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DRAFT RISK ANALYSIS REPORT

APPLICATION A410

PHYTOSTEROL ESTERS DERIVED FROM VEGETABLE OILS

Note:

This report is the "Full Assessment" as referred to in Section 15 of the *Australia New Zealand Food Authority Act (1991)*.

Public comments are now sought before completion of a Final Risk Analysis Report (referred to as the 'Inquiry' under Section 16 of the Act). See under 'Invitation for Public Submissions' for details.

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DRAFT RISK ANALYSIS REPORT

A410 – PHYTOSTEROL ESTERS DERIVED FROM VEGETABLE OILS

EXECUTIVE SUMMARY

Background

An application was received from Unilever Foods on 14 March 2000 to amend the Food Standards Code to approve the use of phytosterol esters derived from vegetable oils as novel food ingredients under Standard A19 – Novel Foods. Phytosterol esters are prepared by the reaction of phytosterols with fatty acid methyl esters or free fatty acids. The free phytosterols are structurally related to cholesterol and occur naturally at low levels (up to 0.9%) in common vegetable oils. Phytosterol esters are considered to be novel food ingredients because they do not have a history of significant human consumption by the broad community in Australia and New Zealand at the proposed levels of dietary exposure.

Phytosterol esters, when incorporated into the table spreads at levels of 13.7% are reported to be an effective way of lowering total and LDL-cholesterol levels in the blood.

Issues addressed

Current and proposed use

At the time of gazettal of the Novel Food Standard (December 1999), phytosterol esters were being used in two brands of table spreads, one marketed by Unilever and one by Goodman-Fielder. Phytosterol esters can continue to be used in foods until clause 2 of Standard A19, which deals with the prohibition of the sale of novel foods, comes into force on 16 June 2001. By October 2000, there was also a phytosterol ester-containing mayonnaise product, a coleslaw dressing product and a breakfast bar product being marketed by Goodman-Fielder. In the future, Goodman-Fielder has indicated they wish to market a phytosterol ester-containing white, fibre-increased bread product and a mixed flake breakfast cereal product. Dairy Farmers have expressed an interest in marketing phytosterol ester-containing low fat yoghurt product, a low fat soy beverage product, and a low and reduced fat cheese product. Arnott's Biscuits/Campbell's Soups have indicated an interest in marketing phytosterol ester-containing biscuit and soup products.

Safety evaluation

The safety of phytosterol esters has been examined in studies in experimental animals as well as in humans. The studies in animals indicate that these substances are poorly absorbed from the gastrointestinal tract, have low toxicity, are not genotoxic, and have no effect on reproductive parameters. Excretion is via the faeces both as free phytosterol and phytosterol esters. The human studies did not provide any evidence of adverse effects associated with consumption of table spreads containing 13.7% (w/w) phytosterol esters at dietary intakes of 3.3 g/day for 3.5 weeks and 1.6 g/day for one-year (calculated as free phytosterols). There is no long-term data to demonstrate the safety of phytosterol esters at higher levels of exposure. There is also evidence from these studies that the plasma levels of carotenoids, which have antioxidant activity, are reduced although the levels are still considered to be within normal variation. This includes β-carotene, which is a precursor for vitamin A. While not a concern

per se at these levels of exposure , there is no data on the effect on carotenoid levels at higher phytosterol exposure levels.

Estimated dietary exposure

Dietary modelling was conducted on the existing and proposed uses of phytosterol esters in various foods. The results showed that the level of exposure in the 3.5-week and one-year human studies was approximately the estimated intake for mean consumption consumers and 95th percentile consumption consumers of table spread enriched with phytosterol esters at 13.7% (w/w). Consumption of other foods containing phytosterol esters in addition to table spreads would lead to higher levels of dietary exposure. The use of phytosterol esters in some foods such as cereals, biscuits and breads may lead to higher levels of intake than necessary to achieve the cholesterol-lowering effect in the target groups, since the intake of these foods is not as self-limiting as the intake of table spread. Use of phytosterol esters in these foods may also provide a greater potential for intake in the non-target groups.

Effect on cholesterol absorption

The effectiveness of phytosterol esters to reduce blood cholesterol has not been specifically assessed as part of this application. However, the human studies that have been examined do indicate that plasma cholesterol was reduced by approximately 5% and LDL-cholesterol by 7-8% over a one-year period by the consumption of table spread containing 13.7% (w/w) phytosterol esters. No studies have been conducted using other foods containing phytosterol esters. Given that phytosterol esters exert their effect in the gut, the food matrix may be critical to their efficacy, and therefore should be tested before any labelling statements are used which imply a beneficial effect of phytosterol esters in foods other than table spreads.

Advice to consumers

With phytosterol ester-enriched foods, there is a clear intention to market the reported beneficial effects of phytosterol esters on blood cholesterol and therefore it is important that these foods be consumed as part of a healthy diet that is low in saturated fats. A mandatory advisory statement to this effect is proposed. There is also a need to protect at-risk groups in relation to any potential reduction in plasma carotenoid levels as a result of phytosterol ester intake. These at-risk groups are children, pregnant and lactating women. A mandatory advisory statement indicating that phytosterol ester-enriched foods are unsuitable for these groups is also proposed. The use of phytosterol ester-containing table spreads should not be considered a substitute for cholesterol-lowering medication and a mandatory advisory statement is proposed that will indicate the need for individuals on such medication to seek medical advice regarding the use of the product. In relation to the ability of phytosterol esters to reduce cholesterol absorption, this will be considered in the light of the current review of the framework for health claims.

Risk assessment

Overall, the data support the safety of phytosterol esters at the level of intake which would be achieved by their addition to table spreads at 13.7% (w/w), but there is insufficient data to demonstrate their safety at the higher levels of intake which could occur from their use in a broader range of foods.

Risk management

Given the limited data on the potential long-term effects of phytosterol esters on clinical pathology parameters and specifically on plasma carotenoid levels at exposure levels higher than 1.6 g free phytosterols/day, dietary exposure should be restricted to the level which would be obtained from the consumption of table spreads containing 13.7% (w/w) phytosterol esters. Phytosterol ester preparations must also comply with the established specifications. This approach is consistent with the conclusions of the regulatory impact assessment.

In order to ensure that phytosterol ester-enriched table spreads are used appropriately by consumers, the following mandatory advisory statements should be used:

- *A statement to the effect that the product should be consumed in moderation as part of a diet low in saturated fats.*

- *A statement to the effect that the product is unsuitable for infants, children, and pregnant or lactating women.*

- *A statement to the effect that consumers already on cholesterol-lowering medication should seek medical advice about using the product.*

Conclusions

- There are no public health and safety concerns associated with the use of phytosterol esters in table spreads at a maximum concentration of 13.7% (w/w). The available data is insufficient to assess the safety of phytosterol esters at higher levels of exposure.

- There is some evidence from the available data that phytosterol esters when incorporated into a table spread at 13.7% (w/w) can reduce the level of plasma cholesterol. There is no data available in relation to their effectiveness in this regard when present in other foods.

- Permission for use should be limited to table spreads at a maximum concentration of 13.7% (w/w) phytosterol esters. Mandatory advisory statements are required to ensure that consumers use phytosterol ester-enriched table spreads appropriately.

- The proposed changes to the Food Standards Code are consistent with the section 10 objectives of the *Australia New Zealand Food Authority Act* 1991 and the regulatory impact assessment.

INVITATION FOR PUBLIC SUBMISSIONS

The Authority has completed a Draft Risk Analysis Report on this application (referred to as the 'Full Assessment' in section 15 of the Act), which includes a draft Safety Assessment Report and draft variation to the Australian *Food Standards Code.* The Authority now seeks public comment on the draft Safety Assessment Report, the draft variation to the *Food Standards Code,* and the Regulatory Impact Assessment before preparing a Final Risk Analysis Report (referred to as the 'Inquiry' under section 16 of the Act).

Written submissions containing technical or other relevant information that will assist the Authority in preparing the Final Risk Analysis Report for this application are invited from interested individuals and organizations. Technical information presented should be in sufficient detail to allow independent scientific assessment.

Submissions providing more general comment and opinion are also invited. The Authority's policy on the management of submissions is available from the Standards Liaison Officer upon request.

The processes of the Authority are open to public scrutiny, and any submissions received will ordinarily be placed on the public register of the Authority and made available for inspection. If you wish any information contained in a submission to remain confidential to the Authority, you should clearly identify the sensitive information and provide justification for treating it as commercial-in-confidence. The *Australia New Zealand Food Authority Act 1991* requires the Authority 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.

All correspondence and submissions on this matter should be addressed to the **Project Manager - Application A410** at one of the following addresses:

Australia New Zealand Food Authority

PO Box 7186	PO Box 10559
Canberra Mail Centre ACT 2610	The Terrace WELLINGTON 6036
AUSTRALIA	NEW ZEALAND
Tel (02) 6271 2222 Fax (02) 6271 2278	Tel (04) 473 9942 Fax (04) 473 9855
Email info@anzfa.gov.au	Email nz.reception@anzfa.gov.au

Submissions should be received by the Authority **by 10 January 2001**
General queries on this matter and other Authority business can be directed to the Standards Liaison Officer at the above address or by Email on slo@anzfa.gov.au. Submissions should not be sent by email as the Authority cannot guarantee receipt. Requests for more general information on the Authority can be directed to the Information Officer at the above addresses.

INTRODUCTION

The Australia New Zealand Food Authority (ANZFA) is a joint statutory body responsible for making recommendations on food standards which, when approved by the Australia New Zealand Food Standards Council (ANZFSC), are adopted by reference and without amendment into food laws of the Australian States and Territories. In the near future, there will be a joint Australia New Zealand Food Standards Code that will apply in both countries. In the interim, for the majority of the food standards, there is a system of dual standards operating. In the case of Standard A19 – Novel Foods, this Standard has not yet been declared as a mandatory standard in New Zealand and therefore will only operate in New Zealand as part of the joint Code.

Standard A19 - Novel Foods – came into effect on 16 December 1999[1]. The standard prohibits the sale of novel foods or novel food ingredients unless they are listed in the Table to clause 2 and comply with any special conditions stipulated in that Table.

The purpose of the Standard is to ensure that an assessment will be undertaken under the *Australia New Zealand Food Authority Act* (1991) for those non-traditional foods for which, in the words of the Standard, there is 'insufficient knowledge in the broad community to enable safe use'. Because the Standard has a definition of a novel food that is based on the level of knowledge about the safe use of a food in the community, a preliminary assessment of this level of knowledge for a particular non-traditional food is needed in order to assess whether an application under the Standard is necessary. The Standard provides some assistance in this regard by indicating the factors to be taken into account in this decision-making process. Guidelines for assessing the novelty of a non-traditional food are provided in the ANZFA document *Guidelines for amending the Food Standards Code: Standard A19 – Novel Foods*, and a decision is made in consultation with the Senior Food Officers in each of the States, Territories and New Zealand.

BACKGROUND TO THE APPLICATION

General

Phytosterol esters as novel foods

Free phytosterols are structurally related to cholesterol and occur naturally at low levels (up to 0.9%) in common vegetable oils. Phytosterol esters are prepared by the reaction of phytosterols with fatty acid methyl esters or free fatty acids. At the time the Novel Food Standard was gazetted (December 1999), there were two brands of table spread on the market in Australia that contained phytosterol esters. In line with the then *Format for applying to amend the Food Standards Code: Standard A19 – Novel Foods*, the novelty of these food ingredients was considered by ANZFA in consultation with the Senior Food Officers (SFOs) in each of the States/Territories and New Zealand.

The outcome of this consultation was that phytosterol esters should be considered novel food ingredients because they do not have a history of significant human consumption by the

[1] Clause 2 of the Standard, which deals with the prohibition on the sale of novel foods, does not come into effect until 16 June 2001. This is to allow the identification of any novel foods currently on the market and to allow time for any applications related to these foods to be considered by ANZFA.

broad community in Australia or New Zealand at the proposed levels of dietary exposure. A letter indicating this information was forwarded to the manufacturers of these table spreads in December 1999.

Application to ANZFA

ANZFA subsequently received an application from Unilever Foods on 14 March 2000 to amend the Food Standards Code to include phytosterol esters in the Table to Clause 2 of Standard A19 – Novel Foods. The manufacturers claim that incorporation of phytosterol esters into the diet may be an effective way of lowering total and LDL cholesterol levels in the blood. The naturally occurring levels of free phytosterols in table spreads are 0.3-0.4%. Nutritional and/or health claims made by the applicant in relation to phytosterol esters will not be assessed as part of this application.

Current and proposed uses

Phytosterol esters can continue to be used in foods until clause 2 of Standard A19, which deals with the prohibition of the sale of novel foods, comes into force on 16 June 2001. By October 2000, there was also a phytosterol ester-containing mayonnaise, coleslaw dressing and breakfast bar being marketed by Goodman-Fielder. In the future, Goodman-Fielder has indicated they wish to market a phytosterol ester-containing white, fibre-increased bread and a mixed flake breakfast cereal.

Dairy Farmers have expressed an interest in marketing phytosterol ester-containing low fat yoghurt, low fat soy beverage, and low and reduced fat cheese.

Arnott's Biscuits/Campbell's Soups have indicated an interest in marketing phytosterol ester-containing biscuits and soups.

Assessment of the application

The focus of the assessment of novel foods is primarily related to safety although, as for all applications to change the Food Standards Code, all of the objectives identified in section 10 of the ANZFA Act must be considered, including (i) the provision of adequate information relating to food to enable consumers to make informed choices; and (ii) the prevention of misleading or deceptive conduct.

Approval for phytosterol ester use in other countries

USA

Vegetable oil phytosterol esters have 'generally recognized as safe' (GRAS) status as an ingredient in vegetable oil spreads in amounts not to exceed 20%.

Switzerland

A vegetable oil spread containing 8% phytosterols (13.7% phytosterol ester) is approved.

Brazil

A vegetable oil spread containing 8% phytosterols (13.7% phytosterol ester) is approved.

European Union

Phytosterol esters are currently being considered under the EU Novel Food regulations.

Public consultation

The Authority conducted a Preliminary Assessment on A410 – Phytosterol esters derived from vegetable oils – and public comments on the application were called for on 5 July 2000. A total of 13 submissions were received and are summarised in **Attachment 7**. The majority of the submissions are supportive of the application. The issues raised in these submissions relate mainly to safety and efficacy. The safety of phytosterol esters is considered below. The efficacy of phytosterol esters to reduce plasma cholesterol has not been specifically addressed but some issues in relation to this matter are discussed below. The request for an extension to the application by Goodman-Fielder is considered below.

Australia and New Zealand are members of the World Trade Organization (WTO) and are signatories to the agreements on the Application of Sanitary and Phytosanitary Measures (SPS Agreement) and on Technical Barriers to Trade (TBT Agreement) (for further details on WTO Agreements, see **Attachment 6**). In some circumstances, Australia and New Zealand have an obligation to notify the WTO of changes to food standards to enable other member countries of the WTO to make comments.

In the case of application A410, this application has not been notified to the WTO because permission to use phytosterol esters would not have a negative trade effect. There are no international standards in relation to phytosterol esters.

Extension of the application

Goodman-Fielder in their submission requested extension of the application to include the free phytosterols derived from vegetable oils as well as phytosterol esters. While there is evidence that the phytosterol esters are rapidly hydrolysed to free phytosterols in the gastrointestinal tract, there are outstanding questions regarding the use of free phytosterols in foods. Free phytosterols have very low solubility in both water and oil compared to the esterified phytosterols, and given the need for free phytosterols to be solubilized and incorporated into the bile salt micelles in the gut to be effective at an equivalent dose level, their efficacy in reducing cholesterol absorption needs to be demonstrated. Given the potential for misleading consumers regarding the purpose of using free phytosterol-enriched table spreads, and the potential to slow the progression of the current application until this matter is resolved, this request for an extension to the application was not agreed.

ISSUES ADDRESSED DURING ASSESSMENT

1. Safety of phytosterol esters and free phytosterols

A detailed report on the safety of phytosterol esters is provided at **Attachment 2**.

The animal studies available on free phytosterols and phytosterol esters indicate that these substances are poorly absorbed from the gastrointestinal tract, have low toxicity, are not genotoxic, and have no effect on reproductive parameters. There was also no evidence of oestrogenic activity in both *in vitro* and *in vivo* studies. Excretion is via the faeces both as free phytosterols and as phytosterol esters.

The available human studies provide evidence of reduced cholesterol absorption resulting in lower plasma cholesterol levels following intakes up to 3.3 g/day in the 3.5-week studies and at an intake level of 1.6 g/day in the 1-year study (calculated as free phytosterols). There is no evidence of adverse health effects in these studies, but the reduction in the levels of plasma β-carotene (approximately 10%), and possibly vitamin K, raises a concern, particularly for groups that may be at risk of vitamin deficiency such as children or lactating women. Carotenoids are important precursors for the synthesis of vitamin A. Carotenoids and other lipid-soluble micronutrients, such as vitamin E, play important physiological roles in systems such as vision, maintenance of differentiated epithelia, mucus secretion, reproduction, and antioxidant activity. Epidemiological studies have shown that diets poor in carotenoids and antioxidants may result in increased risk of some cancers. It is important, therefore, that an adequate and regular supply of these substances is maintained.

There is evidence that plasma levels of carotenoids can vary seasonally by up to 30% depending on the availability of fruit and vegetables (see Lux & Naidoo, 1994; Olmedilla *et al.* 1994; Saintot *et al*. 1995 Scott *et al*. 1996). The reductions seen in the 1-year human study are well within this variation and do not raise concerns *per se*. The available data, however, does not address the potential for β-carotene levels to be reduced further at high levels of phytosterol ester intake.

The applicant has indicated that the level of phytosterol esters in table spreads will be limited to 13.7% (w/w) and, therefore, exposure from this source is probably self-limiting. However, there is some potential risk of over-exposure if phytosterols are widely used in other foods, and also increased potential for exposure of non-target groups, eg, other family members.

Conclusion from the safety assessment report

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

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

3. The available data indicates that plasma cholesterol levels are reduced by approximately 5% and LDL cholesterol levels by 7-8% when phytosterol esters are consumed in table spreads at an intake equivalent to 1.6 g free phytosterols/day of over a 1-year period.

There is no data available in relation to their effectiveness in this regard when present in other foods.

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

2. Use of phytosterols and phytosterol esters in food products

A detailed report on the food technology aspects of this application is provided at **Attachment 3**.

Plant sterols are natural components of edible vegetable oils. Plant sterols have a role in cell membrane structures in plants. The most common plant sterols are β-sitosterol, campesterol and stigmasterol, all of which are structurally very similar to cholesterol. When edible oils undergo normal refining, plant sterols are partially extracted – it is estimated that 2500 tonnes of vegetable oil needs to be refined to produce 1 tonne of plant sterols.

Free phytosterols have only low solubility in oil and are esterified with fatty acids to increase their solubility in oils to 20%. Esterified phytosterols give a more uniform distribution in a table spread product. Phytosterols and their fatty acid esters are very stable compounds and undergo only minimal change during processing. Even under severe conditions, such as deep-frying, sterol oxidation products are only formed at ppm concentrations.

Specifications for phytosterol esters are provided in Attachment 3.

3. Potential dietary exposure to phytosterol esters

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

Dietary modelling was conducted to estimate the dietary intake of phytosterol esters as a result of current and projected uses. The dietary modelling was conducted for both Australian and New Zealand populations using DIAMOND, ANZFA's dietary modelling computer program. Dietary data were obtained from the Australian 1995 National Nutrition Survey (NNS), which surveyed 13,858 people aged from 2 years and above, and the New Zealand 1997 NNS, which surveyed 4,636 people aged 15 years and above. Both surveys used a 24-hour food recall methodology.

The target age group for these products has been specified as 25 years and above, but more so from 40 years and above. The likelihood that children may also consume these foods was also a concern. Therefore, modelling was conducted for all of the population as well as for the target age group of 40 years and above.

Additional modelling was performed for children (2-12 year olds, Australia only), teenagers (13-19 year olds for Australia, 15-19 years for New Zealand), and younger adults (20-39 years.

The dietary intakes for the mean consumer and the 95th percentile consumer have been determined for each of the foods in which it is proposed that phytosterol esters be added. Consumption of one of these foods alone is enough to reach the level of intake of phytosterols reported to reduce cholesterol intake. If more than one of these foods were consumed regularly, the intake of phytosterols would be higher than the intake level that has been shown to be safe.

The dietary modelling has been performed using the figures provided by the food industry. In all cases, the calculations have used the free phytosterol equivalent concentration, as provided by the industry. In some cases, it is not clear whether phytosterol esters or free phytosterols are proposed to be added to the product. The data indicates that consumption of table spreads containing phytosterol esters at 13.7% (w/w) would lead to a consumer mean free phytosterol intake of approximately 1.3 g/day in Australia and 1.0 g/day in New Zealand. The same spread would lead to a consumer 95th percentile free phytosterol intake of approximately 3.6 g/day in Australia and 2.8 g/day in New Zealand.

