Notification Dossier

PHYTOSTEROLS AND THEIR ESTERS FOR USE AS AN INGREDIENT IN A RANGE OF FOOD APPLICATIONS

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PHYTOSTEROLS AND THEIR ESTERS FOR USE AS A FOOD INGREDIENT IN A RANGE OF FOOD APPLICATIONS

SUMMARY

Under Regulation (EC) No 258/97 on Novel Foods and Food Ingredients, Unilever received approval for the use of phytosterol esters as a novel food ingredient in yellow fat spreads in European Commission Decision 2000/500/EC of 24 July 2000 (EC, 2000).

On March 31, 2004 the Commission Decision 2004/335/EC also approved the application of phytosterols and phytosterol esters in

- Milk type products, such as semi-skimmed and skimmed milk type products, yoghurt type products, and milk/yoghurt type products where the milk fat has been partly or fully replaced by vegetable fat.

This Decision was addressed to Unilever.

Cognis hereby applies for a favourable opinion regarding the substantial equivalence of its phytosterols and their esters to the phytosterols and their esters referred to in Commission Decisions 2000/500/EC and 2004/335/EC, in order to put phytosterols and phytosterol esters on the market as ingredients for the food applications described above.

The phytosterols and phytosterol esters to be used in said food applications are identical in composition and manufacture to those currently approved.

Cognis is a major supplier of phytosterol esters to a holder of a Novel Food marketing authorisation for phytosterols in fat spreads. Cognis' phytosterol esters have been on the European market in significant amounts, via this food use, since the year 2000.

The innocuousness of phytosterols that comply with certain purity criteria has been shown by numerous toxicological and clinical investigations. Cognis proved compliance with the new phytosterol (ester) profile and purity criteria as required by the latest Decisions of the European Commission on the use of plant sterols in foods (2004/333/EC, 2004/334/EC, 2004/335/EC, and 2004/336/EC). This is considered to be not only a proof of 'substantial equivalence' but a proof of identity to the legally authorised phytosterols and, respectively, their esters.

No additional source of phytosterols or their esters is added to the food chain by allowing the marketing of said Cognis ingredients for the use in milk and yoghurt type products, and yellow fat spreads. The overall intake of phytosterols will not be affected, since there will be only alternative, not cumulative, consumption of multiple foods within one food category. Consequently, it can be guaranteed that the safe daily dose of 3 g is not exceeded by introducing said products to the market.

Products made from these phytosterols or their esters shall be presented in such a manner that they can be easily divided into portions that contain either a maximum of 3 g or a maximum of 1 g of added phytosterols/phytostanols or its esters. The amount of phytosterols/phytostanols added to a container of beverages shall not exceed 3 g. The margarine/vegetable oil spread produced from said ingredients shall contain up to 8 % w/w of added phytosterols (equivalent to 14 % w/w phytosterol esters) as currently laid down in Commission Decisions 2000/500/EC and 2004/335/EC.

The cholesterol-lowering effect of phytosterol esters has been demonstrated in various food types including yellow fat spreads, milk, yoghurt and salad dressings.

The food products produced form said phytosterols or their esters shall be labelled according to the requirements of Commission regulation 608/2004/EC as follows:

(1) In the same field of vision as the name under which the product is sold there shall appear, easily visible and legible, the words: “with added plantsterols”.
(2) The content of added phytosterols, phytosterol esters (expressed in % or as “xg of free plantsterols per 100 g or 100 ml” of the food) shall be stated in the list of ingredients.
(3) There shall be a statement that the product is intended for people who want to lower their blood cholesterol level.
(4) There shall be a statement that patients on cholesterol lowering medication should only consume the product under medical supervision.
(5) There shall be an easily visible and legible statement that the product may not be nutritionally appropriate for certain sections of the population (pregnant and breast feeding women and children under the age of five years).
(6) Advice shall be included that the product is to be used as part of a healthy diet, including regular consumption of fruit and vegetables (to help maintain carotenoid levels).

If consumers use the product as recommended on the labelling, then it is anticipated that the intake will be 1-3g of free phytosterols per day. This level of intake does not exceed that originally anticipated by the EC.

In conclusion, this application demonstrates the substantial equivalence of the notified phytosterols and phytostanols and its esters compared to the legally authorised phytosterols and phytosterol esters referred to in Commission Decisions 2000/500/EC and 2004/335/EC.

Furthermore, this application shows that the consumption of Cognis’ phytosterols and phytosterol esters, if used as intended, will not result in adverse public health effects.

