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**DRAFT ASSESSMENT
(Full Assessment – section 15)**

APPLICATION A417

**TALL OIL NON-ESTERIFIED PHYTOSTEROLS
DERIVED FROM TALL OILS**

00P-1275

RPT 1

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EXECUTIVE SUMMARY

Background

An Application was received from Novartis Consumer Health Australasia Pty Ltd on 5 June 2000 to amend Standard A19 of the Australian *Food Standards Code* (now known as Volume 1 of the *Food Standards Code*) to approve the use of tall oil non-esterified phytosterols derived from tall oils as Novel Food ingredients in a broad range of products.

Since receiving the Application, Volume 2 of the *Food Standards Code* was gazetted in Australia and New Zealand, consequently Standard 1.5.1- Novel Foods in Volume 2 of the *Food Standards Code* will also require an amendment to approve the use of tall oil non-esterified phytosterols derived from tall oils as Novel Food ingredients.

The Applicant further amended the Initial Application on three subsequent occasions – reducing the range of products in which the use of tall oil non-esterified phytosterols derived from tall oils were sought to be approved. Details specifying the amendments to the Initial Application by the applicant are contained in **Attachment 7** to this Draft Assessment. The final Application amendment occurred on 22 August 2001, and sought approval for the use of tall oil non-esterified phytosterols in edible table-spreads only, at a level of 8%(w/w).

For convenience in this Draft assessment tall oil non-esterified phytosterols derived from tall oils will be referred to as tall oil phytosterols (TOPs).

TOPs are extracted from tall oil soap, a by-product of the pulping process used for coniferous trees and then purified in a three-step process. The free phytosterols are structurally related to cholesterol and occur naturally at low levels (up to 0.9%) in common vegetable oils. TOPs are reported to reduce plasma cholesterol levels.

Under Standards A19 and 1.5.1 of the *Food Standards Code*, for a food to be considered novel it must be a non-traditional food, as defined in the Standard. TOPs are considered to be Novel Foods for the purposes of the Standards because they are non-traditional foods that do not have a history of significant human consumption by the broad community to enable safe use of this food in the form or context in which they are proposed to be presented.

During the Draft Assessment (Full assessment – section 15) period evaluations were performed on the safety of TOPs, estimated dietary exposure for mean and high level consumers and an assessment of the likely implications for consumers, industry, and government agencies if approval for use of TOPs as a Novel Foods ingredient is granted.

Consequently in this Application it is proposed that TOPs be added to edible oil spreads only, at a level of 8% (w/w). Phytosterol esters are currently being used in table-spread products following a recent approval as novel foods in June 2001 by the Ministerial Council.

Issues addressed

Current and proposed use

In the USA, vegetable oil-based spreads containing TOPs at a level up to 12 % have self-affirmed Generally Recognised As Safe (GRAS) status. In the European Union, an application for use in table-spreads is being considered under the Novel Food legislation.

TOPs cannot be used in foods by virtue of clause 2 of Standard A19 and Standard 1.5.1 (Volume 1 and Volume 2 of the *Food Standards Code* respectively). This clause specifies that a novel food must not be sold by way of retail sale as a food or for use as a food ingredient unless it is listed in the table to clause 2 of the above Standards, and complies with the conditions of use, if any specified in the table. The above prohibition came into force on 16 June 2001. At present the only novel food that has been approved for inclusion in the Novel Food Standards are Phytosterol esters (which have specific conditions of use).

The Applicant originally sought permission to use TOPs as Novel Food ingredients in a broad range of foods. Following discussions and correspondence with the Applicant in relation to the available data, the Applicant amended the application on a number of occasions (details of amendments to the initial application are contained in Attachment 7). The final amendment to the initial Application, by the applicant, occurred on 22 August 2001 in which the applicant sought to limit the permission for the use of TOPs in:

- Edible oil spreads (less than 80% fat) at a maximum of 8% (w/w).

Safety evaluation

The safety of TOPs has been evaluated in animals and well as in humans. The available animal studies on TOPs indicate that these substances are poorly absorbed from the gastrointestinal tract, have low toxicity, are not genotoxic and demonstrate no reproductive or developmental toxicity. There was also no evidence of oestrogenic activity in the *in vitro* and *in vivo* studies evaluated.

The studies provided by the Applicant can be used to demonstrate the safety of TOPs up to and including a level of 3.6g/day (i.e. total exposure to TOPs, irrespective of the particular food matrix). There is no evidence of adverse health effects in the human studies, apart from some minor non-significant reductions in vitamin A at doses of 0.9, 1.8 and 3.6g/day without a dose-relationship. There is, however, a decrease in the plasma levels of carotenoids as a result of exposure to TOPs in a milk-based beverage at the highest dose (3.6g/day) over a 4-week period. While the decrease in carotenoid levels observed following exposure 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 TOPs exposure.

Estimated dietary exposure

Dietary modelling was conducted on the proposed uses of TOPs in spreads to determine the dietary intakes for the mean and the 95th percentile consumers (Australian and New Zealand consumers). The maximum level of exposure for a consumer in the target group of 40 years and above was the following:

- Mean consumers (1.3g/day Australian consumers; 1g/day New Zealand consumers) and 95th percentile consumption (3.5 g/day Australian consumers; 2.8 g/day New Zealand consumers).

Effect on cholesterol absorption

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

Advice to consumers

With phytosterol enriched foods, there is a clear intention to market the reported beneficial effects of TOPs on blood cholesterol and therefore it is important that these foods be consumed as part of a healthy and varied diet that is low in saturated fats and high in fruit and vegetables (by virtue of potential to reduce plasma carotenoid levels). A mandatory advisory statement to this effect is proposed.

There is also a need to protect at-risk groups (children, pregnant and lactating women) in relation to any potential reduction in plasma carotenoid levels as a result of phytosterol intake, although there are limited effects noted from available studies in humans with TOPs. Additionally, in these groups it may not be generally appropriate to reduce cholesterol levels without medical supervision. A mandatory advisory statement indicating that TOP-enriched foods are not recommended for these groups is also proposed.

The use of phytosterol containing spreads should not be considered a substitute for cholesterol-lowering medication and a mandatory advisory statement is proposed that will indicate the need for consumers on such medication to seek medical advice regarding the use of the product in conjunction with their medication. In relation to the ability of phytosterols to reduce cholesterol absorption, this will be considered in the light of the current review of the framework for health claims.

Conclusions

Overall, the data supports the safety of TOPs at the level of intake, which would be achieved by their addition to edible oil spreads at 8% (w/w).

The conclusions from the risk assessment are as follows:

- There is no public health and safety concern associated with the use of TOPs in edible oil spreads at a maximum concentration of 8% (w/w).
- There is some evidence from the available data that TOPs, when incorporated into edible oil spreads at 8% (w/w), can reduce the level of plasma cholesterol in humans.

- Mandatory advisory statements are required to ensure that consumers use phytosterol enriched edible oil spreads appropriately.
- The proposed changes to Volume 1 and Volume 2 of 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.

Recommendations arising from the Risk Assessment and Regulatory Impact Analysis

On the basis of the available data on the safety of TOPs, permission should not be broadened to foods other than edible oil spreads at this stage. The level in edible oil spreads should be limited to 8% (w/w). TOP 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 edible oil spreads containing TOPs are used appropriately by consumers the following mandatory advisory statements should be used:

- *A mandatory advisory statement to the effect that the product should be consumed in moderation as part of a diet low in saturated fats and high in fruit and vegetables.*
- *A mandatory advisory statement to the effect that the product is not recommended for infants, children, and pregnant or lactating women.*
- *A mandatory advisory statement to the effect that consumers on cholesterol-lowering medication should seek medical advice about using the product in conjunction with their medication.*

INTRODUCTION

The Australia New Zealand Food Authority (ANZFA) is a joint statutory body responsible for developing draft food standards and draft variations of standards, to make recommendations to the Australia New Zealand Food Standards Council (ANZFSC) in relation to those drafts, and to review standards. ANZFSC may then decide to adopt the draft standards or draft variations of standards, which when approved by ANZFSC are adopted by reference and without amendment into food laws of the Australian States and Territories.

On 24 November 2000, ANZFSC adopted the *Australia New Zealand Food Standards Code* (known as Volume 2 of the *Food Standards Code*) that will apply in both Australia and New Zealand. A two-year transitional period has been implemented at the conclusion of which Volume 2 of the *Food Standards Code* will be the sole Code for both countries. In the interim, for the majority of the food standards, there is a system of dual standards operating in both Australia and New Zealand.

Standard A19 - Novel Foods – was gazetted on 16 December 1999 and came into effect on 16 June 2001 following an 18-month implementation period. The Novel Foods Standard is incorporated in both Volume 1 (as Standard A19) and Volume 2 (as Standard 1.5.1) of the *Food Standards Code*. Standard A19 and Standard 1.5.1 prohibit a novel food being sold by way of retail sale as food, or for use as a food ingredient, unless it is listed in the Table to clause 2 of the Standard, and complies with any special conditions specified in that Table. This Draft assessment includes proposed draft variations for both Volume 1 and Volume 2 of the *Food Standards Code*.

The purpose of the Standard A19 and Standard 1.5.1 is to ensure that non-traditional foods that have features or characteristics that may raise safety concerns will undergo a risk-based safety assessment before they are offered for retail for consumption in Australia or New Zealand. Because the Standards have 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 Standards 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/Standard 1.5.1 – Novel Foods*. A decision in this regard is made in consultation with the Senior Food Officers in each of the States, Territories and New Zealand.

BACKGROUND

The focus of the assessment of Novel Foods is primarily related to safety, to ensure that non-traditional foods that have features or characteristics, which raise safety concerns will undergo a risk-based safety assessment before they are offered for retail for direct consumption in Australia and New Zealand. Consequently the Authority assess the safety for human consumption of each novel food prior to its inclusion in the Table to clause 2 of Standards A19 and 1.5.1. 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: (a) the protection of public health and safety; (b) the provision of adequate information relating to food to enable consumers to make informed choices; and (c) the prevention of misleading or deceptive conduct.

Previous Phytosterol application-Application A410-Phytosterol esters as Novel Foods

ANZFA received an Application (A410) 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 and Standard 1.5.1 – Novel Foods of the *Food Standards Code*. The manufacturers claimed that incorporation of phytosterol esters into the diet was an effective way of lowering total and Low Density Lipoprotein (LDL) cholesterol levels in the blood.

The Application was subsequently assessed by ANZFA according to the *Australia New Zealand Food Authority Act 1991*, and the Ministerial Council approved the addition of phytosterol esters as novel food ingredients in oil spreads in June 2001.

Tall oil phytosterols as Novel Foods-Application A417 (Current Application)

For convenience in this report tall oil non-esterified phytosterols derived from tall oils will be referred to as tall oil phytosterols (TOPs).

This current application was received from Novartis Consumer Health Australasia Pty Ltd on 5 June 2000 to amend Standard A19 Novel Foods – to approve the use of TOPs derived from tall oils as Novel Foods ingredients in a broad range of products.

Since receiving the Application, Volume 2 of the *Food Standards Code* was gazetted in Australia and New Zealand, consequently Novel Foods Standard 1.5.1-Novel Foods in Volume 2 of the *Food Standards Code* will also require an amendment to approve the use of TOPs as Novel Food ingredients.

The Applicant further amended the initial Application on three subsequent occasions – reducing the range of products in which TOPs was sought to be used. Details specifying the amendments to the initial Application by the applicant are contained in **Attachment 7**. The final Application amendment by the applicant on 22 August 2001, sought approval of the use of TOPs as Novel Foods in edible table spreads only, at a level of 8%(w/w).

Under Standards A19 and 1.5.1 of the *Food Standards Code*, for a food to be considered a novel it must be a non-traditional food, as defined in the Standard. TOPs are considered to be Novel Foods for the purposes of the Standards because they are non-traditional foods that do not have a history of significant human consumption by the broad community to enable safe use of this food in the form or context in which they are proposed to be presented. TOPs are predominantly a mixture of four phytosterols: sitosterol, sitostanol, campesterol and campestanol, extracted from tall oil soap which is a bi-product of the pulping process used for coniferous trees in North America and Europe.

Phytosterols are found naturally in plant foods at low levels. TOPs are extracted from tall oil soap, a by-product of the pulping process used for coniferous trees and then purified in a three-step process. The free phytosterols are structurally related to cholesterol and occur naturally at low levels (up to 0.9%) in common vegetables. TOPs are reported to reduce plasma cholesterol levels. It is claimed by the applicant that incorporation of additional phytosterols, namely, TOPs into the diet may be an effective way of lowering total and LDL cholesterol levels.