4. Nutritional and/or health claims associated with phytosterol esters

Health claims are currently prohibited under Standard A1 clause 19 of the Food Standards Code and therefore products containing phytosterol esters cannot make health claims, as defined under Standard A1. ANZFA has before it a proposal (P153) to review the current regulations in this area. The ANZFA Board has recently considered the Full Assessment Report on Proposal P153 –Review of Health and Related Claims – and this paper was available for public comment until 25 October 2000.

There is no evaluation of any health claim being considered as part of this application. Any application for a health claim for phytosterol esters in the future would need to be considered in the context of the proposed changes arising out of Proposal P153. Irrespective of whether any statement is considered a health claim, all statements on the label should be true and not mislead consumers.

5. Effect of phytosterols on absorption of cholesterol

The currently marketed food products that contain phytosterol esters carry statements on the label, such as: 'With natural plant sterols which reduce cholesterol uptake' or 'With plant-derived ingredients that lower cholesterol absorption'. Advertisements for these products have used the statement: 'Logical® will reduce your cholesterol absorption'. The manufacturers have indicated that all current and future products will continue to carry this or a similar message. Therefore, in assessing this application, there is a need to establish whether such a message might be considered to be inconsistent with the above objective.

The effectiveness of phytosterol esters incorporated into food products to reduce cholesterol absorption has not been specifically assessed as part of this application, although the human studies that have been examined do provide some information in this regard. Plasma cholesterol was reduced by approximately 5% and LDL-cholesterol by 7-8% over a one-year period by the incorporation of phytosterol esters into a table spread (see Attachment 2).

A more thorough examination of the evidence to substantiate a 'health claim' in relation to phytosterols will, however, be required under the proposed changes to the health claim regulations detailed in Proposal P153.

The human studies with phytosterol esters provided to date have been conducted using only phytosterol ester-enriched table spreads – no studies have been conducted using other foods containing phytosterol esters. For a compound that exerts its effect in the gut, the food matrix may be critical to its efficacy. Therefore, there is no justification for the proposed labelling statement in relation to phytosterols when administered in foods other than table spreads.

While it may be argued that the presence of phytosterol esters in any food will reduce cholesterol absorption, there are also reasons why this may not be the case:

- While the exact mechanism by which phytosterol esters inhibit cholesterol absorption is unknown (probably by reducing cholesterol solubilization in bile salt micelles), it is clear that the maximum effectiveness is realised when they are present in the intestine simultaneously with cholesterol;
- Phytosterol esters needs to be dispersed in the diet in a physical state that allow them to be optimally effective; and
- There is considerable individual variation in the effectiveness of phytosterol esters to reduce plasma cholesterol.

Thus, there are factors such as the time of consumption, the frequency of consumption and the nature of the food matrix that could influence the effectiveness of phytosterol esters to achieve the desired effect. While it may not be necessary to establish that phytosterol esters reduce cholesterol absorption when present in each of the foods proposed to be marketed, further studies to show the influence of both the food matrix and pattern of consumption of phytosterol esters on plasma cholesterol and LDL-cholesterol levels are warranted.

The above requirement is justified under the third objective of the Authority in developing food regulatory measures, namely, *the prevention of misleading or deceptive conduct.*

6. Labelling information for consumers

(1) Use of phytosterol esters as part of a healthy diet

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 phytosterol esters can lead to lower plasma cholesterol by reducing the absorption of cholesterol from the diet, phytosterol esters are ineffective in preventing the elevation of plasma cholesterol that is a consequence of the ingestion of saturated fatty acids.

Where phytosterol esters have been intentionally added at high enough levels to have an effect on cholesterol absorption, the following mandatory advisory statement is proposed:

> *A statement to the effect that the product should be consumed in moderation as part of a diet low in saturated fats.*

This requirement is justified under the third section 10 objective 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 Food Standards Code. It is also consist with the second section 10 objective of the Authority, namely, *the provision of adequate information relating to food to enable consumers to make informed choices.*

(2) Restriction for at-risk groups

As indicated in the safety assessment report, the groups in the population considered to be at risk regarding plasma carotenoid levels are children, pregnant and lactating women. A clear statement on the label should indicate that this novel food ingredient is inappropriate for these population groups.

The following mandatory advisory statement is proposed:

> *A statement to the effect that the product is unsuitable for infants, children, and pregnant or lactating women*

The above requirement is justified under the first objective of the Authority, namely, *the protection of public health and safety*.

Another at-risk group are 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) Use by individuals on cholesterol-lowering medication

While the use of phytosterol-enriched table 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 following mandatory advisory statement is proposed:

A statement to the effect that consumers already on cholesterol-lowering medication should seek medical advice about using the product.

The above requirement is justified under the first objective of the Authority, namely, *the protection of public health and safety*.

(4) Effect on plasma cholesterol levels

While there is some evidence from the data presented as part of the safety assessment that phytosterol esters when incorporated in table spreads can reduce plasma cholesterol levels, the ability of phytosterol esters generally to reduce cholesterol absorption and thus reduce lower plasma cholesterol has not been specifically addressed as part of this application.

Any labelling statements in relation to this aspect of the use of phytosterol esters will be considered in the light of the current review of the framework for health claims. If the regulations are changed in the future to allow health claims, a specific application in relation to phytosterol esters will be required.

RISK ANALYSIS

Risk assessment

The safety assessment has concluded that phytosterol esters are substances of low toxicity that are poorly absorbed and efficiently excreted via the faeces. The human studies conducted with phytosterol esters are of limited duration and scope, but provide no evidence of adverse health effects. There is, however, a dose-related decrease in the plasma levels of carotenoids as a result of exposure to phytosterol esters in a table spread preparation. While the decrease in carotenoid levels observed following exposure to table spreads containing 13.7% (w/w) phytosterol esters is well within the natural variation of carotenoid levels in humans, and not considered to be a concern *per se*, there is a paucity of data on the potential effect on plasma carotenoids at higher levels of phytosterol ester exposure.

The dietary modelling shows that the level of exposure in the human studies (1.6 g/day for one-year and 3.3 g/day for 3.5 weeks, calculated as free phytosterol) is approximately the estimated intake for mean consumption consumers and 95th percentile consumption consumers of table spread enriched with phytosterol esters at 13.7% (w/w). Consumption of other foods containing phytosterol esters in addition to table spreads containing phytosterol esters would lead to higher levels of dietary exposure.

Overall, while there is no evidence of adverse effects as a result of exposure to phytosterol esters when incorporated into table spreads at a level of 13.7% (w/w), there is insufficient data to demonstrate the safety of phytosterol esters at the higher levels of exposure which could occur if these compounds were available in a range of food products.

Risk management

Given the limited data on the potential long-term effects of phytosterol esters on clinical pathology parameters and specifically on plasma carotenoid levels at exposure levels higher than 1.6 g/day, dietary exposure should be restricted to the level which would be obtained from the consumption of table spreads or margarines containing 13.7% (w/w) phytosterol esters. Phytosterols esters added to these foods should also conform to the specifications indicated in Attachment 3.

In order to ensure that phytosterol ester-enriched table spread or margarines are used appropriately by consumers, the following mandatory advisory statements should be used:

- *A statement to the effect that the product should be consumed in moderation as part of a diet low in saturated fats.*

- *A statement to the effect that the product is unsuitable for infants, children, and pregnant or lactating women.*

- *A statement to the effect that consumers already on cholesterol-lowering medication should seek medical advice about using the product.*

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

REGULATORY IMPACT ANALYSIS

The Authority is required to consider the impact of various regulatory (and non-regulatory) options on all sectors of the community, which includes consumers, food industry and government in both Australia and New Zealand. The benefits and costs associated with the proposed amendment to the Food Standards Code have been analysed in a draft Regulatory Impact Assessment (Attachment 5). For the preferred option, namely, the approval of phytosterol esters in table spreads only; the benefits of the proposed amendment outweigh the costs.

CONCLUSIONS

- There are no public health and safety concerns associated with the use of phytosterol esters in table spreads at a maximum concentration of 13.7% (w/w). The available data is insufficient to assess the safety of phytosterol esters at higher levels of exposure.

- There is some evidence from the available data that phytosterol esters when incorporated into a table spread at 13.7% (w/w) can reduce the level of plasma cholesterol. There is no data available in relation to their effectiveness in this regard when present in other foods.

- Permission for use should be limited to table spreads at a maximum concentration of 13.7% (w/w) phytosterol esters. Mandatory advisory statements are required to ensure that consumers use phytosterol ester-enriched table spreads appropriately.

- The proposed changes to the Food Standards Code are consistent with the section 10 objectives of the *Australia New Zealand Food Authority Act* 1991 and the regulatory impact assessment.

ATTACHMENTS

1. Draft variation to the *Food Standards Code*
2. Safety assessment report
3. Food technology report
4. Dietary exposure assessment report
5. Draft regulatory impact assessment
6. World Trade Organization Agreements
7. Summary of public submissions

ATTACHMENT 1

DRAFT VARIATION TO THE FOOD STANDARDS CODE

A410 – PHYTOSTEROL ESTERS DERIVED FROM VEGETABLE OILS

To commence: On gazettal

The Food Standards Code and Australia New Zealand Food Standards Code are varied by -

(1) *deleting the Table to clause 2 in Standard **A19** of the Food Standards Code, substituting –*

Table to clause 2

Column 1 **Novel Food**	**Column 2** **Conditions of Use**
phytosterol esters	May only be added to food **-** (1) according to Standards G2 or G5, and Standard A11; and (2) where the total fatty acid present in the food is not more than 200g/kg of saturated fatty acids. The name 'phytosterol ester' must be used when declaring the ingredient in the ingredient list, as prescribed in clause 5 of Standard A1. The label on or attached to a package of food containing phytosterol esters must include statements to the effect that - 1. that the product should be consumed in moderation as part of a diet low in saturated fats; 2. the product is unsuitable for infants, children, and pregnant or lactating women; and 3. consumers already on cholesterol-lowering medication should seek medical advice about using the product.

(2) *inserting immediately after ADDENDUM 7* ***in Standard A11*** *of the Food Standards Code, the following –*

ADDENDUM 8

SPECIFICATION FOR PHYTOSTEROL ESTERS DERIVED FROM VEGETABLE OILS

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

Phytosterol esters + free phytosterols (%)	min.	94
Free phytosterols (%)	max.	10
Steradienes (%)	max.	0.3
Fatty acid methylester (%)	max.	0.5
Iron, Fe (ppm)	max.	1.0
Copper, Cu (ppm)	max.	0.5
Moisture (%)	max.	0.1
Trans fatty acids (%)	max.	1.0

Sterol profile (%) as below:

Cholesterol	min.	0.0	max.	2.0
Brassicasterol	min.	0.0	max.	6.0
Campesterol	min.	20.0	max.	29.0
Campestanol	min.	0.0	max.	6.0
Stigmasterol	min.	12.0	max.	23.0
β-Sitosterol	min.	42.0	max.	55.0
β-Sitostanol	min.	0.0	max.	2.5
D5-Avenasterol	min.	0.0	max.	4.0
D7-Stigmastenol	min.	0.0	max.	2.0
D7-Avenasterol	min.	0.0	max.	2.0
Other	min.	0.0	max.	6.0

(3) *inserting immediately after subparagraph (1)(b)(ii)(i) in* ***Standard G2*** *of the Food Standards Code, the following -*

(j) not more than 137 g/kg of phytosterol esters.

(4) *inserting immediately after paragraph 2(3)(n) in* ***Standard G2*** *of the Food Standards Code, the following - –*

(o) not more than 137 g/kg of phytosterol esters.

(5) *inserting into Columns 1 and 2 respectively of the Table to clause 2 in* ***Standard 1.2.3*** *of the Australia New Zealand Food Standards Code, the following –*

Food regulated in Standard 2.4.2 containing phytosterol esters	Statements to the effect that - 1. that the product should be consumed in moderation as part of a diet low in saturated fats; 2. the product is unsuitable for infants, children, and pregnant or lactating women; and 3. consumers already on cholesterol-lowering medication should seek medical advice about using the product.

(6) *deleting the Table to clause 2 in* ***Standard 1.5.1*** *of the Australia New Zealand Food Standards Code, substituting –*

Table to clause 2

Column 1 **Novel Food**	**Column 2** **Conditions of Use**
phytosterol esters	The requirements in clause 2 of Standard 1.2.3. The name 'phytosterol ester' must be used when declaring the ingredient in the ingredient list, as regulated by 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.

(7) *inserting immediately after Item 8 (testing Requirements for Nucleotides) in* ***Standard 1.3.4*** *of the Australia New Zealand Food Standards Code, the following –*

SPECIFICATION FOR PHYTOSTEROL ESTERS DERIVED FROM VEGETABLE OILS

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

Phytosterol esters + free phytosterols (%)	min.	94
Free phytosterols (%)	max.	10
Steradienes (%)	max.	0.3
Fatty acid methylester (%)	max.	0.5
Iron, Fe (ppm)	max.	1.0
Copper, Cu (ppm)	max.	0.5
Moisture (%)	max.	0.1
Trans fatty acids (%)	max.	1.0

Sterol profile (%) as below:

Cholesterol	min.	0.0	max.	2.0
Brassicasterol	min.	0.0	max.	6.0
Campesterol	min.	20.0	max.	29.0
Campestanol	min.	0.0	max.	6.0
Stigmasterol	min.	12.0	max.	23.0
β-Sitosterol	min.	42.0	max.	55.0
β-Sitostanol	min.	0.0	max.	2.5
D5-Avenasterol	min.	0.0	max.	4.0
D7-Stigmastenol	min.	0.0	max.	2.0
D7-Avenasterol	min.	0.0	max.	2.0
Other	min.	0.0	max.	6.0

(8) *inserting immediately after paragraph 2(1)(f) in* ***Standard 2.4.2*** *of the Australia New Zealand Food Standards Code, the following –*

(g) no more than 137g/kg of phytosterol esters.

ATTACHMENT 2

SAFETY ASSESSMENT REPORT

A410 – PHYTOSTEROL ESTERS DERIVED FROM VEGETABLE OILS

SUMMARY

Phytosterol-esters are a novel food ingredient currently being used in a vegetable oil spread at a level 13.7% (w/w). This is equivalent to 8% (w/w) free phytosterols. Phytosterols are naturally occurring plant compounds that have been reported to reduce blood cholesterol levels by inhibiting the absorption of dietary cholesterol. Phytosterols are naturally found in common vegetable oils and spreads at a level of 0.1 – 0.9% (w/w).

Absorption, Distribution, Metabolism and Excretion

The major sterols and sterol esters in the proposed phytosterol ester product were tested in rats *in vivo* to compare their uptake, tissue distribution, metabolism and excretion with those of cholesterol and cholesterol esters. 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.

Acute Studies

No acute study data were submitted.

Short-term Studies

A short-term (14-day) palatability study in rats was submitted. In this study, plant sterols at up to 5% w/w in diet were well tolerated by rats with no reduction in growth rates.

Sub-chronic Studies

A 13-week dietary toxicity study in rats treated with up to 5% free sterols (representing final doses in males and females up to 3.9 and 4.2 g/kg bodyweight/day, respectively). There were no significant clinical findings, nor treatment related effects on clinical chemistry, haematology, or macroscopic and histological assessment of organs and tissues at the highest dose levels tested.

Chronic Studies

No chronic studies or carcinogenicity studies were submitted.

Reproduction Studies

A 2 generation reproduction study in rats dosed with up to 5% phytosterol in 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.

Developmental Studies

No developmental studies were submitted, although the detailed clinical examination of F_1 and F_2 litters in the preceding 2 generation feeding study in rats would suggest little potential for birth defects with phytosterols.

Genotoxicity

Phytosterols and their esters were found to be negative in a battery of bacterial and mammalian genotoxicity test systems at doses *in vivo* up to 2 g/kg and concentrations *in vitro* up to 200 μg/ml. These suggest that phytosterols and phytosterol esters are non-genotoxic both with and without metabolic activation).

Other Studies

In vitro oestrogenic potential

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. These data confirm the *in vivo* reproductive toxicity assays finding no uterotrophic activity of phytosterols.

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.

Human Studies

In a series of human studies, both cholesterol-lowering properties and physiological effects of phytosterol esters were examined in normal healthy individuals following dietary exposure through enriched vegetable oil-based spreads. Four studies were performed with dietary

intake of 25, 25, 40 or 20 g/day of phytosterol ester-enriched spreads for 3.5, 3.5, 3-4 or 52 weeks, respectively.

Equivalent sterol dose levels were 1.7 - 3.2 g free sterol/day (3.5 week study), 0.9 - 3.3 g free sterol/day (3.5 week study), 8.6 g free sterol/day (males - 3 weeks, females - 4 week study), and 1.6g free sterol/day (52 week study). Studies were double-blind placebo-controlled designs. None of these studies found any significant adverse effects in relation to clinical observations, clinical chemistry, haematology, urinalysis or gut flora parameters.

Mean total faecal bile acid excretion, total secondary bile acids and lithocholic acid excretion were slightly but statistically significantly reduced. While some forms of colon cancer are associated with increased bile acid excretion, decreased bile excretion is not considered an adverse outcome.

The available human studies provide evidence that daily intake of 1.6 g plant sterols/day reduces total plasma cholesterol and LDL plasma cholesterol by up to 5 and 8% respectively. Dietary uptake of some carotenoids (α and β-carotenes and lycopene) was also significantly reduced with phytosterol intake (by up to 22% with soybean oil enriched spread). Blood levels of vitamin K1 were also reduced in those consuming phytosterol-enriched spread although this did not reach statistical significance, whereas vitamin E levels were slightly (3%) depressed and this was statistically significant. This was apparent in both short (3 - 4 week) and long-term (52 week) studies, although in neither case were these effects associated with any clinical evidence of vitamin deficiency.

Discussion

The animal studies available on phytosterols and phytosterol esters indicate that these substances are poorly absorbed from the gastrointestinal tract, have low toxicity, are not genotoxic, and have no effect on reproductive parameters. There was also no evidence of oestrogenic activity in both *in vitro* and *in vivo* studies. Excretion is via the faeces both as free phytosterols and as phytosterol esters.

The available human studies provide evidence of reduced cholesterol absorption resulting in lower plasma cholesterol levels following intakes up to 3.3 g/day in the 3.5-week studies and at an intake level of 1.6 g/day in the 1-year study (calculated as free phytosterols). There is no evidence of adverse health effects in these studies, but the reduction in the levels of plasma β-carotene (approximately 10%), and possibly vitamin K, raises a concern, particularly for groups that may be at risk of vitamin deficiency such as children or lactating women. Carotenoids are important precursors for the synthesis of vitamin A. Carotenoids and other lipid-soluble micronutrients, such as vitamin E, play important physiological roles in systems such as vision, maintenance of differentiated epithelia, mucus secretion, reproduction, and antioxidant activity. Epidemiological studies have shown that diets poor in carotenoids and antioxidants may result in increased risk of some cancers. It is important, therefore, that an adequate and regular supply of these substances is maintained.

There is evidence that plasma levels of carotenoids can vary seasonally by up to 30% depending on the availability of fruit and vegetables (see Lux & Naidoo, 1994; Olmedilla *et al.* 1994; Saintot *et al*. 1995 Scott *et al*. 1996). The reductions seen in the 1-year human study are well within this variation and do not raise concerns *per se*. The available data, however, does not address the potential for β-carotene levels to be reduced further at high levels of phytosterol ester intake.

The applicant has indicated that the level of phytosterol esters in table spreads will be limited to 13.7% (w/w) and, therefore, exposure from this source is probably self-limiting. However, there is some potential risk of over-exposure if phytosterols are widely used in other foods, and also increased potential for exposure of non-target groups, eg, other family members.

Conclusions

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

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

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

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

PHYTOSTEROL-ESTERS
A NOVEL FOOD INGREDIENT

INTRODUCTION

Phytosterol esters are manufactured from edible vegetable oils by a process of refining, purification, and re-esterification with fatty acids using methods and techniques commonly used in the food manufacturing industry. Phytosterol esters are currently being used in vegetable oil spreads, a salad oil product, a mayonnaise product and a breakfast bar. In the vegetable oil spread, the phytosterol esters are present at 13.7% (w/w), which represents 8% free phytosterols, compared with 0.1 – 0.9% in common vegetable oils and spreads. Phytosterols are naturally occurring plant compounds that have been reported to reduce blood cholesterol levels by inhibiting the absorption of dietary cholesterol. An intake of 1.6 g phytosterols per day is reported to reduce blood cholesterol by 10%. Food spreads and vegetable oils containing phytosterol esters are currently approved and available in the USA and Switzerland, and other products based on phytostanol esters are marketed in the USA and European Union.

ABSORPTION, DISTRIBUTION, METABOLISM AND EXCRETION

The fate in the male rat of [14C]β-sitosterol and [14C] β-sitosterol linolate following gavage administration. Minter, H. and Sanders, D. (1997**)** Unilever Research, Bedford, England. Study AM960460. July 1997.

Test material: [14C]β-sitosterol (Amersham International, ≥96.3%; 134 uCi/mg), and [14C]β-sitosterol linoleate (synthesized by Unilever, >99.9%; 13.14 uCi/mg).