INTRODUCTION

This notification dossier is partly based on data from the Novel Food authorisation dossier of Unilever (Unilever 2002) and the relevant SCF opinions on the safety of phytosterols and phytosterol esters (SCF 2000, SCF 2002, SCF 2003, SCF 2003a, SCF 2003b).

Under Regulation (EC) No 258/97 on Novel Foods and Food Ingredients, Unilever submitted an application seeking approval for the use of phytosterol esters as a novel food ingredient in yellow fat spreads. This application was approved in European Commission Decision 2000/500/EC of 24 July 2000. Under Article 3 of this decision, Unilever was required to establish a surveillance programme to accompany the marketing of the product. A document reporting the findings of this Post Launch Monitoring was submitted to the European Commission (EC) in January 2002. This report concluded that yellow fat spreads containing phytosterol esters were being bought by the target population, intakes were <20g/day and there was no evidence of adverse health effects, expected or unexpected. At <20g spread/day, intakes were lower than the assumptions made in the original Novel Foods application.

On September 26, 2002 the Scientific Committee on Food concluded that the data from these studies provided valuable information, in particular with respect to product consumption, which complemented that obtained in the pre-market safety evaluation studies (SCF 2002).

On March 31, 2004 the Commission Decision 2004/335/EC approved the application of phytosterols and phytosterol esters in

- Milk type products, such as semi-skimmed and skimmed milk type products, yoghurt type products, and milk/yoghurt type products where the milk fat has been partly or fully replaced by vegetable fat.

This Decision was addressed to Unilever.

Based on these Decisions and Scientific Opinions, Cognis applies for a favourable opinion in order to notify its phytosterols and phytosterol esters respectively, as ingredients in the range of product types into which phytosterol esters may currently be added, according to Decisions 2000/500/EC and 2004/335/EC. With appropriate labelling of the resulting food products, the intake of phytosterol esters will not exceed that previously considered by the EC to be safe.

The phytosterols/esters manufactured by Cognis are identical in composition and manufacturing process to those currently used in phytosterol ester containing foods (yellow fat spreads, milk and yoghurt type products).

This application seeks approval for the inclusion of phytosterol esters as a cholesterol-lowering ingredient in ‘milk’ and ‘yoghurt’ type products and yellow fat spreads, also referred to as spread(s) in this document.

The ‘milk’ type products would include skimmed, semi-skimmed and vegetable oil based

milk variants. The ‘yoghurt’ type products would include a range of natural and fruit flavoured yoghurts. It is understood that, under EU Milk legislation (EC Directive 95/2) and the varying national legislation across member states on Yoghurt, the addition of phytosterol esters, and other ingredients required to stabilise the products, will prevent the use of the term ‘milk’ and ‘yoghurt’, to describe these Novel Foods. Therefore, to ensure clarity in this document, phytosterol esters are considered to be the Novel Food Ingredient and the ‘milk’ and ‘yoghurt’ type products with added phytosterol esters the Novel Foods.

This document provides evidence to confirm the composition and safety of phytosterols and phytosterol esters in these products.

2. SPECIFICATION OF THE NOVEL INGREDIENT

The novel ingredient is phytosterols and phytosterol esters. The esters are identical to the ingredient used in Unilever's yellow fat spreads that were previously approved under Regulation (EC) No 258/97 on Novel Foods and Food Ingredients.

The specification will meet the requirements as set by Decision 2004/335/EC:

Composition (with GC-FID or equivalent method):
< 80% β-sitosterol
< 15% β-sitostanol
< 40% campesterol
< 5% campestanol
< 30% stigmasterol
< 3% brassicasterol
< 3% other sterols/stanols

Contamination/Purity (GC-FID or equivalent method)
Phytosterols and phytostanols extracted from sources other than vegetable oil suitable for food have to be free of contaminants, best ensured by a purity of more than 99% of the phytosterol/phytostanol ingredient.

The specifications set for Cognis' phytosterol esters regarding quality and potential contaminants are summarized in Appendix I. Cognis phytosterols and their esters are specified according to current food grade requirements in terms of heavy metals and microbiology.

Appendix II shows a compilation of GC-FID analyses performed on four individual batches of Cognis' phytosterol esters, proving compliance with the legally required purity profile and proving substantial equivalence with the profile of the phytosterols that are approved for the use in fat spreads according to Commission Decision 2000/500/EC. The method of analysis DGF F III-1 (DGF 1998) has been validated in a ring trail in which Cognis successfully participated. Both the original certificates of Cognis' analyses, as well as the ring trail certificate, are attached as Appendix II.