During the assessment period, ANZFA requested the Applicant to provide further data to support the Application. The final data was received on 6 July 2001 and this led to the applicant advising ANZFA that they wished to further amend their initial application to allow the use of TOPs as Novel Foods in edible table spreads only, at a level of 8% (w/w). Application amendment details are contained in Attachment 7. The amendments to the Initial Application were in light of the fact that the safety data supplied by the applicant could not support all the proposed food uses, other than edible oil spreads.

In summary, this Application is similar to Application A410 in that permission is sought for use of a novel food ingredient, namely, TOPs in edible oil spreads at a level of 8% (w/w).

Approval of TOPs in other countries

Codex

There are no Codex standards in relation to TOPs.

Approval in other Countries

In the USA, vegetable oil-based spreads containing TOPs at a level up to 12 % have self-affirmed Generally Recognised As Safe (GRAS) status. In the European Union, an application for use in table spreads is being considered under Novel Food legislation.

SCIENTIFIC ASSESSMENT

Detailed reports on the safety, technological aspects and expected dietary exposure to TOPs have been prepared (Attachments 2,3 and 4).

Overall Summary of Safety Assessment

The safety assessment has concluded that TOPs have low toxicity, are poorly absorbed and are efficiently excreted via the faeces. The human studies conducted with TOPs although of limited duration, provide no evidence of adverse health effects at the dose level studied. There is no data to demonstrate safety at higher levels of exposure, which would occur from use in a broader range of foods.

The studies provided by the Applicant can be used to demonstrate the safety of TOPs up to and including a level of 3.6g/day (i.e. total exposure to TOPs, irrespective of the particular food matrix). There is, however, a decrease in the plasma levels of carotenoids as a result of exposure to TOPs in a milk-based beverage at the highest dose (3.6g/day) over a 4-week period. While the decrease in carotenoid levels observed following exposure 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 TOPs exposure.

Overall Summary of Food Technology Report

The applicant has indicated, in the currently available data, confidence in the technology to overcome the limited lipid solubility of free phytosterols and stanols that has previously been the biggest obstacle to efficiently blending these products into foods.

Overall Summary of Dietary Exposure Assessment

Dietary modelling was conducted on the proposed uses of TOPs in table spreads to determine the dietary intakes for the mean and the 95th percentile consumers (Australian and New Zealand consumers). The maximum level of exposure for a consumer in the target group of 40 years and above was the following:

- Mean consumers (1.3g/day Australian consumers; 1g/day New Zealand consumers) and 95th percentile consumption (3.5 g/day Australian consumers; 2.8 g/day New Zealand consumers).

The Issue

The issue is to consider whether approval should be recommended for the use of TOPs in edible oil spreads.

Objectives

ANZFA's overarching section 10 objectives in the *Australia New Zealand Food Authority Act 1991* (section 10 objectives of ANZFA) are:

1. Protection of public health and safety;
2. Provision of adequate information relating to food to enable consumers to make informed choices; and
3. Prevention of misleading or deceptive conduct.

The specific objectives for this application are:

1. To provide reasonable assurance that TOPs consumed from edible oil spreads is safe for consumers;
2. To provide adequate information to consumers that will contribute to their safe consumption of TOPs.

Options

Option 1: To not allow the use of TOPs as novel food ingredient.

Option 2: To allow TOPs as a novel food ingredient in edible oil spreads.

If Option 2 were chosen, there would be requirements for labelling. See discussion in the Impact Analysis and Conclusion sections.

Impact Analysis

The affected parties in this application are -

- Manufacturers of food products in Australia and New Zealand that are intending to market new table spreads with TOPs as an ingredient.
- Consumers, particularly those that have or are at risk of developing high cholesterol levels in the blood. Consumers taking cholesterol-reducing medication. Infants, children and pregnant or lactating women.

- Governments of the States, Territories, Commonwealth and New Zealand.

Option 1: To not allow the use of TOPs as novel food ingredient.

Impact on Consumers and the Community

Consumers would incur a cost from the inability to choose edible oil spreads, particularly those consumers that may be brand loyal to a particular product or company. The community would incur a cost from the loss of a food, which may lower blood cholesterol levels.

Consumers and the community would incur no benefit,

Impact on Business

The impact on business of this option is the loss of a potential market for TOPs in table spreads and business will lose the ability to introduce new products. This option would result in a cost to business. As there is already a table spread marketed with phytosterol esters as an ingredient, this option may be in breach of fair trading considerations and allow a market in one particular product (phytosterol esters) to be the sole products available.

Option 2: To allow TOPs as a novel food ingredient in edible oil spreads.

This option imposes a maximum limit on the amount of TOPs in spreads in accordance with the available safety data.

Impact on Consumers and the Community

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

A cost to consumers would be the lack of choice in spreads that contain phytosterols – except current ones that are esterified. This disadvantages consumers who may be ‘brand loyal’ and prefer to purchase a specific product.

A major issue is what information consumers need to make informed choices about TOPs in spreads, and how it should be presented. There are three sets of behaviours that would be outside the specified conditions of the evidence and where advice would avoid costs to consumers and the community.

1. Where consumers believe that this product can compensate for an unhealthy diet and use this product rather than changing their dietary habits. Costs to consumers in general would flow from a diet that increased or maintained high cholesterol in the blood. To address this cost, mandatory advice in this situation to the effect that *the product should be consumed in moderation as part of a diet low in saturated fats and high in fruit and vegetables* is recommended.
2. Where the product is consumed by infants, children and pregnant or lactating women. The consequence of reducing blood cholesterol in these population groups is unclear and may in some cases be adverse.

To address this consequence, mandatory advice in this situation to the effect that *the product is not recommended for infants, children and pregnant or lactating women unless under medical supervision* is recommended.

3. Where consumers are using cholesterol-lowering medication. There is a potential cost to these consumers from poor management of their high blood cholesterol. To address this potential cost, mandatory advice in this situation to the effect that *consumers on cholesterol-lowering medication should seek medical advice on the use of this product in conjunction with their medication* is recommended.

The manufacturers of spreads have commercial incentives to provide this kind of information to consumers and indeed they are already providing it on product labels (eg table spreads containing phytosterol esters). These labelling requirements relate to the information to be provided and do not mandate uniform wording. These requirements will confirm good practice in the industry.

Impact on Business

Businesses that currently manufacture phytosterol ester table spreads may be advantaged by a prohibition on adding TOPs to other products. This may lead to an ability of these businesses to increase their prices. The extent of this effect depends on whether consumers are specifically demanding phytosterol esters – where there could be an increase in demand for table spreads if other products are not allowed– or whether consumers prefer phytosterol esters in the usual basket of goods that they buy – where withdrawal of other products would have no impact on table spreads.

The impact of the labelling requirements on table spreads should be minimal because manufacturers already provide the required information on their packaging.

Impact on Government

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

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

CONSULTATION AND ISSUES RAISED IN PUBLIC SUBMISSIONS

ANZFA conducted a Preliminary Assessment on A417 – Tall oil non-esterified phytosterols derived from tall oils – and public comments on the application were called for on 23 August 2000. A total of 14 submissions were received and are summarised in **Attachment 6**. **Eight** submissions **supported** the application, **5 did not support** it and one was non-committal until ANZFA had performed a risk analysis.

The issues raised below in these submissions relate mainly to safety and efficacy. The safety of TOPs is considered below. The efficacy of phytosterols to reduce plasma cholesterol has not been specifically addressed but other issues in relation to this matter are discussed below.

1. Safety of TOPs

Submissions received raised concerns over the general safety of phytosterols and whether there was any potential for oestrogenic effects.

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

The safety of TOPs has been evaluated in animals and well as in humans. The available animal studies on TOPs indicate that these substances are poorly absorbed from the gastrointestinal tract, have low toxicity, are not genotoxic and demonstrate no reproductive or developmental toxicity. There was also no evidence of oestrogenic activity in the *in vitro* and *in vivo* studies evaluated.

There is no evidence of adverse health effects in the human studies, apart from some minor non-significant reductions in vitamin A at doses of 0.9, 1.8 and 3.6g/day without a dose-relationship. There is, however, a decrease in the plasma levels of carotenoids as a result of exposure to TOPs in a milk-based beverage at the highest dose (3.6g/day) over a 4-week period. While the decrease in carotenoid levels observed following exposure 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 TOPs exposure.

2. Potential dietary exposure to TOPs

Submissions raised the issue that dietary exposure evaluations should be undertaken by ANZFA to determine the amount of likely phytosterol intake in the general population.

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

Dietary modelling was conducted to estimate the dietary intake of TOPs as a result of proposed 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.

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 edible oil spreads to which TOPs, are proposed to be added.

The data indicates that consumption of edible oil spreads containing TOPs at 8% (w/w) in the target group of 40 years and above, would lead to a mean consumption of 1.3g/day in Australia and 1g/day in New Zealand. The same spread would lead to a 95th percentile intake of approximately 3.5 g/day in Australia and 2.8 g/day in New Zealand.

The maximum level of exposure in the 4-week study in a milk-based beverage (3.6g/day) exceeded the intake of TOPs at (8% w/w) from table spreads for a consumer at the mean and 95th percentile consumption. High consumers (Australian only) in the age group 20-39 years marginally exceeded the 3.6g/day level with a 95th percentile intake of 3.8g/day; however, this is unlikely to be a safety issue as this is not the proposed target group.

3. Nutritional and/or health claims associated with tall-oil phytosterols

Submissions suggested that there must be supporting data if a 'claim' is being made that TOPs reduce cholesterol levels.

Health claims are currently prohibited under Standard A1 clause 19 of Volume 1 of the *Food Standards Code* and Standard 1.1.3 of Volume 2 of the *Food Standards Code*, therefore products containing TOPs are prohibited from making health claims (unless specific permission is given under the *Food Standards Code*).

ANZFA has recently (31 July 2001) made a recommendation to the Ministerial Council on Health Claims (Proposal P153); however, the Council has deferred its decision until further considerations by ANZFA are made.

There is no evaluation of any health claim being considered as part of this application. Any application for a health claim for TOPs in the future would need to be considered in the context of 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.

4. Effect of phytosterols on absorption of cholesterol

Submissions indicated that proof should be supplied that TOPs actually lower blood cholesterol.

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'. These statements are consistent with the third objective in section 10 of the Australia New Zealand Food Authority Act 1991, namely, *the prevention of misleading or deceptive conduct*.

The effectiveness of TOPs 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. Total plasma cholesterol was reduced by 5-7% and low density lipoprotein (LDL) by 5-14% at a dosage of 1.5g/day with a vegetable oil matrix (10-days); 9% and 14% respectively with margarine at 1.5g/day (30-days); 4% and 3% with a cereal bar at 1.8g/day (8-weeks) and; 9% and 13% respectively with a milk based beverage at 3.6 g/day (4-weeks) (see **Attachment 2**).

A more thorough examination of the evidence to substantiate a 'health claim' in relation to TOPs will, however, be required under any future standard established to regulate health claims.

5. Labelling information for consumers

Submissions raised concerns over the indiscriminate use of TOPs, particularly, in high-risk groups (eg children and pregnant women).

(1) Use of tall-oil phytosterols 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 TOPs can lead to lower plasma cholesterol by reducing the absorption of cholesterol from the diet, phytosterols are ineffective in preventing the elevation of plasma cholesterol that is a consequence of the ingestion of saturated fatty acids.

Where TOPs 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 and high in fruit and vegetables.

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

(2) Restriction for at-risk groups

Specific groups in the population considered to be at risk regarding reduced plasma carotenoid levels are infants, children, pregnant and lactating women. Additionally, it may be inappropriate to reduce cholesterol in these groups without consulting a medical practitioner that could conduct a thorough clinical evaluation to determine the needs.

Therefore, ANZFA is proposing that 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 not recommended for infants, children, and pregnant or lactating women unless under medical supervision.

The above requirement is justified under the first section 10 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 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 on cholesterol-lowering medication should seek medical advice on the use of this product in conjunction with their medication.

The above requirement is justified under the first section 10 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 TOPs when incorporated in edible oil spreads can reduce plasma cholesterol levels, the ability of TOPs 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 TOPs 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 TOPs will be required.

CONCLUSIONS

- There are no public health and safety concerns associated with the use of TOPs in edible oil spreads at a maximum concentration of 8% (w/w). The available data is insufficient to assess the safety of TOPs at higher levels of exposure.
- There is some evidence from the available data that TOPs when incorporated into a spread at 8% (w/w) can reduce the level of plasma cholesterol.
- Mandatory advisory statements are required to ensure that consumers use TOP-enriched 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.

Assessment of regulatory options

Option 1 would not allow TOPs as an ingredient in spreads. While it avoids the possibility of over-consumption of edible oil spreads containing TOPs and phytosterol esters, it imposes costs on consumers of loss of choice of new products where their safety and efficacy have been established.