Test Species:	Male Charles River CD rat (Charles River UK Ltd, Margate), 146 –172 g, 10 per test material, administration orally by cannula
Dose:	[^{14}C]β-sitosterol: mean 0.56 mg/kg (at approx 77 uCi/kg); [^{14}C]β-sitosterol linoleate: 5.86 mg/kg (at approx 65 uCi/kg).
GLP:	Environmental Safety Laboratory, Unilever, policy on GLP

Study conduct

Ten male rats were each treated with a single gavage dose of either [^{14}C]β-sitosterol and [^{14}C]β-sitosterol linoleate at approximately 0.6 and 6 mg/kg body weight respectively. Animals were housed in a climate-controlled facility for 4 days prior to and for up to 96 hours following administration. Metabolism cages were designed to permit the collection of urine, faeces and expired carbon dioxide. Diets were restricted for 24 hours prior to dosing and for 2 hours after, when *ad libitum* food was provided. Water was available *ad libitum* throughout the procedure. A single rat from each treatment group was sacrificed at 4, 8, 24, 48, 72 and 96 hours after dosing for analysis by whole body autoradiography. Radiolabelled CO_2 was analysed at 2, 4, 8 and 24 hours. At sacrifice and at 8, 24, 48, 72 and 96 hours urine and faeces were analysed for ^{14}C content. At 96 hours, remaining animals were sacrificed and samples of blood, stomach and intestine contents, selected organs and the remaining carcasses were analysed for ^{14}C.

Formulation stability

The [^{14}C]-labelled test materials were assessed by radio-HPLC. This showed that the test materials were both stable and of high radiochemical purity.

Analysis

Analysis in duplicate or triplicate was by liquid scintillation counting of tissue and sample extracts and CO_2 absorber. Faecal extracts were also analysed by TLC and radio-HPLC. Selected animals were analysed by whole body autoradiography.

Results

No animals displayed clinical signs of adverse effect during the study. Absorption of [^{14}C]β-sitosterol and [^{14}C]β-sitosterol linoleate after oral administration was low with approximately 93% of the administered dose excreted in faeces within 96 hours. A small proportion (less than 1%) was excreted in urine during this time. The total absorbed (the amount in all tissues, carcass and urine at 96 hours) was approximately 1.5% of the dose for both the sterol and its ester, and recovered [^{14}C] was absent or very low in all tissues evaluated. Although this under-represents the likely true absorbed dose, there was little evidence for significant biliary excretion of absorbed material from whole body autoradiography. No evidence was found of excretion of in expired air as [$^{14}CO_2$]. Autoradiography showed that levels of [^{14}C] from either test material were generally associated with the intestinal tract, with small amounts appearing in the liver, adrenals and other tissues from 4 hours, declining to background levels at 72 hours. The adrenal gland retained small amounts of radioactivity at 96 hours.

HPLC and TLC analysis of faecal extracts showed that both sitosterol and sitosterol linoleate were excreted in both free and esterified forms. That is, free sterol and esterified sterol products were seen with both materials administered. The identity of these products, although they co- chromatographed with authentic [^{14}C]β-sitosterol and [^{14}C]β-sitosterol linoleate, was not determined and they may have represented other trans-esterified products formed in the gut. There was insufficient evidence to suggest that these represented hepatic metabolites. A third, minor, unidentified metabolite was also detected in faecal samples.

[^{14}C]β-sitosterol and [^{14}C]β-sitosterol linoleate: the distribution and metabolism in the rat following gavage administration Sanders, D., Minter, H. and Dilley, S. (1997) Unilever Research, Bedford, England. Study AM970152. December 1997.

Test material:	[^{14}C]β-sitosterol (Amersham International, ≥96.3%; 134 uCi/mg), and [^{14}C]β-sitosterol linoleate (synthesised by Unilever, >99.9%; 13.14 uCi/mg)
Test Species:	Charles River CD rat (Charles River UK Ltd, Margate) 152 – 193 g, 11 per test material plus 2 coconut oil control, administration orally by cannula
Dose:	[^{14}C]β-sitosterol: mean 3 mg/kg (at approx 69 uCi/kg in experiment 1) and 50 mg/kg (approx 35 uCi/kg in experiment 2); [^{14}C]β-sitosterol linoleate: 5.7 mg/kg (at approx 65 uCi/kg in experiment 1) and 30 mg/kg (15 uCi/kg in experiment 1).
GLP:	UK GLP Regulations 1997/OECD GLP Guidelines

Study conduct

In experiment 1, 6 male and 6 female rats were each treated with a single gavage dose of either [^{14}C]β-sitosterol or [^{14}C]β-sitosterol linoleate at approximately 3 and 6 mg/kg body weight respectively. Doses were administered in sunflower oil vehicle. Animals were housed in a climate-controlled facility for 4 days prior to and for up to 96 hours following administration. Metabolism cages were designed to permit the collection of urine and faeces. Diets were restricted for 24 hours prior to dosing and for 2 hours after, when *ad libitum* food was provided. Water was available *ad libitum* throughout the procedure. A single rat from each sex and treatment group was sacrificed at 24, 48 and 96 hours after dosing for analysis by whole body autoradiography. At sacrifice and at 8, 24, 48, 72 and 96 hours urine samples were collected for ^{14}C content. At 24 hour intervals, faecal samples were also collected for analysis.

In experiment 2, groups of 2 male and 2 female rats were treated with a single gavage dose of either [^{14}C]β-sitosterol (approx. 55 mg/kg) or [^{14}C]β-sitosterol linoleate (approx. 30 mg/kg) in coconut oil. A further animal of each sex received coconut oil treatment as controls. Animals were housed in a climate-controlled facility for 4 days prior to and for up to 96 hours following administration. Metabolism cages were designed to permit the collection of urine and faeces. Diets were restructured for 24 hours prior to dosing and for 2 hours after, when *ad libitum* food was provided. Water was available *ad libitum* throughout the procedure. Animals were sacrificed at 24 hours after dosing for analysis for total ^{14}C content. At 8 and 24 hours urine and faeces were collected for ^{14}C content. At 96 hours, remaining animals were sacrificed and samples of blood, stomach and intestine contents, selected organs and the remaining carcasses were analysed for ^{14}C.

Formulation stability

The [^{14}C]-labelled test materials were assessed by radio-HPLC. This showed that the test materials were both stable and of high radiochemical purity.

Analysis

Analysis in duplicate or triplicate was by liquid scintillation counting of tissue and sample extracts. Faecal sample extracts were also analysed by TLC and radio-HPLC. Selected HPLC fractions were recovered and analysed by mass spectroscopy.

Results

No animals showed signs of ill effect in this study. In experiment 1, excretion of [^{14}C]β-sitosterol and [^{14}C]β-sitosterol linoleate after oral administration was predominantly (over 89%) via faeces within 96 hours, with over 70% of each test material in females and 80% in males excreted in the first 24 hours. A small proportion (0.2% and less than 0.1% in females and males respectively) was excreted in urine by 96 hours. Autoradiography at 24 hours showed that levels of ^{14}C from either test material were generally associated with the intestinal tract, with smaller amounts appearing in the adrenal gland and ovary, and less in the bone marrow, liver, intestinal lining and spleen. At 96 hours, ^{14}C had declined to near background levels in all tissues except the adrenal gland and ovary.

The proportion of sterol and sterol ester absorbed was not determined in this study, since all animals were examined by whole body radiography and carcass and tissue recoveries were not performed.

However, based on the urinary excretion data, and the small amounts of radioactivity appearing in tissues, it was considered that the absorption of sterol and sterol ester were consistent with those of the preceding study (approximately 5% of dose).

Chromatography of faecal extracts from experiment 1 showed that both sitosterol and sitosterol linoleate were excreted in both free and esterified forms. The identity of these products was not determined. The identity of a third minor metabolite in some faecal extracts was not determined. In experiment 2, there were free and esterified fatty acids present, although the degree of esterification of free sitosterol was greater with coconut oil dosing than with sunflower oil. This, together with chromatographic evidence of the fatty acid profiles in samples, suggested that trans-esterification of sterols occurred in the gut. The predominant sterols in faeces were β-sitosterol, campesterol and cholesterol.

The fate in the rat of [^{14}C]β-sitostanol and [^{14}C]β-sitosterol following gavage administration Sanders, D. and Minter, H. (1997) Unilever Research, Bedford, England. Study AM970054. October 1997.

Test material:	[^{14}C]β-sitostanol (synthesised by Unilever, >99%; 15 uCi/mg), and [^{14}C]β-sitosterol (Amersham International, ≥96.3%; 134 uCi/mg)
Test Species:	Charles River CD rat (Charles River UK Ltd, Margate), 10 per test material, administration orally by cannula
Dose:	[^{14}C]β-sitostanol: mean 3.7 mg/kg (at approx 54 uCi/kg); [^{14}C]β-sitosterol linoleate: 7.3 mg/kg (at approx 90 uCi/kg).
GLP:	UK GLP Regulations 1997/OECD GLP Guidelines

Study conduct

Two groups (each of 5 male and 5 female rats) were each treated with a single gavage dose of either [^{14}C]β-sitostanol or [^{14}C]β-sitosterol at approximately 3.6 and 7.2 mg/kg body weight respectively. Animals were housed in a climate-controlled facility for 24 hours prior to and for up to 24 hours following administration. Metabolism cages were designed to permit the collection of urine and faeces. Diets were restricted for 24 hours prior to dosing and for 2 hours after, when *ad libitum* food was provided. Water was available *ad libitum* throughout the procedure. A single rat of each sex from each treatment group was sacrificed at 24 hours after dosing for analysis by whole body autoradiography. At 8 hours after dosing urine was collected and at 24 hours urine and faeces were collected for ^{14}C analysis. At 24 hours, remaining animals were sacrificed and samples of blood, stomach and intestine contents, selected organs and the remaining carcasses were analysed for ^{14}C.

Formulation stability

The [^{14}C]-labelled test materials were assessed by radio-HPLC. This showed that the test materials were both stable and of high radiochemical purity.

Analysis

Analysis in duplicate or triplicate was by liquid scintillation counting of tissue and sample extracts. Faecal extracts were also analysed by TLC and radio-HPLC. Selected animals were analysed by whole body autoradiography.

Results

No animals showed signs of ill effect in this study. Excretion of [^{14}C]β-sitostanol and after oral administration was mainly via faeces with approximately 88% of administered dose of sitostanol excreted in faeces within 24 hours in both male and female rats. For [^{14}C]β-sitosterol, females excreted 85% and males 96% by this route in 24 hours. A small proportion of both sitostanol and sitosterol was excreted in urine during this time. Whereas male and female rats did not differ with respect to urinary sitostanol excretion (0.01%), males dosed with sitosterol excreted 0,02% and females 0.07%. The total sterol absorbed (the amount in all tissues, carcass and urine at 24 hours) was approximately 1.2% of the dose of [^{14}C]β-sitostanol in females and 0.5% in males. For [^{14}C]β-sitosterol, the estimated absorption was 4.3% and 1.9% in females and males respectively. For animals dosed with [^{14}C]β-sitosterol, ^{14}C was found in all tissues dissected except the brain and testes. For [^{14}C]β-sitostanol, ^{14}C was found in all tissues dissected except the brain. ^{14}C was found in the liver (0.9% and 0.3% of dose in females and males), small intestine (0.4% and 0.16%), large intestine (0.4% and 0.2%), and all other tissues contained less than 0.1% of the dose. Expressed relative to wet weight of tissues, the organ concentrations of ^{14}C in females were generally higher than in males with both [^{14}C]β-sitostanol and [^{14}C]β-sitosterol administration. The adrenal glands and stomach of both sexes, and the ovaries of females were the primary sites of ^{14}C accumulation. Brain, heart kidney, uterus and testes had tissue levels below that of blood. Whole body autoradiography showed a wider tissue distribution of ^{14}C with sitosterol than with sitostanol, although this may be a consequence of the higher dose of sitosterol administered.

HPLC and TLC analysis of faecal extracts showed that both sitosterol and sitostanol were excreted in modified forms. These products did not co-chromatographed with authentic [^{14}C]β-sitosterol and [^{14}C]β-sitostanol, and it was suggested that they may have represented other trans-esterified products formed in the gut. A third minor HPLC peak seen following sitosterol dosing was also not characterised but may represent a product of oxidation, either in vivo or during sample storage.

The fate in the rat of [^{14}C]cholesterol following gavage administration Sanders, D. and Minter, H. (1998) Unilever Research, Bedford, England. Study AM970180. January 1998.

Test material:	[^{14}C]β-cholesterol (Amersham International, 98.1%; 141 uCi/mg)
Test Species:	Charles River CD rat (Charles River UK Ltd, Margate), 10 per test material, administration orally by cannula
Dose:	[^{14}C]β-cholesterol: mean 34 mg/kg (at approx 68uCi/kg.
GLP:	UK GLP Regulations 1997/OECD GLP Guidelines

Study conduct

Ten male and 10 female rats were each treated with a single gavage dose of [^{14}C]cholesterol at approximately 34 mg/kg body weight. Animals were housed in a climate-controlled facility for 24 hours prior to and for up to 96 hours following administration. Metabolism cages were designed to permit the collection of urine and faeces. Diets were restricted for 24 hours prior to dosing and for 2 hours after, when *ad libitum* food was provided. Water was available *ad libitum* throughout the procedure.

A single rat from each treatment group was sacrificed at 8, 24, 48, 72 and 96 hours after dosing for analysis by whole body autoradiography. At sacrifice and at 8, 24, 48, 72 and 96 hours urine and faeces were collected for ^{14}C analysis. At 24 hours and 96 hours, 3 and 2 animals respectively of each sex were sacrificed and samples of blood, stomach and intestine contents, selected organs and the remaining carcasses were collected for ^{14}C analysis.

Formulation stability

The [^{14}C]-labelled test materials were assessed by radio-HPLC. This showed that the test material was both stable and of high radiochemical purity.

Analysis

Analysis in duplicate or triplicate was by liquid scintillation counting of tissue and sample extracts. Faecal extracts were also analysed by TLC and radio-HPLC. Selected animals were analysed by whole body autoradiography.

Results

Absorption of [^{14}C]cholesterol 24 hours after oral administration was approximately 27% in females and 24% in males. After 96 hours, the residual absorbed dose was 11.7% in females and 10.0% in males. The loss of ^{14}C was unlikely to be due to exhalation as $^{14}CO_2$, but more probably in bile, although this was not measured. ^{14}C was found in all tissues evaluated at 24 and 96 hours, with tissue ^{14}C declining over this time. The liver contained the greatest amount of radiolabel, expressed as a percentage of dose, followed by the large and small intestine. In females there were relatively high levels in the ovaries, but none detected in the testes of male rats. The carcass contained significant ^{14}C at 24 and 96 hours. When expressed as ^{14}C per gram wet weight of tissue, the adrenal glands contained the greatest proportion of radiolabel at 24 and 96 hours. Autoradiography showed that highest levels of ^{14}C] were generally associated with the stomach and intestinal tract, with observations at 8 – 96 hours revealing a progression of activity from stomach to intestine to faecal pellet. Elimination of radioactivity from tissues over this period was described as "*slow and steady*". There was evidence of ^{14}C in the bile ducts from 8 to 96 hours, suggesting that biliary excretion may have accounted for the loss of administered label. Small amounts appeared in the liver, adrenals and other tissues from 24 hours, declining to background levels at 72 hours. The adrenal gland retained small amounts of radioactivity at 96 hours.

HPLC and TLC analysis of faecal extracts showed that [^{14}C]cholesterol was eliminated as three metabolites, with free cholesterol as a high proportion, and probably esterified cholesterol as the second major metabolite. A third minor metabolite was not characterised but was thought to be an oxidation product from either *in vivo* gut metabolism or *ex vivo* during storage.

[^{3}H]Campestanol: the fate in the rat following gavage administration Sanders, D. and Minter, H. (1998) Unilever Research, Bedford, England. Study AM970306. March 1998.

Test material:	[^{3}H]campestanol (Amersham International, ≥98%; 91.4 mCi/mg) diluted with β-sitastanol
Test Species:	10 Female Charles River CD rat (Charles River UK Ltd, Margate), administration orally by cannula
Dose:	[^{3}H]campestanol: mean 4.2 mg/kg (at approx 225 uCi/kg).
GLP:	UK GLP Regulations 1997/OECD GLP Guidelines

Study conduct

Ten female rats were each treated with a single gavage dose of [^{3}H]campestanol at approximately 4.2 mg/kg body weight. Animals were housed in a climate-controlled facility for 24 hours prior to and for up to 96 hours following administration. Metabolism cages were designed to permit the collection of urine and faeces. Diets were restricted for 24 hours prior to dosing and for 2 hours after, when *ad libitum* food was provided. Water was available *ad libitum* throughout the procedure. A single rat from the group was sacrificed at 8, 24, 48, 72 and 96 hours after dosing for analysis by whole body autoradiography. At sacrifice and at 8, 24, 48, 72 and 96 hours urine was collected, and at 24 hour intervals faeces were collected for analysis. At 24 hours, 3 animals were sacrificed and samples of blood, stomach and intestine contents, selected organs and the remaining carcasses were analysed for ^{3}H.

Formulation stability

The [^{3}H]-labelled test materials were assessed by radio-HPLC. This showed that the test materials were both stable and of high radiochemical purity.

Analysis

Analysis in duplicate or triplicate was by liquid scintillation counting of tissue and sample extracts. Faecal extracts were also analysed by TLC and radio-HPLC. Selected animals were analysed by whole body autoradiography.

Results

No animals displayed any ill effects during this study. Absorption of [^{3}H]campestanol after oral administration was low with approximately 1.9% of the administered dose retained at 24 hours and 0.9% after 96 hours. Approximately 96% of the dose was excreted in faeces within 96 hours, with 90% of the dose eliminated by this route in the first 24 hours. A small proportion (less than 0.1%) was excreted in urine in 96 hours. Autoradiography showed that ^{3}H was found in all tissues except the brain, albeit at low levels with highest ^{3}H in the liver at 24 hours after dosing (0.2%). At 96 hours all tissue levels had declined to below 0.1% of the dose. The gastrointestinal tract contained less than 0.4% of dose at 24 hours, with caecum and rectum containing 5%. At 96 hours trace amounts of ^{3}H were found in the intestinal tract. Expressed relative to wet weight of tissues 24 hour concentrations of ^{3}H were highest in the adrenals and the liver, and 96 hour concentrations were highest in the liver. Tissues with ^{3}H at levels higher than those of blood were liver, adrenals, lungs, ovaries, stomach, small and large intestine and the caecum and rectum (at 24 hours) and were liver, adrenals, lungs, ovaries, stomach, small and large intestine and the caecum and rectum, uterus heart and kidneys (at 96 hours), although there was a decline in tissue concentrations from 24 to 96

hours. Autoradiography showed that ^{3}H was predominantly found in the adrenal gland, ovaries and intestinal epithelia.

HPLC and TLC analysis of faecal extracts showed that both free campestanol and esterified campestanol were the major excreted products.

Comments

The test preparation was found to contain 7.8 mg/ml β-sitostanol, resulting in an overall dose of free stanol of 54 mg/kg bodyweight.

[^{3}H]Campesterol: the fate in the rat following gavage administration Sanders, D. and Minter, H. (1998) Unilever Research, Bedford, England. Study AM970307. March 1998.

Test material:	[^{3}H]campesterol (Amersham International, ≥97%; 44.78 mCi/mg)
Test Species:	10 Female Charles River CD rat (Charles River UK Ltd, Margate), administration orally by cannula
Dose:	[^{3}H]campesterol: mean 1.68 mg/kg (at approx 236 uCi/kg).
GLP:	UK GLP Regulations 1997/OECD GLP Guidelines

Study conduct

Ten female rats were each treated with a single gavage dose of [^{3}H]campesterol at approximately 1.7 mg/kg body weight. Animals were housed in a climate-controlled facility for 24 hours prior to and for up to 96 hours following administration. Metabolism cages were designed to permit the collection of urine and faeces. Diets were restricted for 24 hours prior to dosing and for 2 hours after, when *ad libitum* food was provided. Water was available *ad libitum* throughout the procedure. A single rat from each treatment group was sacrificed at 8, 24, 48, 72 and 96 hours after dosing for analysis by whole body autoradiography. At 8, 24, 48, 72 and 96 hours urine was collected and at 24 hour intervals faeces were collected for analysis. At 24 and 96 hours, 3 and 2 rats respectively were sacrificed and samples of blood, stomach and intestine contents, selected organs and the remaining carcasses were analysed for ^{3}H.

Formulation stability

The ^{3}H-labelled test materials were assessed by radio-HPLC. This showed that the test materials were both stable and of high radiochemical purity.

Analysis

Analysis in duplicate or triplicate was by liquid scintillation counting of tissue and sample extracts and CO_2 absorber. Faecal extracts were also analysed by TLC and radio-HPLC. Selected animals were analysed by whole body autoradiography.