Cognis is a major supplier of phytosterol esters to a holder of a Novel Food authorisation for the use of phytosterol esters. Cognis' phytosterol esters have been on the European market in significant amounts, via these approved novel foods, since 2000 (see Appendix III).

3. PRODUCTION METHODS

3.1 Production methods for the phytosterols and their esters

The production methods used to produce the phytosterols and their esters are identical to those used to produce the phytosterol esters currently allowed in yellow fat spreads and milk and yoghurt type products. They have been produced in significant amounts by Cognis, for the holder of a Novel Food authorisation in Europe, since the year 2000.

The production process is described in Appendix IV.

3.2 Description of the Novel Foods

The Novel Foods are standard 'milk' and 'yoghurt' type products with added phytosterols or phytosterol esters. The application covers the addition of phytosterols or phytosterol esters, and agents used to stabilise them in:

- Milk type products, such as semi-skimmed and skimmed milk type products, yoghurt type products, and milk/yoghurt type products where the milk fat has been partly or fully replaced by vegetable fat.
- yellow fat spreads

The 'milk' type product will be sold in standard milk cartons containing 1.6g phytosterol ester or 1g free phytosterols per 250mL serving.
The 'yoghurt' type product will be sold in individual pots or multipacks. Each yoghurt pot, regardless of size, will contain 1.6g phytosterol ester (or 1g free phytosterols).
The yellow fat spreads shall contain 1g (free) phytosterols in 12.5g spread.

The recommended intake will be 2-3 servings per day from the range of foods containing phytosterol esters. This will be equivalent to a daily intake of 2-3 g free phytosterols.

3.3 Process for producing milk products containing phytosterol esters

Milk products containing phytosterol esters are made using the same process and procedures as conventional fortified milks/milk drinks and no additional controls are considered necessary. Storage and distribution temperatures used are the same as conventional milks, fortified milk and milk drinks and the same Hazard Analysis and Critical Control Point (HACCP) schemes are used to control product safety and quality. The only additional process required is to control the amount and quality of phytosterol esters added.

3.4 Process for producing yoghurt products containing phytosterol esters

Yoghurt products containing phytosterol esters are made using the same process and procedures as conventional yoghurts and no additional controls are considered necessary. Storage and distribution temperatures used are the same as conventional yoghurts and the same Hazard Analysis and Critical Control Point (HACCP) schemes are used to control product safety and quality. The only additional process required is to control the amount and quality of phytosterol esters added.

3.5 Process for producing yellow fat spread products containing phytosterol esters

Yellow fat spread products containing phytosterol esters are made using the same process and procedures as conventional yellow fat spreads and no additional controls are considered necessary. Storage and distribution temperatures used are the same as conventional yellow fat spreads and the same Hazard Analysis and Critical Control Point (HACCP) schemes are used to control product safety and quality. The only additional process required is to control the amount and quality of phytosterol esters added.

4. ANTICIPATED INTAKE/EXTENT OF USE OF THE NOVEL FOOD INGREDIENT

4.1 Current intake of phytosterol esters from cholesterol-lowering spreads

In Unilever's original application for the use of phytosterol esters in yellow fat spreads a number of assumptions were made about use. These were:

- Typical daily intake of the product would be 20-30g
- Upper 95th percentile intake levels would be around 57g in the UK and 70g in the Netherlands
- Consumers would be mainly over 45 years old and concerned about their cholesterol level

The typical daily intake and upper intake levels were based on published information on the consumption of yellow fat spreads in the UK and the Netherlands and were consistent with product marketing information from Unilever commissioned trials.

The assumption about the consumer profile was based on Unilever's market research and the sales performance of Raisio's Benecol (yellow fat spread containing phytostanol-esters) in Finland.

As part of Unilever's Post Launch Monitoring scheme, the estimated daily intake of phytosterol esters (as free phytosterols) from its cholesterol-lowering spreads (Flora/Becel/Fruit d'or/pro.activ) was obtained using independent market research (consumer purchase) data. This showed that the amount of spread purchased per household across the five main markets in Europe varied between 15 and 18g/day for regular users of the product (intakes were higher for regular users than for occasional/once only users). Upper (95th percentile) intakes varied between 27 and 45g/day. In general, intakes per household were similar irrespective of the number of people in the household, indicating that usage was predominately by one person in each household. Phytosterols are added to the spread at a level of 8% (as free phytosterols) as approved by the EC. Therefore, at these levels of spread use, individual intake of phytosterols is estimated to be 1.2-1.4g/day with 95th percentile intakes of 2.2-3.6g/day. As can be seen from *Table 2* (below) all intakes are lower, in particular the 95th percentiles, than the pre-market assumptions.