Option 2 allows TOPs in spreads, which by virtue of the data submitted, have been shown to be safe, and their blood-cholesterol lowering substantiated. Option 2 does not subject consumers, the community or Governments to other costs.

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

Recommendations

It is concluded that the safety of TOPs from the consumption of spreads containing 8% (w/w) TOPs has been established. TOPs added to these foods should also conform to the specifications indicated in **Attachment 1**.

However, in order to ensure that TOPs – enriched 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 and high in fruit and vegetables.*
- *A statement to the effect that the product is not recommended for infants, children, and pregnant or lactating women unless under medical supervision.*
- *A statement to the effect that consumers on cholesterol-lowering medication should seek medical advice on the use of this product in conjunction with their medication.*

The proposed drafting in Volume 1 and Volume 2 of the *Food Standards Code* is shown in **Attachment 1**.

Implementation and Review

Monitoring and review of the impact of this regulatory change would be highly desirable. A cost-effective method to do so has not been identified. However, this application will be referred to a project within ANZFA to improve the monitoring and evaluation of changes to food standards.

WORLD TRADE ORGANIZATION

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 5**). 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.

This application will be notified to the WTO because permission to use TOPs would have a liberalising effect on trade via removal of the prohibition on the sale of these novel food ingredients. There are no international standards in relation to TOPs.

FOOD STANDARDS SETTING IN AUSTRALIA AND NEW ZEALAND

The Governments of Australia and New Zealand entered an Agreement in December 1995 establishing a system for the development of joint food standards. On 24 November 2000, Health Ministers in the Australia New Zealand Food Standards Council (ANZFSC) agreed to adopt the new *Australian New Zealand Food Standards Code*. The new Code was gazetted on 20 December 2000 in both Australia and New Zealand as an alternate to existing food regulations until December 2002 when it will become the sole food code for both countries. It aims to reduce the prescription of existing food regulations in both countries and lead to greater industry innovation, competition and trade.

Until the joint *Australian New Zealand Food Standards Code* is finalised the following arrangements for the two countries apply:

- **Food imported into New Zealand other than from Australia** must comply with either Volume 1 (known as *Australian Food Standards Code*) or Volume 2 (known as the joint *Australia New Zealand Food Standards Code*) of the *Australian Food Standards Code*, as gazetted in New Zealand, or the *New Zealand Food Regulations 1984*, but not a combination thereof. However, in all cases maximum residue limits for agricultural and veterinary chemicals must comply solely with those limits specified in the *New Zealand (Maximum Residue Limits of Agricultural Compounds) Mandatory Food Standard 1999*.
- **Food imported into Australia other than from New Zealand** must comply solely with Volume 1 (known as *Australian Food Standards Code*) or Volume 2 (known as the joint *Australia New Zealand Food Standards Code*) of the *Australian Food Standards Code*, but not a combination of the two.
- **Food imported into New Zealand from Australia** must comply with either Volume 1 (known as *Australian Food Standards Code*) or Volume 2 (known as *Australia New Zealand Food Standards Code*) of the *Australian Food Standards Code* as gazetted in New Zealand, but not a combination thereof. Certain foods listed in Standard T1 in Volume 1 may be manufactured in Australia to equivalent provisions in the *New Zealand Food Regulations 1984*.
- **Food imported into Australia from New Zealand** must comply with Volume 1 (known as *Australian Food Standards Code*) or Volume 2 (known as *Australia New Zealand*

Food Standards Code) of the Australian *Food Standards Code*, but not a combination of the two. However, under the provisions of the Trans-Tasman Mutual Recognition Arrangement, food may **also** be imported into Australia from New Zealand provided it complies with the New Zealand *Food Regulations 1984*.

- **Food manufactured in Australia and sold in Australia** must comply with Volume 1 (known as Australian *Food Standards Code*) or Volume 2 (known as *Australia New Zealand Food Standards Code*) of the Australian *Food Standards Code* but not a combination of the two. Certain foods listed in Standard T1 in Volume 1 may be manufactured in Australia to equivalent provisions in the New Zealand *Food Regulations 1984*.

In addition to the above, all food sold in New Zealand must comply with the New Zealand *Fair Trading Act 1986* and all food sold in Australia must comply with the Australian *Trade Practices Act 1974*, and the respective Australian State and Territory *Fair Trading Acts*.

Any person or organisation may apply to ANZFA to have the *Food Standards Code* amended. In addition, ANZFA may develop proposals to amend the Australian *Food Standards Code* or to develop joint Australia New Zealand food standards. ANZFA can provide advice on the requirements for applications to amend the *Food Standards Code*.

INVITATION FOR PUBLIC SUBMISSIONS

Written submissions containing technical or other relevant information which will assist the Authority in undertaking a draft assessment on matters relevant to the application, including consideration of its regulatory impact, are invited from interested individuals and organisations. 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 confidential information contained in a submission to remain confidential to the Authority, you should clearly identify the sensitive information and provide justification for treating it 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.

Following its draft assessment of the application the Authority may prepare a draft standard or draft variation to a standard (and supporting draft regulatory impact statement), or decide to reject the application. If a draft standard or draft variation is prepared, it is then circulated to interested parties, including those from whom submissions were received, with a further invitation to make written submissions on the draft. Any such submissions will then be taken into consideration during the inquiry, which the Authority will hold to consider the draft standard or draft variation to a standard.

All correspondence and submissions on this matter should be addressed to the **Project Manager - Application A417** at one of the following addresses:

Australia New Zealand Food Authority	Australia New Zealand Food Authority
PO Box 7186	PO Box 10559
Canberra BC ACT 2610	The Terrace WELLINGTON 6036
AUSTRALIA	NEW ZEALAND
Tel (02) 6271 2222 Fax (02) 6271 2278	Fax (04) 473 9942 Fax (04) 473 9855

Submissions should be received by the Authority by: **21 November 2001.**

ATTACHMENTS

1. Draft variation to Volume 1 and Volume 2 of the *Food Standards Code*
2. Safety assessment report
3. Food technology report
4. Dietary exposure assessment report
5. World Trade Organization Agreements
6. Summary of public submissions
7. Amendments of the application by the Applicant

ATTACHMENT 1

DRAFT VARIATION TO VOLUME 1 AND VOLUME 2 OF THE FOOD STANDARDS CODE

To commence: on gazettal

[1] **Standard A19** of Volume 1 is varied by inserting in the Table to clause 2, into Column 1 and Column 2 respectively –

tall oil phytosterols	<p>May only be added to food -</p> <p>(1) according to Standard G2 or G5 and Standard A11; and</p> <p>(2) where the total fatty acid present in the food is not more than 280g/kg of saturated fatty acids.</p> <p>The name 'tall oil phytosterols' must be used when declaring the ingredient in the ingredient list, as prescribed in clause 5 of Standard A1.</p> <p>The label on or attached to a package of food containing Tall oil phytosterols must include statements to the effect that -</p> <ol style="list-style-type: none"> 1. the product should be consumed in moderation as part of a diet low in saturated fats and high in fruit and vegetables; 2. the product is not recommended for infants, children and pregnant or lactating women unless under medical supervision; and 3. consumers on cholesterol-lowering medication should seek medical advice on the use of this product in conjunction with their medication.
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[2] **Standard A11** of Volume 1 is varied by-

[2.1] *inserting after* ADDENDUM 10 –

ADDENDUM 11

SPECIFICATION FOR TALL OIL PHYTOSTEROLS DERIVED FROM TALL OILS

Tall oil phytosterols (non-esterified) are derived from tall oil soap, a by-product of the pulping process, and then purified.

Major phytosterol/phytostanol content (%)	min. 95.0
Minor sterol content (%)	max. 5.0
Loss on drying (water) (%)	max. 5.0
Solvents (%)	max. 0.5
Residue on ignition (%)	max. 0.1

Total heavy metals (ppm)	max. 10
Cadmium (ppm)	max. 1.0
Mercury (ppm)	max. 1.0
Arsenic (ppm)	max. 2.0
Lead (ppm)	max. 0.25
Total aerobic count (CFU/g)	max. 10,000
Combined moulds and yeasts (CFU/g)	max. 100
Coliforms	Negative to test
E. coli	Negative to test
Salmonella	Negative to test

Major Sterol profile (%) as below –		
Campesterol	min. 4.0	max. 25.0
Campestanol	min. 0.0	max. 14.0
β-Sitosterol	min. 36.0	max. 79.0
β-Sitostanol	min. 6.0	max. 34

Minor Sterol profile (%) as below –	min. 0.0	max. 5.0
α-Sitosterol		
Stigmasterol		
Ergosterol		

[2.2] inserting in the Schedule to A11 into Column 1 and Column 2 respectively, after the entry for Talc –

Tall oil phytosterols Addendum 11

[3] **Standard G2** of Volume 1 is varied by omitting subparagraph (1)(b)(ii)(J), and substituting –

- (J) not more than 137 g/kg of phytosterol esters;
- (K) not more than 80 g/kg of tall oil phytosterols.

[4] **Standard G5** of Volume 1 is varied by omitting paragraph 2(3)(o), and substituting –

- (o) not more than 137 g/kg of phytosterol esters;
- (p) not more than 80 g/kg of tall oil phytosterols.

[5] **Standard 1.2.3** of Volume 2 is varied by inserting in the Table to clause 2, into Column 1 and Column 2 respectively –

<p>Food regulated in Standard 2.4.2 containing tall oil phytosterols.</p>	<p>Statements to the effect that -</p> <ol style="list-style-type: none"> 1. the product should be consumed in moderation as part of a diet low in saturated fats and high in fruit and vegetables; 2. the product is not recommended for infants, children and pregnant or lactating women unless under medical supervision; and 3. consumers on cholesterol-lowering medication should seek medical advice on the use of this product in conjunction with their medication.
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[6] **Standard 1.3.4** of Volume 2 is varied by inserting in clause 4, into the Schedule after the entry Specification for phytosterol esters derived from vegetable oils –

Specification for tall oil phytosterols derived from tall oils

Tall oil phytosterols (non-esterified) are derived from tall oil soap, a by-product of the pulping process and then purified.

Major Phytosterol/phytostanol content (%)	min. 95
Minor Sterol content (%)	max. 5.0
Loss on drying (water (%)	max. 5.0
Solvents (%)	max. 0.5
Residue on ignition (%)	max. 0.1
Total Heavy metals (ppm)	max. 10
Cadmium (ppm)	max. 1.0
Mercury (ppm)	max. 1.0
Arsenic (ppm)	max. 2.0
Lead (ppm)	max. 0.25
Total aerobic count (CFU/g)	max. 10,000
Combined moulds and yeasts (CFU/g)	max. 100
Coliforms	Negative to test
E. coli	Negative to test
Salmonella	Negative to test

Major Sterol profile (%) as below –

Campesterol	min. 4.0	max. 25.0
Campestanol	min. 0.0	max. 14.0
β-Sitosterol	min. 36.0	max. 79.0
β-Sitostanol	min. 6.0	max. 34

Minor Sterol profile (%) as below –

α-Sitosterol	min. 0.0	max. 5.0
Stigmasterol		
Ergosterol		

[7] **Standard 1.5.1** of Volume 2 is varied by inserting in the Table to clause 2, into Column 1 and Column 2 respectively –

tall oil phytosterols	<p>The requirements in clause 2 of Standard 1.2.3.</p> <p>The name 'tall oil phytosterols' must be used when declaring the ingredient in the ingredient list, as prescribed in Standard 1.2.4.</p> <p>May only be added to food -</p> <p>(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.</p>
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[8] **Standard 2.4.2** of *Volume 2* is varied by omitting paragraph 2(1)(e) and 2(1)(f), and substituting –

- (e) milk products; and
- (f) no more than 137 g/kg of phytosterol esters; and
- (g) no more than 80 g/kg of tall oil phytosterols.

Safety Assessment of Tall oil non-esterified phytosterols (TOPs).

