at the level of dietary exposure that would be achieved by addition of TOPs to low-fat milk products at the levels proposed to be used by the Applicant (0.9g/250 mL serve). The estimated mean dietary exposure to TOPs did not exceed 1.9 g/day in any population group assessed. The 95th percentile dietary exposure for the target population was 4.8 g/day, the majority of which is derived from edible oil spreads. While this level of exposure is higher than that used in the human studies, FSANZ is proposing additional risk management measures to reduce over-consumption of TOP containing low-fat milks. The overall conclusion of the risk assessment is that low-fat milk enriched with TOPs is not associated with any adverse effects.

Risk management

In order to ensure appropriate use of TOP-enriched low-fat milk by the target group and to discourage use by the non-target groups, the following risk management measures are proposed:

[1] i.e. phytosterols derived from tall oils

1. retain the current mandatory advisory statements in Standard 1.5.1;
2. prescribe an additional labelling statement that indicates that there is no additional benefit by consuming greater than 2-3 serves/day; and
3. prescribe additional conditions of use, namely: (i) that low-fat milk must not contain more than 3.6g/litre of free phytosterols (from a tall oil source); (ii) the fat content must not contain more than 1.5g total fat/100g liquid, and (iii) the maximum container size is to be specified at 1 litre; and (iv) that foods containing added plant sterols must not be used as ingredients in other foods,

Additional risk management strategies have been proposed by the Applicant. Ongoing monitoring (possibly via a survey) of the use of phytosterols in foods would provide additional reassurance of the effectiveness of the proposed risk management measures.

Other issues raised in public submissions

Other issues raised in the public submissions consisted of comments on the specific requirements and intent of the novel foods standard, specifications and labelling for phytosterols in general, the possibility of inequity for consumers of lower socio-economic groups and the issue of medicalisation of the food supply if TOP-containing products are approved.

Impact analysis of regulatory options

The options identified were to permit or not permit the use of TOPs in low-fat milk products, or to permit the use of TOPs generally. The impact analysis shows that the second option (to permit TOPs in low fat milk) satisfies the objectives based on the outcome of the scientific risk assessment and the Regulatory Impact Statement (RIS), taking into account matters raised following the public consultation period.

These matters included the following:

- an assurance of the safety of TOPs;
- the provision of adequate labelling so as to give consumers informed choices for purchases of products containing TOPs;
- advisory statements and conditions of use to manage inappropriate use and over-consumption of products; and
- the provision of benefits to industry and governments, in terms of enhanced market opportunities and trade.

Statement of Reasons

FSANZ recommends the approval of TOPs in low-fat milk subject to specified conditions of use for the following reasons:

- there are no anticipated public health and safety concerns associated with the use of TOPs in low-fat milk when used in conjunction with the recommended risk management measures;

- there is evidence that TOPs when incorporated into low-fat milk products can, following consumption, reduce cholesterol absorption in humans;

- the nutrition assessment indicates that TOPs 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 due to a range of 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 TOP-enriched low-fat milk 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 TOPs as a novel food ingredient in low-fat milks; the benefits of the proposed amendment outweigh the costs.

The proposed drafting to the *Australia New Zealand Food Standards Code* is shown in **Attachment 1**.

1. Introduction

1.1 Nature of Application

An application was received from Parmalat Australia Pty Ltd, on 25 June 2003 seeking approval for the use of tall oil phytosterols (TOPs) as novel food ingredients in low-fat milk products under Standard 1.5.1 – Novel Foods – of the *Australia New Zealand Food Standards Code* (the Code). The Application is cost-recovered and designated as a Category 3.

FSANZ has previously received and approved an Application for permission to add TOPs to edible oil spreads and margarines. There are currently no products on the market containing TOPs. TOPs when added to the diet lead to a reduction in plasma cholesterol levels in the target age group, namely, hypercholesterolaemic individuals aged 45-years plus.

This Application (A 508) is seeking an extension of use of TOPs to low-fat milk products in order that there would be an additional food product available for consumers.

2. Regulatory Problem

In the Code Standard 1.5.1-Novel Foods requires that non-traditional foods which have features or characteristics that may raise safety concerns undergo a safety assessment before they are offered for retail sale in Australia and New Zealand. Novel foods or novel food ingredients that have been assessed under the Standard, when approved, are listed in the Table to clause 2 of the Standard.

TOPs are considered to be novel foods for the purposes of the Standard 1.5.1 because they are a non-traditional food for which there is insufficient knowledge in the broad community to enable safe use of this food in the form or context in which it is presented.

The current permissions to use TOPs as novel food ingredients was limited to edible oil spreads and margarines primarily due to the availability of safety data using these foods, the lack of relevant scientific information relating to their cholesterol lowering effects in other food matrices.

The present Application (A508) seeks permission to add TOPs to low fat milk products as currently there is no permission in the Code.

3. Objective

The objective of this Application is to establish if the food regulations should be changed to allow the use of TOPs in low-fat milk products. Before this food containing TOPs can enter the food supply in Australia and New Zealand, FSANZ must undertake a safety assessment that specifically considers (a) the potential health impact of higher dietary exposure to these compounds on target consumers and (b) the potential effects on non-target consumers. For 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. 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 to approve an extension of the use of TOPs as novel food ingredients, FSANZ is required by its legislation to meet three primary objectives in developing and varying food standards 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

4.1 Historical background

In 1999, following consultation between FSANZ and Senior Food Officers (SFOs) in each of the Australian States and Territories and New Zealand, it was agreed that phytosterol esters derived from vegetable oils ought to be regarded as novel food ingredients because of the lack of a history of significant consumption by the broad community at the proposed levels of dietary exposure. Non-esterified phytosterols derived from tall oils are also considered to be novel for similar reasons.

In June 2000, FSANZ received an Application (A417) to amend the Code to include TOPs in edible oil spreads as novel foods at a level of 8% w/w. Approval was granted by the Ministerial Council in June 2002. The permissions were limited to edible oil spreads at that time primarily because of a lack of information relating to the safety of phytosterols at the higher levels of exposure in a broader range of foods.

4.2 Nature of tall-oil phytosterols

Phytosterols are found naturally in plants at low levels. TOPs are predominantly a mixture of four phytosterols: sitosterol, sitostanol, campesterol and campestanol, extracted from tall oil soap which is a bi-product of the pulping process used for coniferous trees in North America and Europe. They are then purified in a three-step process. The free phytosterols are structurally related to cholesterol and occur naturally at low levels (up to 0.9%) in common vegetables. TOPs are reported to reduce plasma cholesterol levels. It is claimed by the Applicant that incorporation of additional phytosterols, namely, TOPs into the diet may be an effective way of lowering total and low-density lipoprotein (LDL) cholesterol levels.

4.3 Related Applications

FSANZ is currently considering two other related Applications:

- **A434 from Dairy Farmers**-is seeking to extend the approval for the use of phytosterol esters derived from vegetable oils to a low-fat milk product.
- **A433 from Goodman Fielder Ltd**-is seeking to extend the approval for the use of phytosterol esters derived from vegetable oils to breakfast cereals.

4.4 Additional information requested during assessment

During the assessment period, FSANZ requested the Applicant on 12 September and 27 October 2003 to provide further data and information on the safety, efficacy and proposed risk management options with TOPs in low-fat milk products to support the Application. The Applicant supplied this information on 13 October 2003 and 28 November 2003. FSANZ re-commenced assessment of the Application on 5 December 2003.

4.5 International regulations for TOPs and free phytosterols

4.5.1 Codex

There are no Codex standards in relation to TOPs.

4.5.2 Approval in other countries

In the USA, vegetable oil-based spreads containing TOPs at a level up to 12% have self-confirmed Generally Recognised as Safe (GRAS) status.

In the European Union, an application for use in table spreads is still being considered under the Novel Food Directorate.

Other applications for use of free phytosterols in a range of foods[2] (bakery products, grain-based snack products and gum arabic pastilles) are currently being considered under the Novel Food Directorate.

A variety of foods containing plant sterols (both phytosterol and phytostanol esters) are reportedly currently on the market in the EU. 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.

On 25 November 2003 an opinion on the use of TOPs in plant sterol and stanol-containing milk drinks (Reducol ^{TM}) following an Application from Forbes Medi-Tech was expressed by the European Food Safety Authority (EFSA[3]). The EFSA concluded that Reducol ^{TM} could be accepted provided that total phytosterol intakes did not exceed 3g/day and that risk management options were implemented to minimise the likelihood of exceeding 3g/day of phytosterol/phytostanols from a tall oil source.

[2] It could not be ascertained whether the source was from tall oils or vegetable oils.
[3] The EFSA Journal (2003), 15, 1-12.

These risk management options included appropriate information to consumers regarding the need for regular consumption of fruits and vegetables to address the potential β-carotene lowering effect of the product, and the particular circumstances of phytosterolaemic patients, people under cholesterol-lowering medication and of women during pregnancy and lactation.

The specifications for TOPs approved by the EFSA are consistent with the current ones for TOPs in the Code under Standard 1.3.4-Identity and Purity and those requested for approval by the Applicant for Application A508.

5. Relevant Issues

FSANZ has identified and addressed the relevant issues in relation to a broader use of TOPs. These issues include safety, the potential for increased dietary exposure, 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.

The following issues are discussed:

- the purpose and scope of the Application;
- technical properties of TOPs;
- the potential for adverse health effects from consumption of higher amounts of phytosterols in target and non-target consumers;
- the potential nutritional effects of phytosterols on absorption of certain fat-soluble vitamins and carotenoids;
- details of the proposed risk management measures and the proposed manner of marketing of phytosterol-containing products; and
- consideration of other important issues raised in public submissions.

5.1 Purpose and scope of the Application

TOPs are added to foods with the intended purpose of lowering cholesterol absorption in humans. It is proposed that each low-fat milk product will contain 0.9 g of TOPs/250 mL serving size and will carry labelling statements to indicate that consumption of two serves/day is the recommended limit for individual daily consumption and that, further consumption above the recommended levels does not significantly lead to any further reductions in cholesterol absorption (refer to further information below on the labelling of these products in section 5.7 below).

The food matrix may be a determining factor in the efficacy of free sterols and stanols. However, while an assessment of efficacy is not a specific requirement of an assessment under the novel foods standard, truth in labelling is considered relevant to the section 10 objectives of the FSANZ Act. If there is a specific intent to indicate on the label that the consumption of phytosterol-containing low-fat milks will achieve lower cholesterol levels in subjects, then this should be supported by relevant clinical evidence as part of the provision of adequate information relating to food to enable consumers to make informed choices.

In support of this Application to extend the use of TOPs, the Applicant has submitted efficacy studies including the data and results from clinical studies involving mildly hypercholesterolaemic individuals in a variety of food matrixes, including milk[4]. The Applicant has also provided specific detailed marketing information[5] in relation to low-fat milk products that are the subject of this Application. Overall, the available human studies do provide information in relation to the effectiveness of TOPs incorporated into food products to reduce cholesterol absorption.

However, there is no specific evaluation of any health claim being considered as part of this Application. Any application for a health claim for TOPs in the future would need to be considered in the context of PP 293 (Health Claims). Irrespective of whether any statement is considered a health claim, all statements on the label should be true and not mislead consumers.

5.2 Technical properties of TOPs

Tall oil phytosterols as well as phytosterols derived from edible vegetable products are comprised of varying ratios of the same four primary phytosterol substances sitosterol, sitostanol, campesterol and campestanol, with varying amounts of minor components such as stigmasterol and brassicasterol. The physiological activity of phytosterol products is due to the presence of these compounds.

Free tall oil phytosterols such as 'Reducol^{TM}' (commercial name of the tall oil phytosterols of Application A508) vary from phytosterol ester products as a result of the constituent phytosterol profile. These variations arise for the following reasons:

1. the source material differs;
2. fatty acid esterification is not used to modify the solubility properties for product application; and
3. hydrogenation processing is not used.

However, tall oil phytosterols do not need to be esterified to improve their solubility as the Applicant has suggested that they can be incorporated into low-fat milks.

A report on the technical properties of TOPs is provided at **Attachment 2.**

5.3 Safety of TOPs

The majority of submissions (**Attachment 6**) raised issues in relation to the safety of phytosterol-containing foods; in particular, the following concerns were raised:

- The potential for long-term effects;
- The potential for oestrogenic activity; and
- The need for sufferers of homozygous sitosterolaemia to restrict their intakes of plant sterols.

[4] These studies are assessed in Attachment 3.
[5] FSANZ has assessed this as commercially-in-confidence material and as such is not available on the public register

A previous consideration of the safety of TOPs as novel food substances was conducted under Application A417. As well as a cholesterol-lowering effect, consumption of TOPs was found to reduce the plasma levels of plasma β-carotene, a precursor for the synthesis of vitamin A. An updated safety assessment on TOPs incorporating some new data submitted from the Applicant is at **Attachment 3**. A consideration of the nutritional effects of TOPs in low-fat milk products is considered in Section 5.5 below.

FSANZ's safety assessment concluded that TOPs are poorly absorbed in the body, have low toxicity (which is supported by subchronic studies in animals up to 90-days duration), are not genotoxic and demonstrate no reproductive or developmental toxicity. The human studies demonstrated that administration of TOPs in the diet at 3.6g/day for a period up to 28 days or at 1.8g/day for an 8-week period was well tolerated and raised no safety concerns. A study with 4g/day of a phytostanol mixture for 8 weeks further supports the safety of TOPs.

In respect of the long-term safety of phytosterols, there have now been a number of human studies addressing the safety and efficacy of phytosterols orally administered to normal and hypercholesterolaemic subjects from both tall oil and vegetable oil sources. The longest study identified in the scientific literature to date used a dose of 1.6g/day (free phytosterols derived from vegetable oils) for a period of one year, and showed no evidence of adverse toxicological effects. However, there is no evidence to suggest that long-term effects would result from continuous use of these products, based on the overall toxicological studies now available in animals and the safety/efficacy studies in humans.

The submission from Queensland Health cited a 1999 Report from the National Heart Foundation (NHF) which raised some concerns in relation to the safety of phytosterols in foods. However, the most recent report (August 2003) from the NHF's Nutrition and Metabolism Advisory Committee suggests that many of the concerns raised in the earlier report on safety of phytosterols have now been addressed. The NHF (2003) Report cites evidence of oestrogenic activity in Rainbow Trout following oral administration of sitosterols; however, they considered that the relevance of these findings for humans was not known. The report also suggests that there is no evidence of safety concerns with short-term consumption of plant sterols and stanols, although it was noted that long-term safety studies have not been conducted.

From the studies evaluated by FSANZ under Application A417 there was no evidence of *in vitro* or *in vivo* oestrogenic activity in rats or human studies. These studies are more relevant to an assessment of any possible oestrogenic effects in humans compared to studies conducted in Rainbow Trout. In addition, a recent evaluation from the EU Scientific Committee on Food (SCF) Final Report (3 October 2002) stated that newly submitted studies provided sufficient reassurance of the absence of endocrine effects via the oral route (SCF, 2002).

In respect of individuals with the rare inherited lipid storage disorder known as sitosterolaemia characterised by excessive absorption of phytosterols (20% compared with approximately 5% in normal individuals) from the previous FSANZ evaluation under Application A417 it was cited that by 1996, 26 cases had been identified worldwide. People with this condition are under regular medical supervision and must maintain a diet free of phytosterols. However, given the rarity of this disease and the need for individual suffers to be under regular medical supervision, a specific warning on the label for this at-risk group appeared unnecessary.