Results

Absorption of [^{3}H]campesterol after oral administration was low with approximately 13% of the administered dose retained at 24 hours and about 10% after 96 hours. Approximately 83% of the dose was excreted in faeces within 96 hours, with about 75% of the dose eliminated by this route in the first 24 hours. 96 hour data were limited to only 2 animals since 24 – 96 hour

faeces were unavailable for animals 1, 2 and 3. A small proportion (less than 0.2%) was excreted in urine in 96 hours. Autoradiography showed that [^{3}H] was found in all tissues at 24 hours, and at 96 hours all tissue levels had declined. At 24 hours tissues containing greatest amounts of ^{3}H included adrenals, spleen, intestinal epithelia, ovary, liver and bone marrow. Some of these tissues apparently retained ^{3}H at 96 hours , although this was not confirmed by autoradiography and may have represented poor tissue preparation. HPLC and TLC analysis of faecal extracts showed that both free and esterified campesterol were the major excreted products.

[3-^{3}H] stigmasterol: the fate in the rat following gavage administration Sanders, D. and Minter, H. (1998) Unilever Research, Bedford, England. Study AM980013. June 1998.

Test material:	[3-^{3}H]stigmasterol (Amersham International, ≥98%; 41 mCi/mg)
Test Species:	10 Female Charles River CD rat (Charles River UK Ltd, Margate), administration orally by cannula
Dose:	[3-^{3}H]stigmasterol: mean 1.9 mg/kg (at approx 245 uCi/kg).
GLP:	UK GLP Regulations 1997, No. 654/OECD Principles on GLP (1997) ENV/MC/CHEM/(98) 17

Study conduct

Ten female rats were each treated with a single gavage dose of [3-^{3}H]stigmasterol at approximately 1.9 mg/kg body weight. Animals were housed in a climate-controlled facility for 24 hours prior to and for up to 96 hours following administration. Metabolism cages were designed to permit the collection of urine, faeces and expired ^3H_2O. Diets were restricted for 24 hours prior to dosing and for 2 hours after, when *ad libitum* food was provided. Water was available *ad libitum* throughout the procedure. A single rat from each treatment group was sacrificed at 8, 24, 48, 72 and 96 hours after dosing for analysis by whole body autoradiography. Expired ^3H_2O was analysed at 2, 4, 8 and 24 hours. At 8, 24, 48, 72 and 96 hours urine was collected and at 24 hour intervals faeces were collected for ^{3}H analysis. At 24 and 96 hours, 3 and 2 rats respectively were sacrificed and samples of blood, stomach and intestine contents, selected organs and the remaining carcasses were analysed for ^{3}H.

Formulation stability

The ^{3}H-labelled test materials were assessed by radio-HPLC. This showed that the test materials were both stable and of high radiochemical purity.

Analysis

Analysis in duplicate or triplicate was by liquid scintillation counting of tissue and sample extracts and ^3H_2O absorber. Faecal extracts were also analysed by TLC and radio-HPLC. Selected animals were analysed by whole body autoradiography.

Results

No animals displayed any ill effects during this study. Absorption of [3-^{3}H]stigmasterol after oral administration was low with approximately 4% of the administered dose retained at 24 hours and 4.4% after 96 hours. Approximately 87% of the dose was excreted in faeces within

96 hours, with 85% of the dose eliminated by this route in the first 24 hours. A small proportion (less than 1%) was excreted in urine in 96 hours, and air traps for the collection of exhaled ^3H_2O contained less than 0.1% of dose. Autoradiography and tissue sample analysis showed that ^3H was found in all tissues, albeit at low levels with highest ^3H in the liver at 24 hours after dosing (0.4%). At 96 hours all tissue levels were similar to or had declined from 24 hour values. The gastrointestinal tract samples combined (excluding the caecum/rectum) contained less than 0.6% of dose at 24 hours, with caecum and rectum containing 1.3%. At 96 hours trace amounts of ^3H were found in the intestinal tract. Expressed relative to wet weight of tissues 24 hour concentrations of ^3H were highest in the adrenals and the liver, and 96 hour concentrations were highest in the liver.

Tissues with ^3H at levels higher than those of blood were adrenals, lungs, ovaries, stomach, uterus and brain, small and large intestine and the caecum and rectum (at 24 hours) and were these tissues plus heart and kidney (at 96 hours), although there was a decline in tissue concentrations from 24 to 96 hours. Autoradiography showed that ^3H was at very low levels in tissues but predominantly found in the adrenal gland, but also in the spleen, liver, ovaries and intestinal epithelia and bone marrow.

HPLC and TLC analysis of faecal extracts showed that both free and esterified stigmasterol were the major excreted products.

[3-^{3}H]stigmasterol: an investigation into dose dependent absorption in the rat following gavage administration Sanders, D. and Minter, H. (1999) Unilever Research, Bedford, England. Study AM980124. June 1999.

Test material:	[^{3}H]stigmasterol (Amersham International, ≥98%; 41 mCi/mg)
Test Species:	Charles River CD rat (Charles River UK Ltd, Margate); experiment 1 - 5 females per test dose, administration orally by cannula; experiment 2 - 5 females per test dose, administration orally by cannula
Dose:	[^{3}H]stigmasterol: experiment 1 - mean 4.2, 43.7, 418.5 and 4115 mg/kg (at approx 0.17, 1.79, 17.2 and 169 mCi/kg); experiment 2 - mean 4.1 and 3921 mg/kg (at approx 0.17 and 161 mCi/kg).
GLP:	UK GLP Regulations 1997, No. 654/OECD Principles on GLP (1997) ENV/MC/CHEM/(98) 17

Study conduct

Four groups of 5 female rats were each treated with a single gavage dose of [3-^{3}H]-stigmasterol at doses of total sterol of approximately 4, 40, 400 or 4000 mg/kg body weight. In a second experiment, a further 2 groups of 5 female rats were dosed with 4 or 4000 mg/kg. All treatments were of [3-^{3}H]stigmasterol in sunflower oil with added plant sterols containing unlabelled stigmasterol. Animals were housed in a climate-controlled facility for 24 hours prior to and for up to 24 hours following administration. Metabolism cages were designed to permit the collection of urine, faeces and expired ^3H_2O. Diets were restricted for 24 hours prior to dosing and for 2 hours after, when *ad libitum* food was provided. Water was available *ad libitum* throughout the procedure. A single rat from each treatment group was sacrificed at 24 hours after dosing for analysis by whole body autoradiography. Expired ^3H_2O was analysed at 2, 4, 8 and 24 hours. At 8 and 24 hours urine was collected and at 24 hours faeces

were collected for ^{3}H analysis. At 24 hours, remaining animals were sacrificed and samples of blood, stomach and intestine contents, selected organs and the remaining carcasses were analysed for ^{3}H.

Formulation stability

The ^{3}H-labelled test materials were assessed by radio-HPLC. This showed that the test materials were both stable and of high radiochemical purity.

Analysis

Analysis in duplicate or triplicate was by liquid scintillation counting of tissue and sample extracts. Faecal extracts were also analysed by TLC and radio-HPLC. Selected animals were analysed by whole body autoradiography.

Results

No animals displayed any ill effects during this study. Absorption of [3-^{3}H]stigmasterol after oral administration was linear with respect to the dose of plant sterols. The regression line of absorbed stigmasterol upon administered total plant sterols indicates stigmasterol absorption was of the order of 0.25% of total. Counting radiolabel in tissues, carcass and expired air traps gave absorbed doses of between 0.7 and 10% of the administered dose of *stigmasterol* retained at 24 hours. Almost all of the dose was excreted in faeces within 24 hours, with trace amounts excreted in urine and exhaled air. Autoradiography and tissue sample analysis showed that ^{3}H was found in all tissues, although at very low levels with highest ^{3}H in the carcass at 24 hours after dosing. Patterns of distribution of radioactivity were similar in all dose levels, with highest radioactivity in the intestine, caecum and rectum. The adrenals and epithelia of the stomach and intestine contained highest tissue concentrations of ^{3}H with radioactivity also present in the liver, bone marrow and ovary. The tissue concentrations expressed relative to wet weight were seen to increase with dose. HPLC and TLC analysis of faecal extracts showed that both free and esterified stigmasterol were the major excreted products.

Comments

Some transfer of ^{3}H to water is likely to have occurred contributing to sample variations and variation in some tissues.

Plant sterols and [^{14}C]β-sitosterol linoleate: in vitro digestibility of phytosterol-esters (plant sterols) and [^{14}C]β-sitosterol linoleate Sanders, D. (1997) Unilever Research, Bedford, England. Study AE960457. July 1997.

Test material:	[^{14}C]β-sitosterol linoleate (synthesised by Unilever, >99.9%; 13.14 uCi/mg); plant sterols (URL Vlaardingen, mixed fatty acid esters stigmasterol, sitosterol, campesterol, 8.4% free sterols)
GLP:	Environmental Safety Laboratory, Unilever, policy on GLP/OECD

Study conduct

Samples of plant sterols or of [^{14}C]β-sitosterol linoleate, emulsified with bile acid, were incubated with porcine cholesterol esterase or pancreatic lipase enzyme preparations for 1 to 24 hours. Free sterols and remaining esterified sterols were quantitated by HPLC, radio-HPLC and liquid scintillation after solvent extraction. Data were expressed as the proportion of free sterol relative to free sterol plus sterol esters. Analyses were performed in duplicate.

Results

Porcine cholesterol esterase and pancreatic lipase enzyme preparations were able to hydrolyse both [^{14}C]β-sitosterol linoleate and mixed sterol esters. The rate of hydrolysis of both substrates was greater with cholesterol esterase than with the lipase. This indicated that sterol esters will probably be hydrolysed in vivo in the intestinal tract.

ACUTE STUDIES

No acute study data were submitted for evaluation.

SHORT-TERM STUDIES

Since it involves observations made with treatment of less than 90 days, a single study has been considered here as a short term and repeat dose study.

Plant sterols: 14-day palatability study in rats Tinston, D.J. (1997) Central Toxicology Laboratory, Alderley Park, Macclesfield, England. Study KF960464. 19 February 1997.

Test material:	Plant sterols (URL Vlaardingen check AC960458 for composition)
Test Species:	Alpk:AP$_f$SD rat (Zeneca Pharmaceuticals, Alderley Park) 3 males and 3 females per test dose, administration in diet
Dose:	0, 1, 2, 5% w/w in diet, 14 days.
GLP:	UK GLP Compliance Programme, Department of Health 1989/OECD Principles of GLP 1982, EC 87/18/EEC, 88/320/EEC

Study conduct

Twelve male and 12 female rats were treated in groups of 3 with plant sterols in diet at 0, 1, 2 and 5% w/w for 14 days after a 1 week period of acclimatisation. Observations were made daily and detailed clinical examination and body weights were recorded before feeding (day 1) and on days 4, 8, 11 and 15. Food and water consumption were recorded as a weekly mean per animal and food utilisation expressed as increase in bodyweight/100g food consumed. All animals were sacrificed on day 16. No post mortem evaluations were performed.

Results

No animals died during the study and no clinical signs were observed at any plant sterol dose. Bodyweights of either sex did not differ between dose levels. Apart from slight differences in individual groups that were not dose related, there were no differences in food consumption or food utilisation. Changes observed were consistent with chance observations. The results showed that the incorporation of plant sterols in diet at up to 5% w/w was well tolerated by rats of both sexes and there was no reduction in growth rate. These data suggest that these dose levels would be suitable for subchronic and reproduction studies.

SUB-CHRONIC STUDIES

Plant sterols: 13-week dietary toxicity study in rats Horner, S.A. (1997) Central Toxicology Laboratory, Alderley Park, Macclesfield, England. Study KF960455. 14 October 1997.

Test material:	Plant sterols (Unilever ESL)
Test Species:	Alpk:AP_fSD rat (Zeneca Pharmaceuticals, Alderley Park) 3 males and 3 females per test dose, administration in diet
Dose:	0, 0.1, 1, 2, 5% sterols w/w in diet, 90 days.
GLP:	UK GLP Compliance Programme, Department of Health 1997
Guidelines:	OECD Guideline ref. 408 (1981), USEPA, EC.

Study conduct

After 1 to 2 weeks acclimatisation, five groups of rats (20 each sex) were allocated to control or treatment with plant sterols in diet at 0.16, 1.57, 3.19 and 8.09% (equivalent to 0, 77, 781, 1551 and 3910 mg sterol/kg/day in males, and 0, 87, 865, 1770 and 4204 mg sterol/kg/day in females) for 90 days. These doses represented 0.1% to 5% w/w plant sterol esters. Clinical observations and body weight measurements were made prior to the feeding study and animals were observed for clinical condition and behaviour twice daily. Food consumption, body weights and detailed clinical observations were recorded weekly. Food utilisation expressed as increase in bodyweight/100g food consumed. Ophthalmology of all animals was performed before the study and of control and high dose groups at week 13.

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

Results

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

In females, sterol treatment at all levels was associated with slight but significant decreases in prothrombin time, and activated partial prothrombin time was higher in males at 2 and 5% sterol. A variety of clinical chemistry measurements were significantly altered compared with concurrent control. These included increased plasma albumin (in females at 5% sterols), increased LDL (dose related in males but significant at 2 and 5%), increased cholesterol and HDL (in males and females at 1 and 2% but not 5%), increased alkaline phosphatase (in males at 5% and females at 1, 2 and 5%), increased alanine aminotransferase (in males and females at 2 and 5% and in males at 1%), increased aspartate aminotransferase, creatine kinase and lactate dehydrogenase (in females at 5%), increased phosphorus (in males at 2% but not 5%) and increased magnesium (in females at 2 and 5%).The extent of these changes, although in general statistically significant, were considered small and of little biological or toxicological importance. Organ weights, organ morphology and microscopic features were unaffected by sterol treatment in diet at 5% w/w. The lack of histopathological or other findings generally supports the interpretation of the clinical chemistry and haematology data.

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

Comments

Comparing platelet counts in males treated at 5% sterols with controls using 2-tailed student t-test yielded a t of 2.1 and a p<0.04. Notwithstanding the limitations of t-tests in this application (including the non-independence of sample groups) this suggests that there may be some caution in the interpretation of no effect. However, the decrease represented only 4 - 5% of the control activity and may be considered of little toxicological significance. This effect was not seen in other studies so is considered incidental.

CHRONIC STUDIES

No chronic studies were submitted.

REPRODUCTION STUDIES

Oral two generation reproduction study with plant sterols in Wistar rats Waalkens-Berendsen, D.H. and Wolterbeek, A.P.M. (1998) TNO Nutrition and Food Research Institute, Zeist, Netherlands. Study KR970242 (TNO Report V98.627). 24 August 1998.

Test material:	Plant sterols (Unilever ESL)
Test Species:	Wistar (Crl:(WI)WU BR) rat (Charles River Deutschland, Sulzfeld) 28 females and 28 males per F_0 dose group; 28 males and 28 females per F_1 dose group; administration in diet

Dose: 0, 1, 2 and 5% sterols w/w in diet; F0/F1 males - 10 weeks + gestation + 3 weeks; F0/F1 females – 10 weeks + gestation + 6 weeks.

GLP:	OECD/EC
Guidelines:	None

Study conduct

Groups of 28 male and 28 female Wistar derived rats were treated with plant sterols in diet at 0, 1, 2 and 5 % (made up from 0, 1.6, 3.2 and 8.0% test material which was a mixture of sterols and sterol-esters). Treatments began in F_0 animals 10 weeks before mating. The day on which sperm was found in the vaginal smears was counted as day 0 of gestation. Treatments continued for F_0 males and females until weaning when F_0 males were sacrificed. F_0 females were maintained on test diets until weaning (3 weeks post partum) and for 3 weeks following, during which time vaginal smears were performed to establish oestrus cycle length. F_0 females were then sacrificed. During the F_0 mating and parturition phase, clinical examinations were

28 males and 28 females were selected from the F_1 pups and were treated with plant sterols in diet at the same levels as their F_0 parents for 10 weeks premating. The treatment, observation and sacrifice schedule followed that of the F_0 generation. Of the remaining F_1 pups, 10 male and 10 female were subject to necropsy and tissue analysis. F_1 and F_2 litters were examined at 1, 4, 7, 14 and 21 days (weaning).

Data collected included;

- Daily clinical observations
- weekly body weight (and for mated females on days 0, 7, 14 and 21 of gestation, and on days 1, 7, 14 and 21 post partum)
- weekly food consumption (not during mating)
- premating food efficiency (weeks 0-5 and 6-10 premating, days 0-21 gestation and 1-14 postpartum)
- test substance intake
- litter evaluation (size, numbers male and female, still- and live births, malformed offspring on days 4, 7, 14 and 21 post partum)
- pup weight (days 1, 4, 7, 14 and 21 post partum)
- sexual maturation
- oestrus cycle length
- weanling necropsy and histology of abnormal tissues (stillborns and intercurrent deaths)
- weanling necropsy and histology (selected tissues only in 10 males and 10 females)
- necropsy and histology of F_0 and F_1 parental animals (listed in attachment 1 including femur, salivary glands; excluding bone marrow)
- fertility and reproductive performance.

Results

Sterol doses were in the ranges 0.5 - 1.3 g/kg/day (low dose), 1.0 - 2.6 g/kg/day (mid-dose) and 2.8 – 4.4 g/kg/day (high dose) in males and females of F_0 and F_1 groups premating. Doses were similar in F_0 and F_1 females during gestation but were increased to 1.7 – 1.8 mg/kg/day (low dose), 3.4 – 3.5 mg/kg/day (mid-dose) and 8.5 – 9.1 mg/kg/day (high dose) at week 2 of lactation. The latter consumption figures, and those of week 3 of lactation, may include significant food consumption by offspring.

There were no abnormal or dose related clinical observations during the study. Mean body weights in groups of F_0 and F_1 males of all dose groups were consistently lower than in

controls, and this reached statistical significance on some occasions in the highest dose group. Differences in body weights were up to 6% in F_0 and 8.5% in F_1 animals. These differences were reflected in slight, and sometimes statistically significant differences between high dose males and controls in body weight change and food consumption and efficiency. In F_0 females, there were slight (statistically insignificant) increases in body weights at all doses, but no consistent differences were seen in F_1 females.

Fertility and reproductive performance parameters were not significantly altered by sterol treatment in either generation. There were also no dose related changes in litter data. There were no consistent or dose related effects on organ weights or histopathology in either generation.

The NOEL for reproductive effects in rats in a 2-generation feeding study was 5% plant sterols in diet, representing approximately 2.8 - 4.4 g sterol/kg/day.

DEVELOPMENTAL STUDIES

No developmental studies were submitted for evaluation. However, the 2-generation reproduction study (Study KR970242) revealed no significant morphological or pathological findings in pups of F_0 and F_1 matings with plant sterols at 5% of diet.

GENOTOXICITY

The following studies were performed and main features are included in the table. These studies were uniformly well prepared, performed and presented. Protocols were carried out under GLP, with OECD and EC guidelines. Studies were designed with appropriate positive and negative control test substances and appropriate criteria were defined for positive and negative outcomes, including the comparison of data with historic control data. Appropriate pilot data are presented on dose ranging phases of experiments to assess cytotoxicity and solubility of test materials. Where S9 mix has been used as a metabolic activating system of test chemicals, the preparation of S9 is described, and the procedures were appropriate. Where necessary, deviations from defined protocols were identified and these were considered minor and unlikely to influence the outcome of any test.

Phytosterols: bacterial mutation assay Gant, R.A. (1996) Huntingdon Life Sciences Ltd, Huntingdon, England. Study KA960254. 13 November 1996.

Phytosterol-esters: bacterial mutation assay Gant, R.A. (1996) Huntingdon Life Sciences Ltd, Huntingdon, England. Study KA960256. 13 November 1996.

Plant sterols: bacterial mutation assay Kitching, J. and Anderson, D.H. (1998) Huntingdon Life Sciences Ltd, Huntingdon, England. Study KA980008. 10 March 1998.

Phytosterols: metaphase chromosome analysis of human lymphocytes cultured in vitro Akhurst, L.C. and Taylor, K. (1997) Huntingdon Life Sciences Ltd, Huntingdon, England. Study KC960255. 3 February 1997.

Phytosterol-esters: metaphase chromosome analysis of human lymphocytes cultured in vitro Akhurst, L.C. and Taylor, K. (1997) Huntingdon Life Sciences Ltd, Huntingdon, England. Study KC960257. 12 February 1997.

Plant sterols: in vitro mammalian chromosome aberration test in human lymphocytes Akhurst, L.C. and Taylor, K. (1998) Huntingdon Life Sciences Ltd, Huntingdon, England. Study KC980009. 23 April 1998.

Plant sterol ester SSE26698-02: measurement of unscheduled DNA synthesis in rat liver using an in vivo/in vitro procedure Fellows, M. (1999) Covance Laboratories Ltd, Harrogate, England. Study KU990111. November 1999.

Plant sterol ester SSE26698-02: induction of micronuclei in the bone marrow of treated rats Riley, S. (1999) Covance Laboratories Ltd, Harrogate, England. Study KU990110. November 1999.