Summary

Absorption, Distribution, Metabolism and Excretion (ADME)

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

The major sterols and sterol esters in the phytosterol ester product under Application A410-Phytosterol esters derived from vegetable oils were tested in rats *in vivo* to compare their uptake, tissue distribution, metabolism and excretion with those of cholesterol and cholesterol esters. The summary is as follows:

- The rats adequately tolerated dosing with the sterols sitosterol, sitostanol, stigmasterol, campesterol, and campestanol, and also sitosterol-esters. Sitosterol, sitostanol, stigmasterol and campestanol, in addition to the linoleate ester of sitosterol, were poorly absorbed (between 1.2 and 5% of dose in females, and 0.5 - 1.9% in males), whereas a greater proportion of campesterol and cholesterol were absorbed (12 - 27% in females, 24% cholesterol absorbed in males).
- Sterols were found in tissues at low concentrations. Sitosterol and sitostanol were found in the adrenals, ovary and stomach at low concentrations, campestanol in the adrenals, ovaries and intestinal epithelia, and campesterol in the adrenals, spleen, intestinal epithelia, ovaries, liver and bone marrow.
- The greater proportion of each of the phytosterols investigated was eliminated in the faeces, as both the free sterol and sterol esters, suggesting that some esterification of sterols occurs in the gut *in vivo*. A minor faecal metabolite was observed in various studies, but this was not characterised and may have been an oxidation product, although from *in vivo* or *ex vivo* storage was not clear.

Sub-chronic Studies

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

Chronic Studies

No chronic studies or carcinogenicity studies were submitted.

Developmental study

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

Reproduction studies

A 2 generation reproduction study in rats dosed with up to 5% phytosterols in the diet (as a mixture of sterols and sterol-esters at up to 8.0 %) equivalent to up to 4.4 g/kg/day, for 10 weeks prior to mating, then throughout gestation, lactation and weaning, found no significant effect on clinical, growth or reproductive parameters in either the F₀ or F₁ generations.

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

Genotoxicity studies

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

Other animal studies

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

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.

In vivo oestrogenic potential

A series of studies were conducted to examine the uterotrophic potential of the dietary sterols, using various sterols and their mixtures in rats by gavage. The end point determined was the wet weight of uterus. Phytosterols, phytosterol esters, cholesterol and cholesteryl palmitate were all found to be negative in this assay system at doses of up to 500 mg/kg/day for 3 days. Positive controls coumestrol and β-estradiol both gave positive responses (increased uterine weights) at doses of 20 and 0.4 mg/kg/day respectively.

Efficacy Studies in animals

These studies, while primarily efficacy studies, do provide some limited information on the toxicity of TOPs.

A short-term (10-day) study in rats was submitted. In this study, TOPs at up to 1% w/w in diet (1000 mg/kg bw/day) were well tolerated by rats with no reduction in growth rates.

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

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

Human Studies

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

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

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

Vitamin A and E and alpha and beta-carotene levels were compared at the start of treatment and at week 4 post-treatment. Reductions were noted in vitamin A levels post-treatment at doses of 0.9, 1.8 and 3.6g/day (10%, 12% and 9% respectively). However, this lacked a dose-response and there were no other significant differences between treatment groups between day 0 and week 4 post-treatment with respect to either the change or the relative change in vitamin A levels between the start and 4-week treatment levels. There were no significant differences between groups with respect to vitamin E levels.

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

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

Discussion

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

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

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

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

There is evidence that plasma levels of carotenoids can vary seasonally by up to 30% depending on the availability of fruit and vegetables. The reductions seen in the 28-day milk study are well within this variation and do not raise concerns *per se*.

The overall conclusion from the human studies was that administration of TOPs in the diet at 3.6g/day for a period up to 28 days and at 1.8g/day for an 8-week period was well tolerated. The available data is not adequate to assess the safety of TOPs at the higher level of exposure that could result from their use in a broader range of foods.

Introduction

Phytosterols are a group of plant compounds that are naturally occurring at low levels in a variety of foods (oils, margarines and fats) in the human diet. Tall oil non-esterified phytosterols (TOPs) are extracted from tall oil soap, a by-product of the pulping process used for coniferous trees and then purified in a three-step process.

TOPs are predominantly a mixture of sitosterol (36-79%) and campesterol (0-14%), minor quantities of stigmasterol (and other sterols) together with saturated (stanol) compounds, sitostanol (6 to 34%) and campestanol (4-25%).

Most phytosterols are similar to cholesterol in the basic structure, except that they contain methyl, ethyl, di-methyl, di-ethyl or other groups next to the C₂₄ position on the aliphatic side chain.

These structural differences have important physiological implications as it is claimed that increased phytosterol consumption impedes cholesterol absorption with subsequent reductions in plasma total and Low Density Lipoprotein (LDL) cholesterol levels.

The following studies have been considered in support of the safety assessment of TOPs.

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:	[14C]β-sitosterol: mean 0.56 mg/kg (at approx 77 uCi/kg); [14C]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 [14C]β-sitosterol and [14C]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 CO₂ 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 ¹⁴C 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 ¹⁴C.

Formulation stability

The [¹⁴C]-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 CO₂ 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 [$^{14}\text{CO}_2$]. 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, $\geq 96.3\%$; 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, \geq 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-chromatograph 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 $^{14}\text{CO}_2$, 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.

[^3H]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:	[^3H]campestanol (Amersham International, $\geq 98\%$; 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:	[^3H]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 [³H]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 ³H.

Formulation stability

The [³H]-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 [³H]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 ³H was found in all tissues except the brain, albeit at low levels with highest ³H 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 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 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 ³H 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.

[³H]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 [³H]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 ³H.

Formulation stability

The ³H-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 CO₂ absorber. Faecal extracts were also analysed by TLC and radio-HPLC. Selected animals were analysed by whole body autoradiography.

Results

Absorption of [³H]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 [³H] 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 ³H included adrenals, spleen, intestinal epithelia, ovary, liver and bone marrow. Some of these tissues apparently retained ³H 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.

[3-³H] stigmaterol: 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]stigmaterol (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]stigmaterol: 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 [3-³H]stigmaterol 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 ³H₂O. 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 ³H₂O 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 ³H 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 ³H.

Formulation stability

The ³H-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 ³H₂O 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 [3-³H]stigmaterol 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 ³H₂O 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- ^3H]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:	[^3H]stigmasterol (Amersham International, $\geq 98\%$; 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:	[^3H]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 [$3\text{-}^3\text{H}$]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 [$3\text{-}^3\text{H}$]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 $^3\text{H}_2\text{O}$. 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 $^3\text{H}_2\text{O}$ 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 ^3H 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 ^3H .

Formulation stability

The ^3H -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 [$3\text{-}^3\text{H}$]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 ^3H was found in all tissues, although at very low levels with highest ^3H 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 ^3H 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 ^3H 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 [¹⁴C]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.

SUBCHRONIC STUDIES

Three-month dietary toxicity study with FCP-3P1 in Sprague-Dawley rats. Wedig J. Redfield Laboratories. Study Number 115-003. August 31, 2000.

Test material:	Tall-oil non-esterified plant sterols (sitosterol 44%, sitostanol 25%, campesterol 12%, campestanol 6%).
Test Species:	Sprague-Dawley rats 24 males and 20 females per test dose, administration in diet
Dose:	0, 1.25, 2.5, 5% in diet for 91 days.
GLP:	USA GLP Regulations
Guidelines:	

Study conduct

After acclimatisation for at least seven days, four groups of rats (24/sex/group) were treated with TOPs in the diet at 0, 1.25, 2.5 or 5% (equivalent to 0, 999, 2021, 4161 mg/kg bw/day in males and 0, 1163, 2358 or 4839 mg/kg bw/day in females). Control animals were given standard rat diet as provided by the manufacturer.

Clinical observations were recorded twice daily, and bodyweight and food consumption were recorded weekly. A satellite group of rats (4/sex/group) were selected for measurement of phytosterol concentrations pre-dose, and at day 29, 58 and 92. Haematology, clinical chemistry, urinalysis and measurements of vitamin A, D, E, K and beta-carotene were performed prior to necropsy. For a list of parameters measured see Appendix 1. Ophthalmology of all animals was performed before the study and near termination. At the end of the study, all animals were sacrificed and a complete necropsy performed (gross examination, organ weights and tissue sampling). Histopathology was performed on all tissues from the control and high dose groups and the caecum, colon and rectum from low and mid-dose groups were also examined. Appendix 1 lists the histopathological parameters measured.

Results

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

Male rats at high-dose had significant increased feed consumption throughout the whole study.

The reporting ophthalmologist concluded that there were no ocular abnormalities associated with the test material.

There were no treatment related differences in haematology, clinical chemistry or urinalysis at any dose. Serum cholesterol and triglyceride levels in the treated groups were not significantly different to controls.

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

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

In conclusion, no evidence of toxicity was noted following treatment with TOPs in the diet of rats up to doses of 5%. The NOEL was the highest dose tested, namely, 4161mg/kg bw/day for 91 days in male rats.

CHRONIC STUDIES

No chronic studies were submitted.

DEVELOPMENTAL STUDIES

Slesinski RS et al (1999) Developmental toxicity study of vegetable oil-derived stanol fatty acid esters. Regulatory Toxicology and Pharmacology, 29, 227-233.

Test material:	Plant stanol esters (vegetable oil derived stanol ester). 68% sitostanol, 30% campestanol, 2% unsaturated sterol.
Test Species:	SPF Wistar rats (Harland Netherlands) 28 mated females per dose group; administration in diet
Dose:	0, 1, 2.5 and 5% sterols w/w in diet (equivalent to 1.75 4.38 or 8.76% stanol esters respectively).
GLP:	OECD
Guidelines:	OECD

Study conduct

Groups of 28-mated female rats were treated with plant stanols in the diet at 0, 1, 2.5 and 5 % (made up from 0, 1.75, 4.38 and 8.76% test material which was a mixture of stanol-esters). The presence of sperm cells in a vaginal smear was assigned as gestation day 0. Mated females were distributed over the control and treatment groups. The plant esters were administered in the diet from day 0 to 21 gestation. Rats were examined twice daily and bodyweights were recorded on days 0, 7, 14 and 21 of gestation. Food consumption was measured during 0-7, 7-14 and 14-21 days. Rats were sacrificed on day 21 and examined for gross abnormalities. The uteri and ovaries were examined for the number of corporal lutea, number of implantation sites, early and late resorptions, live and dead foetuses, sex of foetuses, and any malformed foetuses. Ovarian and uterine weights were determined.

Results

There were no abnormal or dose related clinical signs and no animals died during the treatment period. A significant decrease in mean bodyweights compared to controls was observed at gestation day 7 (-3%) and 14 (-3%) and in mean bodyweight gains during 0-7 days (-13%) at a dose of 5 %w/w. However, no changes were noted at day 14-21 and as such the changes were considered not to be particularly biologically meaningful. A small but significant increase in food consumption was observed at a dose of 2.5% (+9%) at days 7-14 and at 5% (+9%) at days 14-21.

Gross examination of maternal organs and tissues did not reveal any significant treatment related effects. There were no significant differences in uterine weight, placental weight, foetal weight, number of foetuses, implantation sites or corpora lutea and early and late/late resorptions between treated and control groups. No abnormalities were noted from visceral and skeletal examinations of foetuses.

In conclusion, rats receiving a diet of up to 5% total stanols (equivalent to 2.4-3.5g stanols/kg bw/day) during a gestation period of 21 days showed no adverse treatment-related maternal or foetal developmental effects.

The NOEL for developmental effects in rats was 5 % plant stanols in the diet.

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 (CrI:(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 F₀ 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 F₀ males and females until weaning when F₀ males were sacrificed. F₀ 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. F₀ females were then sacrificed. During the F₀ mating and parturition phase, clinical examinations were

28 males and 28 females were selected from the F₁ pups and were treated with plant sterols in diet at the same levels as their F₀ parents for 10 weeks pre-mating. The treatment, observation and sacrifice schedule followed that of the F₀ generation. Of the remaining F₁ pups, 10 male and 10 female were subject to necropsy and tissue analysis. F₁ and F₂ 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)
- pre-mating food efficiency (weeks 0-5 and 6-10 pre-mating, 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 F₀ and F₁ 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 F₀ and F₁ groups pre-mating. Doses were similar in F₀ and F₁ 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 F₀ and F₁ 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 F₀ and 8.5% in F₁ 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 F₀ females, there were slight (statistically insignificant) increases in body weights at all doses, but no consistent differences were seen in F₁ 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.

Whittaker MH et al (1999) Oral two generation reproductive toxicity of plant stanol esters in rats. *Regulatory Toxicology and Pharmacology*, 29, 196-204.

Test material: Plant stanol esters (vegetable oil derived stanol ester)
Test Species: (CrI:(WI)WU BR) rat (Charles River Deutschland, Sulzfeld)
28 females and 28 males per F₀ dose group; 28 males and 28 females per F₁ dose group; administration in diet

Dose: 0, 1, 2.5 and 5% sterols w/w in diet (equivalent to 1.75 4.38 or 8.76% stanol esters respectively); F₀/F₁ males - 10 weeks (pre-mating)+ gestation + 3 weeks; F₀/F₁ females - 10 weeks + gestation + 3weeks.