5.4 Dietary Exposure Assessment

A dietary exposure assessment was undertaken to determine the impact of allowing TOPs to be added to low-fat milk products. The assessment took into account the existing permission under Standard 1.5.1 to add TOPs to edible oil spreads (the 'baseline' scenario) as well as the proposed addition of TOPs to milks (the 'milk only' scenario) and a combination of these products (the 'milk plus baseline' scenario). In each scenario, addition of phytosterols at a level equivalent to 0.9g free phytosterols per serve was assumed and it was further assumed that all edible oil spreads and low fat milks contained added free phytosterols. Intrinsic levels of phytosterols in foods were not taken into consideration.

Assuming that consumers maintain their existing eating patterns, estimated mean dietary exposure, (expressed as free phytosterols), did not exceed 1.9 g per day in any population group assessed under any of the scenarios considered. At the 95th percentile of exposure, no population group assessed exceeded 4.8 g free phytosterols per day for any of the scenarios modeled. The analysis shows that, for the target population group in particular, edible oil spreads contribute more to dietary exposure to added TOPs (78-84% of exposure) than low fat milks, according to the available data on food consumption patterns.

A detailed report on the potential dietary exposure to TOPs is provided at **Attachment 4.**

5.5 Nutritional issues associated with TOPs

Submissions raised concerns in relation to the loss of fat soluble vitamins, carotenoids and other fat soluble phytochemicals following consumption of TOPs, nutritional concerns in relation to consumption by non-target groups and the lack of evidence that consumers will consume extra fruit and/or vegetables to make up for the loss of carotenoids.

Plant sterols 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 nutritional effects were examined in further studies, reductions in α- and β- carotene, lycopene, lutein and cryptoxanthin were observed, while vitamin E and vitamin A levels 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 (from the studies with phytosterol-esters undertaken by the CSIRO) generally results in a reduction in plasma β-carotene levels of approximately 20-25%. The reduction does not translate into an overt 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.

However, there were no significant reductions in β-carotene levels following administration of TOPs at levels ranging from 0.9 to 3.6 g/day for 30-days. This may reflect differences in design of the study undertaken with TOPs compared to those studies performed by the CSIRO using phytosterol esters.

The data submitted with Applications A 433 and A434 indicate that consumption of foods enriched with phytosterol-esters (10.7 g/day) providing up to approximately 6.6 g free phytosterols/day is safe from a nutritional perspective. Furthermore, other information from other published studies suggests that intake of phytosterol esters at high levels (up to approximately 9 g/day) is not associated with adverse effects arising from a reduction in some carotenoids. However, the effects of TOP consumption above 4.0 g/day on nutritional parameters, or over the long-term, have not been extensively researched, although many of the components are common with phytosterol esters derived from vegetable oils. As there is no additional cholesterol lowering effect with increased phytosterol intake above approximately 3 g/day, there is no additional benefit in consuming unlimited amounts of phytosterol-enriched foods.

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 above average intake of nutrients, these population groups would therefore derive no 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 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.

A separate nutrition report was not undertaken for Application A508, as the report for Applications A433 and A434 (**Attachment 5)** covered the nutritional issues associated with the use of TOPs in low-fat milk products due to the following reasons:

- the report was more comprehensive than previous studies submitted for Application A417 in evaluating the effects of phytosterols on a larger number of carotenoids (other than alpha and beta-carotene) and fat-soluble vitamins via new studies undertaken by the CSIRO evaluating specific nutritional parameters following high doses of phytosterols; and

- the dose levels used in the CSIRO studies of 6.6g free phytosterols/day exceeded the mean and 95th % exposure for consumers in the target group exposed to TOPs in low-fat milk products for any population group assessed. Therefore, overall the above conclusions from the Nutrition report were supportive of TOPs addition in low-fat milks.

5.6 Risk assessment of TOPs

The data support the safety of TOPs in both the target and non-target population[6] at the level of intake that would be achieved by addition of TOPs to low-fat milk (this category includes no-fat milk) at the levels proposed to be used by the Applicant (0.9g/250 mL serve).

The estimated mean dietary exposure to TOPs did not exceed 1.9 g/day in any population group assessed. This included the scenario of the target population being exposed to TOPs from edible oils and margarines and additional TOPs from low-fat milks. The highest potential exposure by age group (consumers aged 40-65 years at the 95th percentile exposure level) was 4.8 g/day from edible oils, margarines and low-fat milks. This is the target group to which the products will be marketed.

However, it is likely that the dietary modelling overestimates the consumption of TOPs for the following reasons:

- the dietary modelling assumes that all foods within a category contain TOPs at the proposed levels, i.e., consumers purchase only TOP-enriched foods;

- the addition of phytosterols to low fat milks as well as to edible oil spreads results in only a slight increase in predicted mean phytosterols exposure compared to baseline exposure; an increase of 0.2 g/day for all Australians and 0.1 g/day for all 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 milk in the milk + edible oil spreads scenario;

- consumption of foods are actual amounts as recorded in the National Nutrition Surveys (NNSs), as opposed to suggested serve sizes that appear on product labels of foods containing phytosterols. This assumes that consumers will not significantly change their eating habits of the foods containing the phytosterols, but follow existing patterns of use; and that consumers are product loyal, and would always consume the products with added phytosterols; and that

- the data used for modelling is a 24-hour record, which overestimates food consumption for high consumers (the use of multiple day records tends to significantly reduce predicted high consumer exposure).

These assumptions are likely to lead to a conservative estimate for phytosterol dietary exposure for high consumers.

The nutrition report suggested that reductions in β-carotene levels of approximately 20-25% occurred at doses of approximately 10.7 g/day phytosterol-esters (6.6g free phytosterols/day). However, this is still considered safe from a nutritional perspective as these reductions are still within a broad natural range and there was no measurable effect on retinol or vitamin A levels.

[6] The target population refers to male and female subjects aged over 45 years with elevated cholesterol levels

The safety of non-target groups, namely, infants, children, pregnant and lactating women has not been specifically demonstrated in these studies on TOPs and whilst FSANZ considers that there will be no specific concerns at the anticipated low doses, it is not appropriate that these products should be consumed by these groups.

High-consumers (95^{th} %) of edible oils and margarines and low-fat milks have a dietary exposure of between 3.5 and 4.8 g/day for all population groups assessed with the target population having the highest exposure at 4.8 g/day. The available data does not indicate any cause for concern regarding the safety of TOPs at these dose levels; however, the highest dose level of TOPs tested in humans was 3.6 g/day. The new studies on phytosterol esters derived from vegetable oils at 6.6 g free phytosterols/day provide some supporting data in this regard. The potential for adverse health effects (including nutritional effects) is considered to be very small, however, risk management strategies should be considered to ensure appropriate use of TOPs in the target group, and to discourage use in the non-target groups.

5.7 Risk management options for TOPs

5.7.1 Risk management issues previously raised

The assessment of previous phytosterol applications had identified a discrepancy between the broad nature of the foods for which approval was sought, and the stated intention of the applicants to target these foods to a particular section of the market i.e. consumers over 40 years with concerns about plasma cholesterol levels. Product descriptions such as low-fat milk and low-fat yoghurt are very broad and may include products that are generally not restricted to a specific population group.

FSANZ considers that phytosterol-enriched foods should be marketed as part of a healthy food choice. Consumers who are concerned about their cardiovascular health are then more likely to make food choices that are consistent with the current public health messages regarding the nutritional benefits of a low-fat, high-fibre diet including multiple daily servings of fruits and vegetables. Certain foods such as those with limited salt (sodium) and sugar content are also compatible with public perceptions of a 'healthy' food profile, especially in relation to dietary risks for obesity and cardiovascular disease. For these reasons, phytosterol enrichment of foods that are already associated with healthy eating habits provides a more consistent health message.

With regard to non-target consumers, consumption of phytosterol enriched products by children and pregnant women is presently considered unnecessary. However, casual consumption by individuals other than those in the target group is considered more likely when phytosterols are added to staple foods such as milk, or to foods that are generally available to a broad range of consumers, such as with yoghurts.

Labelling can assist consumers to use phytosterol-enriched foods appropriately, as part of a healthy food choice. In addition, the availability of a broader range of phytosterol-enriched foods may require some additional advisory statements that would apply to all such foods.

5.7.2 Risk management options considered for A508

FSANZ considers that there are minimal, if any, potential risks to consumers of the target group from the use of TOPs in low-fat milk products. However, 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 included:

- prescribing of maximum limits in the Code for TOPs in low-fat milks;
- specifying a maximum container size;
- an additional labelling statement and other conditions of use to avoid possible over-consumption of TOPs; and
- consideration of the marketing and other strategies proposed by the Applicant to confine exposure to the target population.

5.7.3 Additional information sought from the Applicant on the risk management issues

In light of these issues and in order to clarify the main messages to the specific target group on the label in relation to cholesterol reduction, and to ascertain Parmalat's position on strategies for non-target consumers, FSANZ sought additional information from the Applicant on the following:

1. Specific information as to how these products would be promoted to the identified target consumers, and how the message in relation to achieving an effective daily intake of TOPs would be presented, given that one serve of food per day may not deliver an effective amount for average consumers.

2. The risk management strategy proposed in relation consumption by pregnant women and children as a result of inadvertent use of a phytosterol-containing product in the absence of any specialist dietary advice for this population group.

3. Information on how the effectiveness of the milk products is to be ensured, and hence the truthfulness of the proposed labelling statement regarding the reduction in cholesterol absorption.

FSANZ met with the Applicant on 22 September 2003 to discuss these questions and received letters in response to the above questions on 13 October 2003 and 28 November 2003.

The Applicant submitted information on the proposed labelling and packaging of low-fat milks, which highlighted the following information on the label of a 1-litre carton of low-fat milk:

- there was clear advice on the label that a minimum level of consumption was required (e.g., two 250 mL serves at 0.9g) for a cholesterol-lowering effect;

- the intent stated on the label was that consumption above the recommended two serves/day does not significantly improve effect. This statement on the label also serves to confine consumption to a maximum daily amount;
- the label indicated that the product is not recommended for infants, children, pregnant or lactating women except on medical advice; and that;
- a healthy lifestyle should be maintained.

In respect of the promotion of the products to the target groups, Parmalat submitted survey data from Roy Morgan Brand Planner[7] indicating that 74% of 1-litre milk purchases are from households with no children compared to 13% with one child in the home, 9% with 2 children, and 3% with 3 or more children. This suggested that the majority of purchases of 1 litre milk would be in households without children and that the proposed marketing of these products confined to 1 Litre cartons would be expected to further confine exposure to specific target groups.

Other strategies proposed by the Applicant are as follows:

- a consumer information line will be established to assist consumers with advice on purchasing and consumption of phytosterol-containing foods;
- advertising will be specific for the target audience; and
- educational material will be distributed to medical and diet related professionals.

5.7.4 Current labelling information for consumers

Submissions raised concerns over the use of TOPs, particularly, in non-target groups (e.g. children and pregnant women) and use by individuals already prescribed cholesterol-lowering medication by a medical practitioner.

These concerns have also been raised in previous Applications and are addressed below.

Currently labelling requirements (1-3 below) are as follows for TOPs in edible oils and spreads:

1. A statement to the effect that the product should be consumed in moderation as part of a diet low in saturated fats and high in fruit and vegetables.

For a food for which there is a clear intention to market its beneficial effects, consuming the food as part of a healthy diet is an important dietary message. While there is evidence that TOPs can lead to lower plasma cholesterol by reducing the absorption of cholesterol from the diet, phytosterols are ineffective in preventing the elevation of plasma cholesterol that is a consequence of the ingestion of saturated fatty acids.

This requirement is justified under the third of the section 10 objectives of the Authority, namely, the prevention of misleading or deceptive conduct, and is consistent with the policy on mandatory advisory statements developed during the review of the Code. It is also consistent with the second section 10 objectives of the Authority, namely, the provision of adequate information relating to food to enable consumers to make informed choices.

[7] January to December 2001 (Australian data)

2. A statement to the effect that the product is not recommended for infants, children, and pregnant or lactating women unless under medical supervision.

For specific groups in the population (e.g. children, pregnant and lactating women) it may be inappropriate and unnecessary to reduce cholesterol in these groups without consulting a medical practitioner who could conduct a thorough clinical evaluation to determine the needs. Therefore, a clear statement on the label should indicate that this novel food ingredient is inappropriate for these population groups. The above requirement is justified under the first section 10 objective of the FSANZ, namely, the protection of public health and safety.

Another group is individuals with the rare inherited lipid storage disorder known as sitosterolaemia, which is characterised by excessive absorption of phytosterols (20% compared with approximately 5% in normal individuals). This disorder leads to premature atherosclerosis and by 1996, 26 cases had been identified worldwide. People with this condition are under regular medical supervision and must maintain a diet free of phytosterols. Given the rarity of this disease and the need for individual suffers to be under regular medical supervision, a specific warning on the label for this at-risk group seems unnecessary.

3. A statement to the effect that consumers on cholesterol-lowering medication should seek medical advice on the use of this product in conjunction with their medication.

While the use of phytosterol-enriched spreads may assist in the reduction of plasma cholesterol, its use should not be considered a substitute for cholesterol-lowering medication unless advised by a medical practitioner. The above requirement is justified under the first section 10 objectives of the Authority, namely, the protection of public health and safety.

5.7.5 Proposed labelling information for consumers

In order to ensure that the target groups receive the appropriate amount of phytosterols in order to achieve the desired effects, and has adequate information to make an informed choice, the following labelling option is proposed in addition to the above current labelling requirements:

A statement to the effect that there is no additional benefit by consuming greater than 2-3 serves/day of phytosterol-containing products

This will serve to limit over-consumption of TOPs and is consistent with the data that demonstrates the safety of phytosterols for high consumers within the target group. It will also provide a consistent message for consumers of all phytosterol-containing foods.

5.7.6 Conditions of use

In order to ensure appropriate use of phytosterols in the target group and discourage use in the non-target group, the following conditions of use are proposed:

Foods containing added plant sterols must not be used as ingredients in other foods.

Low-fat milk must not contain more than 3.6 g/litre of free phytosterols (from a tall oil source) and the fat content must not contain more than 1.5 g total fat/100 g liquid

The maximum container size is to be specified at 1 litre.

This will limit over-consumption by prescribing a maximum limit consistent with available safety studies and a maximum container size, be consistent with recent dietary guidelines in relation to healthy food choices (to consume products low in saturated fats) and prohibit the use of TOP enriched foods in mixed foods, other than those specified, will limit the ability of manufacturers to extend the product range beyond the products now under assessment.

5.7.7 Recent international surveys on the consumption of phytosterol-containing foods

As part of the European Commission's decision to allow Unilever to place 'yellow fat spread with added phytosterols' on the market they were obliged to collect data to assess the extent to which the product was reaching its target market. In 2001, Unilever undertook post launch monitoring. The study was undertaken using independent market research companies in The Netherlands, UK, France, Germany and Belgium. The results from about 2000 households are as follows (SCF):

- 75-95% of purchasers were over 45 years old
- 66-90% of regular purchaser came from 1-2 person households
- 62-82% of all people purchasing the product came from 1-2 person households.
- 79-91% of household purchasing the products had no children
- 87-96% of regular purchasers had no children living at home

With the exception of Germany, intake estimated for one person households were similar to those of two to four person households, indicating that the product was predominantly used by one person per household. Actual intakes of the product by the target population of 15-18g/day were lower than the 20-30 g/day predicted. Users of Unilever margarines were also consuming other plant stanol ester products (spread and non spread products).

As part of an on-going surveillance of novel food use in food in Australia and New Zealand, a survey of phytosterol use in food in 3-5 years time may provide useful information with regard to the effectiveness of the proposed risk management measures.