Table 2. A summary of the daily phytosterol intakes for regular users from the Post Launch Monitoring report (SCF 2002)

	Pre-market assumptions		***Post launch Monitoring***	
	Daily spread intake	Daily free phytosterol intake (g/day)	Daily spread intake (g/day)	Daily free phytosterol intake (g/day)
Average intake	20-30g	1.6-2.4g	15-18g	1.2-1.4g
95th percentile intake	57g (UK) 70g (NL)	4.6g (UK) 5.6g (NL)	27-45g	2.2-3.6g

Furthermore, the Post Launch Monitoring also confirmed that pro.activ is being bought predominately by the target consumers, i.e. those over 45 years old. Extension of the product range will provide an opportunity for consumers to optimise the cholesterol-lowering benefit of phytosterol esters by providing additional or alternative products. It will also provide consumers who do not eat spreads, the cholesterol-lowering benefit of phytosterol esters in food products. Evidence from consumer carelines confirms that consumers would like additional or alternative products to spreads. To maximise the benefit of any additional products, these should also be foods that are used on a regular basis as part of a healthy diet. 'Milk' and 'yoghurt' type products are considered suitable alternative products and are consistent with consumers' requests.

4.2 Phytosterol and Phytostanol Foods on the EU market

Since Novel Foods Approval in 2000, Unilever has launched yellow fat spreads containing phytosterol esters in 12 EU countries (Netherlands, Austria, Belgium, Finland, France, Germany, Greece, Ireland, Portugal, Spain, Sweden and the UK). In addition to this, foods containing Raisio phytostanol esters, the hydrogenated forms of phytosterols, which are not covered by EC258/97, are available in many EU countries. Examples of these currently on the market in the EU include:

- yellow fats spreads (fat contents from 32 and 63%)
- Yoghurts (Natural, Vanilla, Toffee and fruit flavours including strawberry, raspberry, apricot and black cherry)
- Semi-Skimmed Milk
- Chicken Balls
- Chicken Gratin (Chicken dish with potatoes and cream)

- Sausages
- Salads (Potato and Beetroot, mayonnaise based)
- Cereal Bars (Chocolate Chip, Raisin Nut and Apricot)
- Soft Cream Cheeses (Natural and Garlic & Herbs)

4.3 Potential market for the Novel Food(s)

The Post Launch Monitoring on yellow fat spreads has confirmed the pre-market assumptions about the type of consumer that will purchase cholesterol-lowering products. The pro.activ yellow fat spread has a low market share (0.1-2.5%) across most EU yellow fat spread markets. The approved range extensions of 'milk' and 'yoghurt' type products, like the spread, will be targeted at the 'cholesterol concerned' and, due to the cost of phytosterols, will carry premium prices significantly above standard '(unfortified)' variants. The 'milk' and 'yoghurt' type products are expected to be purchased by a similar target population to the spread, with market shares estimated at less than 1.0% in each category. This assumption is supported by marketing information, showing a low market share for comparable phytostanolester products in EU countries, where these are available.

4.4 Product Labelling

In the past, yellow fat spreads were labelled in accordance with the Scientific Committee on Foods decision (SCF, 2000) and European Commission Decision 2000/500/EC of 24 July 2000. Although the translations vary slightly, in general the pack label states:

- Pro.activ may not be nutritionally appropriate for people with special dietary needs (pregnant and breast feeding women, and children under five);
- If taking cholesterol-lowering medication seek your doctor's advice.

Since April 21, 2004 Regulation 608/2004/EC requires the following labelling information for every food with added phytostanols/phytosterols:

> (1) In the same field of vision as the name under which the product is sold there shall appear, easily visible and legible, the words: "with added plantsterols".
> (2) The content of added phytosterols, phytosterol esters (expressed in % or as "xg of free plantsterols per 100 g or 100 ml" of the food) shall be stated on the list of ingredients.
> (3) There shall be a statement that the product is intended for people who want to lower their blood cholesterol level.
> (4) There shall be a statement that patients on cholesterol lowering medication should only consume the product under medical supervision.
> (5) There shall be an easily visible and legible statement that the product may not be nutritionally appropriate for certain sections of the population (pregnant and breast feeding women and children under the age of five years).