GLP: OECD
Guidelines: OECD

Study conduct

Groups of 28 male and 28 female rats were treated with plant sterols in diet at 0, 1, 2.5 and 5 % (made up from 0, 1.75, 4.38 and 8.76% test material which was a mixture of stanol-esters). Treatments began in F₀ animals 10 weeks before mating. The day on which sperm was found in the vaginal smears was counted as day 0 of gestation. The females were housed individually for the birth and rearing of their young (F₁ generation). F₀ males were sacrificed after the mating period and necropsied. The morning after the birth was considered postnatal day 1 (PN day1).

On PN day 21, the F₁ pups were weaned and 28 males and females selected for the F₂ generation. Following weaning the F₀ females were sacrificed and necropsied. The F₁ litters were treated at the same dose as their parents from the day of weaning until sacrifice. The F₁ rats were mated at the end of a pre-mating period of about 10 weeks and the F₂ generation raised according to the same procedures as the F₁ generation.

Gross necropsy was carried out on all animals that died or were moribund and in all surviving male and female parents of the F₀ and F₁ generation.

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) and food consumption of mated females during pregnancy (days 0-7, 7-14 and 14-21) and postpartum (days 1-7, 7-14 and 14-21).
- 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)
- weanling necropsy and histology of abnormal tissues (stillborns and intercurrent deaths)
- necropsy and histology of F₀ and F₁ parental animals
- fertility and reproductive performance.

Results

There were no abnormal or dose related clinical observations during the study. There were no treatment related effects on bodyweights in male and females rats of the F₀ and F₁ generation except for an increased weight in F₁ females at the highest dose during weeks 3 and 4 of the pre-mating period.

Increased food consumption was observed in high-dose F₀ males, F₀ females at mid and high dose and in F₁ males (high dose) and females (all doses) at specific time periods during pre-mating. Increased food consumption was observed in F₀ and F₁ females (mid and high dose groups) throughout different time intervals during gestation and lactation (with the exception of F₁ females not being an increase during lactation).

There were no effects noted on absolute or relative organ weights in males and females of the F₀ generation. At a dose of 2.5% (stanols) both absolute and relative weight of the testes were increased and also the relative weight of the epididymis was significantly increased in the F₁ males (not observed at high dose). This later finding was not considered treatment related.

Fertility and reproductive performance parameters were not significantly altered by 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.

A treatment-related decrease in bodyweights was observed in F₁ and F₂ male and female pups at the highest dose (4.48%), particularly, on PN days 14-21. This was attributed to consumption of the test substance, which is not absorbed and reduces the caloric value of the test diet compared to controls.

The NOEL for reproductive effects in rats in a 2-generation feeding study was 4.48% plant stanols in the diet.

GENOTOXICITY STUDIES

The following studies were performed and main features are included in the table. Protocols were carried out under GLP, with OECD, USFDA guidelines.

Studies were designed with appropriate positive and negative control test substances and appropriate criteria were defined for positive and negative outcomes. Reference to historical control data was also included. Appropriate 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.

Bacterial mutation assay-*In vitro* genotoxicity assays with Phytrol TM (tall oil non-esterified phytosterols) Pant KJ (2000) Sitek Research Laboratories, Vancouver, Canada. Study 0521-2140. 23 February, 2000.

Mutation assay in mouse lymphoma cells. *In vitro* genotoxicity assays with Phytrol TM (tall oil non-esterified phytosterols) Pant KJ (2000) Sitek Research Laboratories, Vancouver, Canada. Study 0521-2400. 23 February, 2000.

***In vitro* genotoxicity assays with Phytrol TM (tall oil non-esterified phytosterols) for detection of chromosomal aberrations in human peripheral blood lymphocyte cells.** Xu J (2000) Sitek Research Laboratories, Vancouver, Canada. Study 0521-3300. 23 February, 2000.

***In vivo* genotoxicity assays with Phytrol TM-(tall oil non-esterified phytosterols) – induction of micronuclei in mouse bone marrow cells.** Xu J (2000) Sitek Research Laboratories, Vancouver, Canada. Study 0521-1521. 23 February, 2000.

Turbull et al (1999) Genotoxicity evaluation of wood-derived and vegetable-oil derived stanol esters. *Regulatory Toxicology and Pharmacology*, 29, 205-210.

<i>Test</i>	<i>Test material</i>	<i>Concentration</i>	<i>Test object</i>	<i>Result</i>
Reverse point mutation (In vitro)	TOPs	104-1667µg/plate (+/- S9)	<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537 and <i>E. Coli</i> WP2uvrA	-ve
Forward point mutation (In vitro)	TOPs	5 to 167µg/mL (+/-S9)	L5178Y TK Mouse lymphoma cells	-ve
Chromosome aberrations (In vitro)	TOPs	100 to 1200 µg/mL (+/-S9)	Human peripheral lymphocytes	-ve
<i>In vivo</i> micronucleus test (oral gavage)	TOPs	500 to 2000mg/kg bw	Rat bone marrow <i>in vivo</i> (CD-1 mice)	-ve

OTHER ANIMAL STUDIES

Reproduction study with plant sterols in Wistar rats FCP-3P1: oral gavage uterotrophic assay in immature female rats. Dearlove GE. Primedica argus protocol 706-001A. Argus Research Laboratories. Study Number TXF 9904. 27 January 2000.

Test material: Plant sterols (TOPs), vehicle methylcellulose
Test Species: Crl:CD SD IGS BR VAF/plus immature female rats (Charles River USA).

Animal species	Vehicle and positive control	GLP	Dosage (mg/kg /day)	Methodology and Phytosterol mixtures
Rats	0.5% w/v Methylcellulose as vehicle 17- α -ethinyl oestradiol in sesame oil as positive control	USFDA OECD	0, 1000, 2500 and 5000 30mcg/kg/day	Groups of 10 rats were administered phytosterol mixture (FCP-3P1) via gavage twice daily at 0, 500, 1250 and 2500mg/kg on day 19 postpartum (<i>equivalent to 0, 100, 250 or 500 mg/kg bw/day</i>) for 4 consecutive days.

Study conduct

Following administration of test compound any resulting clinical signs were assessed daily and body weights were recorded daily during the treatment period and at sacrifice (day 5).

Following sacrifice gross necropsy was performed on the thoracic, abdominal and pelvic viscera. Each uterus was dissected out and weighed after removal of the ovaries.

Results

There were no deaths of either control or treated animals. Clinical signs consisted of gasping and/or rales in three rats at the highest dose (5000mg/kg/day). It was suggested that these signs were due to an intubation problem as there was absence of these signs in other rats.

No gross lesions were observed at necropsy in controls of treated groups; however, 4/10 rats had enlarged uterine horns in the positive control group.

There were significant reductions in bodyweight gains for the entire treatment period at doses of 2500 (77% of controls; $p < 0.05$) and 5000 mg/kg/day (69% of controls; $P < 0.01$). Absolute uterine weights were unaffected by treatment up to the highest dose; however, significant ($p < 0.01$) increases in the uterine weights were observed in positive controls (527% of the control value). The ratio of the uterine weight/terminal body weight was unaffected in all dose groups except the positive controls whereby it was increased 547% ($p < 0.01$).

In conclusion, dosages of up to 5000mg/kg/day for four consecutive days to immature rats did not lead to an increase in absolute uterine weight or in the uterine weight/terminal body weight compared to normal controls (significant increases were noted in the positive control groups). There were significant reductions in bodyweight gains at 2500 and 5000mg/day.

The NOEL for the study was 1000mg/kg/day (equivalent to 100 mg/kg bw/day) with effects on bodyweight gains at the next highest dose.

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.

<i>Test</i>	<i>Test material</i>	<i>Conc.</i>	<i>EC₅₀*</i>	<i>% ³H-E₂ bound</i>	<i>Relative activity**</i>
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 x 10 ⁻⁵
	Oryzanol	≤ 100 μM	> 100 μM		< 3.2 x 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 ³H-17 β -estradiol (³H-E₂). For yeast assay represents concentration for 50% absorbance (amount β -galactosidase produced)

** ratio of EC₅₀ 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 (CrI:(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 (CrI:(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 (CrI:(WI)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 (CrI:(WI)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 (CrI:(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 (CrI:(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 decrease (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 (CrI:(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 (CrI:(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.

EFFICACY STUDIES IN ANIMALS

Comment: It is noted that the primary purpose of these studies was to examine efficacy rather than to examine potential toxicity and as such they are deficient in reporting of any features normally associated with toxicology studies. However, they are reported here since they provide some limited information relevant to the toxicity of tall-oil phytosterols.

Plant sterols: 10-day administration of Forbes versus soybean phytosterols in male rats: effect on hepatic cholesterol synthesis. Jones PJH. School of Dietetics and Human Nutrition, McGill University, Montreal, Quebec. Study PHF 9401. Ref: McGill Annual Report 1993-94

Test material:	Controls -1% cholesterol only, Tall-oil non-esterified plant sterols (TOPs)-Group 1*, Group 2** Group 3***
Test Species:	Male Wistar rats-6 males per test dose, administration in diet
Dose:	1% w/w in diet phytosterols, and 1% cholesterol for 10 days (as above)
GLP:	Not stated

*TOPs-sitosterol 51%, sitostanol 21%, campesterol, 18%.

**TOPs-sitosterol 45%, sitostanol 16%, campesterol 15%.

***Soybean mixture-sitosterol 55%, sitostanol 0%, campesterol 33%, dihydrobrassicasterol 11%.

Study conduct

Six male rats were treated with TOPs in the diet at 1% w/w for 10 days (1000 mg/kg bw/day) in three separate experiments after a 4-day period of acclimatisation. Control groups for the three separate experiments received cholesterol only. Food intakes were calculated daily whilst bodyweights were measured every second day. These were recorded as food intake and bodyweight gain in grams/10 days. Animals were anaesthetised after the experiment and blood was collected via heart puncture for analysis of lipid, phytosterol and hepatic cholesterol synthesis.

Results

It was not stated in the report whether any animals died during the experiment or whether there were any clinical signs of toxicity were observed at any dose levels. There was no significant difference in food consumption or body weight gain at any dose compared to controls.

The results showed that the incorporation of TOPs in diet at up to 1% w/w was well tolerated by male rats over a period of 10 days and there was no reduction in growth rate. However, an assessment of any clinical parameter was not undertaken.

Plant sterols: 45-day administration of phytosterol mixtures in male hamsters: effect on cholesterol metabolism. Jones PJH. School of Dietetics and Human Nutrition, McGill University, Montreal, Quebec. Study PHF 9502. Ref: McGill Annual Report 1993-94.

Test material:	Tall-oil non-esterified plant sterols (TOPs). Group 1 (controls 0.25% cholesterol); Group 2 (0.25% cholesterol and pure sitostanol); Group 3 (0.25% cholesterol and 1% TOPs*); Group 4 (0.25% cholesterol and 1% soybean phytosterols**); Group 5 (0.25% cholesterol and sitosterol plus artificial phytosterol subspecies***) administered in diet.
Test Species:	Hamsters-10 males per test dose, administration in diet.
Dose:	1% w/w in diet TOPs and 0.25% cholesterol for 45 days.
GLP:	Not stated

*TOPs: sitosterol 65%; campesterol 18% and sitostanol 17%.

**Soybean phytosterols: sitosterol 65%; campesterol (% not stated) and dihydrobrassicasterol (% not stated).

***Artificial phytosterol subspecies-composed of soybean and added sitostanol to produce plant sterol ratio similar to TOPs.

Study conduct

After 1 to 2 weeks acclimatisation, five groups of male hamsters received doses of cholesterol as a control group and cholesterol and mixtures of phytosterols as described above (dosage 680 mg/kg bw/day).

It was not stated in the report whether any animals died during the experiment or whether there were any clinical signs observed at any dose levels. Food intake was monitored daily and animals were weighed weekly. After 45 days, animals were anaesthetised and blood withdrawn by cardiac puncture for analysis of lipid and phytosterol content. Tissue samples including the liver, small and large intestine, heart were removed, weighed and stored for later analysis.

Results

No significant differences were observed in food intake or bodyweight gain between groups over the dose period. There were significant reductions in liver weight in all treated groups compared to controls with the TOPs and sitostanol (group 2) treated groups having a mean reduction of 15-20% less than controls. None of the histopathological data was presented in this report.

The results showed that the incorporation of TOPs in diet at up to 1% w/w was well tolerated by hamsters over a period of 6 weeks and there was no reduction in growth rate.