5.7.8 Summary of proposed risk management strategies

In conclusion, in order to manage any possible associated risk from additional consumption the following risk management options are proposed:

Retain the current mandatory advisory statements in Standard 1.5.1.

Prescribe an additional labelling statement that there is no additional benefit by consuming greater than 2-3 serves/day.

Prescribe additional conditions of use (as described above).

5.8 Other issues raised in public submissions

5.8.1 Issue 1

The AFGC considers that FSANZ has erred in not making a decision at the time of initial assessment to determine whether the application warrants a variation to a regulatory measure.

5.8.1.1 Evaluation

The AFGC are not taking account of the context of section 13 which is the Initial stage - the assessment that FSANZ is required to make is a preliminary one only, and is therefore limited by those circumstances. Thus whether a variation is warranted can only be assessed within that limited and restricted circumstance.

5.8.2 Issue 2

The AFGC disputes that efficacy in relation to labelling statements has anything to do with a safety assessment under Standard 1.5.1-Novel Foods as there is no requirement for provision of efficacy data.

5.8.2.1 Evaluation

FSANZ agrees that this has nothing to do with a safety assessment *per se* under the provisions of Standard 1.5.1. However, there is a broader issue of provision of adequate information to consumers under the requirements in the FSANZ Act on how the effectiveness of TOP containing milk products is to be ensured, and hence the truthfulness of the proposed labelling statement regarding the reductions in cholesterol absorption observed in studies in humans.

The dietary modelling undertaken by FSANZ suggests a significant potential for intake by the target group below the level reported to provide a benefit for average consumers where no change is made to dietary habits. Therefore, FSANZ has required that the Applicant provided data to support the labelling statements that reductions in cholesterol absorption can be achieved by appropriate use of both TOPs containing low-fat milk products.

5.8.3 Issue 3

AFGC considers that conditions of use are an essential part of fulfilling the requirements of being a novel food.

5.8.3.1 Evaluation

Specific conditions of use will be required to use TOPs in low-fat milk products as detailed in Section 5.7, which addresses this comment.

However, the AFGC submission does not adequately take into account the whole of standard 1.5.1. Taken as a whole, Standard 1.5.1 makes clear that the imposition of restrictions or conditions is not a necessary prerequisite to a food being considered novel. Clause 2 of 1.5.1 provides:

> A novel food must not be sold by way of retail sale as a food or for use as a food ingredient unless it is listed in column 1 of the Table to this clause and complies with the conditions of use, **if any**, specified in column 2 (emphasis added).

The words 'if any' clearly leave open that a novel food may be sold in the manner contemplated in the clause without any condition or restriction whatsoever. That must include any condition or restriction relating to safety. This permits, in FSANZ's view, the sale of a novel food without any conditions or restrictions whatsoever. It is self-evident that the absence of public health and safety concerns, and consequent absence of restrictions upon use cannot prevent a food from being regarded as a novel food.

5.8.4 Issue 4

NZFSA and Food Technology Association of Victoria considered that approval for phytosterol-containing foods should all be addressed at one time rather than approving an increasing number of product categories on a case-by-case basis.

5.8.4.1 Evaluation

The intent of Standard 1.5.1 is prohibition on the sale of novel foods and novel food ingredients unless they are listed in the Table to clause 2. This was a policy issue decided during formulation of the standard in order that an appropriate and adequate risk-based safety assessment could be undertaken before approval was granted. This policy is also consistent with other international regulatory bodies approach to assessment of novel foods.

5.8.5 Issue 5

The editorial note in Standard 1.5.1 and Clause 2 (1) of Standard 2.4.2 require review and revision as it is unclear what the situation is with the use of phytosterols and phytosterol esters in the same products (Food Technology Association of Victoria).

5.8.5.1 Evaluation

The requirements of the editorial note and Clause 2 (1) of Standard 2.4.2 is that no mixing of phytosterol esters and TOPs are permitted. They both have separate specifications in order to alleviate manufacturers mixing the two ingredients leading to the possibility of higher doses of phytosterols for consumers.

5.8.6 Issue 6

Request consideration of specifications accepted by other regulatory agencies to be included as part of this application and also requested FSANZ to review the current specifications to include both the free sterol and ester forms. Questioned the need for minimum limits of sterols (Unilever).

5.8.6.1 Evaluation

As there are currently numerous specifications approved and products currently being considered for approval in the EU, it is too early to give consideration to specifications from other regulatory agencies.

The minimum limits relates to the issue of truth in labelling and ensures that TOPs are efficacious. Additionally, the Applicant has indicated that the current specifications for TOPs in edible oil a spread is appropriate for use of TOPs in low-fat and no-fat milk products.

5.8.7 Issue 7

Suggest that consideration is given to a common labelling format for multiple foods containing phytosterols (Unilever).

5.8.7.1 Evaluation

FSANZ is currently giving strong consideration to labelling that provides meaningful information to consumers and is always conscious of international labelling considerations. Refer to section 5.7 above.

5.8.8 Issue 8

FSANZ needs to use previous information on the labelling costing study following the introduction of mandatory nutrition labelling to determine the impact on target groups consumers (Nestle Australia Ltd).

5.8.8.1 Evaluation

The results of the original costing study undertaken by Peat Marwick was contentious and its validity was questioned by another study commissioned by FSANZ and undertaken by the Allen Consulting Group. As the costing concerned the change over costs from old to new labels the original costing studies would be of limited relevance to the introduction of a new product in which the design and printing of a label would represent part of the normal development costs. Parmalat have already considered the issue of the impacts of labelling on the target groups and have presented evidence of labelling of TOPs in low-fat milks to FSANZ.

5.8.9 Issue 9

The cost of phytosterol-containing foods is high and raises issues of inequity for some consumers (e.g. from lower socio-economic groups). (Queensland Health)

5.8.9.1 Evaluation

Cost is always an issue with lower socio-economic groups because of competing demands on their resources. However, FSANZ does not have a role in determining the price of food. This is a commercial decision on the part of the manufacturer and is subject to the influence of the marketplace. The higher prices for products containing phytosterols reflect the costs of researching and developing products initially, the high costs of extracting the phytosterols, together with production and marketing costs. If consumers are unwilling to pay the price set the product may not be established in the market place.

5.8.10 Issue 10

The Environmental Health Unit of Queensland Health expressed 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.

5.8.10.1 Evaluation

Phytosterol esters derived from vegetable oils and TOPs are already approved novel foods for use in edible oil spreads and margarines 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 consumption would provide.

Several identified risk factors for major diseases such as cardiovascular disease and stroke can be correlated in varying degrees with the 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 Britain, 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. Over time, similar dietary recommendations have prevailed in broad nutritional health policy development.

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 Australian and New Zealand 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 children because of the requirement for a full complement of dietary fats necessary for 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. In this regard, mandatory labelling, in combination with manufacturer's 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 TOPs 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, consumption of these foods may interfere with the bioavailability of some carotenoids and they therefore 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. In addition, because of the costs associated with production, phytosterol enriched foods are more expensive to purchase compared with the non-enriched counterparts, providing a potent commercial barrier to general consumption. Restricting package sizes of phytosterol enriched foods further reduces their appeal to entire households.

These combined marketing features of phytosterol enriched products places them in a similar retail position to reduced fat products which do not provide benefits to all consumers and whose unsuitability to certain subgroups within the population is managed through appropriate labelling. With phytosterol enriched spreads already on the market for several years, consumers who are sufficiently motivated to purchase these products have demonstrated that their use of them is likely to be informed and appropriate, and therefore restriction to broadening the choice of products is unwarranted.

6. Regulatory Options

6.1 Option 1 – prohibit the use of TOPs in low-fat milks

This option maintains the status quo by not including these foods in the Table to clause 2 of Standard 1.5.1, thereby retaining the current limitations on the use of TOPs to edible oil spreads only.

6.2 Option 2 – approve the use of TOPs in low-fat milks

This option will result in an amendment to the Code to permit the sale and use of TOPs at specified levels in low-fat milks.

6.3 Option 3 – approve the general use of TOPs

This option will result in an amendment to the Code to permit the use of TOPs as ingredients in any food to a maximum permitted level.

7. Impact Analysis

7.1 Affected parties

- consumers, especially target groups such as adults over 45 years of age with health concerns about high serum cholesterol and non-target groups such as pregnant and lactating women and children;

- 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 assessment of the costs and benefits of the three regulatory options identified so far is based on an assessment of the information supplied by the applicant, knowledge of previous considerations relating to the use of phytosterols in the food supply and public submissions.

7.2.1 Option 1

There is a potential cost to consumers with this option in terms of the lack of availability and choice of TOPs-enriched milk products. Similarly, there is an identifiable cost to the food industry in terms of a loss of product range and marketing opportunities. There would be no immediate impact on government. There are no benefits to consumers or government.

7.2.2 Option 2

7.2.2.1 Impact on Consumers and the Community

There is a reported benefit to consumers from consuming TOPs in milk products, leading to a reduction in their blood cholesterol. The community would also benefit from any improvement in health status. The evidence also shows that consumption of TOPs under specified conditions, which equate to normal and informed use by consumers, is safe.

A cost to consumers would be the lack of choice of phytosterols in full fat milk products. This disadvantages consumers who may be 'brand loyal' and prefer to purchase a full-milk product for taste etc. rather than a low-fat product.

Another cost would be that a wider range of foods containing added phytosterols may lead to over consumption of phytosterol-containing foods.

7.2.2.2 Impact on Industry

This option would provide an alternative novel food ingredient and would increase market opportunities for other manufactures of low-fat milk beverages. There may be some impact of the labelling requirements for some manufacturers in terms of costs associated with labelling.

7.2.2.3 Impact on Government

In the short-term, this option would not have a material impact on the enforcement activities of the State, Territory and New Zealand Governments.

However, it would have an impact on the resources required to provide dietary advice to consumers from organisations within governments charged with this role.

In the long-term, governments may benefit in terms of health expenditure from lower blood cholesterol in the community associated with the normal and informed use of TOPs in low-fat milks, although the extent of this benefit cannot be measured at present.

7.3 Option 3

This option was not considered appropriate due to the possible expansion of phytosterols in a range of foods that would effectively expose non-target consumers to phytosterol-containing foods. This option overall received no strong support from public submissions.

7.4 Evaluation

Option 1 would not allow TOPs as an ingredient in low-fat milks. This option cannot be justified on the basis of public health and safety. It also imposes costs on consumers of loss of choice of new products where their safety has been established.

Option 2 allows TOPs in low-fat milks, which by virtue of the data submitted, have been shown to be safe. Option 2 does not subject consumers, the community or governments to other costs.

Overall, **Option 2** is preferred because of the two options it most clearly achieves the objectives of this assessment: providing a reasonable assurance of the safety of consuming TOPs products, providing information to consumers that will contribute to the safe consumption of TOPs and provides a fair trading aspect to allow manufacturers and businesses a new source of phytosterols for inclusion in low-fat milks.

8. Consultation

FSANZ conducted an Initial Assessment on A508 and public comments on the application were called for from the period 13 August to 24 September 2003. A total of 13 submissions were received and are summarised in **Attachment 6.**

The Initial Assessment Report sought early input from the general community on a range of issues concerning the availability of a low-fat milk products containing TOPs. Comment was also invited on a broader permission for food products containing phytosterols than is currently permitted in the food supply in Australia and New Zealand. These comments have been addressed in the Draft Assessment Report.

All individuals, groups or organisations who made a submission in relation to this application were included on a mailing list to receive further FSANZ documents pertaining to this application.

FSANZ sought public comment to assist with assessment of the application on the following:

- scientific aspects of the application, in particular, any information relevant to the safety assessment;

- information that would assist in an assessment of the appropriateness and effectiveness of current labelling statements on edible spreads containing phytosterol esters derived from vegetable oils and/or phytosterols derived from tall oils;

- parties that might be affected by having this application approved or rejected;

- potential costs and benefits to consumers, industry and government.

8.1 World Trade Organization (WTO)

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.

There are not any relevant international standards, namely a Codex standard for TOPs. Amending the Code to allow TOPs as novel food ingredients may have a liberalising effect on international trade via removal of the prohibition on the sale of these novel food ingredients. However, at this stage of the assessment this does not appear to warrant notification to the WTO as either a TBT or SPS issue.

9. Conclusions

The conclusions from the draft assessment are as follows:

- there are no anticipated public health and safety concerns associated with the use of TOPs in low-fat milk when used in conjunction with the recommended risk management measures;

- there is evidence that TOPs when incorporated into low-fat milk products can, following consumption, reduce cholesterol absorption in humans;

- The nutrition assessment indicates that TOPs 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 due to a range of 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 TOP-enriched low-fat milk 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 TOPs as a novel food ingredient in low-fat milks; the benefits of the proposed amendment outweigh the costs.

ATTACHMENTS

1. Draft variations to the *Australia New Zealand Food Standards Code*

2. Food Technology Report

3. Safety Assessment of Tall Oil Non-Esterified Phytosterols (TOPs)

4. Dietary Exposure Assessment Report

5. Nutrition Assessment Report for Application A433 – Phytosterol esters in breakfast cereal bars and Application A434 – Phytosterol esters in low-fat milk and low-fat yoghurt

6. Summary of Submissions received

Attachment 1

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

To commence: On gazettal

[1] ***Standard 1.2.3*** *of the Australia New Zealand Food Standards Code is varied by –*

[1.1] *omitting from the* Table to clause 2 –

Food regulated in Standard 2.4.2 containing tall oil phytosterols	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.

substituting –

Foods containing added tall oil phytosterols	Statements to the effect that - 1. the product should be consumed 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; 3. consumers on cholesterol-lowering medication should seek medical advice on the use of this product in conjunction with their medication; and 4. foods containing added plant sterols do not provide additional benefits when consumed in excess of three serves per day.

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

[2.1] *omitting from the* Table to clause 2 –

Tall oil phytosterols	The requirements in clause 2 of Standard 1.2.3. The name 'tall oil phytosterols' or 'plant sterols' must be used when declaring the ingredient in the ingredient list, as prescribed in Standard 1.2.4. May only be added to food - (1) according to Standards 1.3.4 and 2.4.2; and (2) where the total saturated and trans fatty acids present in the food is no more than 28% of the total fatty acid content of the food.

substituting –

Tall oil phytosterols	The requirements in clause 2 of Standard 1.2.3. The name 'tall oil phytosterols' or 'plant sterols' must be used when declaring the ingredient in the ingredient list, as prescribed in Standard 1.2.4. May only be added to edible oil spreads – (1) according to Standard 2.4.2; and (2) where the total saturated and trans fatty acids present in the food is no more than 28% of the total fatty acid content of the food. May only be added to milk in accordance with Standard 2.5.1. Foods to which tall oil phytosterols have been added may not be used as ingredients in other foods.

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

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

5. Tall oil phytosterols

(1) Tall oil phytosterols may only be added to milk –

(a) that contains no more than 1.5 g/100 g 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 (from a tall oil source) added is 3.6 g/litre of milk.

Attachment 2

FOOD TECHNOLOGY REPORT

A508 – PHYTOSTEROLS DERIVED FROM TALL OILS AS INGREDIENTS IN LOW FAT AND NO FAT LIQUID MILK

Introduction

Phytosterols (plant sterols) belong to a group of plant compounds that are found in a variety of foods in the human diet. The tall oil non-esterified phytosterols are sourced from tall soap, a by-product that is formed in the pulping process of coniferous trees [1].

Tall oil phytosterols as well as phytosterols derived from edible vegetable products are comprised of varying ratios of the same four primary phytosterol substances sitosterol, sitostanol, campesterol and campestanol, with varying amounts of minor components such as stigmasterol and brassicasterol. The physiological activity of phytosterol products is due to the presence of these compounds[1].