(6) Advice shall be included that the product is to be used as part of a healthy diet, including regular consumption of fruit and vegetables (to help maintain carotenoid levels).

The labelling of the range of spreads, 'milk' and 'yoghurt' type products will include the information from the original approval indicating target group, lack of suitability for children, pregnant and lactating women and advice to those receiving cholesterol-lowering medication. All product types applied for will be labelled according to Regulation 608/2004/EC.

For example:

A yoghurt pot will be labelled:

- *with added plantsterols*
- *1 g of plantsterol esters per 100 g*
- The product is intended for people who want to lower their blood cholesterol level.
- Recommended: 2-3 servings daily for optimum cholesterol reduction. This 150g pot is one serving. Extra servings will not provide additional cholesterol-lowering benefit.
- To continue the cholesterol-lowering effects, keep eating two or three servings everyday as part of your healthy diet;
- Patients on cholesterol lowering medication should only consume the product under medical supervision.
- A healthy diet should contain plenty of fruit and vegetables.
- The product may not be nutritionally appropriate for pregnant and breast feeding women and children under the age of five years.

Where it is not possible to include large amounts of text on labels, further details will be available through carelines, websites and accompanying leaflets.

4.5 Anticipated intake of phytosterol esters from the Novel Food(s)

A comprehensive evaluation on the anticipated intake of phytosterol esters has been carried out by Unilever (Unilever 2002).

It is anticipated that the intake of phytosterol esters from the range of food products will be consistent with the product labelling/consumer information that will accompany the marketing of the products and be in the range of 3.2-4.8g of phytosterol esters per day (2-3g of free phytosterols).

Current experience indicates that consumers will follow the advice on product usage. However, should consumers not follow this advice and replace all current spread, 'milk' and 'yoghurt' type product usage with phytosterol ester containing products, then the

potential intake of phytosterols has been modelled based on available information on intakes of these product types. This would represent a worst case scenario phytosterol intake.

Since Cognis' phytosterol source will not add further phytosterol containing products to the market, the total human intake of phytosterols via normal nutrition will not be affected. Cognis' products represent an alternative to existing products, not an additional contribution.

The overall amount of phytosterols resulting from a cumulative consumption of said food products is not expected to exceed 3 g/day as shown by Unilever (Unilever 2002).

5. PREVIOUS HUMAN EXPOSURE TO THE NOVEL FOODS

There has been no previous exposure to phytosterol esters in 'milk' and 'yoghurt' products in the EU. However, there has been significant human exposure to phytosterols in the diet and, as cholesterol-lowering preparations and phytosterol esters, in yellow fat spreads. In addition, there has been significant human exposure to the hydrogenated forms of phytosterols, and phytostanols in a range of food products including yellow fat spreads, 'milk' and 'yoghurt' type products in a number of EU countries. Phytostanols have the same safety profile (Slesinski *et al*., 1999; Turnbull *et al*., 1999a; 1999b; 1999c; Whittaker *et al*., 1999) and cholesterol-lowering efficacy as phytosterols (Hallikainen *et al*., 2000; Weststrate and Meijer 1998).
A summary of the previous exposure to phytosterols, phytosterol esters phytosterol esters and phytostanol-esters in the EU is given in Table 3 below.

Table 3. Previous exposure to phytosterols, phytosterol esters phytosterol esters and phytostanol-esters in the EU (cited from Unilever 2002)

Ingredient	***Source***	***Average Daily Intake***	***How long***
Phytosterols	Diet: vegetable oils, fats, fruits, vegetables and seeds	Typical Western diet 200-400 mg/day Vegetarians up to 680 mg/day	Lifetime exposure
Phytosterols, mainly ß-sitosterol	Cholesterol-lowering preparations, eg Sito-Lande	3-6 g/day	Long term use (years)
Phytosterol-esters	yellow fat spreads	1.2-1.5 g/day	2 years in EU
Phytostanol-esters	yellow fat spreads, soft cream cheese, yoghurt, milk, salads, cereal bars, salads, meat products	Estimated to be up to 3 g/day based on the labelling recommendation of 2-3 servings/day	6+ years in Finland 3+ years in other EU countries

6. NUTRITIONAL ASPECTS

Phytosterols lower serum cholesterol by decreasing cholesterol absorption in the small intestine, with a consequential increase in faecal excretion of cholesterol. The principal mechanism of serum cholesterol-lowering is considered to be competition between cholesterol and the phytosterols for micellar solubilisation in the small intestine. The first studies demonstrating the cholesterol-lowering effect of phytosterols in humans were reported by Pollak in the early 1950's. Since then a large number of clinical studies with phytosterols have been conducted (Pollak, 1985). In these studies, the phytosterols

were given as the free sterols and not as fatty acid esters and both short term and long term (>1 year studies) are reported with many of the studies using large amounts of phytosterol preparations (up to 53g / day).