Plant sterols: 50-day administration of Forbes versus soybean phytosterols in male New Zealand white rabbits: effect on cholesterol metabolism. Jones PJH. School of Dietetics and Human Nutrition, McGill University, Montreal, Quebec. Study PHF 9503. Ref: McGill Annual Report 1993-94

Test material:	Tall-oil non-esterified plant sterols (TOPs). Group 1 (controls 0.5% cholesterol); Group 2 (0.5% cholesterol and 1% soybean phytosterols*); Group 3 (0.5% cholesterol and 1% TOPs**), Group 4 (0.5% cholesterol and 1% sitostanol) administered in diet.
Test Species:	New Zealand White rabbits 6 males per test dose, administration in diet.
Dose:	1% w/w in diet TOPs, and 0.5% cholesterol for 50 days.
GLP:	Not stated

*Soybean phytosterol constituents not stated.

**TOPs mixtures not stated.

Study conduct

After 2 weeks acclimatisation, four groups of male rabbits (6/group) received doses of cholesterol as a control group and cholesterol and mixtures of phytosterols as described in the table above (dosage 370 mg/kg bw/day).

It was not stated in the report whether any animals died during the experiment or whether there were any clinical signs observed at any dose levels. Food intake was monitored every third day and animals were weighed weekly. Blood was collected for analysis of lipid content, however, the method and time of collection was not stated.

Results

No significant differences were observed in bodyweight gain between groups over the dose period. No data was provided on food intakes. The results showed that the incorporation of TOPs in the diet at up to 1% w/w was well tolerated by rabbits over a period of 7 weeks and there was no reduction in growth rate.

Plant sterols: Toxicology Report:-50-day administration of Forbes versus soybean phytosterols in male New Zealand white rabbits: effect on cholesterol metabolism. Stejskal R (1995) Bio-Research Laboratories. Study No. TXF 9503. December 20, 1995.

A histopathological report was undertaken, presumably on samples taken from the previous study (Study PHF 9503) at those specific dose levels. However, it was not stated in study No. PHF 9503 that histopathological samples were taken.

In this report TXF 9503 a histopathological examination has been undertaken on samples from the liver and small intestine.

Results

No significant histopathological changes were noted in sections taken from the liver or small intestine.

Plant sterols: 90-day administration of Forbes versus soybean phytosterols in male and female hamsters: effect on cholesterol metabolism. Jones PJH. School of Dietetics and Human Nutrition, McGill University, Montreal, Quebec. Study PHF 9501. Ref: McGill Annual Report 1993-94

Test material:	Tall-oil non-esterified plant sterols (TOPs). Group 1 (controls 0.025% cholesterol); Group 2 (0.25% cholesterol); Group 3 (0.25% cholesterol and 0.5% TOPs*); Group 4 (0.25% cholesterol and 1% TOPs*); Group 5 (0.25% cholesterol and 0.5% soybean phytosterols**); Group 6 (0.25% cholesterol and 1% soybean phytosterols**) administered in diet.
Test Species:	Hamsters 20 male and female per test dose, administration in diet.
Dose:	0.5 and 1% w/w TOPs in diet, and 0.25% cholesterol for 90 days.
GLP:	Not stated

*TOPs: sitosterol 52%; campesterol 18% and sitostanol 21%.

**Soybean phytosterols: sitosterol 55%; campesterol 33% and dihydrobrassicasterol 11%.

Study conduct

After 1 to 2 weeks acclimatisation, six groups of hamsters (20 each sex) received doses of cholesterol as a control group and cholesterol and mixtures of phytosterols as described in the table above (dosage 340 or 680 mg/kg bw/day).

It was not stated in the report whether any animals died during the experiment or whether there were any clinical signs were observed at any dose levels. Food intake was monitored daily and animals were weighed weekly.

After 90 days animals were anaesthetised and blood withdrawn from the abdominal aorta for analysis of lipid, phytosterol and hepatic cholesterol synthesis.

Tissue samples including the liver and small intestine were removed, weighed and stored for later analysis.

Results

Animals that consumed non-soy phytosterols at both 0.5 and 1% levels had increased mean food intakes compared to other groups ($p < 0.05$). However, no significant differences were observed in bodyweight gain between groups over the dose period.

The results showed that the incorporation of TOPs in the diet at up to 1% w/w was well tolerated by hamsters over a period of 13 weeks and there was no reduction in growth rate.

None of the histopathological data was presented in this report.

Plant sterols: Histopathological examination of Forbes versus soybean phytosterols-treated hamsters fed an atherogenic diet for 90 days. Jolette J and Lamer N (1997) Bio-research laboratories, McGill University. Study Number TXF 9501.

A histopathological report was undertaken on samples taken from the previous study (Study PHF 9501).

However, there appears to be a discrepancy in the dose used with controls presented as basal diet (group 1; no cholesterol) and basal diet plus 1% cholesterol (group 2) compared to the doses stated in the previous study of 0.025 and 0.25% cholesterol respectively.

Study conduct

No detailed methodology was included in the report. However, liver and small intestine tissues were received frozen and standard histopathological processing was performed. These were presumably from the 90-day Hamster study No. PHF 501.

Results

In controls, fatty changes were evident from receiving an atherogenic diet; whereas, when treated groups received phytosterols there was a dose-related reduction in incidence and severity of fatty change. No other significant histopathological changes were noted in sections taken from the liver or duodenum.

Plant sterols: Tall oil-derived phytosterols reduce atherosclerosis in ApoE¹-deficient mice. 126-day study. Moghadasian FJJ (1996) Department of Pathology and Laboratory Medicine. Study No.PHF 9601. Published: in *Arteriosclerosis Thrombosis Vascular Biology*, 17: 119-226, 1997.

Test material:	Tall-oil non-esterified plant sterols (TOPs). Group 1 (controls 0.15% cholesterol), Group 2 (0.15% cholesterol and 2% TOPs*) administered in diet.
Test Species:	ApoE-deficient mice-6 controls and 13 males for treated group, administration in diet.
Dose:	2% w/w TOPs in diet, and 0.25% cholesterol for 126 days.
GLP:	Not stated

*TOPs-sitosterol 69%, campesterol 15% and sitostanol 16%.

Study conduct

After 10 days acclimatisation, mice were divided into 2 groups, one group received doses of cholesterol as a control group and the second group cholesterol and TOPs as described in the table above (dosage 3340 mg/kg bw/day).

Three control animals and one treated died during the experiment. A specific assessment of any clinical signs following treatment was not stated. Blood was obtained from the tail vein and at the end of the experiment from the right ventricle in anaesthetised animals for lipid analysis and histopathology. The heart and aorta were sectioned for analysis. Bodyweight was monitored throughout the experimental period.

¹ Apo-E-deficient mice develop severe hypercholesterolaemia and atherosclerotic lesions similar in appearance and distribution to those observed in humans.

Results

Animals that consumed TOPs showed a significant ($p < 0.05$) increase in bodyweight compared to controls at week 5 and throughout the remainder of the study. Histopathological analysis showed that less atheromatous plaques developed in treated animals compared to controls. This was consistent with lowered cholesterol levels.

The results showed that the incorporation of plant sterols in diet at up to 2% w/w was well tolerated by mice over a period of 18 weeks and there was no reduction in growth rate.

Plant sterols: Tall oil-derived phytosterol mixture lowers plasma cholesterol levels and prevents atherosclerosis in ApoE-deficient mice in the absence of dietary cholesterol. 20-week study. Study number PHF 9602. Author and research facility not stated.

Test material:	Tall-oil non-esterified plant sterols (TOPs). Group 1 (controls-no cholesterol added to diet mix); Group 2 (2% TOPs*) administered in diet.
Test Species:	ApoE-deficient 8 male mice/group, administration in diet.
Dose:	2% w/w TOPs in diet for 126 days.
GLP:	Not stated

*TOPs-sitosterol 69%, campesterol 15% and sitostanol 16%.

Study conduct

Mice were divided into 2 groups, one group received mouse diet with cholesterol as a control group and the second group TOPs as described in the table above (dosage 3340 mg/kg bw/day).

It was not stated whether any animals died during the study or whether clinical signs were assessed. Plasma lipid levels and atherosclerotic lesions were measured and assessed as per the previous study (above). Blood was obtained from the tail vein and at the end of the experiment from the right ventricle in anaesthetised animals for lipid analysis and histopathology. The heart and aorta were sectioned for analysis. Bodyweight was monitored throughout the experimental period.

Results

Histopathological analysis showed that less atheromatous plaques developed in treated animals compared to controls. This was consistent with lowered cholesterol levels. It was stated that there were no differences in mean body weights between the controls and treated groups (supporting data was not provided for independent assessment).

The results showed that the incorporation of plant sterols in diet at up to 2% w/w was well tolerated by mice over a period of 20 weeks and there was no reduction in growth rate.

Moghasdasian H et al (1999) Histologic, hematologic and biochemical characteristics of ApoE-deficient mice: effects of dietary cholesterol and phytosterols. Laboratory Investigation, 79, 355-364.

Test material:	Tall-oil non-esterified plant sterols (TOPs). Group 1 (controls-0.15% cholesterol added to diet mix); Group 2 (0.15% cholesterol and 2% TOPs*) administered in diet.
Test Species:	ApoE-deficient 8 male mice/group, administration in diet.
Dose:	2% w/w TOPs in diet for 18-weeks.
GLP:	Not stated

*TOPs-sitosterol 69%, campesterol 15% and sitostanol 16%.

Study conduct

Mice were divided into 2 groups, one group received doses of cholesterol as a control group and the second group cholesterol and phytosterol mixture as described in the table above (dosage 3340 mg/kg bw/day).

A specific assessment of any clinical signs following treatment was not stated. Blood was obtained from the tail vein and at the end of the experiment from the right ventricle in anaesthetised animals for lipid and hepatic enzyme analysis, haematology and determination of erythrocyte fragility. Urine was collected directly from the bladder for urinalysis and an examination of the thoracic and abdominal organs was undertaken. Histopathological analysis was performed on specimens from the heart, lung, brain, kidney, skeletal muscle, skin, oesophagus, stomach, small and large intestines, liver, adrenal gland, spleen, pancreas and bladder.

Results

There were no reports of any animal deaths during the study or any adverse clinical signs. There were no changes in blood glucose and urinalysis was unremarkable. There were no differences in haemoglobin concentration, red cell counts and hematocrit between controls and treated groups; however, a significant reduction (20%; $p < 0.05$) in mean platelet counts in the treated group was noted compared to controls. No abnormalities were observed upon gross examination except for 2 controls having skin lesions. Histological examination demonstrated focal and non-specific inflammation in the pelvic calyces of the kidneys and hepatic vacuolation in livers of control mice. Arrested spermatogenesis and atrophy in the seminiferous tubules was observed to a variable extent in controls and treated groups.

The results showed that the incorporation of a plant phytosterol mixture in the diet at up to 2% w/w was well tolerated by mice over a period of 18 weeks and there was no reduction in growth rate. Some slight non-specific histological changes in the kidney and reductions in platelet counts were observed in treated groups; however, this did not appear to be clinically significant and the histological changes were also observed in the control group.

Further Histo-pathological Analysis

Histopathological examination of selected tissues from hamster receiving phytosterol orally or via subcutaneous injection for 60 days, or phytosterol (regular or derivative) orally for 8 days. Lambert AJ and Besner J, Bioresearch Laboratories. October 29, 1997. Study Number TXF 9605

Summary of 60-day oral study conduct:

Test material:	Tall-oil non-esterified plant sterols (TOPs). Group 1 (No cholesterol; 4 females- oral); Group 2 (0.25% cholesterol; 4 males, 3 females- oral); Group.3 (0.25% cholesterol and 0.1% TOPs*-1 male, 5 females- oral). Administered in diet.
Test Species:	Hamsters
Dose:	1% w/w TOPs in diet (680 mg/kg bw/day) and 0.25% cholesterol for 60 days.
GLP:	Not stated

*Specific phytosterol content not stated.

Histopathological analysis was carried out on the duodenum, epididymides, liver, ovaries, tested and uterus.

There were no significant differences between controls and treated groups other than mild to moderate mononuclear cell infiltration in the liver. This was not considered related to treatment.

HUMAN STUDIES

In a series of 5 human studies, provided by the applicant both cholesterol lowering and physiological effects of TOPs were examined in normal and hyper-cholesterolaemic subjects. The phytosterols were administered in four forms, vegetable oil, margarine, a cereal based nutritional bar and milk.

10-day oral administration of sitostanol-enriched FCP-3P1 phytosterols versus sitostanol free soybean phytosterols in male and female normolipidaemic humans: effect on sterol metabolism. Jones PJH. School of Dietetics and Human Nutrition, McGill University, Montreal, Quebec. Study No. CLF 9601. McGill Annual Report, 1995-96.