Free tall oil phytosterols such as 'Reducol^{TM}' (commercial name of the tall oil phytosterols of this Application) vary from phytosterol ester products as a result of the constituent phytosterol profile. These variations arise for the following reasons:

1. the source material differs;
2. fatty acid esterification is not used to modify the solubility properties for product application; and
3. hydrogenation processing is not used.

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. The sterol ring is common to all sterols, with differences in the side chain accounting for different properties in sterol compounds. Phytosterols 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 β-sitosterol, campesterol and stigmasterol and structurally these are very similar to cholesterol, belonging to the class of 4-desmethylsterols (Fig. 1)[1].

Plant stanols are hydrogenation products of the respective plant sterols, e.g. campestanol/campesterol and sitostanol/sitosterol, and are found in nature at very low levels. Stanols have no double bonds in the sterol ring and belong to the group of 4-desmethylsterols (Fig. 1).

All plant sterols and stanols are closely related in structure to cholesterol. The main difference is the presence of a methyl or ethyl group in their side chains (again see Fig. 1).

Solubility

Free phytosterols or stanols in free form; exhibit limited lipid solubility. Some manufacturers elect to esterify them with fatty acids from edible oils. The solubility of free sterols in oil is around two percent, but the solubility of sterol esters in oil exceeds twenty per cent.

The esterification of phytosterols improves their solubility in oil and facilitates their incorporation into certain foods. However, esterification does not materially affect the physiological properties of the phytosterol components. Once ingested, the esters are rapidly cleaved by endogenous lipases, releasing the free phytosterols that are then able to interact with cholesterol absorption[6].

On a molar basis, free and esterified phytosterols exhibit similar physiological activity. This equivalence means the extensive safety and efficacy data for esterified phytosterol forms is directly and appropriately applicable to non-esterified forms. Difference in molecular weight of phytosterols needs to be taken into account when assessing safety and efficacy i.e. 1.6 grams esters is approximately equivalent to 1.0 grams of free phytosterols[2].

Phytosterol esters are fat soluble, and the main reason for esterification is to allow incorporation into fatty foods such as margarines. The Applicant has advised FSANZ that tall oil non-esterified phytosterols are able to blend with non-fat matrices, although details of this specific process remain intellectual property of the company. The free phytosterols are therefore able to be incorporated into non-fat foods such as low-fat and non-fat milks as requested in the Application.

Stability

Phytosterols and their fatty acid esters are basically very stable compounds and experience only limited damage during oil processing [3]. Only under specific conditions, such as high temperatures (>100 °C) in the presence of air, may some oxidation of phytosterols occur, in the same way as for cholesterol [4]. Phytosterols are mono-unsaturated compounds (double bond in the B-ring), which are much more stable than the mono-unsaturated fatty acids (e.g. oleic acid), because of steric hindrance by the ring structure. Therefore even under severe conditions, such as during deep frying, sterol oxidation products are only formed at ppm concentrations [5].

The Applicant states that there are some losses (approximately 5-10%) of the tall oil phytosterols content with heating at high temperatures (100˚C) for several hours, which appear to be as the result of oxidation.

Production methods

Tall oil soap is the lipid layer skimmed off when wood chips are digested at pH 14 and 50˚C, to free wood fibres. Phytosterols are extracted directly from the tall oil soap and purified in a three-step process.

1. The first step is a solvent extraction of the tall oil soap. Organic solvents, water and tall oil soap are mixed while heating in stainless steel reactors. The mixture is allowed to separate into distinct aqueous and organic phases. The organic phase contains extracted organic materials, and 15-25% sterols, which is used in the next step of the process.

2. The second step consists of a complexation-washing process that removes the bulk of the organic material. The extract from Step 1 is mixed while heating with a solvent, and complexing agent. The sterols rapidly bind to the agent, which are then separated from the solvent phase by centrifugation. Next, the complexing agent is dissolved from the crude complex by heating in water. The water is removed and the resulting material contains 60-75% sterols, that are referred to as crude sterols.

3. Crude sterols are dissolved in alcohol at elevated temperature. The temperature of the mixture is reduced to allow for crystallisation of the sterols. The crystals are recovered and then dried. The mixture is assayed for the content of sterols. If the desired purity is not achieved, then the mixture is re-crystallised.

Legislation

Recent tall oil phytosterol FDA GRAS notifications (where the FDA raised no questions) are:

- GRN 000039 (2000) – Tall oil phytosterols – for use in vegetable oil spread.
- GRN 000112 (2002) – Phytosterols from vegetable oils or tall oil – for use in spreads, various dairy products (including milks, ice-cream, cream cheese), snack bars, various dressings and various breads and rolls.

CONCLUSIONS

Tall oil phytosterols are sourced as a by-product from pulping of coniferous trees. The ratio of primary phytosterol substances and minor compounds of tall oil phytosterols is different to that of other oils and this in turn determines physiological activity. The major limitation to this point, on the wider use of phytosterols and stanols, has been their limited solubility in food matrices and consequently this has affected the efficacy with which they could be blended into foods.

However, tall oil phytosterols do not need to be esterified to improve their solubility as the Applicant has suggested that they are able to be incorporated into the non-fat food media of this Application, namely low-fat and non-fat milks.

REFERENCES

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

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Other relevant references

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

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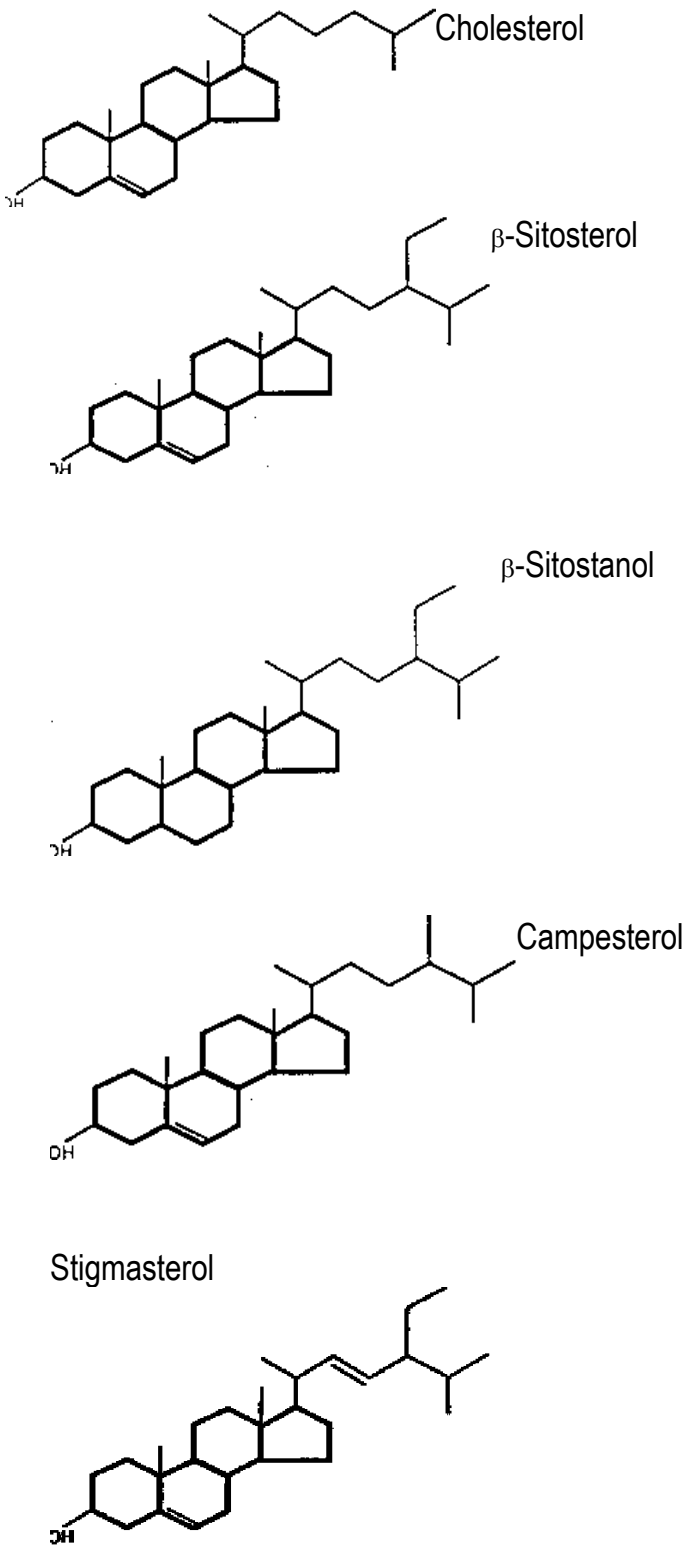
Figure 1. Structure of cholesterol and some common phytosterols and phytostanols (taken from Reference 1).



Attachment 3

SAFETY ASSESSMENT OF TALL OIL NON-ESTERIFIED PHYTOSTEROLS (TOPS)

The safety of TOPs were reviewed under Application A417 following a previous application from Novartis Pty Ltd. A summary of the safety assessment is included below. Additional studies submitted on the safety and efficacy in humans by Parmalat for A508 has also been included in the safety assessment below.

Summary of the safety assessment for TOPs in Application A417

Absorption, Distribution, Metabolism and Excretion (ADME)

Studies on ADME were reviewed under Application A410-Phytosterol esters derived from vegetable oils and were considered appropriate for the assessment of tall oil non-esterified phytosterols (TOPs), as these studies covered the specifications of the individual phytosterol components.

The major sterols and sterol esters derived from vegetable oils were tested in rats *in vivo* to compare their uptake, tissue distribution, metabolism and excretion with those of cholesterol and cholesterol esters. The summary is as follows:

- The rats adequately tolerated dosing with the sterols sitosterol, sitostanol, stigmasterol, campesterol, and campestanol, and also sitosterol-esters. Sitosterol, sitostanol, stigmasterol and campestanol, in addition to the linoleate ester of sitosterol, were poorly absorbed (between 1.2 and 5% of dose in females, and 0.5 - 1.9% in males), whereas a greater proportion of campesterol and cholesterol were absorbed (12 - 27% in females, 24% cholesterol absorbed in males).

- Sterols were found in tissues at low concentrations. Sitosterol and sitostanol were found in the adrenals, ovary and stomach at low concentrations, campestanol in the adrenals, ovaries and intestinal epithelia, and campesterol in the adrenals, spleen, intestinal epithelia, ovaries, liver and bone marrow.

- The greater proportion of each of the phytosterols investigated was eliminated in the faeces, as both the free sterol and sterol esters, suggesting that some esterification of sterols occurs in the gut *in vivo*. A minor faecal metabolite was observed in various studies, but this was not characterised and may have been an oxidation product, although from *in vivo* or *ex vivo* storage was not clear.

Sub-chronic Studies

In a 13-week subchronic study in rats, there was no evidence of toxicity following treatment with TOPs in the diet of rats up to doses of 5%. The No Observed Effect Level (NOEL) was 4161 mg/kg bw/day in male rats.

Chronic Studies

No chronic studies or carcinogenicity studies were submitted.

Developmental study

A published paper of a developmental/teratology study where rats received up to 5% plant *stanol esters* in the diet for a gestation period of 21 days demonstrated no teratological potential.

Reproduction studies

A 2 generation reproduction study in rats dosed with up to 5% phytosterols in the diet (as a mixture of sterols and sterol-esters at up to 8.0 %) equivalent to up to 4.4 g/kg/day, for 10 weeks prior to mating, then throughout gestation, lactation and weaning, found no significant effect on clinical, growth or reproductive parameters in either the F_0 or F_1 generations.

A published paper of a 2 generation reproduction study in rats dosed up to 4.38% plant *stanol esters* for 10-weeks prior to mating, then throughout gestation, lactation and weaning found no significant effect on clinical or reproductive parameters in either F0, F1 or F2 generations. A treatment related decrease in bodyweights in male and female pups of the F0 and F1 generation was observed at the highest dose of 4.38%. However, this decrease in bodyweight was attributed to the lack of absorption of phytosterols and the resulting reduction in the caloric value of the test diet compared to controls.

Genotoxicity studies

TOPs were found to be negative in a battery of bacterial and mammalian genotoxicity test systems at doses in vivo up to 2000 mg/kg bw and concentrations in vitro up to 1200 μg/ml. These suggest that TOPs are non-genotoxic both with and without metabolic activation.

Other animal studies

A study was conducted to evaluate the oestrogenic potential of TOPs. Doses of up to 5000 mg/kg/day for four consecutive days to immature female rats did not lead to an increase in absolute uterine weight or in the uterine weight/terminal body weight compared to normal controls (significant increases were noted in the positive control groups). There were significant reductions in bodyweight gains at 2500 and 5000 mg/day.

<u>In vitro oestrogenic potential</u>

Two *in vitro* studies on the oestrogenic potential of phytosterols were performed, using binding to rat uterine cytosol oestrogen receptors and binding to and activation of human oestrogen receptor in yeast cells. These studies used phytosterols at up to 100 and 129 μM, with no binding evident in either test system. Positive controls (β-estradiol) performed as expected in these assays.

In vivo oestrogenic potential

A series of studies were conducted to examine the uterotrophic potential of the dietary sterols, using various sterols and their mixtures in rats by gavage. The end point determined was the wet weight of uterus.

Phytosterols, phytosterol esters, cholesterol and cholesteryl palmitate were all found to be negative in this assay system at doses of up to 500 mg/kg/day for 3 days. Positive controls coumestrol and β-estradiol both gave positive responses (increased uterine weights) at doses of 20 and 0.4 mg/kg/day respectively.

Efficacy Studies in animals

These studies, while primarily efficacy studies, do provide some limited information on the toxicity of TOPs. A short-term (10-day) study in rats was submitted. In this study, TOPs at up to 1% w/w in diet (1000 mg/kg bw/day) were well tolerated by rats with no reduction in growth rates.

A number of subchronic efficacy studies were submitted in mice, hamsters and rabbits. In these studies TOPs at up to 2% w/w in the diet were well tolerated (representing doses of up to 3340 mg/kg bw/day in males) for a period of up to 20 weeks. There appeared to be no significant clinical findings (although the studies did not specifically state this or present clinical data), effects on bodyweights or food intakes and growth. Histopathological analysis (albeit limited) was unremarkable even at the highest doses.

An assessment by FSANZ of the efficacy of treatment with TOPs on reductions in cholesterol was not undertaken, although the Applicant has presented numerous published and unpublished studies in which the efficacy of oral doses of TOPs in reducing blood cholesterol has been demonstrated in animals.

Human Studies

The Applicant supplied original data on available studies in humans on TOPs. The phytosterols were administered in four forms, vegetable oil, margarine, milk and a cereal based nutritional bar. Two of the studies were for a period of 10 days in normal and hypercholesterolaemic subjects at 1.5g/day (medium was vegetable oil), one 30-day study in normal subjects with phytosterol-containing table spreads at 1.5g/day, a 28-day study in normal subjects with phytosterol-containing milk at 0.9, 1.8 and 3.6g/day and an 8-week study in hypercholesterolaemic subjects with phytosterol-containing snack bars at 1.8g/day.

Total plasma cholesterol was reduced by 5-7% and low density lipoprotein (LDL) by 5-14% at a dosage of 1.5g/day with a vegetable oil matrix (10-days); 9% and 14% respectively with margarine at 1.5g/day (30-days); 4% and 3% with a cereal bar at 1.8g/day (8-weeks) and; 9% and 13% respectively with a full-milk (3.6%) based beverage at 3.6 g/day (4-weeks).

The studies demonstrated that in normal healthy human subjects and in subjects with hypercholesteraemia, doses of TOPs up to a level of 1.5 g/day over a 10-day period were well tolerated. In the third study, subjects tolerated doses of TOPs up to a level of 1.5g/day over a period of 30 days.