Test	*Test material*	*Concentration*	*Test object*	*Result*
Reverse point mutation	Phytosterols (Roche)	≤ 5000 μg/plate (+/- S9)	*S. typhimurium* TA98, TA100, TA1535, TA1537	-ve
Reverse point mutation	Phytosterol-esters (Roche)	≤ 5000 μg/plate (+/- S9)	*S. typhimurium* TA98, TA100, TA1535, TA1537	-ve
Reverse point mutation	Plant sterols	≤ 5000 μg/plate (+/- S9)	*S. typhimurium* TA98, TA100, TA1535, TA1537; E. coli CM891 WP2	-ve
Chromosome aberrations	Phytosterols (Roche)	≤ 160 μg/ml (+/- S9)	Fresh human lymphocyte	-ve
Chromosome aberrations	Phytosterol-esters (Roche)	≤ 100 μg/ml (+/- S9)	Fresh human lymphocyte	-ve
Chromosome aberrations	Plant sterols	≤ 200 μg/ml (+/- S9)	Fresh human lymphocyte	-ve
Unscheduled DNA synthesis	Plant sterol ester SSE26698-02	≤ 2000 mg/kg by gavage	Han Wistar rat in vivo treatment/in vitro hepatocyte assessment of UDS*	-ve
In vivo micronucleus test	Plant sterol ester SSE26698-02	≤ 2000 mg/kg/day (2 days) by gavage	Rat bone marrow *in vivo* (Han Wistar)	-ve

- Rats were treated with test article for either 12 – 14 hours or 2 – 4 hours.

OTHER STUDIES

In vitro oestrogenic potential

The following *in vitro* studies were performed to determine whether the test materials possessed oestrogenic activity. These studies were well prepared, performed and presented. Protocols were carried out under in house GLP guidelines. Studies were designed with appropriate positive and negative control test substances and appropriate criteria were defined for positive and negative outcomes. The test substances included phytosterols (28.8% campesterol, 23.3% stigmasterol, and 47.9% β-sitosterol) and oryzanol (25.7% campesterol, 24.2% cycloartenol, 38.8% 24-methylene cycloartenol, and 11.2% β-sitosterol).

These data indicate that phytosterols and oryzanol did not bind to rat oestrogen receptor nor bind to or activate human oestrogen receptor *in vitro*.

***In vitro* detection of oestrogenic potential using oestrogen receptor binding** Baker, V. (1997) Central Toxicology Laboratory, Alderley Park, Macclesfield, England. (Based on study XC960417). October 1997.

***In vitro* detection of oestrogenic potential using the recombinant yeast assay** Williams, J. (1997) Central Toxicology Laboratory, Alderley Park, Macclesfield, England. (Based on study XC960417). 21 April 1997.

Test	*Test material*	*Conc.*	*EC_{50}**	*% ^3H-E_2 bound*	*Relative activity***
Rat uterine cytosol oestrogen receptor binding assay	Phytosterols (Roche)	≤ 100 μM	> 100 μM	107.3 ± 2.7	
	Oryzanol	≤ 100 μM	> 100 μM	111.8 ± 13.2	
	β-estradiol	≤ 1 μM	130 pM	-	
Human estrogen receptor binding and activation in yeast cells	Phytosterols (Roche)	≤ 129 μM	> 129 μM		$< 2.5 \times 10^{-5}$
	Oryzanol	≤ 100 μM	> 100 μM		$< 3.2 \times 10^{-5}$
	β-estradiol	≤ 100 nM	32 pM		100.0
	Testosterone	≤ 1 μM	> 1 μM		< 0.0032

* for RUC represents concentration for 50% displacement of ^{3}H-17β-estradiol (^{3}H-E_2). For yeast assay represents concentration for 50% absorbance (amount β-galactosidase produced)
** ratio of EC_{50} for β-estradiol/test substance, expressed as a percentage

In vivo oestrogenic potential

Phytosterols: uterotrophic assay in immature rats Williams, J. (1997) Central Toxicology Laboratory, Alderley Park, Macclesfield, England. Study KP960245. 21 April 1997.

Test material:	Phytosterols (Roche)
Test Species:	Wistar (Crl:(W1)BR) rat (Charles River, Margate) 10 females per dose group, gavage administration in peanut oil
Dose:	0, 5, 50, 500 mg sterols/kg/day and β-estradiol positive control, 3 days.
GLP:	UK GLP Compliance Programme, Department of Health 1989/OECD 1982/EC

Study conduct

Groups of 10 immature female Wistar-derived rats were treated with phytosterols by gavage at 0, 5, 50, and 500 mg/kg on 3 consecutive days after a 24 hour acclimatisation period. After treatment, clinical observations and body weights were made daily, and on the day of sacrifice. Uteri were dissected post mortem and uterus weights were recorded for each animal. There were no treatment related adverse clinical observations, nor changes in body weights. β-estradiol increased mean uterus weights by over 2-fold, whereas sterol treatment had no effect on either mean absolute organ weights or organ weights expressed relative to body weight.

Comments

One rat from the control group had a uterus weight 2 – 3 times that of other control animals. Omission of this animal's data did not alter the conclusion of this study

Phytosterol-esters: uterotrophic assay in immature rats Williams, J. (1997) Central Toxicology Laboratory, Alderley Park, Macclesfield, England. Study KP960246. 21 April 1997.

Test material:	Phytosterol-esters (Roche)
Test Species:	Wistar (Crl:(W1)BR) rat (Charles River, Margate) 10 females per dose group, gavage administration in peanut oil
Dose:	0, 5, 50, 500 mg sterols/kg/day and β-estradiol positive control, 3 days.
GLP:	UK GLP Compliance Programme, Department of Health 1989/OECD 1982/EC

Study conduct

Groups of 10 immature female Wistar-derived rats were treated with phytosterol esters by gavage at 0, 5, 50, and 500 mg/kg on 3 consecutive days after a 24 hour acclimatisation period. After treatment, clinical observations and body weights were made daily, and on the day of sacrifice. Uteri were dissected post mortem and uterus weights were recorded for each animal. There were no treatment related adverse clinical observations, nor changes in body weights. β-estradiol increased mean uterus weights by over 2-fold, whereas sterol treatment had no effect on either mean absolute organ weights or organ weights expressed relative to body weight.

Comments

One rat from the control group had a uterus weight 3 – 4 times that of other control animals. Omission of this animal's data did not alter the conclusion of this study.

Cholesterol: uterotrophic assay in immature rats Williams, J. (1997) Central Toxicology Laboratory, Alderley Park, Macclesfield, England. Study KP960252. 17 April 1997.

Test material:	Cholesterol (Sigma)
Test Species:	Wistar (Crl:(W1)BR) rat (Zeneca Pharmaceuticals, Alderley Park) 10 females per dose group, gavage administration in peanut oil
Dose:	0, 5, 50, 500 mg sterols/kg/day and β-estradiol positive control, 3 days.

GLP:	UK GLP Compliance Programme, Department of Health 1989/OECD 1982/EC

Study conduct

Groups of 10 immature female Wistar-derived rats were treated with cholesterol by gavage at 0, 5, 50, and 500 mg/kg on 3 consecutive days after a 24 hour acclimatisation period. After treatment, clinical observations and body weights were made daily, and on the day of sacrifice. Uteri were dissected post mortem and uterus weights were recorded for each animal. There were no treatment related adverse clinical observations. Apart from a transitory (day 2) decrease in mean body weight of the 50 mg/kg/day group, there were no treatment-related changes in body weights. β-estradiol increased mean uterus weights by over 2-fold, whereas sterol treatment had no significant effect on either mean absolute organ weights or organ weights expressed relative to body weight. Due to the observation of 1 or 2 animals in each of the cholesterol treated groups exhibiting uterus weights higher than the vehicle control range, it was concluded that cholesterol may have weak oestrogenic activity. Due to the equivocal nature of these results, the study was repeated (KP960421).

Comments

Since the previous 2 studies each found one rat from the control group with a uterus weight greater than those of other control animals, this finding may have been an artefact. It was prudent of the testing laboratory to confirm this finding in a repeat study.

Cholesterol: 2nd uterotrophic assay in immature rats Williams, J. (1997) Central Toxicology Laboratory, Alderley Park, Macclesfield, England. Study KP960421. 17 April 1997.

Test material:	Cholesterol (Sigma)
Test Species:	Wistar (Crl:(W1)BR) rat (Charles River, Margate) 10 females per dose group, gavage administration in peanut oil
Dose:	0, 5, 50, 500 mg sterols/kg/day and β-estradiol positive control, 3 days.
GLP:	UK GLP Compliance Programme, Department of Health 1989/OECD 1982/EC

Study conduct

Groups of 10 immature female Wistar-derived rats were treated with cholesterol by gavage at 0, 5, 50, and 500 mg/kg on 3 consecutive days after a 24 hour acclimatisation period. After treatment, clinical observations and body weights were made daily, and on the day of sacrifice. Uteri were dissected post mortem and uterus weights were recorded for each animal. There were no treatment related adverse clinical observations. There were no treatment-related changes in body weights. β-estradiol increased mean uterus weights by over two-fold, whereas sterol treatment had no significant effect on either mean absolute organ weights or organ weights expressed relative to body weight.

Cholesteryl palmitate: uterotrophic assay in immature rats Williams, J. (1997) Central Toxicology Laboratory, Alderley Park, Macclesfield, England. Study KP960253. 3 July 1997.

Test material:	Cholesteryl palmitate (Sigma)

Test Species:	Wistar (Crl:(W1)BR) rat (Charles River, Margate) 10 females per dose group, gavage administration in peanut oil
Dose:	0, 5, 50, 500 mg sterols/kg/day and β-estradiol positive control, 3 days.
GLP:	UK GLP Regulations 1997/OECD 1981

Study conduct

Groups of 10 immature female Wistar-derived rats were treated with cholesteryl palmitate by gavage at 0, 5, 50, and 500 mg/kg on 3 consecutive days after a 24 hour acclimatisation period. After treatment, clinical observations and body weights were made daily, and on the day of sacrifice. Uteri were dissected post mortem and uterus weights were recorded for each animal. There were no treatment related adverse clinical observations. There were no treatment-related changes in mean body weights, although slight (4%) decreases in body weights of the positive controls and 500 mg cholesteryl palmitate/kg/day group. β-estradiol increased mean uterus weights by almost 3-fold, whereas cholesteryl palmitate treatment had no significant effect on either mean absolute organ weights or organ weights expressed relative to body weight.

Cholesteryl palmitate: 2nd uterotrophic assay in immature rats Williams, J. (1997) Central Toxicology Laboratory, Alderley Park, Macclesfield, England. Study KP960422. 3 July 1997.

Test material:	Cholesteryl palmitate (Sigma)
Test Species:	Wistar (Crl:(W1)BR) rat (Charles River, Margate) 10 females per dose group, gavage administration in peanut oil
Dose:	0, 5, 50, 500 mg sterols/kg/day and β-estradiol positive control, 3 days.
GLP:	UK GLP Regulations 1997/OECD 1981

Study conduct

Groups of 10 immature female Wistar-derived rats were treated with cholesteryl palmitate by gavage at 0, 5, 50, and 500 mg/kg on 3 consecutive days after a 24 hour acclimatisation period. After treatment, clinical observations and body weights were made daily, and on the day of sacrifice. Uteri were dissected post mortem and uterus weights were recorded for each animal. There were no treatment related adverse clinical observations. There were no treatment-related changes in mean body weights of groups treated with 50 and 500 mg cholesteryl palmitate/kg/day, although there was a slight decreases (3-4%) in body weights of the positive controls and 5 mg cholesteryl palmitate/kg/day group. β-estradiol increased mean uterus weights by over 2-fold, whereas cholesteryl palmitate treatment had no significant effect on either mean absolute organ weights or organ weights expressed relative to body weight.

Coumestrol: uterotrophic assay in immature rats Williams, J. (1998) Central Toxicology Laboratory, Alderley Park, Macclesfield, England. Study KP960412. 23 January 1998.

Test material:	Coumestrol (Unilever)
Test Species:	Wistar (Crl:(W1)BR) rat (Charles River, Margate) 10 females per dose group, gavage administration in peanut oil

Dose:	0, 5, 20, 40, 80 mg coumestrol/kg/day and β-estradiol positive control, 3 days.
GLP:	UK GLP Regulations 1997/OECD 1981

Study conduct

Groups of 10 immature female Wistar-derived rats were treated with coumestrol by gavage at 0, 5, 20, 40, and 80 mg/kg on 3 consecutive days after a 24 hour acclimatisation period. After treatment, clinical observations and body weights were made daily, and on the day of sacrifice. Uteri were dissected post mortem and uterus weights were recorded for each animal. There were no treatment related adverse clinical observations. There were no treatment-related changes in mean body weights of groups treated with 5, 40 and 80 mg coumestrol/kg/day, although there was a slight, transient decrease (3%) in mean body weights of the 20 mg coumestrol/kg/day group. β-estradiol increased mean uterus weights by over 2-fold. Coumestrol treatment at 5 mg/kg/day had no significant effect on either mean absolute organ weights or organ weights expressed relative to body weight. Coumestrol at 20, 40 and 80 mg/kg/day significantly increased uterus weights by up to 100% over controls.

Baseline study: uterotrophic assay in immature rats Williams, J. (1997) Central Toxicology Laboratory, Alderley Park, Macclesfield, England. Study KP960396. 21 April 1997.

Test material:	Peanut oil (Sigma), Corn oil (Mazola), deionised water
Test Species:	Wistar (Crl:(W1)BR) rat (Charles River, Margate) 10 females per dose group, gavage administration
Dose:	10 ml/kg/day each vehicle and untreated control, 3 days.
GLP:	UK GLP Compliance Programme, Department of Health 1989/OECD 1982/EC

Study conduct

Groups of 10 immature female Wistar-derived rats were untreated or treated with peanut oil, corn oil or water vehicles by gavage at ml/kg on 3 consecutive days after a 24 hour acclimatisation period. After treatment, clinical observations and body weights were made daily, and on the day of sacrifice. Uteri were dissected post mortem and uterus weights were recorded for each animal. There were no treatment related adverse clinical observations. Slightly decreased mean body weights were seen in peanut oil and corn oil treated groups on day 4 and in the peanut oil treated group on day 3. These decreases were less than 3% and are considered biologically insignificant. There were no significant vehicle related effects on either mean absolute organ weights or organ weights expressed relative to body weight.

HUMAN STUDIES

A comparison of the effects on blood cholesterol level of margarines enriched in soybean oil, rice bran oil or sheanut oil derived sterols, Benecol and Flora Weststrate, J.A. and Meijer, G.W. (1997) Unilever Research Laboratory, Vlaardingen, Netherlands. Report VD 97 0144. August 1997.

Test material:	Margarine spreads enriched with phytosterol-esters derived from soybean (equivalent to 10.8 % sterol), ricebran (equivalent to 5.6 % sterol), sheanut (equivalent to 7.9 %

	sterol); Positive control Benecol spread (sterol ester mainly as sitostanol equivalent to 9.1 % sterol); negative control Flora spread (0.4 % sterol)
Test groups:	50 male and 50 female healthy volunteers, aged 18 – 65
Dose:	30 g spread per day in diet, uncooked.3.5 weeks each spread, in a double blind, randomised latin square design.
GLP:	Not stated

Study conduct

One hundred human volunteers consumed four different spreads consecutively as part of the diet, each for a 3.5-week period (lunch and dinner, 30 g/day). In each study period, fasting blood samples were collected at 2.5 and 3.5 weeks for analysis of lipids (total cholesterol, HDL and LDL), enzymes (plasma glutamyl transferase, GGT; alanine aminotransferase, ALT; aspartate aminotransferase, AST; serum alkaline phosphatase, ALP), albumin, urea, glucose, creatinine, total bile acids, sterols, cholesterylester fatty acids, carotenoids (α-carotene, β-carotene, lycopene). Standard haematology parameters were determined. The study assessed all relevant confounding factors by questionnaire repeated 12 times during the administration period, including lifestyle factors, bodyweight, disease status and medicine use.

Results

Between 75 and 77 subjects completed each of the dietary phases of the study. Average spread consumption was between 29.8 g/day (ricebran spread) and 30.4 g/day for sheanut spread. The doses of sterol or sterol ester were not stated, but were calculated as 3.3g/day from soybean, 1.7g/day from ricebran, 2.4g/day from sheanut, 2.7g/day from the positive control, Benecol, and 0.12g/day from the negative control, Flora.

Average plasma lipid data for each 2.5 and 3.5 week measurement were reported. Total cholesterol and LDL-cholesterol were lowered by Benecol (7.3% and 12% respectively) and soybean spread (8.3% and 13%, respectively) in comparison with the Flora spread diet. LDL/HDL ratios were also reduced by 12-13% with these diets. The extent of reduction of these parameters by Benecol and soybean spreads was the same in subjects entering the study with low, medium or high total and LDL-cholesterol. Ricebran and sheanut derived spreads did not affect blood lipids. All haematological parameters remained within normal ranges and there were no significant treatment-related changes. Plasma α+β carotenes were reduced by all dietary treatments relative to Flora, with the greatest reduction with sheanut spread (43%), followed by soybean (22%), Benecol (22%), and ricebran (8%). Lycopene was reduced to a similar extent, with the reduction caused by ricebran spread being statistically insignificant. This pattern of differences remained when plasma carotenes were expressed relative to total lipids.

Plasma plant sterol levels were highest after soybean spread (mean sitosterol approximately 4.4 mg/L, mean campesterol approximately 12 mg/L) compared with Benecol (mean sitosterol approximately 2.1 mg/L, mean campesterol approximately 5.8 mg/L) and Flora (mean sitosterol approximately 3.2 mg/L, mean campesterol approximately 7 mg/L). Fatty acid composition of plasma cholesterylesters showed treatments had roughly the same fatty acid intake profile.

Under the conditions of this study, there was a total cholesterol lowering effect of the soybean-enriched spread (containing esters of sitosterol, campesterol and stigmasterol) and a sitostanol-ester enriched spread of about 8 – 13%. These enriched spreads had no effect on routine haematological parameters, but reduced plasma levels of carotenes, probably by sequestration in the gut lumen. It is possible that the fat-soluble dietary vitamins and other micronutrients may remain in the more lipid-rich milieu of the gastrointestinal tract, suggesting micronutrient deficiencies are a possible consequence of consumption of these products.

Comments

This study was not clear about the nature of the sterols administered in spreads. The descriptions of the test and control margarines indicated that the soybean-derived enrichment was 65% esterified, but that the sheanut oil and ricebran oil-enriched spreads were produced from phytosterol concentrates without defining the level of esterification. It is only in the discussion that it is stated "… the ricebran and sheanut margarines contained sterols composed primarily of phenolic acid esters of 4,4'-dimethylsterols …". It is assumed that all margarine enrichments were of sterol-esters, but there is no statement of the percentage of sterol-ester in spreads.

A double-blind placebo-controlled trial of the efficacy of phytosterol enriched spreads to lower blood cholesterol levels in healthy humans Hendriks, H.F.J. (1997) TNO Nutrition and Food Research Institute, Zeist, Netherlands. TNO Report V97.658. 4 November 1997.

Test material:	Margarine spreads enriched with phytosterol derived from soybean at 3 concentrations; 13.1 % sterol, 6.5 % sterol, 3.4 % sterol; control butter; negative control Flora spread (0.4 % sterol)
Test groups:	42 male and 58 female healthy volunteers, aged 18 – 65
Dose:	25 g spread per day in diet, uncooked.3.5 weeks each spread, in a double blind, incomplete block crossover.
GLP:	EC principles of Good Clinical Practice/OECD Principles of GLP

Study conduct

One hundred human volunteers consumed three different spreads consecutively as part of the diet, each for a 3.5-week period (lunch and dinner, 12.5 g/meal). Fasting blood samples were collected at the end of each study phase for analysis of lipids (total cholesterol, HDL and LDL, triglycerides), and enzymes (plasma glutamyl transferase, GGT; alanine aminotransferase, ALT; aspartate aminotransferase, AST; serum alkaline phosphatase, ALP). The study assessed all relevant confounding factors, including food intake parameters, by questionnaire 11 times during the administration period, and adverse effects were reported using a standard questionnaire.

Results

All subjects completed each of the dietary phases of the study. Average spread consumption was between 24.7 g/day (3.4% soybean spread) and 24.9 g/day (6.5% soybean spread). Intake of sterols was approximately 0.9, 1.6 and 3.3 g/day with the three phytosterol-containing

spreads. Non-compliance (not consuming any or all of any single spread portion on the day assigned) was estimated to be approximately 1%. The mean body weights of the subjects ranged from 71.4 ± 10.9 kg (Flora) to 72.3 ± 11.0 kg (butter).