Test material:	Group 1-Corn-oil (controls); Group 2-Olive oil (controls); Group 3-Olive oil plus soybean mixture*; Olive oil plus TOPs**
Test groups:	6 male and 5 females with normal cholesterol and triglyceride levels.
Dose:	1.5g per day in diet-two intervals of 10-days with 14-day washout interval-double blind crossover design,
GLP:	Not stated.

*Sitosterol 62%, campesterol 24% and stigmasterol 14% (Nu-life mixture).

**TOPs-Sitosterol 62%, campesterol 16% and sitostanol 21%.

On day 9 and 10 of each dietary phase, fasting blood samples were collected (before and 24 hours after dosing with deuterium oxide) for determination of plasma lipid levels, sterol levels and deuterium incorporation into cholesterol.

Results

Determinations were made of total, LDL and HDL cholesterol levels, triglyceride and phytosterol concentrations. Body weights of subjects remained constant throughout the treatments.

The mean dose achieved was 1600mg/kg /day for the Nulife mixture (sitostanol free) and 1500mg/kg/day of the TOPs mixture. Reductions in mean total and LDL cholesterol of 6.9% and 13.9% respectively were achieved in the sitostanol enriched TOPs treated group; whereas, in the sitostanol-free group reductions of 0.3% were achieved and there was an increase in LDL cholesterol of 9.5%.

No adverse effects in subjects were reported, although an extensive analysis of blood chemistry parameters (eg to determine liver and kidney function) was not undertaken.

In conclusion, this study demonstrated that normal healthy human subjects appeared to tolerate doses of TOPs mixtures in a vegetable oil based product up to a level of 1.5g/day over a 10-day period.

10-day oral administration of FCP-3P1 phytosterol versus placebo in hypercholesterolemic males: effect on sterol metabolism. Jones PJH. School of Dietetics and Human Nutrition, McGill University, Montreal, Quebec. Study No. CLF 9602. McGill Annual Report, 1995-96.

Test material:	Group 1-Olive-oil (controls), Group 2-Olive oil plus TOPs*
Test groups:	12 male subjects with cholesterol levels >5.2mmol/L aged 25-60 years.
Dose:	1.5g per day in diet-two intervals of 10-days with 14-day washout interval-double blind crossover design,
GLP:	Not stated.

*TOPs-Sitosterol 62%, sitostanol 21%, campesterol 16%, and campestanol 0.9%.

Prior to treatments, subjects received a complete physical examination, and fasting blood and urine samples were collected for blood chemistry, haematology and urine analyses. Blood was also collected on day 1 and 9 of treatment to measure cholesterol and triglycerides and at that time subjects were orally dosed with deuterium oxide at 0.7g/kg body water weight to measure deuterium incorporation into cholesterol.

On day 10 post-treatment a fasting blood sample was collected to measure plasma cholesterol, phytosterol, testosterone and triglyceride levels.

Results

Based on a purity of 94%, the TOPs mixture provided an average intake over the basal diet of 0.27g campesterol, 1.04g sitosterol and 0.3 g sitostanol/day in subjects.

There was a significant ($p<0.05$) reduction in mean bodyweights in both placebo and phytosterol administered subjects during the treatment period. However, the weight loss did not appear to influence the effect of phytosterol administration on sterol metabolism.

With the exception of an elevated plasma total cholesterol level, blood chemistry, haematology, urine analysis and testosterone levels were normal prior to and following treatment.

Reductions in the mean total and LDL cholesterol of 4.9% compared to controls were observed, although the treatment had no effect on plasma HDL and triglycerides.

In conclusion, this study demonstrated that human subjects with hypercholesteremia tolerated doses of TOPs mixtures in a vegetable oil based product up to a level of 1.5g/day over a 10-day period.

30-day FCP-3P1/margarine dietary study in human subjects. Jones Peter. Director, School of Dietetics and Human Nutrition, McGill University, Study No. CLF 9701. 2 December 1997.

Test material:	Group 1-Margarine (controls); Group 2-Margarine plus TOPs*
Test groups:	32 male subjects with cholesterol levels >6.4mmol/L aged 25 to 60 years.
Dose:	1.5g per day in diet-over 3 meal periods/day for 30-days,
GLP:	Not stated.

*TOPs-Sitosterol 55%, sitostanol 21%, campesterol 14%, and campestanol 3%.

On days 0, 1, 10, 20, 29 and 30 of treatment blood samples were obtained for lipid and cholesterol analysis. Post-treatment 12 subjects in each group consumed a normal diet and provided blood samples on day 40 and 50.

Results

There were no differences in mean bodyweight during the treatment period between placebo and phytosterol treated subjects. Subjects in both test groups reported occasional headaches, gas, constipation and nocturia which could not specifically be attributed to treatment.

Reductions in the mean total and LDL cholesterol of 9% and 14% respectively compared to controls were observed at day 30 with a small decrease in HDL occurring in both control and treated groups.

In conclusion, this study demonstrated that human subjects tolerated doses of TOPs mixtures in a margarine based product up to a level of 1.5g/day over a period of 30 days.

To determine the effect of increasing doses of tall oil derived phytosterols (Phytrol™) on the plasma lipid levels of hypercholesterolemic patients. Belsey EM (2000) Novartis Consumer Health. Study Number CLF 9904

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

* TOPs-sitosterol 60%, sitostanol 20%, campesterol 14% and campestanol 3%.

Study conduct

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

One hundred and thirty two human volunteers consumed four different TOPs mixtures (including a placebo group) in a milk-based drink, for a 4-week period ranging in doses from 0 to 3.6g/day. Nine subjects discontinued the treatment due to various factors (withdrew consent, adverse event, protocol deviation, did not receive treatment or unable to drink 3 drinks/day).

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

Results

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

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

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

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

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

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

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

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

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

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

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 28-day milk study are well within this variation and do not raise concerns *per se*.

In conclusion, this study demonstrated that human subjects tolerated doses of TOPs mixture in a milk-based beverage up to a level of 3.6 g/day over a period of 28 days.

Effect of a phytosterol preparation derived from Tall oil on cholesterol metabolism in hypercholesterolaemic subjects. Donazzolo Y (13 March 2000) Novartis Nutrition Research. Study Number CLF 9805

Test material:	Group 1-cereal-based nutritional bar (control); Group 2-cereal-based nutritional bar with TOPs*
Test groups:	150 subjects (2 groups of 60 males and 15 post menopausal females) with primary hypercholesterolaemia and no other health concerns aged 21 to 70 years.
Dose:	1.8g/day (divided into 3 doses/day) for 8-weeks-double blind placebo controlled study.
GLP:	Not stated.

* TOPs-sitosterol (50-60%), sitostanol (16-21%), campestanol (3%) and campesterol (15-18%).

Study conduct

In a 5-week period before treatment subjects underwent a physical examination and a detailed medical history was taken and blood chemistry was performed. Various criteria for inclusion/exclusion in the study were ascertained. At the start of treatment subjects were instructed to take the nutritional bar with the main meals 3-times/per day for the 8-week period.

A physical examination and blood chemistry analysis was performed pre-treatment and at various times during treatment and also at post treatment.

One hundred and fifty subjects human volunteers (males and females aged 21 to 70 years old) with blood cholesterol levels between 5.2 and 9 mmol/L were administered a TOPs mixture in a cereal based nutritional bar at a dose of 1.8g/day (divided in 3 doses/day) with main meals (including a placebo group). This was a multicentric study performed in three study centres, France, Germany and the United Kingdom.

113 subjects completed the study. Reasons for non-completion included incorrect cholesterol levels, a biological or clinical abnormality, personal reasons or forbidden treatment.

Study periods consisted of a screening/run in phase (-5 weeks), active treatment (8 weeks) and a follow up period of 4 weeks in which no treatment was administered. At -5, -1, 1, 5, 8, 9, 13 and 16 patients underwent a clinical assessment and biomedical profiling in which extensive blood chemistry was performed.

Main parameters measured were fasting plasma lipids, serum glucose, apolipoprotein A and B, plasma phytosterols, fat soluble nutrients, liver function, urea and electrolytes, blood pressure and weight.

Results

A thorough critical evaluation of the efficacy of treatment was not undertaken by ANZFA. However, as an overall conclusion at a dose of 1.8g/day reductions in total and LDL cholesterol were 3.8 and 3.4% respectively after 8-week treatment.

During the study a total of 55/134 subjects reported one adverse event (31 in the placebo group and 24 in the active treatment period). Clinical effects reported ranged from general symptoms, skin, respiratory, cardiovascular, gastrointestinal and musculoskeletal effects and psychiatric troubles. However, these clinical signs and symptoms were not confined to a specific sex and were generally considered unrelated to treatment as there was no dose response and there was no significant difference between treatment groups.

No significant differences were noted in any of the clinical chemistry parameters between placebo and treatment groups.

In conclusion, this study demonstrated that human subjects tolerated doses of TOPs mixture in a cereal based nutritional bar up to a level of 1.8g/day over a period of 8-weeks.

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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 Activated partial thromboplastin time (APTT)	Albumin Albumin/globulin ratio Alkaline phosphatase (AP) Alanine aminotransferase (ALT, GPT) Bilirubin (total) Calcium Chloride Cholesterol Creatinine Creatine kinase (CK) Gamma glutamyltransferase Globulin Glucose Potassium Protein (total) Sodium Triglycerides Urea nitrogen	Appearance Bilirubin Chloride Glucose Ketones Leucocytes Nitrite Occult blood pH Protein sediment Potassium Sodium Specific gravity Urobilinogin Volume
Organs Weighed	Tissues Examined Microscopically	
Adrenals brain heart kidneys liver ovaries spleen testes thymus thyroid	Adrenals Aorta Bone marrow (sternum) Brain (3 levels) Cervix Epididymes Eyes with optic nerve Femur Heart Intestine (small) Intestine (large) Kidneys Lacrimal gland Liver Lungs and bronchi Lymph nodes Mammary gland Oesophagus Ovaries pancreas Pituitary Peripheral nerve (sciatic) Prostate Salivary gland Seminal vesicle Skeletal muscle Skin Spinal cord Spleen Stomach Testes Thymus Thyroid Trachea urinary bladder Uterus Urinary bladder Vagina Tissues with gross lesions	

FOOD TECHNOLOGY REPORT

A417 – TALL OIL NON-ESTERIFIED PHYTOSTEROLS

Introduction

Phytosterols belong to a group of plant compounds that are found in a variety of foods in the human diet. The tall oil non-esterified phytosterols are sourced from tall soap, a by-product that is formed in the pulping process of coniferous trees¹.

Tall oil phytosterols as well as phytosterols derived from edible vegetable products are comprised of varying ratios of the same four primary phytosterol substances sitosterol, sitostanol, campesterol and campestanol, with varying amounts of minor components such as stigmasterol and brassicasterol. The physiological activity of phytosterol products is due to the presence of these compounds¹.

Tall oil phytosterols such as “Phytrol” vary from approved and presently marketed phytosterol products as a result of the constituent phytosterol profile. These variations arise for the following factors:

- 1) The source material differs;
- 2) Fatty acid esterification is not used to modify the solubility properties for product application; and
- 3) Hydrogenation processing is not used.

Structure of plant sterols and stanols

Sterols are an essential component of cell membranes, and both animals and plants produce them. The sterol ring is common to all sterols, with differences in the side chain accounting for different properties in sterol compounds. Phytosterols fall into one of three categories: 4-desmethylsterols (no methyl groups); 4-monomethylsterols (one methyl group) and 4,4-dimethylsterols (two methyl groups). The most common plant sterols are β -sitosterol, campesterol and stigmasterol and structurally these are very similar to cholesterol, belonging to the class of 4-desmethylsterols¹.

Plant stanols are hydrogenation products of the respective plant sterols, e.g. campestanol/campesterol and sitostanol/sitosterol, and are found in nature at very low levels. Stanols have no double bonds in the sterol ring and belong to the group of 4-desmethylsterols².

All plant sterols and stanols are closely related in structure to cholesterol. The difference is the presence of a methyl or ethyl group in their side chains. Dissimilarity to cholesterol results in plant sterols and stanols either not being absorbed, or being minimally absorbed³.

Solubility

Free phytosterols or stanols in free form; exhibit limited lipid solubility. Some manufacturers elect to esterify them with fatty acids from edible oils. The solubility of free sterols in oil is around two percent, but the solubility of sterol esters in oil exceeds twenty percent⁶.

The esterification of phytosterols improves their solubility properties and facilitates their incorporation into certain foods. However, esterification does not materially affect the physiological properties of the phytosterol components. Once ingested the esters are rapidly cleaved by endogenous lipases, releasing the free phytosterols that are then able to interact with cholesterol absorption⁵.