In the fourth 28-day study at 0.9, 1.8 and 3.6g/day, clinical signs and symptoms were not confined to a specific sex and were generally considered unrelated to treatment as there was no dose response and there was no significant difference between treatment groups. There were no differences between treatment groups in weight post treatment, blood pressure, pulse rate, blood chemistry and haematology parameters or urinalysis other than isolated increases in platelet counts, eosinophils, red blood cell count, haemoglobin and haematocrit during treatment, although no dose-response relationship was evident.

Vitamin A and E and alpha and beta-carotene levels were compared at the start of treatment and at week 4 post-treatment. Reductions were noted in vitamin A levels post-treatment at doses of 0.9, 1.8 and 3.6g/day (10%, 12% and 9% respectively).

However, this lacked a dose-response and there were no other significant differences between treatment groups between day 0 and week 4 post-treatment with respect to either the change or the relative change in vitamin A levels. There were no significant differences between groups with respect to vitamin E levels.

There were no significant differences between treatment groups at day 0 or 4-weeks post-treatment in either alpha or beta-carotene levels. A significant ($p<0.01$) reduction in subjects dosed at 3.6g/day in mean alpha-carotene levels was observed between day 0 and week 4 (23% reduction) compared to placebo values. It was concluded that human subjects tolerated doses of TOPs in a milk-based beverage up to a level of 3.6g/day over a period of 28 days.

In the fifth study, although there were some reports of adverse symptoms, these were also reported in the placebo group. It was concluded that human subjects tolerated doses of TOPs in a cereal based nutritional bar up to a level of 1.8g/day over a period of 8-weeks.

International reviews of the safety of phytosterols

In April 2000, the Scientific Committee on Food (SCF) of the EU concluded that addition of phytosterol esters derived from vegetable oils in yellow fat spreads, as a novel food was safe at levels up to 8% free phytosterols/100g provided the phytosterol profile was as follows: 30-65% β-sitosterol, 10-40% campesterol, 6-30% stigmasterol and a total of 5% other phytosterols.

Since that time further applications for use in novel foods enriched with non-esterified phytosterols and also containing some phytostanols (bakery products, snack products, meat products, margarine, soft cheese, yoghurt or fruit-based milk drinks) have been submitted for approval and the SCF recently expressed an opinion on the safety of non-esterified phytosterols (SCF, March 2003).

They concluded that, provided that intakes of phytosterol enriched foods did not exceed 3g/day then the use of phytosterols in the above specific foods were safe provided the phytosterol/stanol profile was as follows: up to 80% β-sitosterol, 15% β-sitostanol, 40% campesterol, 5% campestanol, 30% stigmasterol, 3% brassicasterol and 3 % other phytosterols.

Additional studies in humans submitted by Parmalat

The Applicant supplied published papers on TOPs administered in three forms, margarine and shortening, edible oils, full milk (3%) and chocolate to both normal and hypercholesterolaemic subjects (**Table 1**). The studies in edible oils and chocolate demonstrated that reductions in mean total and LDL cholesterol could be achieved at doses of 1.7g/day (edible oils) or 1.8g/day (chocolate) over a 30 day period; however, an independent assessment of whether any adverse clinical effects, changes in clinical chemistry parameters and/or urinalysis was not possible, as the data was not presented in the paper. These studies essentially concentrated on the efficacy of TOPs rather than an assessment of the safety of TOPs.

However, the study by Pritchard and Beer (2000**)** in full milk suggested that human subjects tolerated doses of TOPs mixture in a full milk-based beverage up to a level of 3.6 g/day over a period of 28 days without significant adverse effects being experienced by subjects, other than reductions in alpha-carotene levels observed at the highest dose used. No reductions in β-carotene levels were observed.

A study by Plant and Mensink (1998) in margarine and shortening suggested that subjects tolerated plant stanol esters up to a dose of 6.8 g/day (4 g free phytostanols), without any evidence of adverse clinical effects or effect on blood haematology/chemistry parameters, although the data presented was not as comprehensive as the Pritchard and Beer (2000) study.

A recent published study submitted by Parmalat with low-fat food items (bread, meat products, jam and yoghurt) with incorporated TOPs at doses of 0.91 to 4.17g/day for 15-weeks suggested that a reduction of total cholesterol of 8% and LDL of 13% could be achieved. However, as the percentage of fat in these low-fat foods was not stated in the study, FSANZ cannot verify what exact percentage of fat (referred to by the Authors as low-fat) would achieve the above reductions[8]. This is also supported by another study with a low-fat containing margarine derived from a tall oil source, which achieved reductions in total cholesterol of 10.6% and LDL of 13.7%[9].

Overall conclusions of the safety assessment

The available animal studies on TOPs mixtures indicate that these substances are poorly absorbed from the gastrointestinal tract and excretion is via the faeces (entero-hepatic cycling). They have low toxicity are not genotoxic and demonstrate no reproductive or developmental toxicity.

No evidence of adverse effects were noted following administration of TOPs up to 5% in the diet for 13 weeks in rats study (this later study being a detailed toxicological study in accordance with international toxicological testing requirements). There was also no evidence of oestrogenic activity from the available studies.

[8] Tikkanen et al (2001) Effect of a diet based on low-fat foods enriched with non-esterified plant sterols and mineral nutrients on serum cholesterol. American Journal of Cardiology, 88, 1157-1162.
[9] Hallikainen MA and Uusitupa M (1999) Effects of low-fat stanol-ester-containing margarines on serum cholesterol concentrations as part of a low-fat diet in hypercholesterolaemic subjects. American Society for Clinical Nutrition, 69, 403-10.

Efficacy studies were performed in mice, rabbits and hamsters to determine the cholesterol lowering effects of TOPs. The results suggested that TOPs were well tolerated in animals up to 2% (w/w) in the diet for a period of 20 weeks. The absence of any histopathological changes is also reassuring as if any clinical signs were present these may have been of minor nature.

Efficacy studies including the data and results from clinical studies in humans involving mildly hypercholesterolaemic individuals in a variety of food matrixes, including milk demonstrated the effectiveness of TOPs incorporated into food products to reduce cholesterol absorption.

There is no evidence of adverse health effects in these human studies, apart from some minor reductions in vitamin A at doses of 0.9, 1.8 and 3.6 g/day and reductions in subjects dosed at 3.6 g/day in mean alpha-carotene levels observed between day 0 and week 4 (23% reduction) compared to placebo values in a 28 day study in which tall-oil non-esterified phytosterols were administered in a milk based beverage.

The overall conclusion from the human studies was that administration of TOPs in the diet at 3.6 g/day for a period up to 28 days was well tolerated. The study by Plant and Mensink (1998) using 4 g/day of a phytostanol mixture for 8 weeks further supports the safety of TOP which contain significant amounts of phytostanols.

Table 1 Summary table of efficacy/safety studies in humans submitted by Parmalat with Application A508

Tall-oil Phytosterol mixture	Dose/Duration	Food matrix	Efficacy	Adverse effects	Reference
Sitostanol ester 70% and 30% campestanol-ester	4g/day for 8 weeks in 34 subjects (mean age 33 years) with normal cholesterol levels	Margarine	Not examined	No adverse effects noted and blood chemistry and haematology was normal.	Plat and Mensink (1998)
Sitostanol 20% and a mixture of sitosterol and campesterol[10]	1.7g/day for 30 days in 32 males (aged 25-60 years) with hypercholesterolaemia	Edible oil	Mean total cholesterol reduced 20%; LDL 24%	1 subject with diarrhoea associated with influenza. No blood chemistry, urinalysis or clinical data available for independent review.	Jones (1999)

[10] Exact percentages not stated in the methods

Sitosterol 51%, sitostanol 25%, campesterol 13% and campestanol 4%.	0, 0.9, 1.8 or 3.6g/day in 4 groups of 33 subjects with hypercholesterolaemia	Full milk	4.3 to 9.1% reduction in mean total cholesterol; 7.4 to 13.2% in LDL	Some minor changes in blood chemistry and haematology parameters. Clinical signs and symptoms were not confined to a specific sex and were generally considered unrelated to treatment as there was no dose response and there was no significant difference between treatment groups. Reductions in mean levels of alpha-carotene at doses of 3.6g/day.	Pritchard and Beer (2000)
Sitosterol 60%, sitostanol 18%, campesterol 14% and campestanol 3%.	0 or 1.8g/day for 30 days in males and females (aged 21-75 years).	Chocolate	6.4% reduction in mean total cholesterol and 10.3% in mean total LDL	No reported adverse clinical effects. No blood chemistry, urinalysis or clinical data available for independent review.	deGraf et al (2002)

1. Jones PJH (1999) Cholesterol-lowering efficacy of a sitostanol-containing phytosterol mixture with a prudent diet in hyperlipidemic men. Am. J. Clin. Nutr., *69*, 1140-50.

Test material:	Group 1-controls-North American diet considered to be healthy in terms of macronutrient and fat content*. Group 2-diet plus TOPs** in a margarine matrix (edible oils).
Test groups:	32 males aged 25-60 years. 16 males/group with primary hypercholesterolaemia (6 to 10 mmol/L).
Dose:	1.7 g/day for 30 days double blind study
GLP:	Not stated.

*Protein 15%; carbohydrate 50% and fat 35%.

**TOPs-sitostanol 20% and a mixture of sitosterol and campesterol (exact % not stated in the methods).

Subjects underwent a routine physical examination and detailed blood chemistry before and on day 30 of the study. Blood samples were collected on days 0, 10, 20, 29 and 30 in order to measure total, LDL and HDL cholesterol and triglycerides. Samples were also collected at days 40 and 50 post treatment. The parameters measured from the blood chemistry analysis other than the lipid analysis, were not stated in the methods section.

Results

All 32 subjects completed the study and the authors reported that all subjects tolerated the experimental diet without any adverse effects (other than 1 subject who reported diarrhoea associated with a bout of influenza) and that the blood chemistry and urinalysis was normal throughout the treatment period. This data was not available for independent review and as such cannot be confirmed by FSANZ.

There was no significant difference between controls and treated groups in mean body weights.

Reductions in mean total (20%) and LDL cholesterol (24%) were achieved in the sitostanol enriched TOPs treated group; whereas, reductions in controls was 10% (total) and 9% (LDL) respectively at day 30. HDL and triglyceride concentrations did not change significantly during the study in control or treated groups.

In conclusion, this study demonstrated that in human subjects with hypercholesterolaemia that significant reductions in total and LDL-cholesterol could be achieved at doses of TOPs mixtures in a margarine based product up to a level of 1.7g/day over a period of 32 days.

An independent assessment of whether any adverse clinical effects, changes in clinical chemistry parameters and/or urinalysis was not possible, as the data was not presented in the paper. It could not be determined from the methods the relative percentages of sitosterol and campesterol in the TOP mixture.

2. Pritchard H and Beer M (2000) To determine the effect of increasing doses of tall oil derived phytosterols (Phytrol ^{TM}) on the plasma lipid levels of hypercholesterolaemic patients. Novartis Consumer Health and Forbes Meditech Inc. Final study. 14 November 2000.

Test material:	Group 1-Lactose-free milk* (controls), Groups 2-4 milk with TOPs**
Test groups:	132 subjects (33/group) with primary hypercholesterolaemia and no other health concerns aged 25 to 60 years.
Dose:	0.9, 1.8 or 3.6 g/day over 28-days-double blind placebo controlled study.
GLP:	Not stated.

* 3.6 g fat/100g

** TOPs-sitosterol 51.3%, sitostanol 25.3%, campesterol 13.3% and campestanol 4.4%.

Study conduct

In a 2-week period before treatment subjects underwent a physical examination and a detailed medical history was taken and blood chemistry was performed. Various criteria for inclusion/exclusion in the study were ascertained. At the start of treatment subjects were instructed to take three drinks per day for the 4-week period. A physical examination and blood was collected at each visit (at 3.5 and 4 weeks).

One hundred and thirty two human volunteers consumed four different TOPs mixtures (including a placebo group) in a milk-based drink, for a 4-week period ranging in doses from 0 to 3.6
g/day.

Nine subjects discontinued the treatment due to various factors (withdrew consent, adverse events, protocol deviation, did not receive treatment or unable to drink 3 drinks/day). The adverse effects reported were pain in the kneecap (unrelated to treatment), moderate constipation and elevations in ALT and AST, which could have been related to treatment.

In each study period, fasting blood samples were collected at -2, -1, 0, 3.5 and 4 weeks for analysis of lipids (total cholesterol, HDL and LDL and triglycerides), enzymes (alkaline phosphatase, LDH, SGOT, SGPT), glucose, creatinine, BUN, uric acid and total bilirubin. Standard haematology and urinalysis parameters were determined. The study assessed all relevant confounding factors during the administration period, including lifestyle factors, bodyweight, disease status and medicine use.

Results

At a dose of 1.8g/day reductions in total and LDL cholesterol were 5.5 and 8.6% respectively; and at 3.6g/day reductions of 9 and 13% respectively.

At all dose levels (including the placebo group) mild to moderate adverse clinical effects were reported (ranging from general symptoms, skin, respiratory, cardiovascular, gastrointestinal and musculoskeletal effects) in 52% of subjects. However, these clinical signs and symptoms were not confined to a specific sex and were generally considered unrelated to treatment as there was no dose response and there was no significant difference between treatment groups.

There were no differences between treatment groups in weight post treatment. However, overall there were significant increases in weight among all subjects at all doses. There were no significant increases in blood pressure or pulse rate post treatment at all doses and no differences between groups other than an increase in systolic blood pressure at a dose of 0.9 g/day compared to placebo.

Subjects who were treated at a dose of 0.9 g/day had significantly increased platelet counts and eosinophils at the end of treatment. At a dose of 1.8 g/day significant increases were noted in red blood cell counts, haemoglobin and haematocrit during treatment. Increases were noted in alanine transaminase (ALT) and decreases in uric acid in placebo subjects post-treatment. At a dose of 1.8 g/day a significant increase in alkaline phosphatase was observed during treatment, however, at the highest dose this was not significant. However, none of the changes in blood chemistry differed between the four treatment groups.

Subjects on whom urinalysis were performed was small and as such no statistical tests of significance other than specific gravity and pH were performed.

In the placebo group there was a significant increase in urinary pH at the end of treatment; however, no significant differences in specific gravity were noted. No treatment related effects were noted in the parameters measured form the available data.

Vitamin A and E and alpha and beta-carotene levels were compared at the start of treatment and at week 4 post-treatment.

At the start of treatment there were no differences between treatment groups except in subject's dosed at 1.8g/day who had significantly ($p<0.05$) lower mean levels of vitamin A when compared to placebo (11% reduction). At week 4 post-treatment significant reductions in vitamin A of 10% ($p<0.005$), 12% ($p<0.001$) and 9% ($p<0.01$) compared to placebo controls for that group were observed at doses of 0.9, 1.8 and 3.6g/day respectively. However, this lacked a dose-response and there were no other significant differences between treatment groups between day 0 and week 4 post-treatment with respect to either the change or the relative change in vitamin A levels between the start and 4-week treatment levels.

There were no significant differences between groups with respect to vitamin E levels.

There were no significant differences between treatment groups at day 0 or 4-weeks post-treatment in either alpha or beta-carotene levels. A significant ($p<0.01$) reduction in subjects dosed at 3.6g/day in mean alpha-carotene levels was observed between day 0 and week 4 (23% reduction) compared to placebo controls.

In conclusion, this study demonstrated that human subjects tolerated doses of TOPs mixture in a milk-based beverage up to a level of 3.6 g/day over a period of 28 days without significant adverse effects being experienced by subjects. However, reductions in alpha-carotene levels were observed at the highest dose used.