Body weight was higher after the 13.1% soybean sterols spread than after 6.5% soybean sterols spread or butter. None of the plasma enzyme activities were affected by any of the spreads. Mean total plasma cholesterol was higher after butter (5.27 mM) and Flora spread (5.16 mM) than after the 3 phytosterol-containing spreads (4.94, 4.84 and 4.81 mM after 3.4, 6.5, and 13.1% phytosterol spreads). Similarly, mean LDL-cholesterol was higher after butter (3.15 mM) and Flora spread (3.05 mM) than after the 3 phytosterol-containing spreads (2.86, 2.77 and 2.75 mM after 3.4 6.5, and 13.1% phytosterol spreads). Mean HDL-cholesterol was higher after butter (1.65 mM) than after the 2 highest phytosterol-containing spreads (1.63 and 1.61 mM after 6.5 and 13.1% phytosterol spreads). There was no difference between the lowest level soy spread (1.65 mM), the higher 2 levels and Flora spread (5.16 mM). LDL/HDL ratios were also reduced following phytosterol-containing spreads when compared with butter and Flora.

Only minor adverse effects were reported. Of the 164 reported adverse effects, 4 only were likely to be related to dietary treatment, including moderate constipation, mildly increased appetite, and increased defecation frequency (2 occasions). These suggest that although there are some possibly related effects, these are minor and the dose of 25 g/day of a 13.1% phytosterol enriched spread is well tolerated. The results suggest that the efficacy of cholesterol reduction may depend on the dose, the existing serum cholesterol level and the nature of the phytosterols ingested.

Phytosterol enriched spreads and impact on plasma concentrations of carotenoids and vitamin E in healthy adults: a comparison of different doses of phytosterols (Addendum 1 to TNO Report V97.658) Weststrate, J.A. (1998). TNO Nutrition and Food Research Institute, Zeist, Netherlands. TNO Report VD 98 0263. August 1998.

Study conduct

Experimental conditions were described in the previous study (TNO Report V97.658, 4 November 1997). Plasma samples collected during that study and stored at –70°C were analysed for the level of carotenoids (α-carotene, β-carotene, lycopene) and vitamin E using normal phase HPLC and UV absorbance detection.

Results

Spreads containing 3.4%, 6.5% and 13.1% phytosterols reduced plasma α- plus β-carotenoids in a concentration dependent manner by approximately 9, 11 and 21%, respectively, compared with plasma levels after Flora spread. These three spreads also reduced plasma vitamin E by 3.5, 6.0, and 6.1%, respectively, compared to the plasma levels after Flora spread.

After standardising the results relative to plasma lipid (total cholesterol + total glycerol), there was no significant reduction of plasma vitamin E following consumption of any of the phytosterol-enriched spreads, and plasma levels of α- plus β-carotenoids were reduced 3.7, 5.5 and 16% respectively. Only for the 13.1% phytosterol-enriched spread was this considered a significant difference. The average sterol intake from each of the three spreads was about 0.9, 1.6 and 3.3g/day, respectively.

A double-blind placebo-controlled trial of the efficacy of phytosterol enriched spreads to lower blood cholesterol levels in healthy humans: vitamin D and K analysis (addendum 2 to TNO Report V97.658) Hendriks, H.F.J. (1998)). TNO Nutrition and Food Research Institute, Zeist, Netherlands. TNO Report V98.1183. 11 November 1998.

Study conduct

Experimental conditions were described in the earlier study (TNO Report V97.658, 4 November 1997). Plasma samples collected during that study and stored at –70°C were analysed for the level of vitamin K1 (by HPLC with post column reduction and fluorimetric detection) and vitamin D using a competitive protein-binding assay.

Results

Phytosterol-enriched spreads had no effect on the mean blood levels of vitamins D and K1. Individual values were well within published normal ranges.

The physiological effects of daily consumption of dietary phytosterols on both the gut microflora and oestrogen metabolism in healthy normolipidaemic volunteers Drewitt, P. (1998) BIBRA International, Carshalton, England. BIBRA Study No. 3222/1. 19 February 1997.

Test material:	Test margarine spread enriched with 34 % phytosterol-ester derived from soybean (equivalent to 21.6 % free sterols); control spread with 1% cholesterol ester.
Test groups:	12 male and 12 female healthy volunteers, mean age 36 years
Dose:	40 g spread per day in diet, uncooked. 21 days (male) or 28 days (female) assigned randomly to test or control spread, in a double blind, two group, two period, parallel dose randomised placebo-controlled design.
GLP:	EC principles of Good Clinical Practice/RCP/ABPI/Declaration of Helsinki 1996

Study conduct

24 human volunteers (12/sex) commenced a study with two consecutive 21 (males) or 28 (females) day periods of spread in diet (lunch and dinner, 40 g/day). In the first period, all subjects received control spread, and I the second, half of each sex group received test or control spread. A controlled diet was also provided for all subjects for the duration of the study.

All male subjects provided a blood and urine sample at the commencement of the first control spread phase, and a blood, urine and faecal sample at both the commencement and conclusion of the second control or test spread phase. Blood samples were analysed for haematology (listed in attachment 1 including blood film but excluding prothrombin time) and clinical chemistry parameters (attachment 1 including γ-glutamyl transferase, LDL- and HDL-cholesterol, excluding phospholipids and ornithine carbamyltransferase). Urine samples were analysed for parameters listed in attachment 1 (excluding bile, sediment and volume). Faecal samples were analysed for bacteriological parameters (total faecal short chain fatty acids, SCFA; bacterial enzymes azo-reductase, β-glucosidase, β-glucuronidase, and nitrate

reductase; bacterial count of total anaerobes, total aerobes, enterobacteria, bacteroides, streptococci, staphylococci, bifidobacteria, clostridia and lactobacilli).

Female subjects provided a blood and urine sample at the commencement of the first control spread phase, a weekly blood sample in this phase to measure follicular, mid cycle and luteal phase hormones, and a faecal sample. This sequence was repeated in the second control or test spread phase. Urine samples and blood samples were analysed for haematology, clinical chemistry and urinalysis parameters as for males but female blood samples included measurement of hormones (estradiol, oestrone (E_1), progesterone, luteinising hormone, follicle stimulating hormone and sex hormone-binding globulin). A final blood and urine sample at the conclusion of the study were analysed for haematology, clinical chemistry and urinalysis parameters. The study assessed all adverse events and their severity.

Results

Intakes of sterol esters based on 40 g/day of 34% sterol-ester were approximately 13.6 g/day (equivalent to 8.6 g free sterol/day). Faecal SCFA did not differ between test and control spreads, except for lactic acid, which was reduced in the test spread group (from 0.85 mM to 0.11 mM) and the ratio of N-butyric/total SCFA which was reduced from 0.14 – 0.11. There was no significant change in faecal bacterial enzyme activities, nor in bacterial organism counts.

Test spread reduced mean total and LDH-cholesterol from 4.84 to 4.07 mM and 3,15 to 2.52 mM respectively, representing 16 and 20% reduction in total and LDH-cholesterol. There were no effects of dietary spread on haematology, clinical chemistry or urinalysis parameters, all of which remained within normal ranges.

Female hormone measurements showed a test spread-related difference for only progesterone, which decreased from 16.9 to 13.8 nM. This decrease is small considering the range of progesterone concentrations seen.

In general, both test and control spreads were well tolerated. Thirty of 35 reported adverse events were considered to be unrelated to treatment, with 24 reported incidences in the control group and 11 in the test group. Three incidences of skin rash and one of itchiness and swollen eyes were reported by a single subject consuming the test spread, and one incidence of rash was reported by another subject of the same group. It was unclear whether this response may have been caused by the phytosterol content of the spread or some other component.

Faecal bile acids and sterols in subjects fed controlled diets with or without added vegetable oils sterols (Addendum to BIBRA Study No. 3222/1) Weststrate, J.A., Bauer-Plank, C., Bruin de, Y., Engels, H.A. and Wiersma, A. (1998) Unilever Research Laboratory, Vlaardingen, Netherlands. Report VD 98 0091. February 1998.

Study conduct

Experimental conditions were described in the previous study (BIBRA Study No. 3222/1. 19 February 1997). Faecal samples collected from that study were freeze dried and analysed here for bile acids, cholesterol, plant sterols and their metabolites. Extracted bile acids, sterols and their metabolites were analysed by gas chromatography.

Results

Mean total faecal sterol concentration was increased from approximately 40 mg/g in controls and at the commencement of the study to approximately 190 mg/g after a diet enriched with phytosterols. The sterol metabolites (initially 75% of sterols excreted mainly as coprostanol with control spread) after test spread were mainly coprostanol and metabolites of sitostanol, stigmasterol and probably campesterol, although the latter co-eluted with cholesterol. Mean faecal bile acid excretion (total) was reduced in subjects receiving test spread from 7.93 to 6.21 mg/g. Total secondary bile acids was reduced from 7.57 to 6.00 mg/g and lithocholic acid reduced from 2.99 to 2.26 mg/g. This study has shown that consumption of 8.6 g/day phytosterols in an enriched spread does not adversely affect bile acid excretion.

Comments

Increased faecal content of bile acids has been associated with higher rates of some colon cancers, with secondary bile acids acting in vitro as comutagens and in vivo as colon cancer promoters. It is not clear whether the reduced faecal bile acid excretion in this study has the potential to confer some protection against some cancers.

Investigation of an adverse reaction to a margarine containing plant sterol esters experienced by a panellist of a BIBRA International clinical trial (Follow up to BIBRA Study No. 3222/1) Fairweather, F.A. (1999) BIBRA International, Carshalton, England. BIBRA Study No. 3394/1. 30 July 1999.

Study conduct

A subject who reported 4 of 5 adverse incidences (skin rash) in a previous study (BIBRA Study No. 3222/1. 19 February 1997) after consuming phytosterol-enriched spread was followed up to determine whether the response was allergic and related to test material administration. In this study, the subject was rechallenged with the same test material in a double blind crossover dietary design. Exposure to test or control material was for 7 days. Blood samples were taken for clinical chemistry, haematology, and IgE measurement. Adverse events were reported in each phase of the study, and clinical observations were made to ensure safety of the subject under challenge, including ECG.

Results

The subject completed the study, consuming all portions of test and control spread. No clinical signs were observed related to test spread or control spread ingestion. Haematology, clinical chemistry and serum IgE were unaffected by test spread exposure. There were 5 adverse events reported by the subject, none of them serious. Three were during the test spread phase. These included non-specific itch on head and shoulder of transient duration, irritation from ECG electrode placement, and mouth ulcer. Two events were during the control spread phase, including headache and sore throat neither of which was related to product. There were no adverse events related to the use of test or control spreads.

Long-term follow-up study on the use of a spread enriched with plant sterols Brink, E.J. and Hendriks, H.F.J. (2000) TNO Nutrition and Food Research Institute, Zeist, Netherlands. TNO Report V 99.869. 2 March 2000.

Test material:	Margarine spread enriched with 8.1 % plant sterol; negative control spread
Test groups:	98 male and 108 female healthy volunteers, aged 35 – 65 years
Dose:	20 g spread per day in diet, uncooked, up to 52 weeks, in a double blind, randomised placebo controlled study.
GLP:	EC Principles of Good Clinical Practice/OECD Principles of GLP

Study conduct

Male and female subjects were randomly assigned to test or control spread. At day 1, week 13, week 26, week 39 and week 52 blood and urine samples were collected for a variety of analyses, which included serum lipids (total cholesterol, LDL- and HDL-cholesterol, triacylglycerols and lipoprotein a), haematology (attachment 1), clinical chemistry (attachment 1), urinalysis (attachment 1). At various times measurements were also made of carotenoids and macronutrients (α- + β-carotene, lycopene, lutein, β-cryptoxanthin, zeaxanthin, retinol, vitamin E, 25-OH-vitamin D, vitamin K1), hormones (oestradiol, FSH, LH, progesterone, total and free testosterone), zinc and MDA, vitamin B_{12} and folic acid, osteocalcin decarboxylation, aPTT and PTT. At week 3 in addition to lipids, carotenoids were measured in 50 selected subjects only. Adverse events were recorded and a food frequency questionnaire completed at 13 week intervals.

Results

Ninety males and 95 females completed the 52 week dietary phase of the study. Mean total cholesterol decreased by approximately 5% in test spread consumers compared with control spread at all analysis times except week 13. These paralleled LDL-cholesterol decreases of 7 – 8% at the same times. HDL, triacylglycerol and lipoprotein a were not affected by treatment. Carotenoid levels were variously decreased in subjects consuming phytosterol-enriched spread at different time points. Where no difference existed between control and test subjects, in general there was a reduction in blood carotenoid concentration between day 1 and later weeks in subjects consuming test spread, but not control spread.

Blood β-carotene levels were significantly depressed in subjects consuming test spread from 410 nmol/l at week 0 to 322 and 310 nmol/l in weeks 26 and 52 respectively. In this group, α-carotene levels were significantly depressed from 91 nmol/l at week 0 to 82 and 78 nmol/l in weeks 26 and 52 respectively. Lycopene was also depressed in weeks 26 (283 nmol/l) and 52 (309) relative to week 0 (338 nmol/l) with test spread. Fat soluble vitamins A and D were not reduced in subjects consuming test spread, although vitamin D levels declined over time more in this group than in controls. Vitamin E was slightly but significantly decreased (by less than 3%) after phytosterol consumption. Vitamin K1 at week 26 was 504 pg/l compared with 598 pg/l at week 0, although this decrease was not statistically significant.

There were no treatment related effects on haematology, clinical chemistry or urinalysis parameters. Adverse events were reported in consumers of both control spread (371) and phytosterol enriched spread (414). Of these, 12 events were possibly related to control spread administration, and 9 possibly related to test spread. These were similar in each group, including gastrointestinal symptoms (diarrhoea, nausea, flatulence, increased defaecation frequency) and dermal effects (itch, dermatitis).

Comments

Intake rates were approximately 1.6 g free sterol/day.
The data reported here confirm significant lowering of blood levels of some fat soluble micronutrients with long term consumption of phytosterol-enriched spreads. There were no reports of symptoms associated with deficiencies of carotenoids or vitamins E and K1. However, the subject group were healthy, aged 35 - 65, and may not represent groups at risk of either dietary deficiency in these micronutrients or of impaired absorption of these micronutrients from diet.

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Appendix 1

LABORATORY INVESTIGATION PARAMETERS

Haematology	**Clinical Chemistry**	**Urinalysis**
erythrocyte count (RBC) haematocrit (Hct) haemoglobin (Hb) leucocyte count (WBC) leucocyte differential platelet count mean corpuscular haemoglobin (MCH) mean corpuscular haemoglobin concentration (MCHC) mean corpuscular volume (MCV) prothrombin time	albumin alkaline phosphatase (AP) alanine aminotransferase (ALT, GPT) aspartate aminotransferase (AST, GOT) bilirubin (total) calcium chloride cholesterol creatinine creatine kinase (CK) globulin glucose magnesium ornithine carbamyltransferase phospholipids phosphorus (inorganic) potassium protein (total) sodium triglycerides urea	bile bilirubin glucose ketones nitrite occult blood pH protein sediment specific gravity urobilinogin volume

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

ATTACHMENT 3

FOOD TECHNOLOGY REPORT

A410 – PHYTOSTEROL ESTERS DERIVED FROM VEGETABLE OILS

Introduction

Phytosterols are natural components of edible vegetable oils such as sunflower seed oil and, as such are natural constituents of the human diet. It is difficult to incorporate free sterols into edible fats/oils because of their insolubility, whereas sterols esterified to fatty acids are more fat soluble. In the intestine, most sterol esters are hydrolysed to free sterols as part of the normal digestive process. Plant stanols are the hydrogenated counterparts of the plant sterols but are less abundant in nature than the corresponding plant sterols. Consequently, the normal dietary intake of plant stanols is much less than that of plant sterols [1].

Structure of plant sterols and stanols

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

Plant stanols belong to the group of 4-desmethylsterols. 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.

When edible oils undergo normal refining, plant sterols are partially extracted together with some tocopherols (in the process of natural vitamin E production). It is estimated that 2500 tonnes of vegetable oil needs to be refined to produce 1 tonne of plant sterols [1].

Plant stanols are obtained by hydrogenation of the plant sterols. Another source of plant sterols is tall oil, derived from the process of paper production from wood and approximately 2500 tonnes of pine is required to produce 1 tonne of plant sterols. Tall oil also contains a higher proportion of plant stanols (primarily ß-sitostanol) than do vegetable oils [1].

In nature, plant sterols can be in the free form or predominantly esterified with long chain fatty acids or with phenolic acids as in rice bran oil (ferulates) and shea butter (cinnamates). In the intestine, most sterol esters are hydrolysed to free sterols as part of the normal digestive process [1].

Solubility

The solubility of free sterols in oil is around 2 percent, but the solubility of sterol esters in oil exceeds 20 percent. Therefore, the free plant sterols are esterified with fatty acids from sunflower to improve solubility.

The improved solubility of plant sterols creates a palatable product and is associated with more uniform distribution in the product and in the gastrointestinal tract. In vegetable oils, typically between 25 and 80 percent of the sterol is in the ester form. One gram of plant sterols is equivalent to about 1.6 g of plant sterol esters[2].

Stability

The physical and chemical properties of phytosterols are similar to cholesterol, since they differ only with respect to the side chain. Phytosterols and their fatty acid esters are basically very stable compounds and experience only limited damage during oil processing [3.]. Only under specific conditions, such as high temperatures (>100 °C) in the presence of air, may some oxidation of phytosterols occur, which will occur in the same way as cholesterol [4.]. Phytosterols are mono-unsaturated compounds (double bond in the B-ring), which are much more stable than the mono-unsaturated fatty acids (eg. 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.].

Legislation

In the United States a panel of independent experts has concluded that vegetable oil sterol esters meeting appropriate food-grade specifications and produced by current good manufacturing practice (21 C.F.R. § 182.1(b)), are safe for use as an ingredient in vegetable oil spreads in amounts not to exceed 20%. It is the panel's opinion that qualified experts in the field would generally recognise that vegetable oil sterol esters are safe for this use, i.e. that vegetable oil sterol esters are generally recognised as safe (GRAS). The US Food and Drug Administration (FDA) have also cleared a spread containing up to 20% of plant sterol ester, and one containing plant stanol ester, on the basis of the GRAS recognition [1.]. The FDA has not conducted an independent assessment of these compounds.

In Switzerland, authorisation for Becel pro.activ, a spread enriched with plant sterols, was given in September 1999 by the Swiss Health Office (Bundesamt fur Gesundheit; BAG) [1.].

In the European Union (EU) plant sterol esters for use in margarines/spreads has been reviewed under the EU Novel Foods Regulation (Regulation (EC) No 25 8/97) and in a recently published opinion the Scientific Committee on Food concluded that the use of phytosterol-esters in yellow fat spreads (maximum level of 8% free phytosterols) is safe for human use (European Commission Health & Consumer Protection Directorate-General, April 2000) [1.]. However, a yellow fat spread containing plant stanol esters is already legally on the market in the European Union without being subjected to review, because it was marketed in a Member State before the Novel Foods Regulation came into force. A similarly-based cheese spread by the same manufacturer considering it as merely a variant of the yellow fat spread, is also on the market in the UK, but was withdrawn from the shops in The Netherlands on instructions from the Dutch authority which regarded it as a separate novel food requiring prior approval [1.].

The UK Advisory Committee on Novel Foods and Processes (ACNFP), responsible for assessing the safety of these products, asked the Food Advisory Committee (FAC) to advise on the associated labelling issues.

FAC concluded that the products should be clearly labelled so that those with an inborn error in the metabolism of phytosterols (phytosterolaemia) could avoid them. Only a few dozen cases are known worldwide and, for example, it has been reported that in The Netherlands there is only one known case. Due to the nature of the disease, sufferers are aware of their condition and should avoid additional intake of phytosterols. The FAC also agreed that consumers should be informed that phytosterol ester-containing products are not nutritionally appropriate for young children and breast feeding women (as they did not need to reduce their blood cholesterol levels and there was a possibility that the products could affect vitamin A status) [1].

It stressed that this did not present a safety hazard and that the advice was purely for nutritional benefit rather than a health warning. Initially it recommended that the most appropriate way to do this would be through information circulated via GPs and other health professionals and through suitable magazines. Subsequently, however, it modified its earlier view and recommended that this information should also be provided on the product labels. This advice was welcomed by the Committee on Medical Aspects of Food and Nutrition Policy (COMA) [1].

Specifications

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

The proposed specification for the phytosterol component of phytosterol esters is as follows:

Specification for phytosterol esters derived from vegetable oils

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

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

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ATTACHMENT 4

DIETARY EXPOSURE REPORT

A410 – PHYTOSTEROL ESTERS DERIVED FROM VEGETABLE OILS

Dietary modelling was conducted by ANZFA to estimate potential dietary intakes of phytosterol esters for Australia and New Zealand when these substances are added to a variety of foods. Currently there are only a small number of foods on the market containing phytosterol esters (spreads, salad dressing and mayonnaise). However, the submissions suggested a number of other foods where phytosterol esters may potentially be added. Therefore, the modelling was conducted for individual foods where phytosterol esters were proposed to be added, as well as for the total diet including all of the nominated food groups from submissions.

The levels of phytosterol esters used in the models were derived using information from the application and submissions on proposed levels of use in various foods. These proposed levels are displayed below in **Table 1**. While it is the esterified phytosterol that is added to the food, the concentration levels were provided in terms of the equivalent level of free phytosterols. The free phytosterols levels were used in the dietary intake estimates.