On a molar basis, free and esterified phytosterols exhibit similar physiological activity. This equivalence means the extensive safety and efficacy data for esterified phytosterol forms is directly and appropriately applicable to non-esterified forms. Difference in molecular weight of phytosterols needs to be equilibrated when assessing safety and efficacy i.e. 1.6 grams esters is approximately equivalent to 1.0 grams of free phytosterols⁶.

Blending non-esterified phytosterols into Table Spreads

As tall oil non-esterified phytosterols efficiently deliver a high quantity of free phytosterols and stanols, the primary deterrent on wider use has been the efficacy with which it can be blended into foods. The applicant states a number of possibilities for incorporating non-esterified phytosterol ingredients into table spreads, at the 8% level needed to achieve a cholesterol lowering effect:

- 1) Dispersing the phytosterols into a fat mixture of temperatures greater than 85°C so that the ingredient will partially dissolve and recrystallise upon cooling. With the mixing and emulsifying processes completed at elevated temperatures to produce a level of finely dispersed sterol within the fat phase and ultimately within the whole product.
- 2) Dispersing the sterol within the water phase using a well-defined stabilising system to prevent any separation, and then proceeding with normal emulsifying and crystallisation processes.
- 3) Melt the phytosterol compounds at a concentration of 17% into fat and use this as a source solution to prepare the fat phase.

Stability

Phytosterols and their fatty acid esters are basically very stable compounds and experience only limited damage during oil processing⁷. 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 for cholesterol⁸. 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 concentrations⁹.

Production Methods

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

- 1) The first step is a solvent extraction of the tall oil soap. Organic solvents, water and tall oil soap are mixed while heating in stainless steel reactors. The mixture is allowed to separate into distinct aqueous and organic phases. The organic phase contains extracted organic materials, and 15-25% sterols, which is used in the next step of the process.
- 2) The second step consists of a complexation-washing process that removes the bulk of the organic material. The extract from Step 1 is mixed while heating with a solvent, and complexing agent. The sterols rapidly bind to the agent, which are then separated from the solvent phase by centrifugation. Next, the complexing agent is dissolved from the crude complex by heating in water. The water is removed and the resulting material contains 60-75% sterols, that are referred to as crude sterols.
- 3) Crude sterols are dissolved in alcohol at elevated temperature. The temperature of the mixture is reduced to allow for crystallisation of the sterols. The crystals are recovered and then dried. The mixture is assayed for the content of sterols. If the desired purity is not achieved, then the mixture is re-crystallised a second time.

Conclusions

Tall oil phytosterols have a distinctive profile that sets them apart from previously appraised phytosterols. The ratio of primary phytosterol substances and minor compounds of tall oil phytosterols is different to that of other oils and this in turn determines physiological activity. The major limitation to this point, on the wider use of phytosterols and stanols, has been their limited solubility in food matrices and consequently this has affected the efficacy with which they could be blended into foods. The applicant has described a number of possibilities to overcome this drawback, and combine non-esterified phytosterol ingredients into table spread. The described methods of improving free phytosterol solubility have the capacity to deliver free phytosterols more efficiently to the digestive system.

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DIETARY EXPOSURE ASSESSMENT REPORT

A417 – TALL OIL NON-ESTERIFIED PHYTOSTEROLS

A dietary exposure assessment was conducted for Application A417 – Tall oil non-esterified phytosterols in order to determine the impact of allowing non-esterified phytosterol addition to foods. There are a number of assumptions made in the dietary modelling process; therefore, the dietary exposure assessment is used as a guide for risk management decisions regarding food regulation.

Background information

Phytosterol ester containing spreads are currently on the market and have claims on the label stating that phytosterols assist in lowering cholesterol absorption. They also contain advice from the manufacturers on the label recommending that 2-3 serves of phytosterol-ester containing foods be consumed each day in order to achieve the recommended level of intake.

This Application is seeking approval to use non-esterified phytosterols from tall oils in food at levels formulated to provide approximately 1.5 grams per day to the average consumer (through 2-3 serves of products).

Reported average exposures of naturally-occurring phytosterols from unfortified foods vary in the range of 160 to 500 mg per day (Thurnham, 1999; Sierksma *et al*, 1999). These are not exposures for the Australian population.

Summary of the applicant's dietary exposure assessment for phytosterols

The applicant provided information on natural sources of phytosterols, the estimated exposures based on naturally occurring phytosterols and the different chemical structures of different sterols.

The applicant included consumption figures for the foods proposed to include non-esterified phytosterols, from the 1995 National Nutrition Survey (NNS) for people aged 19 years and over, at the major food group level. They state that the increase in phytosterol exposure over current exposures from non-fortified foods would only be 1.5 grams per day. The applicant concluded that, based on clinical trials of phytosterol exposure at this level (1.5 g/d), this level of exposure would be safe for the population.

This information was not sufficiently detailed to allow conclusions to be drawn on projected exposures to phytosterols, assuming their addition to food. ANZFA therefore conducted a detailed dietary exposure assessment.

Dietary modelling

Dietary modelling is the process of combining food consumption data with food chemical concentration data to estimate exposures to the food chemical by populations or distinct age/sex groups.

Dietary modelling was conducted by ANZFA to estimate potential dietary exposure to non-esterified phytosterols for Australian and New Zealand populations, using ANZFA's dietary modelling computer program, DIAMOND. 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 applicant requested use of non-esterified phytosterols in oil based spreads (<80% fat) at a concentration of 8% (or 80 000 mg/kg). This was the concentration used in the dietary modelling.

Estimated dietary exposures to phytosterols do not include phytosterols from naturally occurring sources as data on naturally occurring levels in foods were not readily available; estimated dietary exposure from naturally occurring sources in the Australian or New Zealand populations was not available; and it was assumed that intakes of phytosterols from naturally occurring sources would make little impact on the estimated dietary exposures.

As no target group is specified in the application, it is assumed that the target group is adults, particularly those 40 years and above, who have concerns about their cholesterol levels. The products are not intended for use by infants, children, pregnant or lactating women. However, there is a likelihood that children may also consume these foods. Therefore potential dietary exposure to phytosterols for children was estimated to determine any possible risks associated with their level of exposure. 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.

Assumptions used in dietary modelling

Assumptions made in the dietary modelling include:

- all foods within a category contain non-esterified phytosterols at the proposed levels;
- consumption of foods are actual amounts as recorded in the NNSs, as opposed to suggested serve sizes that appear on product labels of foods containing phytosterols.

This assumes that consumers will not significantly change their eating habits of the foods containing the phytosterols, but follow existing patterns of use; and that consumers are product loyal, and would always consume the products with added phytosterols.

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

Limitations of dietary modelling

A limitation of estimating dietary exposure over a period of time associated with this dietary modelling is that only 24-hour dietary survey data were available, and these tend to over-estimate habitual food consumption amounts for high consumers. Therefore, predicted high percentile exposures are likely to be higher than actual high percentile exposures over a lifetime.

Estimated dietary exposures to phytosterols

How were the dietary exposures calculated?

For each food group specified to contain non-esterified phytosterols, the DIAMOND program multiplies the food's concentration of non-esterified phytosterols by the amount of food that an individual consumed from the group in order to estimate the dietary exposure to each food group. The food consumption amounts for individuals include where the spread was consumed as a spread (on bread for example) and where spreads were consumed as a part of mixed foods (such as spreads used in a stew recipe). Population statistics (mean and high percentile dietary exposures) are then derived from the ranked individual dietary exposures.

Results

The estimated dietary exposures to phytosterols are summarised below in Table 1 for Australia and New Zealand.

The dietary modelling suggests that for both Australia and New Zealand populations, mean dietary exposures for consumers of oil based spreads containing phytosterols are between 0.9 and 1.3 grams per day, assuming all oil based spreads contain phytosterols at the specified concentration. Estimated 95th percentile phytosterol exposures for populations in both countries range between 2.3 and 3.8 grams per day. The highest mean and 95th percentile exposures were for 13-19 year old Australians.

For the target group of 40 years and above, the intake of TOPs from table spreads was 1.3g/day (mean exposure) and 3.5g/day (95th percentile consumers) for Australian consumers and 1g/day and 2.8g/day respectively, for mean and 95th percentile New Zealand consumers.

Table 1: Estimated non-esterified phytosterol exposures for Australia and New Zealand, for different age groups

Country	Age Group (years)	Number of consumers*	Mean exposure consumers (g/d)	95 th percentile exposure consumers (g/d)
Australia	2-12	1 187	0.88	2.38
	13-19	647	1.28	3.57
	20-39	2 621	1.32	3.82
	40 +	3 319	1.26	3.50
	All	7774	1.22	3.47
New Zealand	15-19	81	1.06	3.13
	20-39	558	0.90	2.26
	40 +	812	0.97	2.75
	All	1 451	0.95	2.58

* Consumers – people consuming the products assumed to contain phytosterols (80,000 mg/kg in oil based spreads <80% fat)

Summary

In summary, the dietary modelling for TOPs added to oil based spreads showed that mean consumers of phytosterols have estimated exposures at 1.3 grams per day or below; and, high consumers of phytosterols have estimated dietary exposures between 2.3 and 3.8 grams per day.

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WORLD TRADE ORGANIZATION (WTO) 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 that 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.

This application will be notified to the WTO because permission to use TOPs would have a liberalising effect on trade via removal of the prohibition on the sale of these novel food ingredients. There are no international standards in relation to TOPs.

SUMMARY OF PUBLIC SUBMISSIONS

A417 – NON-ESTERIFIED PHYTOSTEROL ESTERS DERIVED FROM TALL OILS

WA Health Department

Support the application but considered that if approved that additional information should be provided on the nutritional panel which states the non-esterified phytosterol content of the food.

New Zealand Dairy Board

Support the application provided that products have statements indicating that they are not nutritionally appropriate for young children, pregnant women and nursing mothers.

Consumers Association of South Australia Inc.

Support the submission made by Ms Elaine Attwood.

Pauls Limited

Pauls **supports** the application and requests approval for use in a range of soy/dairy based products in the following food groups:

- Reduced fat or low fat liquid milk-up to 3.6g/L;
- Reduced fat or low fat fresh yoghurt-up to 6g/kg;
- Reduced fat or low fat liquid soy drink-up to 3.6g/L;
- Reduced fat or low fat soy yoghurt-up to 6g/kg.

Australian Food and Grocery Council

- **Support** the approval of non-esterified phytosterols as food ingredients subject to a satisfactory safety assessment.
- Supports any conditions that ANZFA may place in the standard, provided that it can be justified under sound science and COAG principles.

Food Technology Association.

Supported the application and will submit final comments after reviewing the full assessment report and that the safety assessment report endorses public health and safety.

Mr Richard James, Whangarei, New Zealand

Opposed to unconditional approval of applications for phytosterol approvals. Provided information on potential adverse effects of phytosterols, including original publications for application A410. Believes that phytosterols will add another level of oestrogenic risk from another toxin added to 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 non-esterified phytosterols may lower blood cholesterol and that there was sufficient supporting data.
Concerned about the possible effects of a higher level of non-esterified phytosterols on levels of other nutrients.
Concerned that there is over-reliance on the US FDA's GRAS status for phytosterols.

Dietitians Association of Australia

Supports the application as it may have potential benefits for some consumers and will provide further comment on the full assessment report when it becomes available.

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 phytosterols in soups, vegetable drinks, crackers and biscuits.

Goodman Fielder Group Services Pty Ltd

Supports the application for the approval of phytosterols as novel food ingredients. Believes that the safety of non-esterified phytosterols has been demonstrated.

National Council of Women of New Zealand

Believe that a health claim needs to be clearly substantiated and not used as a marketing strategy. Require proof of the health claim that phytosterols 'may' lower blood cholesterol.

Valerie James, Whangarei, New Zealand

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

NZ Ministry of Health

ANZFA should assess the appropriateness of non-esterified phytosterols at full assessment.
The exposure of children should be considered as a special group

ATTACHMENT 7

Initial application	Amended by Applicant (19 December 2000)	Amended by Applicant (6 July 2001)	Amended by Applicant (22 August 2001)
<p>Cereals (eg, breakfast cereals, breakfast bars);</p> <p>Low and reduced fat liquid milk, low and reduced fat yoghurt and yoghurt products, and dairy based desserts;</p> <p>Low and reduced fat soy beverages and low and reduced fat soy-based yoghurts;</p> <p>Edible fats and oils (eg, mayonnaise, salad dressings);</p> <p>Margarine; and</p> <p>Table spreads.</p>	<p>Breakfast bars;</p> <p>Low and reduced fat liquid milk, including milk based beverages; and</p> <p>Edible table spreads (less than 80% fat).</p>	<p>Low and reduced fat liquid milk, including milk based beverages; and</p> <p>Edible table spreads (less than 80% fat).</p>	<p>Edible table spreads (less than 80% fat).</p>