3. deGraf J, Pernette RW, de Sauvage Nolting et al (2002) Consumption of tall oil-derived phytosterols in a chocolate matrix significantly decreases plasma total and low-density lipoprotein-cholesterol levels. British Journal of Nutrition, *88*, 479-488.

Test material:	Group 1 controls received chocolate alone. Group 2-chocolate plus TOPs*.
Test groups:	31 males or females aged 21-75 years with primary hypercholesterolaemia (5.5 to 8 mmol/L).
Dose:	1.8g/day for 30 days double blind placebo-controlled study
GLP:	Not stated.

*TOPs-sitosterol 60%, sitostanol 18%, campesterol 14% and campestanol 3%.

Study conduct

In a 4-week period before treatment subjects underwent a physical examination and a detailed medical history was taken and blood chemistry was performed. Various criteria for inclusion/exclusion in the study were ascertained. At the start of treatment subjects were instructed to have three chocolate servings per day for the 4-week period. A physical examination and blood was collected at 3 and 4 weeks.

Seventy human volunteers consumed either a placebo chocolate (31 subjects) or a chocolate containing the TOP mixture (31 subjects) with meals at a total dose of 1.8g/day for 4 weeks.

Two subjects discontinued the treatment; the first in the placebo group (due to an adverse event which was not stated in the paper) and the other subject withdrew their consent after treatment had started. Eight subjects were excluded from the study based on the pre-protocol exclusion criteria.

At weeks 3 and 4 recordings of lipid levels (total cholesterol, HDL and LDL and triglycerides) and routine blood chemistry, haematology and urinalysis was performed. Any adverse clinical effect was recorded.

Results

At a dose of 1.8 g/day reductions in mean total and mean LDL cholesterol were 6.4% and 10.3% respectively at following 4 weeks of treatment. HDL and triglyceride concentrations did not change significantly between control and treated groups.

It was stated in the study that no significant changes were observed between controls and treated groups in blood chemistry, haematology or urinalysis parameters and no significant adverse clinical effects were observed. An independent assessment was not possible, as the data was not presented in the paper.

4. Plat J and Mensink RP (1998) Safety aspects of dietary plant sterols and stanols. In Post-graduate Medicine, Special Report: New Developments in the Dietary Management of High Cholesterol, 32-38.

Test material:	Group 1-oil-based margarine and shortening (controls), Group 2 Margarine and shortening with a tall oil phytostanol ester mixture or a vegetable oil ester mixture*
Test groups:	112 subjects (42 controls and either 34 or 36 treated subjects) with normal cholesterol levels.
Dose:	4g/day of free phytosterols for eight weeks.
GLP:	Not stated.

* Sitostanol ester 70% and 30% campestanol- ester

Study conduct

Fasting blood samples were taken at week 0 and then 112 subjects with normal cholesterol levels consumed a baseline diet for 4 weeks and then one of the following three test diets for the remaining eight weeks:

- 42 subjects consumed unsupplemented diets (controls);
- 36 subjects consumed spread and shortening with 6.4 g/day plant stanol esters derived from tall oil; or
- 34 subjects consumed spread and shortening with 6.4 g/day plant stanol esters derived from vegetable oil.

Treated subjects were instructed replace their usual spread at breakfast and lunch with phytostanol-ester enriched spreads and cooking fat at dinner was replaced with phytostanol-ester enriched shortening.

Clinical chemistry and haematology were recorded at week 4 (baseline) and at week 12 (end of the treatment period). Subjects also completed questionnaires about any possible adverse effects during the treatment period.

Results

There were no significant differences in reported adverse effects or in haematology and blood chemistry parameters between control and treated groups. It is concluded that human subjects tolerated doses of either a phytostanol-ester mixture or a vegetable oil mixture up to a level of 4g/day (free phytostanols) without any significant adverse effects.

Attachment 4

Dietary Exposure Assessment Report

Summary

An application was received by FSANZ requesting the Food Standards Code (the Code) to be amended to allow the use of tall oil phytosterols (TOPs) as a novel food ingredient, under Standard 1.5.1 – Novel Foods, for use in low fat milks (fat content < 1.5%).

A dietary exposure assessment was undertaken to determine the impact of allowing TOPs to be added to the above foods. The assessment took into account the existing permission under Standard 1.5.1 to add TOPs to edible oil spreads (the 'baseline' scenario), the proposed addition of TOPs to low fat milks (the 'low fat milks' scenario) and a combination of these products (the 'low fat milks plus baseline' scenario). In each scenario, addition of phytosterols at a level equivalent to 0.8 g free phytosterols per serve was assumed for edible oil spreads and 0.9 g free phytosterols per serve for low fat milks. It was further assumed that all edible oil spreads and low fat milks contained added free phytosterols. Intrinsic levels of phytosterols in foods were not taken into consideration.

Modelling was conducted assuming that consumers do not change the amounts and general types of foods they eat, simply substituting phytosterol-containing edible oil spreads or low fat milks for their non-phytosterol counterparts. Food consumption data from the most recent Australian and New Zealand National Nutrition Surveys (NNSs) – 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 (those aged 40-64 years and 65 years and above), for the general population, and for two 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.

When it was assumed that consumers maintain their existing eating patterns, simply substituting phytosterol containing spreads or low fat milks for their non-phytosterol counterparts, estimated mean dietary exposure (expressed as free phytosterols) did not exceed 1.9 g per day in any population group assessed under any of the scenarios considered. At the 95th percentile of exposure, no population group assessed exceeded 4.8g free phytosterols per day for any of the scenarios modelled. The analysis shows that, for the target population group in particular, edible oil spreads contribute more to dietary exposure to added free phytosterols (78-84% of exposure) than low fat milks, according to the available data on food consumption patterns.

Introduction

Information supplied by the applicant

The applicant is seeking approval to use TOPs in foods at levels formulated to provide between approximately 2 and 3 grams per day of free phytosterols to target consumers (through 2-3 serves of products). The products containing added TOPs are targeted specifically to consumers over the age of 40 years who have concerns about their blood cholesterol level. However, casual consumption by other non-target population groups, including children, must also be considered.

Since no dietary exposure assessment was provided by the applicant, FSANZ conducted a dietary exposure assessment to estimate the potential exposure to phytosterols if they were added to the proposed foods.

Existing phytosterol-containing products

There are a small number of edible oil spreads currently on the market that contain phytosterols. These products carry label claims stating that phytosterols assist in lowering cholesterol absorption and recommend that 2-3 serves of phytosterol-containing foods be consumed each day in order to achieve the recommended level of intake. Permission for the use of added phytosterols in these products is contained in Standards 1.5.1 and 2.4.2 of the Code, which allows the addition of phytosterol esters at no more than 137 g/kg (equivalent to 1.37 g per 10 g serve of spread), or the addition of tall oil phytosterols at no more than 80 g/kg (equivalent to 0.8 g free phytosterols per 10 g serve).

Natural occurrence of phytosterols

Major sources of naturally occurring phytosterols are vegetable fats and oils, and 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 substantially lower than would result from addition of phytosterols to foods.

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 these esters by the European Commission. For regular users of spreads containing added phytosterol esters, median household consumption was between 15 g and 18 g per day, which represents slightly less than 2 x 10 g serves per day. Research suggested that these consumption amounts represent consumption by a single person in the households and are lower than had been predicted at the time of approval of phytosterol esters, when consumption of the spreads was predicted to be 20 – 30 g per person per day. The ninety-fifth percentile consumption did not exceed 45 g (4.5 serves) per day. 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).

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 TOPs (expressed as free phytosterols) in foods.

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.

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 an 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 may consume these products if available in the household. 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).

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.

Concentration levels and serving sizes

The levels of free phytosterols in low fat milks 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) and 1 glass of milk (250 mL). The foods and proposed levels of use are summarised below in Table 1.

Table 1: Proposed levels of use of non-esterified phytosterols in foods

Food Code	Food Name	Serve size (g)	Proposed level of free phytosterols per serve (g/serve)	Concentration Level used in modelling (mg/kg)
1.1.1.2	Low fat milks (<1.5% fat), unflavoured, including skim milks	250	0.9	3 600
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 phytosterols since neither NNS has specific consumption data for phytosterols containing foods due to such foods being unavailable at the time of the surveys.

Estimating risk

Estimated dietary exposures are compared to a reference health standard in order to determine the potential risk to health of the population or its sub-groups. Free phytosterols do not have an established reference health standard such as an Acceptable Daily Intake (ADI). Therefore, estimated exposures were simply reported in gram amounts per day

Intakes of TOPs up to 3.6 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.

How were the estimated dietary exposures calculated?

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

- phytosterols in edible oil spreads, including margarines only (baseline scenario);
- phytosterols in low fat unflavoured milks only (low fat milks scenario); and
- phytosterols in edible oil spreads and low fat milks combined (low fat milks + 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 spreads or low fat milks that an individual consumed in order to estimate the exposure from 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 to eat low fat milks. In the baseline + low fat milks scenario, the consumer population includes those who consume only edible oil spreads and those who consume only low fat milks as well as those who consume both these foods. Therefore mean consumer exposure in the baseline + low fat milks scenario does not represent the result of simply summing mean consumer exposure from the baseline scenario and from the low fat milks scenario, since the consumer population is not exactly the same.

Percentage contributions of each food group to total estimated exposures are calculated by dividing the sum of consumers' exposures from a food group by the sum of all consumers' exposures from all foods, and multiplying this by 100.

Food consumption amounts for each individual take into account where each food in a classification code is consumed alone and as an ingredient in mixed foods.

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;
3. for the purpose of this assessment it is assumed that 1 mL is equal to 1 g for all foods; and
4. there is no contribution to phytosterols exposure through the use of complementary medicines (Australia) or dietary supplements (New Zealand).

The second assumption will lead 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 actually contain added phytosterols.

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.

Results

Estimated dietary exposures to phytosterols

The estimated dietary exposures of consumers to free phytosterols, for the different food groups, mean and 95th percentile are shown below in Figures 1 and 2 (baseline scenario, 'low fat milks' scenario and 'low fat milks plus baseline' scenario) for Australia and Figures 3 and 4 (baseline scenario, 'low fat milks' scenario and 'low fat milks plus baseline' scenario) for New Zealand. Numerical data are also provided for New Zealand and Australia in Table 2 (edible oil spreads), Table 3 (low fat milks) and Table 4 (edible oil spreads and low fat milks). 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 phytosterols 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

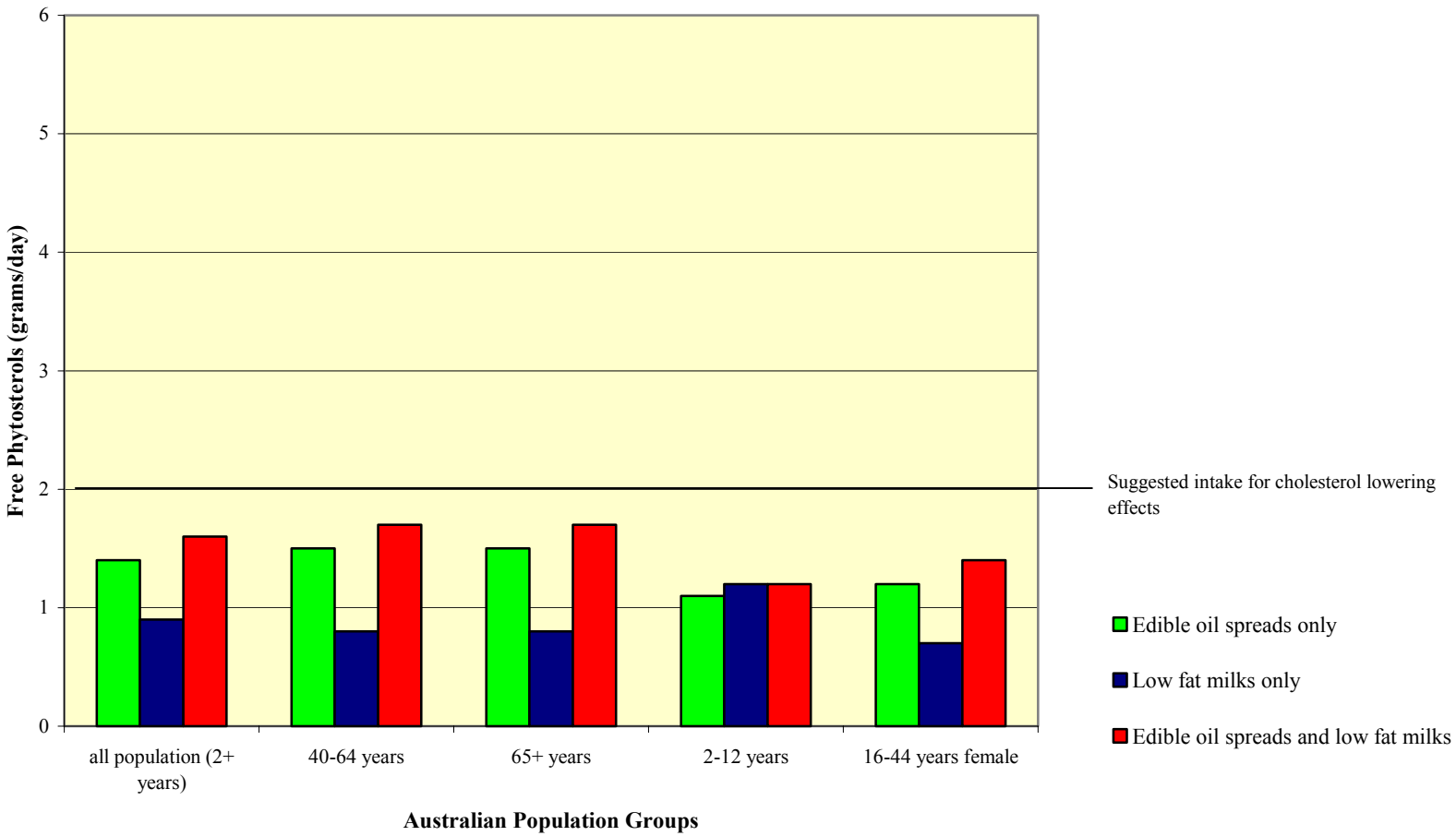


Figure 2: Estimated 95th percentile dietary exposure to free phytosterols for different population groups and scenarios for Australia