Intake assessments do not include intakes of phytosterols from naturally occurring sources because data on naturally occurring levels were not readily available, and it was assumed that they would make little impact on the intake estimates.

The dietary modelling was conducted for both Australian and New Zealand populations using DIAMOND, ANZFA's dietary modelling computer program. Dietary data were obtained from the Australian 1995 NNS, which surveyed 13 858 people aged from 2 years and above, and the New Zealand 1997 NNS, which surveyed 4 636 people aged 15 years and above. Both surveys used a 24-hour food recall methodology.

The target age group for these products has been specified as 25 years and above, with more emphasis on those 40 years and above. However, given there is potential for all population groups to consume these products, modelling was conducted for all of the population as well as for the target age group of 40 years and above, children aged 2-12 years (Australia only), teenagers aged 13-19 years (15-19 years only for New Zealand), and young adults aged 20-39 years in the target age group.

Table 1: Proposed levels of free phytosterol equivalent in foods

Food	Level of use of phytosterols	Concentration (mg/kg)[#]	Submission
Spreads (<80% fat)	8%	80 000	Goodman Fielder
Salad dressing/ mayonnaise, low fat	3.2%	32 000	Goodman Fielder
Soup	0.8g/serve	*4 000	Arnott's
Biscuits	2.3%	23 000	Arnott's
Milk, low fat	3.2 g/L	3 200	Dairy Farmers
Yoghurt, low fat	5.7 g/kg	5 700	Dairy Farmers
Soy beverage, low fat	3.2 g/kg	3 200	Dairy Farmers
Low and reduced fat cheese	16 g/kg	16 000	Dairy Farmers
Bread, white, fibre increased	1.3%	13 000	Goodman Fielder
Breakfast cereal, mixed flakes	1.6%	16 000	Goodman Fielder
Breakfast bars	2.2%	22 000	Goodman Fielder

[#] *Free phytosterols.*
** Based on an estimated serve size of 200 g. No serve size was provided in the submission.*

Estimated intakes from individual foods

Estimated intakes of phytosterols by mean and high consumers from each of the foods listed in Table 1 were determined for both Australian and New Zealand populations for the various age groups listed above. The proposed phytosterol concentration levels were multiplied by the mean or 95th percentile food intake for consumers of each food in each of the specified age groups. It was assumed that each of the foods indicated contained the maximum proposed level of phytosterols.

The food consumption figures used for these calculations were derived using DIAMOND and the Australian and New Zealand National Nutrition Surveys. The consumption figures included mixed foods, where the foods were used as ingredients, except for breakfast bars, biscuits, mayonnaises and salad dressings. The 95th food consumption level was selected in order to estimate the worse case scenario.

The estimated mean and 95th percentile phytosterol intakes, for consumers only, from individual foods are summarised below in **Table 2** for Australia and in **Table 3** for New Zealand. For more comprehensive results, see Appendix 1. Data on consumers (eaters of foods containing phytosterols) only are reported rather than data for the whole population since the purpose of the dietary exposure assessment is to consider the potential intake of phytosterols by individuals expected to eat these foods.

The results show that consumption of each of the individual foods containing phytosterols has the potential to lead to a phytosterol intake sufficient to produce the reported cholesterol-lowering effect. The individual foods that have this potential are spreads, breads and soup for both Australian and New Zealand populations.

Australian, but not New Zealand, consumers of breakfast bars alone can also reach this level of phytosterol intake, however the number of consumers of breakfast bars was low, therefore resulting in an unreliable estimate of 95th percentile food intake and therefore phytosterol intake.

It should be noted that the modelling was conducted using dietary data from a 24-hour food recall survey, therefore the food consumption figures for high consumers (95th percentiles) may be higher than 'habitual intakes' which would tend to be lower when averaged over a long period of time.

Table 2: Estimated free phytosterol equivalent intakes for mean consumers and high consumers from individual foods for Australia, for different age groups

Food Name	Age Group (years)	N^{o} consumers	Consumer mean food intake (g/d)	Consumer mean phytosterol intake (g/d)	Consumer 95th percentile food intake (g/d)	Consumer 95th percentile phytosterol intake (g/d)
Milk, low fat	**2-12**	333	325	1.04	780	2.50
	13-19	248	347	1.11	865	2.77
	20-39	1 363	248	0.79	671	2.15
	40 +	2 416	230	0.74	607	1.94
	All	4 360	250	0.80	650	2.08
Yoghurt, low fat	**2-12**	287	135	0.77	338	1.92
	13-19	103	188	1.07	514	2.93
	20-39	460	148	0.85	378	2.15
	40+	751	147	0.84	259	1.48
	All	1 601	148	0.84	336	1.91
Cheese, low and reduced fat	**2-12**	1 019	30	0.47	78	1.24
	13-19	558	39	0.63	109	1.74
	20-39	2 423	41	0.65	115	1.84
	40+	2 902	33	0.52	90	1.44
	All	6 902	36	0.57	100	1.59
Spreads	**2-12**	1 064	12	0.93	31	2.48
	13-19	599	16	1.31	45	3.58
	20-39	2 466	17	1.34	48	3.83
	40+	3 015	16	1.28	45	3.58
	All	7 144	16	1.25	44	3.48
Soy beverage, low fat	**2-12**	47	298	0.95	777	2.49
	13-19	7	217	0.69	383	*1.22
	20-39	74	246	0.79	686	2.19
	40+	169	205	0.66	510	1.63
	All	297	230	0.74	530	1.70
Breakfast cereal	**2-12**	1 292	44	0.70	100	1.60
	13-19	500	69	1.10	172	2.75
	20-39	1 488	60	0.96	135	2.16
	40+	2 368	41	0.65	90	1.44
	All	5 648	49	0.78	120	1.92
Bread, white	**2-12**	1 877	95	1.24	206	2.68
	13-19	922	129	1.67	288	3.75
	20-39	3 743	128	1.66	300	3.90

	40+	5 527	109	1.42	236	3.07
	All	12 069	114	1.49	257	3.34
Biscuits	**2-12**	1 014	32	0.74	83	1.92
	13-19	327	43	0.99	119	2.73
	20-39	1 328	40	0.93	113	2.60
	40+	2 495	31	0.72	78	1.79
	All	5 164	34	0.79	92	2.12
Breakfast bars	**2-12**	2	28	0.61	37	*0.81
	13-19	3	37	0.81	37	*0.81
	20-39	15	44	0.96	85	*1.87
	40+	8	56	1.22	148	*3.26
	All	28	45	0.99	120	2.63
Mayonnaise & salad dressings	**2-12**	123	12	0.38	4	1.62
	13-19	115	17	0.54	58	1.87
	20-39	494	18	0.58	56	1.80
	40+	624	18	0.57	49	1.57
	All	1 356	17	0.55	51	1.62
Soup	**2-12**	104	305	1.22	764	3.05
	13-19	49	434	1.73	1 026	4.10
	20-39	359	457	1.83	1 040	4.16
	40+	871	385	1.54	780	3.12
	All	1 383	400	1.60	928	3.71

***** Statistically more than 21 consumers are needed for a 95th percentile to be reliable.

Table 3: Estimated free phytosterol equivalent intakes for mean consumers and high consumers from individual foods for New Zealand, for different age groups

Food Name	Age Group (years)	N° consumers	Consumer mean food intake (g/d)	Consumer mean phytosterol intake (g/d)	Consumer 95th percentile food intake (g/d)	Consumer 95th percentile phytosterol intake (g/d)
Milk, low fat	**15-19**	69	221	0.71	645	2.06
	20-39	483	201	0.64	561	1.80
	40 +	958	216	0.69	580	1.86
	All	1 510	211	0.68	576	1.84
Yoghurt, low fat	**15-19***	25	147	0.84	372	2.12
	20-39	162	154	0.88	379	2.16
	40+	291	116	0.66	259	1.48
	All	478	130	0.74	300	1.71
Cheese, low and reduced fat	**15-19**	145	39	0.62	106	1.70
	20-39	863	40	0.63	121	1.94
	40+	1 072	33	0.53	98	1.56
	All	2 080	36	0.58	108	1.72
Spreads	**15-19**	74	14	1.09	40	3.17
	20-39	459	12	0.94	28	2.25
	40+	661	13	1.05	35	2.83
	All	1 194	13	1.01	32	2.59
Soy beverage, low fat	**15-19**	0	0	0.00	0	*0.00
	20-39	12	162	0.52	518	*1.66
	40+	39	204	0.65	518	1.66
	All	51	194	0.62	518	1.66
Breakfast cereal	**15-19**	102	47	0.75	117	1.87
	20-39	580	39	0.63	84	1.34
	40+	1 036	29	0.46	65	1.04
	All	1 718	34	0.54	75	1.20
Bread, white	**15-19**	240	150	1.95	320	4.16
	20-39	1 520	137	1.78	320	4.16
	40+	2 280	125	1.62	274	3.56
	All	4 040	131	1.70	297	3.86
Biscuits	**15-19**	71	54	1.23	167	3.83
	20-39	595	45	1.04	110	2.54
	40+	1 114	35	0.79	85	1.96
	All	1 780	39	0.89	96	2.21
Breakfast bars	**15-19**	0	0	0.00	0	*0.00
	20-39	7	23	0.50	25	*0.55
	40+	0	0	0.00	0	*0.00
	All	7	23	0.50	25	*0.55
Mayonnaise & salad dressings	**15-19**	38	19	0.60	58	1.86
	20-39	301	21	0.66	62	2.00
	40+	427	19	0.62	60	1.92
	All	766	20	0.63	61.3	1.96

Food Name	Age Group (years)	N° consumers	Consumer mean food intake (g/d)	Consumer mean phytosterol intake (g/d)	Consumer 95th percentile food intake (g/d)	Consumer 95th percentile phytosterol intake (g/d)
Soup	**15-19**	30	176	0.71	686	2.75
	20-39	201	273	1.09	728	2.91
	40+	382	312	1.25	632	2.53
	All	613	292	1.17	634	2.53

***** Statistically more than 21 consumers are needed for a 95th percentile to be reliable.

Estimated intakes from the total diet

The manufacturers have proposed that phytosterols would only be used in certain varieties of particular foods, for example, low fat dairy products and soy beverages, and fibre increased white bread. However, food regulations generally give permissions for use to whole categories of foods. Therefore, modelling was conducted assuming either the total food group contained phytosterols (scenario A) or only the sub group of the food contained phytosterols (scenario B). The phytosterol concentrations for each scenario are shown in **Table 4**.

Table 4: Concentrations of free phytosterol equivalents used in the dietary modelling for the total diet estimate scenarios

Food	Scenario A (mg/kg)	Scenario B (mg/kg)
Spreads (<80% fat)	80 000	80 000
Salad dressing/mayonnaise	*2 688	2 688
Salad dressing/mayonnaise (<3% fat)	-	672
Soup	4 000	4 000
Biscuits	**7 360	**7 360
Milk	3 200	-
Milk, low fat	-	3 200
Yoghurt, all types	5 700	-
Yoghurt, low fat	-	2 149
Soy beverage, all types	3 200	-
Soy beverage, low fat	-	672
Cheese, all types	16 000	-
Low and reduced fat cheese	-	1 248
Bread, all types	13 000	-
Bread, white, fibre increased	-	533
Breakfast cereal, mixed flakes	16 000	16 000
Breakfast bars	#1 012	#1 012

** Based on salad dressings and mayonnaise representing 8.4% of the sauces, salad dressings and mayonnaise category in DIAMOND.*

** Based on biscuits representing 32% of the biscuits/cakes/pastries category derived from DIAMOND.

Based on breakfast bars representing 4.6% of the snack foods category derived from DIAMOND.

Scenario A assumes phytosterols are present in the total food group at maximum proposed levels. This means that even though the manufacturers stated that phytosterols would only be in certain varieties of the food, (e.g. low fat dairy and soy beverage, or a fibre increased bread), the whole category of the food was assumed to contain phytosterols to estimate a worse case scenario intake.

Scenario B assumes that phytosterols would only be added to the foods specified in the submissions, for example the low fat dairy products, and the fibre increased white bread. The phytosterol concentration levels for this model were calculated by weighting the concentration level based on the proportion of consumption from the sub category to the consumption of the whole category of the food. Consumption data from the Australian NNS were used to calculate the weighted modelling concentrations. It was assumed that the proportions were the same for New Zealand. The calculations used for the weightings are shown below in Table 5.

Table 5: Calculations for weighted free phytosterol equivalent concentrations for scenario B

Food	Consumption all respondents – all category (g/d)	Consumption all respondents – sub category (g/d)	Sub-category as percent total category	Calculation	Modelling concentration level (mg/kg)
Bread, fibre increased	99.5	4.1	4.1	(0.041 x 13 000) + (0.959 x 0)	533
Soy beverage, low fat	4.9	1.0	21	(0.21 x 3 200) + (0.79 x 0)	672
Cheese, low fat	17.7	1.4	7.8	(0.078 x 16 000) + (0.922 x 0)	1 248
Yoghurt, low fat	17.1	6.4	37.3	(0.373 x 5 700) + (0.627 x 0)	2 149

The results for scenarios A and B are summarised below in **Table 6** for Australia and in **Table 7** for New Zealand. In both cases, for a significant proportion of the population, the intake of phytosterols would be higher than the intake level that has been demonstrated to be safe in long-term human studies.

While the figures for the estimated dietary intakes of phytosterols for the total diet for consumers only do not appear to differ greatly to the results for consumers of the individual foods, this is because the estimates for the total diet are an average of all consumers. A consumer is a person who has consumed one or more of the phytosterol-containing foods.

Therefore the total diet estimates take into account a consumer who may have eaten only one food containing phytosterols, resulting in a relatively low phytosterol intake, and consumers

who may have eaten a number of the phytosterol-containing foods, resulting in a higher individual intake.

Table 6: Estimated free phytosterol equivalent intakes from the total diet for Australia, for different age groups

Age Group (years)	Scenario	N° consumers	Mean intake consumers* (g/d)	95th percentile intake consumers* (g/d)
2-12	A	2 072	2.9	6.0
	B	2 072	1.6	3.9
13-19	A	1 051	3.9	8.7
	B	1 051	2.2	5.7
20-39	A	4 387	3.7	8.3
	B	4 387	2.0	5.3
40 +	A	6 230	3.3	7.1
	B	6 230	1.8	4.7
All	A	13 740	3.4	7.5
	B	13 740	1.9	4.9

* Consumers – people consuming one or more of the products assumed to contain phytosterols.

Highest contributions to total estimated mean phytosterol intakes for the whole food categories model (scenario A) for all of the population for Australia were breads (38%) and spreads (19%). For New Zealand the highest contributors for scenario A were breads (47%) and biscuits (13%).

Highest contributions to total estimated mean phytosterol intakes for scenario B, which were the models run using the sub categories of the foods, for all of the population for Australia were spreads (35%), cereals (17%), biscuits (16%) and low fat milk (14%). For New Zealand, the highest contributors for scenario B were biscuits (28%), spreads (18%), low fat milk (16%), cereals (14%) and soup (11%).

These results indicate that if phytosterols were permitted to be added to a wide range of foods, breads, the major contributors to phytosterols intake from the diet would be low fat milk, biscuits, cereals, spreads and soup.

Table 7: Estimated free phytosterol equivalent intakes from the total diet for New Zealand, for different age groups

Age Group (years)	Scenario	N° consumers	Mean intake consumers (g/d)	95th percentile intake consumers (g/d)
15-19	A	289	3.3	7.0
	B	289	1.4	3.6
20-39	A	1 755	3.2	7.0
	B	1 755	1.4	3.8
40 +	A	2 516	3.1	6.5
	B	2 516	1.5	3.8
All	A	4 560	3.2	6.7
	B	4 560	1.4	3.8

* Consumers – people consuming one or more of the products assumed to contain phytosterols.

If non-target groups (i.e. children and teenagers) were to consume foods containing phytosterols, estimated intakes for these groups would be very similar to, and in some cases higher than, estimated intakes for adults. On a per kilogram body weight basis, the intake by children would be relatively higher than those of adults.

Summary

Consumers of individual phytosterol-enriched foods (spreads, breads and soup) have potential phytosterol intakes in excess of the level needed to obtain the reported cholesterol lowering effect.

Estimated mean intakes of phytosterols from the total diet where whole food categories contain the phytosterols, were generally above the level needed to obtain the reported cholesterol-lowering effect. High consumers of phytosterols from the total diet, on the basis of total diet estimates in both Australia and New Zealand, had up to 5 times the level needed to obtain the reported cholesterol-lowering effect.

References

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Appendix 1: Estimated food intake levels and P95 intake levels of phytosterols for each food – grams per day (g/d)

Australia: All

Food code	Number of Consumers	Percentage: Consumers to Respondents	Respondent mean food intake (g/d)	Respondent median food intake (g/d)	Consumer mean food intake (g/d)	Consumer median food intake (g/d)	*Consumer P95 food intake (g/d)*	Consumer P95 Phytosterol Intake (g/d)	Food Name
1.1.1.1	4 360	31.46	78.53	0.00	249.61	195.00	650.00	2.08	Milk, low fat
1.2	1 601	11.55	17.07	0.00	147.72	129.50	335.89	1.91	Yoghurt, low fat
1.6	6 902	49.81	17.72	0.00	35.57	24.00	99.50	1.59	Low and reduced fat cheese
2.2.2	7 144	51.55	8.05	1.27	15.62	10.75	43.56	3.48	Spreads (<80% fat)
4.3.8.4	297	2.14	4.93	0.00	230.15	198.75	530.00	1.70	Soy beverage, low fat
6.3	5 648	40.76	19.89	0.00	48.80	40.00	120.00	1.92	Breakfast cereal, mixed flakes
7.1.1	12 069	87.09	99.48	83.00	114.23	96.00	257.08	3.34	Bread, white, fibre increased
7.2	5 164	37.30	12.83	0.00	34.43	26.25	92.00	2.12	Biscuits
20.2.3	28	0.20	0.09	0.00	45.11	37.00	119.65	2.63	Breakfast bars
20.2.4	1 356	9.80	1.70	0.00	17.32	11.60	50.71	1.62	Salad dressing/ mayonnaise, low fat
20.2.9	1 383	9.98	39.88	0.00	399.60	337.32	927.84	3.71	Soup

Australia: 2-12 years

Food code	Number of Consumers	Percentage: Consumers to Respondents	Respondent mean food intake (g/d)	Respondent median food intake (g/d)	Consumer mean food intake (g/d)	Consumer median food intake (g/d)	*Consumer P95 food intake (g/d)*	Consumer P95 Phytosterol Intake (g/d)	Food Name
1.1.1.1	333	16.02	52.01	0.00	324.69	260.00	780.00	2.50	Milk, low fat
1.2	287	13.80	18.62	0.00	134.91	120.00	337.50	1.92	Yoghurt, low fat
1.6	1 019	49.01	14.49	0.00	29.56	21.00	77.74	1.24	Low and reduced fat cheese
2.2.2	1 064	51.18	5.92	0.93	11.56	8.00	31.00	2.48	Spreads (<80% fat)
4.3.8.4	47	2.26	6.73	0.00	297.76	255.00	777.24	2.49	Soy beverage, low fat
6.3	1 292	62.15	27.05	21.00	43.53	30.00	100.00	1.60	Breakfast cereal, mixed flakes
7.1.1	1 877	90.28	86.19	74.87	95.47	84.00	206.28	2.68	Bread, white, fibre increased
7.2	1 014	48.80	15.60	0.00	31.98	25.00	83.42	1.92	Biscuits
20.2.3	2	0.10	0.03	0.00	27.80	27.80	37.00	0.81	Breakfast bars
20.2.4	123	5.90	0.70	0.00	11.90	9.28	40.00	1.28	Salad dressing/ mayonnaise, low fat
20.2.9	104	5.00	15.27	0.00	305.34	255.00	763.50	3.05	Soup

Australia: 13-19 years

Food code	Number of Consumers	Percentage: Consumers to Respondents	Respondent mean food intake (g/d)	Respondent median food intake (g/d)	Consumer mean food intake (g/d)	Consumer median food intake (g/d)	*Consumer P95 food intake (g/d)*	Consumer P95 Phytosterol Intake (g/d)	Food Name
1.1.1.1	248	23.33	80.87	0.00	346.63	260.00	865.16	2.77	Milk, low fat
1.2	103	9.69	18.20	0.00	187.85	200.00	514.40	2.93	Yoghurt, low fat
1.6	558	52.49	20.68	4.00	39.39	28.32	108.71	1.74	Low and reduced fat cheese
2.2.2	599	56.35	9.26	3.21	16.43	11.88	44.80	3.58	Spreads (<80% fat)
4.3.8.4	7	0.66	1.43	0.00	216.90	255.00	382.50	1.22	Soy beverage, low fat
6.3	500	47.04	32.25	0.00	68.57	60.00	171.65	2.75	Breakfast cereal, mixed flakes
7.1.1	922	86.74	111.55	95.40	128.61	111.44	288.49	3.75	Bread, white, fibre increased
7.2	327	30.80	13.24	0.00	43.03	33.00	118.88	2.73	Biscuits
20.2.3	3	0.30	0.10	0.00	37.00	37.00	37.00	0.81	Breakfast bars
20.2.4	115	10.80	1.83	0.00	16.94	10.00	58.40	1.87	Salad dressing/ mayonnaise, low fat
20.2.9	49	4.61	19.99	0.00	433.71	330.00	1026.00	4.10	Soup