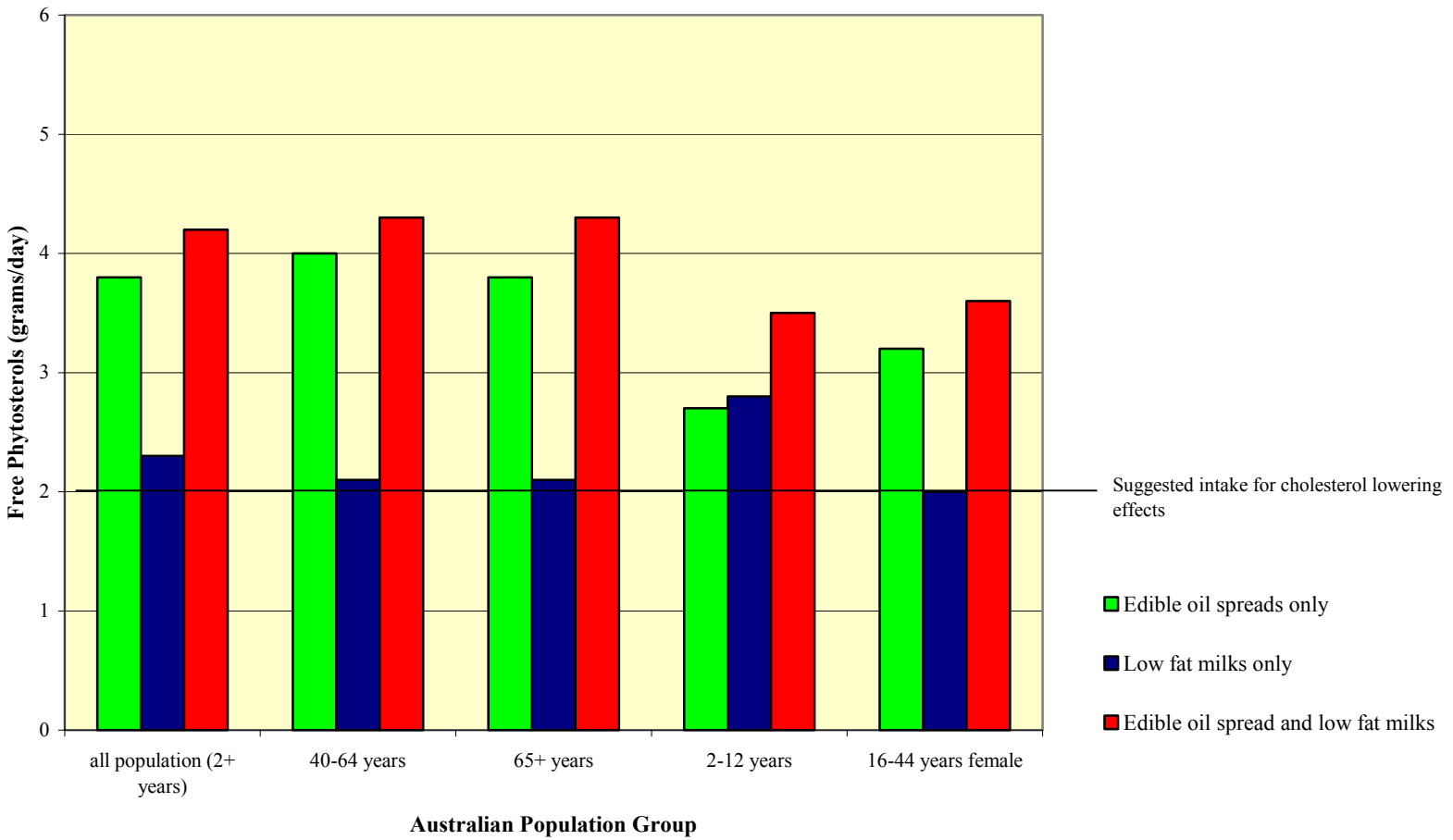


Figure 3: Estimated mean dietary exposure to free phytosterols for different population groups and scenarios for New Zealand

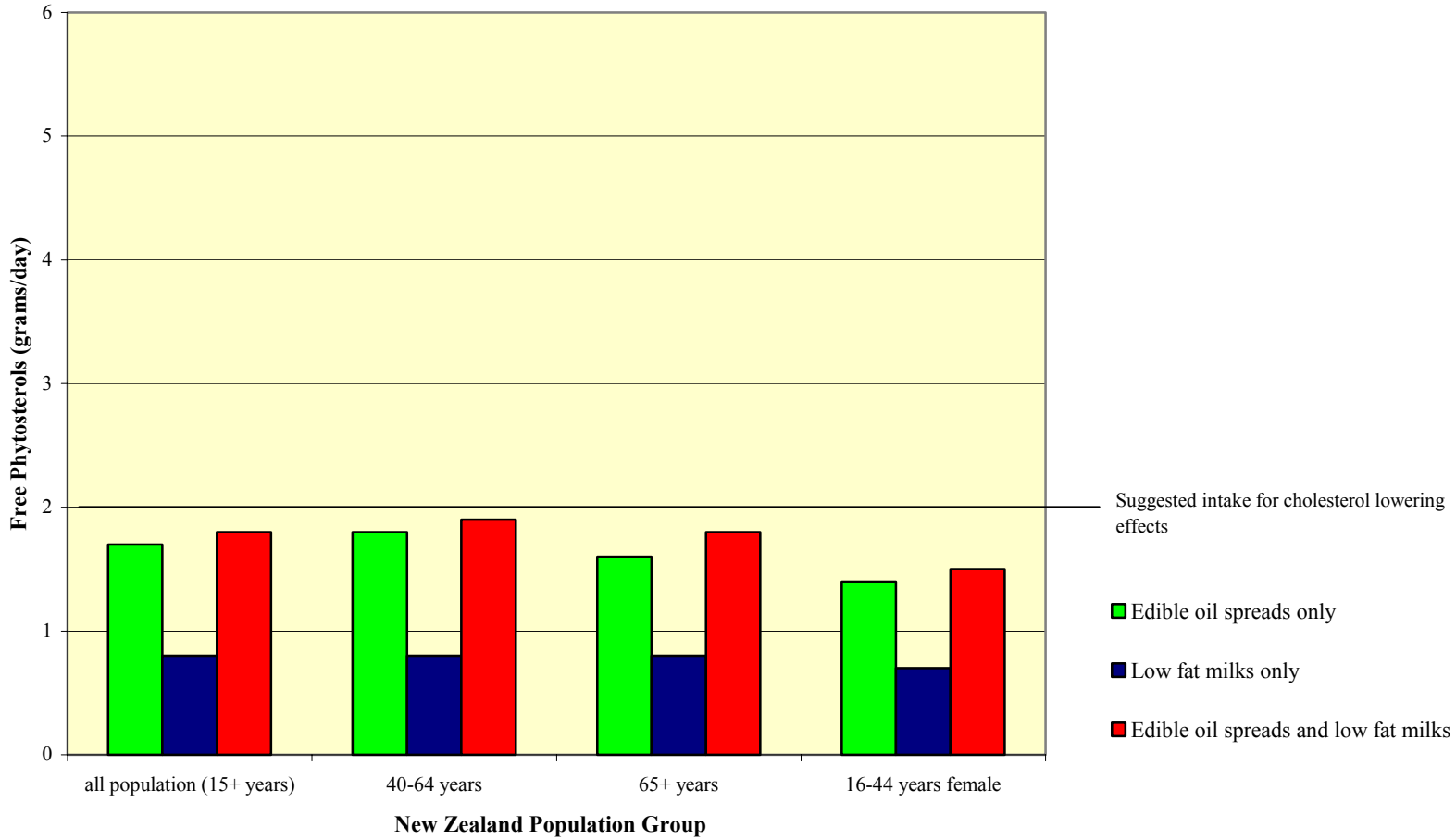
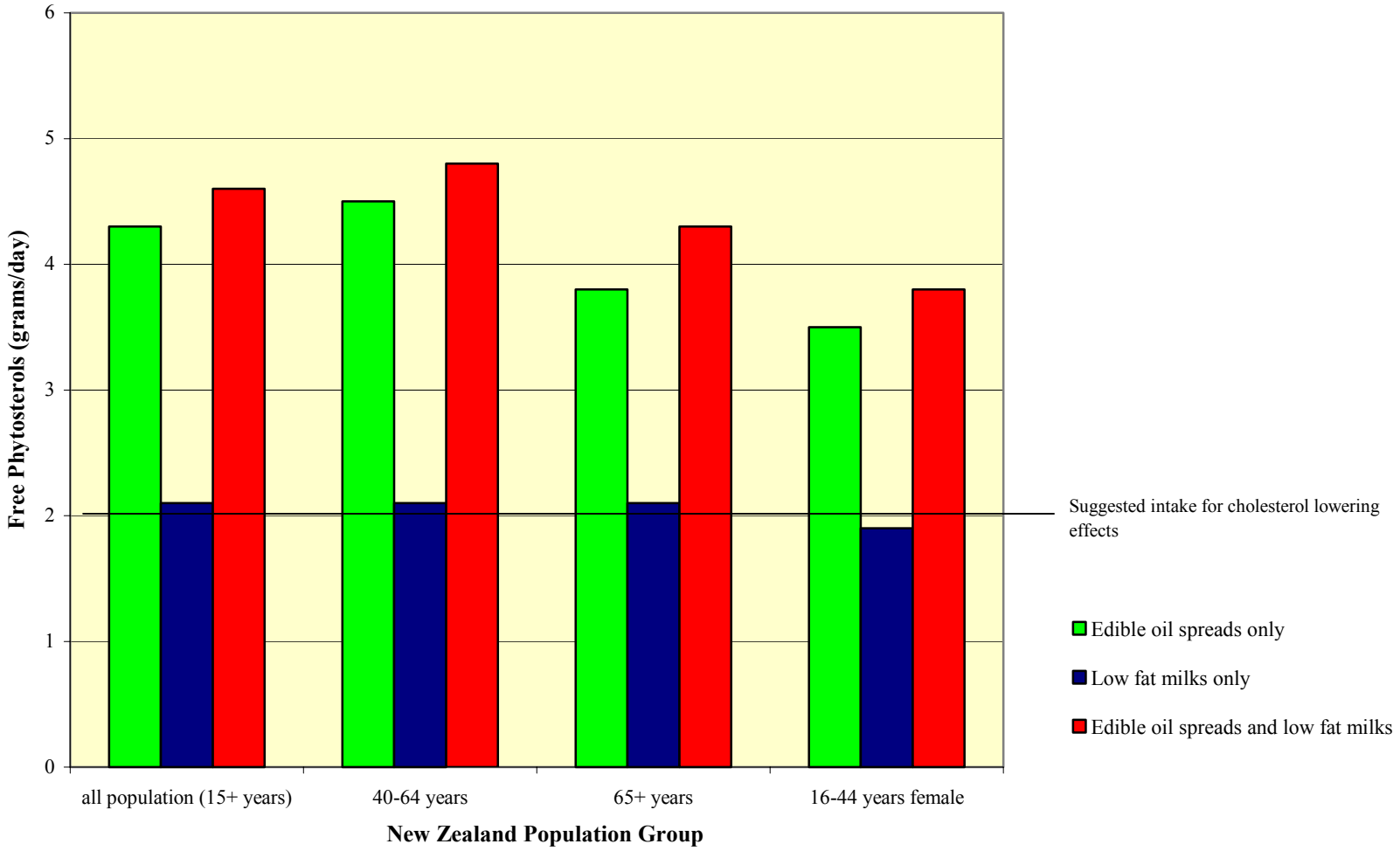


Figure 4: Estimated 95th percentile dietary exposure to free phytosterols for different population groups and scenarios for New Zealand



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

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) = 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) = 4 636, 40-64 years = 1 725, 65+ years = 817, 16-44 years female = 1 509.

Estimated mean exposure to free phytosterols among consumers of edible oil spreads does not exceed 1.8 g per day (see Table 2 above), equivalent to slightly more than 2 serves of phytosterol-containing spreads per day. The highest mean exposures to free phytosterols in Australia were for the target groups of those aged 40-64 years and those aged 65 years and above. For New Zealand, the highest mean dietary exposures were for the target group of those aged 40-64 years. High consumers (95th percentile) of free phytosterols from edible oil spreads have estimated dietary exposures up to 4.5 grams per day, equivalent to approximately 5 1/2 serves of phytosterol-containing spreads per day. The population group with the highest 95th percentile exposures are the 40-64 year age group for both countries and also the whole population group (aged 15+ years) for New Zealand.

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

Table 3: Estimated dietary exposure to free phytosterols from low fat milks, 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+)	4 262	31	0.9	2.3
	40-64 years	1 717	40	0.8	2.1
	65+ years	681	35	0.8	2.1
	2-12 years	303	15	1.2	2.8
	16-44 years female	1 108	35	0.7	2.0
New Zealand	Whole population (15 years+)	1 509	33	0.8	2.1
	40-64 years	649	38	0.8	2.1
	65+ years	310	38	0.8	2.1
	16-44 years female	434	29	0.7	1.9

Total number of respondents for Australia: whole population (2+ years) = 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) = 4 636, 40-64 years = 1 725, 65+ years = 817, 16-44 years female = 1 509.

Estimated mean dietary exposure to free phytosterols among consumers of low fat milks does not exceed 1.2 g per day (see Table 3 above), reflecting the smaller number of serves consumed per day of this food than of edible oil spreads. The highest mean exposure in Australia is for those aged 2-12 years. High consumers (95th percentile) of free phytosterols from low fat milk have estimated dietary exposures up to 2.8 grams per day, equivalent to the phytosterol content of slightly more than 3 serves of low fat milk per day. Again the group with the highest 95th percentile exposure is the 2-12 year age group for Australia.

The proportion of Australians and New Zealanders who consume low fat milks (31% and 33%, respectively) is substantially lower than the proportion who consume edible oil spreads (79% and 67%, respectively).

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

Table 4: Estimated dietary exposure to free phytosterols from edible oil spreads and low fat milks, 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+)	11 885	86	1.6	4.2
	40-64 years	3 737	87	1.7	4.3
	65+ years	1 682	86	1.7	4.3
	2-12 years	1 803	87	1.2	3.5
	16-44 years female	2 691	85	1.4	3.6
New Zealand	Whole population (15 years+)	3 532	76	1.8	4.6
	40-64 years	1 359	79	1.9	4.8
	65+ years	677	83	1.8	4.3
	16-44 years female	1 100	73	1.5	3.8

Total number of respondents for Australia: whole population (2+ years) = 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) = 4 636, 40-64 years = 1 725, 65+ years = 817, 16-44 years female = 1 509;

When free phytosterols are added to low fat milks as well as edible oil spreads, estimated mean exposure to free phytosterols increases slightly from 1.4 g to 1.6 g per day for the Australian consumer population, and also increases slightly from 1.7 g to 1.8 g per day for the New Zealand consumer population (see Table 4 above). Estimated mean dietary exposure does not exceed 1.9 g per day for any population group and is highest for New Zealanders aged 40-64 years. High consumers of free phytosterols (95th percentile) from edible oils spreads and low fat milks have estimated dietary exposures of between 3.5 g to 4.8 g per day for all population groups assessed. The population group with the highest 95th percentile exposure is New Zealanders aged 40-64 years.

The addition of phytosterols to low fat milks as well as to edible oil spreads results in only a slight increase in predicted mean consumer phytosterols exposure compared to baseline exposure; an increase of 0.2 g/day for all Australians and 0.1 g/day for all 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 in the low fat milks + baseline scenario. 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 and low fat milks to estimated exposures to free phytosterols are displayed in Figures 5 and 6. More detailed results are presented in **Attachment 1**.

Foods may be high contributors to phytosterols 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 TOPs than are low fat milks, on a population basis, assuming that eating patterns recorded in 1995 and 1997 (for Australia and New Zealand respectively) are maintained.

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

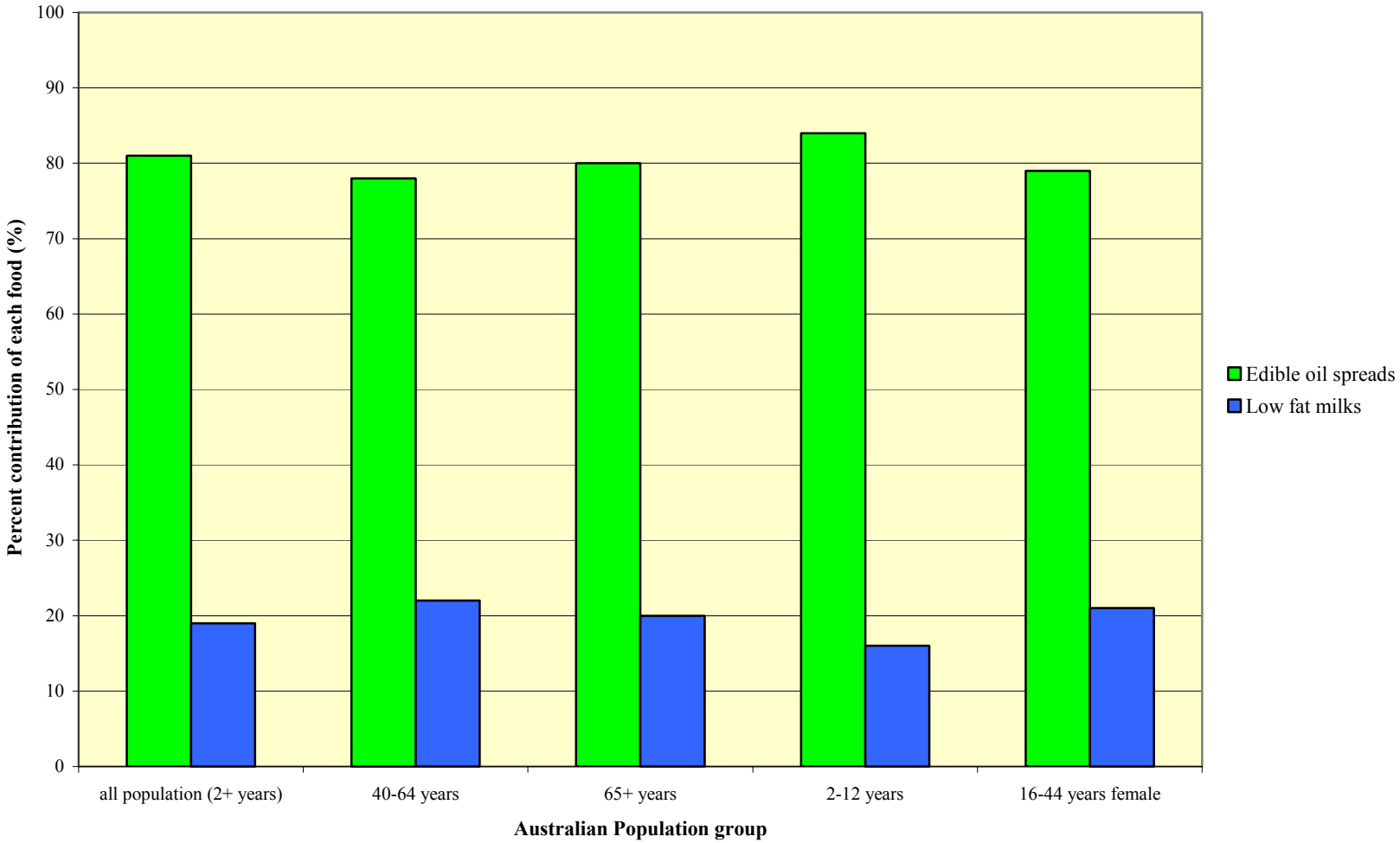
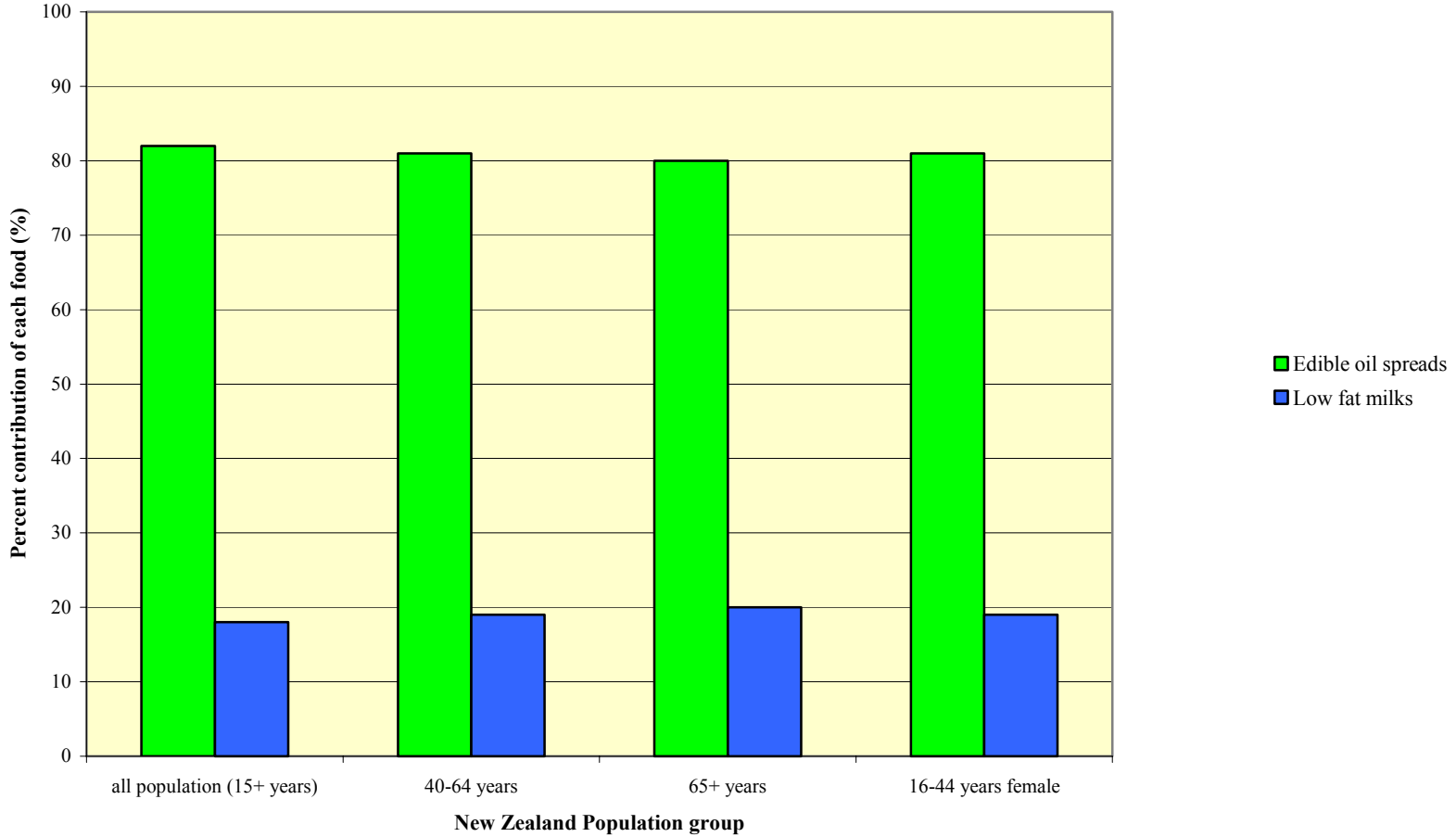


Figure 6: Percent contribution of each food group to free phytosterols dietary exposure for New Zealand



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Annex 1

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

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

Country	Age group	*Major contributing foods and percent of total free phytosterols exposures*
Australia	Whole population (2+ years)	Edible oils and margarines (81%) Low fat milks (19%)
	40- 64 years	Edible oils and margarines (78%) Low and milks (22%)
	65+ years	Edible oils and margarines (80%) Low fat milks (20%)
	2 - 12 years	Edible oils and margarines (84%) Low fat milks (16%)
	16 – 44 years female	Edible oils and margarines (79%) Low fat milks (21%)
New Zealand	Whole population (15+ years)	Edible oils and margarines (82%) Low fat milks (18%)
	40- 64 years	Edible oils and margarines (81%) Low fat milks (19%)
	65+ years	Edible oils and margarines (80%) Low fat milks (20%)
	16-44 years female	Edible oils and margarines (81%) Low fat milks (19%)

Attachment 5

NUTRITION ASSESSMENT REPORT

For Application A433 Phytosterol esters in breakfast cereal bars
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.

The conclusions from this report are directly applicable to Application A508 in which the Applicant applied for permissions to use phytosterols from a tall oil source in low-fat milks, since the individual components of phytosterol esters overlap to some extent with the components of tall oil phytosterols (TOPs). It is proposed that each milk product will contain 0.9g of TOPs/250 mL serving size. The proposed dose levels for TOPs fall within the range of doses that were used in the studies evaluated below.

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 established clearly for α-tocopherol and vitamin C (Handbook of Antioxidants, Eds. E. Cadenzas and L. Packer, 2002). Many hundreds of compounds present in food may act as potential 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 are 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, silverbeet, 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[11] 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 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.

[11] 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[12] 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 this epidemiological evidence regarding the benefits of fruit 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).

[12] 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. Phytosterol ingestion has not been shown to have an impact on plasma vitamin E levels.

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[13].

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.

[13] www.nzgg.org.nz

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). 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 (Attachment 2).

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 10.7 g/day phytosterol esters (equates to 6.6g/day of free phytosterols). 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

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 10.7 g/day phytosterol esters.
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 1.6 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, only β-carotene levels were significantly decreased (by approximately 10%) by milk providing 1.6 g/day phytosterol esters (Table 3).

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 allows 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 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 phytosterol in bread, cereal and margarine did not reduce adjusted plasma β-carotene any more than 1.6 g/day of phytosterol 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 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.

3.2.5 Nutritional issues

The results from both CSIRO studies provide evidence that the effects of free phytosterol consumption up to 6.6 g/day has 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, 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 some fat-soluble nutrients, most significantly β-carotene, with consumption of phytosterol-enriched foods will result in adverse health outcomes. Epidemiological studies show that fruit and vegetable consumption is inversely associated with cardiovascular disease and some cancers (e.g. 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. 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 potential 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.

3.3 Published studies

Table 5 and 6 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 phytosterols up to 3 g/day by mildly hypercholesterolaemic 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.

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 hypercholesterolaemic 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 hypercholesterolaemic 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 (e.g. 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-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-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 2.

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.

Summary – Nutritional effects of phytosterols

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 nutritional effects were examined in further studies, reductions in α- and β- carotene, lycopene, lutein and cryptoxanthin were observed, while vitamin E and vitamin A levels 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 secondary 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 higher levels (up to approximately 9 g/day) is not associated with adverse effects arising from a reduction in some carotenoids. However, the effects of free phytosterol consumption above 4.3 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. Furthermore, as there is no additional cholesterol lowering effect with increased phytosterol intake above approximately 3 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.

International reviews on the nutritional aspects of phytosterols in foods

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).

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 cholestryamine). Therefore, based on currently available information, there is no evidence that decreased levels of β-carotene are associated with increased health risks.

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

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 MUFF 32 g 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 hypercholesterolaemic	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 hypercholesterolaemic 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	hypercholesterolaemic 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

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

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

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/70 kg 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 6

Summary of Submissions received

Submitter	Comment
Australian Food and Grocery Council	The AFGC considers that FSANZ has erred in not making a decision at the time of initial assessment to determine whether the application warrants a variation to a regulatory measure. AFGC disputes that efficacy in relation to labelling statements has anything to do with a safety assessment under Standard 1.5.1-Novel Foods as there is no requirement for provision of efficacy data. AFGC supports that an assessment should be undertaken on the potential nutritional effects of phytosterols. However, they consider that there are options that could be exercised that could adequately protect consumers if adverse effects were found following the assessment. AFGC considers that conditions of use are an essential part of fulfilling the requirements of being a novel food. AFGC supports the option to approve the general use of TOPs as its first preference, followed by approval in all milks and then approval in low-fat and no-fat milk products.
New Zealand Food safety Authority (NZFSA)	NZFSA advocates a cautious approach when assessing applications to extend the use of phytosterols in foods and agree that the issues identified at Initial Assessment should be fully evaluated. Concerned that widening of permissions may lead to over consumption of phytosterols without any additional cholesterol-lowering benefits, yet may affect absorption of some fat-soluble vitamins and carotenoids. NZFSA considers that the product categories should be decided at the outset, rather than approving an increasing number of product categories on a case-by-case basis. Product labels should state the recommended number of serves/day that would provide a benefit and that consumption above recommended servings would not provide additional cholesterol lowering effects.
Dietitians Association of Australia (DAA)	Believes there are some benefits in broadening the range of foods that contain phytosterols. However, the potential for non-target groups (such as children, pregnant and lactating women and people with normal cholesterol levels) to increase their intake of phytosterols is a concern that needs addressing. Supports mandatory advisory statements that are clearly legible and visible to consumers. DAA is unable to support any of the current proposed options without reviewing the safety, efficacy data and dietary exposure data.

Valarie James	A 508 should be rejected because the Applicant has not provided evidence of any benefits for making available a wider choice of phytosterol-containing products.
Richard James	A508 should be rejected and not reconsidered until the applicant proves from controlled, monitored and peer-reviewed studies that its product is safe for adding to foods and beverages.
Food Technology Association of Victoria Inc	Support the application to amend the Code to permit the use of TOPs in low-fat and no-fat milk. Several issues are required to be addressed: • The overall phytosterol issue should be addressed at one time rather than by several different applications which would allow an assessment of the impact on the total diet • The editorial note in Standard 1.5.1 and Clause 2 (1) of Standard 2.4.2 require review and revision as it is unclear what the situation is with the use of phytosterols and phytosterol esters in the same products • The proposed labelling statements from the Applicant require careful consideration as they currently imply a pharmaceutical dose statement. • Option 3 to approve phytosterols in any food to a maximum permitted level is too general at this stage.
Sanitarium Health Food Company	Sanitarium is unable to support any of the options outlined in A508 as inadequate information is available at this stage regarding the safety of higher intakes of phytosterols from a wider variety of sterol fortified foods.
Nestle Australia Ltd	Support option 3 and approve the general use of tall oil phytosterols in a range of foods. FSANZ needs to use previous information on the costing study following the introduction of mandatory nutrition labelling to determine the impact on target groups consumers.
Unilever Australasia	Support option 2 and also the comments from the AFGC. Additional comments: • Request that a level of 1g of TOPs per serving/food group be considered as a more adequate level to allow consumers to meet the recommended levels of 2-3g/day. • Suggest that consideration be given to a common labelling format for multiple foods containing phytosterols. • Request consideration of specifications accepted by other regulatory agencies to be included as part of this application and also reviewing the specifications to include both the free sterol and ester forms. Question the need for minimum limits of sterols.
Dairy Australia	Support the use of TOPs as ingredients in low-fat and no-fat milk provided that careful consideration is given to labelling and usage of foods containing TOPs. Did not support option 3 as this may lead to over consumption by inappropriate groups and may encourage consumption of diets incompatible with Australian Dietary Guidelines.

Environmental Health Unit, Queensland Health	Does not support the option to permit TOPs in low-fat and no-fat milk. Raised the following concerns: • The long-term effects are unknown; • Raises concerns over the use of food as medicines; • The cost of phytosterol-containing foods is high and raises issues of inequity for some consumers (e.g. from lower socio-economic groups); • Phytosterols have oestrogenic activities; • Sufferers of homozygous sitosterolaemia need to restrict their intakes of plant sterols; • The safety of use of these products by non-target consumers needs further consideration.
New Zealand Dietetic Association	Does not support the application due to insufficient knowledge on the safety of higher doses and long-term exposure to phytosterols in foods. This is particularly important in vulnerable groups such as children and pregnant women.
Rosemary Stanton	Does not support the option to permit TOPs in low-fat and no-fat milk. Summary of concern: • loss of fat soluble vitamins, carotenoids and other fat soluble phytochemicals which may be protective against cancers; • lack of research on hundreds of other carotenoids; • lack of need for more phytosterol products and the presumption that they should be given precedence over other health considerations; • ease of over-consumption if more permission are granted; • unlikely restriction by consumers to 2 serves/day; • lack of evidence that consumers will consume extra fruit and/or vegetables to make up for the loss of carotenoids.