Australia: 20-39 years

Food code	Number of Consumers	Percentage: Consumers to Respondents	Respondent mean food intake (g/d)	Respondent median food intake (g/d)	Consumer mean food intake (g/d)	Consumer median food intake (g/d)	*Consumer P95 food intake (g/d)*	Consumer P95 Phytosterol Intake (g/d)	Food Name
1.1.1.1	1 363	30.71	76.30	0.00	248.42	194.25	670.58	2.15	Milk, low fat
1.2	460	10.37	15.39	0.00	148.48	150.00	377.54	2.15	Yoghurt, low fat
1.6	2 423	54.60	22.21	8.28	40.68	29.75	114.90	1.84	Low and reduced fat cheese
2.2.2	2 466	55.57	9.29	3.16	16.71	11.96	47.92	3.83	Spreads (<80% fat)
4.3.8.4	74	1.67	4.10	0.00	245.95	255.00	685.58	2.19	Soy beverage, low fat
6.3	1 488	33.53	20.05	0.00	59.79	45.00	135.00	2.16	Breakfast cereal, mixed flakes
7.1.1	3 743	84.34	107.56	86.00	127.53	108.00	300.00	3.90	Bread, white, fibre increased
7.2	1 328	29.90	12.05	0.00	40.27	30.00	112.83	2.60	Biscuits
20.2.3	15	0.30	0.15	0.00	43.50	37.00	85.00	1.87	Breakfast bars
20.2.4	494	11.10	2.00	0.00	17.99	13.92	56.13	1.80	Salad dressing/ mayonnaise, low fat
20.2.9	359	8.09	36.99	0.00	457.30	379.50	1 040.00	4.16	Soup

Australia: 40+ years

Food code	Number of Consumers	Percentage: Consumers to Respondents	Respondent mean food intake (g/d)	Respondent median food intake (g/d)	Consumer mean food intake (g/d)	Consumer median food intake (g/d)	*Consumer P95 food intake (g/d)*	Consumer P95 Phytosterol Intake (g/d)	Food Name
1.1.1.1	2 416	38.48	88.50	0.00	229.98	191.66	606.66	1.94	Milk, low fat
1.2	751	11.96	17.54	0.00	146.64	129.50	259.40	1.48	Yoghurt, low fat
1.6	2 902	46.22	15.11	0.00	32.68	21.60	89.89	1.44	Low and reduced fat cheese
2.2.2	3 015	48.02	7.68	0.00	16.00	10.86	44.80	3.58	Spreads (<80% fat)
4.3.8.4	169	2.69	5.52	0.00	204.98	168.30	510.00	1.63	Soy beverage, low fat
6.3	2 368	37.72	15.31	0.00	40.59	30.00	90.00	1.44	Breakfast cereal, mixed flakes
7.1.1	5 527	88.04	96.14	83.06	109.20	96.00	236.00	3.07	Bread, white, fibre increased
7.2	2 495	39.70	12.40	0.00	31.19	24.50	78.00	1.79	Biscuits
20.2.3	8	0.10	0.07	0.00	55.50	37.00	148.00	3.26	Breakfast bars
20.2.4	624	9.90	1.78	0.00	17.93	13.92	49.10	1.57	Salad dressing/ mayonnaise, low fat
20.2.9	871	13.87	53.43	0.00	385.15	325.00	780.00	3.12	Soup

New Zealand: All

Food code	Number of Consumers	Percentage: Consumers to Respondents	Respondent mean food intake (g/d)	Respondent median food intake (g/d)	Consumer mean food intake (g/d)	Consumer median food intake (g/d)	*Consumer P95 food intake (g/d)*	Consumer P95 Phytosterol Intake (g/d)	Food Name
1.1.1.1	1 510	32.57	68.80	0.00	211.24	155.71	575.91	1.84	Milk, low fat
1.2	478	10.31	13.44	0.00	130.36	129.50	300.00	1.71	Yoghurt, low fat
1.6	2 080	44.87	16.24	0.00	36.20	26.37	107.51	1.72	Low and reduced fat cheese
2.2.2	1 194	25.75	3.24	0.00	12.58	10.21	32.43	2.59	Spreads (<80% fat)
4.3.8.4	51	1.10	2.13	0.00	194.01	147.80	517.50	1.66	Soy beverage, low fat
6.3	1 718	37.06	12.45	0.00	33.60	30.00	75.00	1.20	Breakfast cereal, mixed flakes
7.1.1	4 040	87.14	114.03	98.00	130.86	115.37	297.00	3.86	Bread, white, fibre increased
7.2	1 780	38.40	14.93	0.00	38.89	29.55	95.97	2.21	Biscuits
20.2.3	7	0.20	0.04	0.00	22.86	25.00	25.00	0.55	Breakfast bars
20.2.4	766	16.50	3.28	0.00	19.83	15.00	61.30	1.96	Salad dressing/ mayonnaise, low fat
20.2.9	613	13.22	38.66	0.00	292.35	264.00	633.60	2.53	Soup

New Zealand: 15-19 years

Food code	Number of Consumers	Percentage: Consumers to Respondents	Respondent mean food intake (g/d)	Respondent median food intake (g/d)	Consumer mean food intake (g/d)	Consumer median food intake (g/d)	*Consumer P95 food intake (g/d)*	Consumer P95 Phytosterol Intake (g/d)	Food Name
1.1.1.1	69	23.23	51.30	0.00	220.81	136.74	645.00	2.06	Milk, low fat
1.2	25	8.42	12.35	0.00	146.77	150.00	371.80	2.12	Yoghurt, low fat
1.6	145	48.82	18.83	0.00	38.57	29.31	106.46	1.70	Low and reduced fat cheese
2.2.2	74	24.92	3.40	0.00	13.63	10.00	39.58	3.17	Spreads (<80% fat)
4.3.8.4	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	Soy beverage, low fat
6.3	102	34.34	16.19	0.00	47.14	45.00	116.85	1.87	Breakfast cereal, mixed flakes
7.1.1	240	80.81	121.49	105.07	150.34	135.40	320.00	4.16	Bread, white, fibre increased
7.2	71	23.90	12.83	0.00	53.68	42.50	166.58	3.83	Biscuits
20.2.3	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	Breakfast bars
20.2.4	38	12.80	2.42	0.00	18.87	14.50	58.23	1.86	Salad dressing/ mayonnaise, low fat
20.2.9	30	10.10	17.82	0.00	176.41	26.49	686.28	2.75	Soup

New Zealand: 20-39 years

Food code	Number of Consumers	Percentage: Consumers to Respondents	Respondent mean food intake (g/d)	Respondent median food intake (g/d)	Consumer mean food intake (g/d)	Consumer median food intake (g/d)	*Consumer P95 food intake (g/d)*	Consumer P95 Phytosterol Intake (g/d)	Food Name
1.1.1.1	483	26.88	53.99	0.00	200.86	144.48	561.46	1.80	Milk, low fat
1.2	162	9.02	13.89	0.00	154.03	150.00	379.18	2.16	Yoghurt, low fat
1.6	863	48.02	19.00	0.00	39.56	28.80	121.46	1.94	Low and reduced fat cheese
2.2.2	459	25.54	2.99	0.00	11.71	10.21	28.15	2.25	Spreads (<80% fat)
4.3.8.4	12	0.67	1.08	0.00	161.63	116.25	517.50	1.66	Soy beverage, low fat
6.3	580	32.28	12.70	0.00	39.36	36.00	84.00	1.34	Breakfast cereal, mixed flakes
7.1.1	1 520	84.59	116.07	97.00	137.22	120.00	320.00	4.16	Bread, white, fibre increased
7.2	595	33.10	14.99	0.00	45.28	32.50	110.26	2.54	Biscuits
20.2.3	7	0.40	0.09	0.00	22.86	25.00	25.00	0.55	Breakfast bars
20.2.4	301	16.80	3.47	0.00	20.73	15.00	62.45	2.00	Salad dressing/ mayonnaise, low fat
20.2.9	201	11.19	30.56	0.00	273.19	260.00	728.00	2.91	Soup

New Zealand: 40+ years

Food code	Number of Consumers	Percentage: Consumers to Respondents	Respondent mean food intake (g/d)	Respondent median food intake (g/d)	Consumer mean food intake (g/d)	Consumer median food intake (g/d)	*Consumer P95 food intake (g/d)*	Consumer P95 Phytosterol Intake (g/d)	Food Name
1.1.1.1	958	37.69	81.32	0.00	215.78	165.60	580.29	1.86	Milk, low fat
1.2	291	11.45	13.25	0.00	115.78	125.00	259.00	1.48	Yoghurt, low fat
1.6	1 072	42.17	13.99	0.00	33.18	23.87	97.64	1.56	Low and reduced fat cheese
2.2.2	661	26.00	3.40	0.00	13.07	10.21	35.33	2.83	Spreads (<80% fat)
4.3.8.4	39	1.53	3.13	0.00	203.97	157.65	517.50	1.66	Soy beverage, low fat
6.3	1 036	40.76	11.84	0.00	29.05	30.00	65.00	1.04	Breakfast cereal, mixed flakes
7.1.1	2 280	89.69	111.73	98.00	124.56	109.16	273.90	3.56	Bread, white, fibre increased
7.2	1 114	43.80	15.13	0.00	34.53	25.40	85.00	1.96	Biscuits
20.2.3	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	Breakfast bars
20.2.4	427	16.80	3.24	0.00	19.27	14.00	60.00	1.92	Salad dressing/ mayonnaise, low fat
20.2.9	382	15.03	46.82	0.00	311.53	283.50	631.65	2.53	Soup

ATTACHMENT 5

DRAFT REGULATORY IMPACT ASSESSMENT

A410 – PHYTOSTEROL ESTERS DERIVED FROM VEGETABLE OILS

Regulatory Impact Analysis

The Authority is required, in the course of development of regulations suitable for adoption in Australia and New Zealand, to consider the impact of various options (including non-regulatory options) on all sectors of the community, including consumers, the food industry and governments in both countries. The regulatory impact assessment will identify and evaluate, though not be limited to, the costs and benefits of the regulation, and its health, economic and social impacts.

Identification of affected parties

1. Governments in Australia and New Zealand
2. Consumers in Australia and New Zealand
3. Manufacturers and producers of food products

Options

Option 1–To prohibit the use of phytosterol esters as a novel food

Benefits

- There is no benefit to government, industry or consumer in not allowing the use of phytosterols or phytosterol esters as novel foods.

Costs

- The cost to government of not allowing the use of phytosterol esters is the lost opportunity to reduce the cost of health care as a result of using a substance reported to reduce plasma cholesterol and, as a consequence, the risk of coronary heart disease.

- The cost to consumers is the loss of an opportunity to use a substance that may reduce the risk of coronary heart disease.

- The cost to industry is the loss of a potential market for a new product.

Option 2 – To permit the use of phytosterol esters in table spreads only

Benefits

- The benefit to government is the opportunity to reduce the cost of health care as a result of using a substance reported to reduce plasma cholesterol and, as a consequence, the risk of coronary heart disease.

- The benefit to consumers is the opportunity to use a substance that may reduce the risk of coronary heart disease.

- The benefit to industry is a potential market for a new product.

Costs

- The cost to government is the enforcement cost only.

- The cost to consumers is the loss of an opportunity to obtain phytosterol esters from a range of food products.

- The cost to industry is the loss of the potential to market phytosterols in a range of food products.

Option 3 – To allow the use of phytosterol esters in a variety of food products

Benefits

- The benefit to government is the opportunity to reduce the cost of health care as a result of using a substance reported to reduce plasma cholesterol and, as a consequence, the risk of coronary heart disease.

- The benefit to consumers is the opportunity to use a substance that may reduce the risk of coronary heart disease.

- The benefit to industry is the opportunity to market phytosterols in a range of food products.

Costs

- The cost to government is the enforcement cost and the potential loss of credibility of allowing consumers to use a novel food ingredient at levels of exposure that have not been shown to be safe.

- The cost to consumers is the uncertainty of using a novel food ingredient which has not been shown to be safe at high levels of consumption. There is also the potential for consumers to be misled and deceived in relation to the labelling, if it has not been shown that phytosterols in foods other than table spreads have not been shown to reduce absorption of cholesterol.

- The cost to industry is the lost of the potential to market phytosterols in a range of food products.

Conclusion of the regulatory impact analysis

Consideration of the regulatory impact for the application to permit the use of phytosterol esters as novel food ingredients concludes that for option 2 only – to permit the use of phytosterol esters in table spreads only – the benefits outweigh the costs for government, consumers and industry.

ATTACHMENT 6

WORLD TRADE ORGANIZATION AGREEMENTS

With the completion of the Uruguay Round of trade negotiations, the World Trade Organization (WTO) was created on 1 January 1995 to provide a forum for facilitating international trade.

The WTO does not engage in any standard-setting activities but is concerned with ensuring that standards and procedures for assessment of and conformity with standards do not create unnecessary obstacles to international trade.

Two agreements which comprise part of the WTO treaty are particularly important for trade in food. They are the;

- Agreement on the Application of Sanitary and Phytosanitary Measures (SPS); and
- Agreement on Technical Barriers to Trade (TBT).

These agreements strongly encourage the use, where appropriate, of international standards, guidelines and recommendations, such as those established by Codex (in relation to composition, labelling, food additives, veterinary drug and pesticide residues, contaminants, methods of analysis and sampling) and the code and guidelines on hygienic practice.

Both Australia and New Zealand are members of the World Trade Organization (WTO) and signatories to the agreements on the Application of Sanitary and Phytosanitary Measures (SPS agreement) and on Technical Barriers to Trade (TBT agreement). Within Australia, the Council of Australian Governments (COAG) has put a memorandum of understanding in place binding all States and Territories to the agreements.

The WTO agreements are predicated on a set of underlying principles that standards and other regulatory measures should be:

- based on sound scientific principles;
- developed using consistent risk assessment practices;
- transparent;
- no more trade-restrictive than necessary to achieve a legitimate objective;
- recognise the equivalence of similar measures in other countries; and
- not used as arbitrary barriers to trade.

As members of the WTO both Australia and New Zealand have an obligation to notify the WTO of changes to food standards to enable other member countries of the WTO to make comment. Notification is required in the case of any new or changed standards which may have a significant trade effect and which depart from the relevant international standard (or where no international standard exists). Matters raised in this proposal may be notified to the WTO as either SPS notifications or TBT notifications, or both.

SPS Notifications

These are primarily health related, and refer to any measure applied:

- to protect animal or plant life from risks arising from the entry, establishment or spread of pests, diseases or disease carrying organisms;

- to protect human or animal life or health from risks arising from additives, contaminants, toxins or disease-carrying organisms in foods, beverages or foodstuffs;

- to protect human life or health from risks arising from diseases carried by animals, plants or products thereof, or from the entry, establishment or spread of pests; and

- to prevent or limit other damage from the entry, establishment or spread of pests.

The Agreement on the Application of Sanitary or Phytosanitary Measures relates to any measure applied to protect animal, plant or human life or health which may directly or indirectly affect international trade. Whether the SPS measure is in the form of a law or mandatory regulation, an advisory guideline, a code of practice or a requirement, it is the purpose of the measure that is important - not its regulatory status. Each WTO member country is entitled to apply SPS measures that are more stringent than the international standards in order to protect the health of its population. In the interests of transparency, each instance of such non-alignment which could result in an impediment to trade must be identified and justified and the documentation of that justification must be readily available

Each member country is also required to apply its methods of risk assessment and management consistently so arrangements under the SPS Agreement do not generate what may really be technical barriers to trade

Under the SPS Agreement, an exporting country can have resort to the WTO's dispute settlement procedures with respect to such a non-alignment. These arrangements mean there is potential for a code of practice to introduce an SPS measure that may bring about non-alignment with international requirements. Such non-alignment would need to be justified scientifically on the grounds that it is necessary to protect human, animal or plant life or health.

TBT Notifications

A technical barrier to trade arises when a mandatory requirement in a country's food regulatory system does not align with the international standard and it is more trade restrictive than is necessary to fulfil a legitimate objective. However, it can be acceptable for a country to have a more stringent requirement than that set internationally for reasons including:

- Maintaining national security;
- Preventing deceptive practices; and
- Protecting human health or safety.

Instances of non-alignment with international standards which could result in trade barriers must be identified and, if questioned, justified. Voluntary codes of practice are not expected to generate technical barriers to trade except where compliance with a code of practice or

some aspect of a code of practice is expected. Consequently, it is possible for a voluntary code of practice to be viewed by the WTO as mandatory and subject to all the notification and other provisions applying to mandatory regulations.

The Agreement on Technical Barrier to Trade relates to requirements covering product characteristics or their related processes and production methods. TBT covers measures that are not SPS, such as requirements relating to terminology, symbols, packaging, marking, labelling, food composition and processing methods.

It is considered that these Full Assessments do constitute a potential Technical Barrier to Trade or a Sanitary/Phytosanitary matter. Matters raised in these Full Assessments therefore will be notified to the WTO.

ATTACHMENT 7

SUMMARY OF PUBLIC SUBMISSIONS

A410 – PHYTOSTEROL ESTERS DERIVED FROM VEGETABLE OILS

Australian Food and Grocery Council

- Support the approval of phytosterol esters as food ingredients.
- Support the establishing standards for identity and purity for phytosterol esters.
- Questioned whether phytosterol esters are novel foods since they have been consumed by the majority of Australians for many years.

Novartis Consumer Health Australia Pty Ltd

- Noted their company's interest in using phytosterol esters derived from tall oils and the similarity of their application to the one from Unilever.
- Suggested that the two application should be considered jointly as the issues raised are similar.
- Support the application to include phytosterol esters from vegetable oils.
- Noted the acceptance worldwide of phytosterols as function food ingredient.

InforMed Systems Ltd

Supports the application as there are no negative effects known.

Food Technology Assoc.

The committee could not reach a conclusion as there was insufficient information presented.
Concerns was expressed in the use of these materials in the market place without approval.
The labels on the current marketed products may not comply with Standard A1 (19).

Mr Richard James, Whangarei, New Zealand

Opposed to unconditional approval of application for phytosterol esters.
Provided information on potential adverse effects of phytosterol esters, including original publications.
Believes that phytosterols will add another level of estrogenic risk from another environmental toxin added the food chain.

National Council of Women of Australia

Concerned that products considered to be novel are currently in the marketplace.
Concerned by the claim made by the applicant that phytosterol esters may lower blood cholesterol.
Concerned about the possible effects of a higher level of phytosterol esters on levels of other nutrients.
Concerned that there is over-reliance on the US FDA's GRAS status for phytosterol esters.
Concerned with the subjective language in the preliminary report, particularly in relation to the company claim of the cholesterol-lowering capacity of phytosterol esters.

Dietitians Association of Australia

Supports the application to allow continued use of phytosterol esters as novel foods in table spread.
Consider such products have proven efficacy for many people with hyperlipidemia.

Nestle Australia Ltd

Supports the application to allow the continued use of phytosterol esters in table spreads.

Dairy Farmers

Supports the application to allow the continued use of phytosterol esters as novel food ingredients.
Requests consideration of extension of the application to permit phytosterol esters in low fat milk, low fat yoghurt, low fat soy beverage and low and reduced fat cheese.
Propose that, in each case, 0.8g of phytosterol esters be allowed per serving and that specific labelling would be used for these products.
Detailed dietary exposure information was provided.

Arnott's Biscuits Ltd/Campbell's Soups Australia

Support the application for the approval of phytosterols as novel food ingredients.
Request consideration of extension of the application to permit phytosterol esters in biscuits and soups.
Extension of the approval of phytosterol esters will assist consumers to construct varied and healthy diets.
Dietary exposure information for biscuits provided.

Valerie James, Whangarei, New Zealand

Expressed concern about the safety, efficacy and reported benefits of phytosterol esters.
Indicated a number of issues which needed to be addressed before approval could be considered.

National Council of Women of New Zealand

Believe that a health claim needs to be clearly substantiated and not used as a marketing strategy.
Until full assessment is available, prefer phytosterol esters not to be approved as novel foods.
Require proof of the health claim that phytosterol esters may lower blood cholesterol.

Goodman Fielder Group Services Pty Ltd

Supports the application for the approval of phytosterol esters as novel food ingredients.
Requests extension of the application to include free phytosterols from vegetable oils.
Requests extension of the application to include permission for phytosterol esters in a wider range of foods.

Considers that this application should be considered together with A417 – Non-esterified phytosterols from tall oils.
Provided detailed information on proposed range of foods which includes: spreads; mayonnaise and creamy dressings; white, fibre-increased bread; mixed flake breakfast cereals; and breakfast bars.
Intention is to add 0.8 g per serving of each food and to recommend consumption of 2-3 serves of any Logical product per day.
Provided detailed dietary exposure information on each food proposed to have phytosterols added.