To date, over 30 clinical trials have been conducted involving over 2000 individuals at study sites in Europe, North America, Latin America, Africa, Asia and Australia.

Different study designs and conditions have been utilised including:

- parallel and cross-over designs,
- variation in the fat level of the spread,
- individuals on both habitual and Step 1 diet (low-fat diet),
- adults and children, males and females,
- normal to hypercholesterolaemics,
- individuals on statin or fibrate cholesterol-lowering drugs,
- diabetics.

In addition, these studies have addressed other issues raised by the European Commission Scientific Committee on Foods and for which information was not available at the time of the original submission. In particular, two studies have been completed that investigate the effects of spreads containing phytosterol esters phytosterol esters when used in conjunction with cholesterol-lowering drugs i.e. statins and fibrates (Neil *et al*., 2001 and Nigon *et al*., 2001, respectively). These studies showed that phytosterol esters phytosterol esters can be used safely, to provide an additional cholesterol-lowering effect to that of the medication alone.

Another study was carried out in a group of children with familial hypercholesterolaemia (Amundsen *et al*., 2001 & 2002). A significant cholesterol lowering effect was seen in these children during the study period. This effect was maintained during a further six month open label follow up period. The spreads were well tolerated by the children with no adverse events reported. Whilst there is no intention to market products containing phytosterol esters phytosterol esters to children, this study confirmed the potential benefit of their use by children with familial hypercholesterolaemia.

In summary, since the original Novel Foods application was made, a significant amount of new clinical data on phytosterol esters phytosterol esters are available, considerably increasing our experience and understanding in this area.

6.1 Effect of the food matrix on the cholesterol-lowering effect of phytosterols

The focus of recent scientific literature has been on the cholesterol-lowering effect of phytosterol esters phytosterol esters in yellow fat spreads. However, their effectiveness in other food matrices has also been demonstrated. In a study reported by Volpe *et al*., (2001) the effects of a yoghurt based drink enriched with 1g/day of plant sterols (soybean extract) for four weeks and 2g/day of plant sterols for eight weeks were

investigated.

At 1g/day of the plant sterol extract, total and LDL cholesterol were reduced by 6.7% and 11.1% respectively, when compared to baseline after four weeks of use. After consuming 2g/day for eight weeks, total and LDL cholesterol were reduced by 11.2% and 15.6% respectively. No significant changes were found in serum levels of fat soluble vitamins (A, D and E) and a number of male and female hormones. In another study the effects of plant stanol-esters in a low fat yoghurt were investigated (Mensink *et al.,* 2002). The plant stanol-esters were emulsified into a low fat (0.7%) yoghurt so as to provide 1g per 150ml cup of yoghurt. Daily consumption was three cups of yoghurt (total of 3g/phytostanol/day) over a three week period. LDL cholesterol was reduced by 13.7% compared to the placebo yoghurt and effects were found to be maximal after one week. There was a decrease of 14.4% in lipid corrected β-carotene levels but other carotenoids were not affected.

A study has been published on the cholesterol-lowering efficacy of phytosterol esters phytosterol esters in milk (Unilever 2002). Thirty nine volunteers consumed both spread and milk in a four way comparison with each intervention lasting three weeks. The interventions were:

- control providing 25g spread and 300ml of milk
- 2g (free) phytosterol/day from 25g phytosterol spread with 300ml of control milk
- 2g (free)phytosterols from 300ml of phytosterol ester milk and 25g/day of control spread;
- 4g (free) phytosterols/day from 25g phytosterol esters spread plus 300ml of phytosterol ester milk;

Phytosterol esters in milk (2g/day), spread (2g/day) and the combination, were equally efficacious in lowering both total and LDL cholesterol by 8-11%. Lipid adjusted serum β-carotene levels were reduced by 10% in the group receiving 2g phytosterol/day in milk and the group receiving 4g phytosterols/day in both spread and milk, but not in the group receiving 2g phytosterols/day in spread. Routine clinical chemistry and haematology parameters were unaffected by any treatment (Clifton, 2002).

The cholesterol-lowering effect of phytosterol esters in salad dressings (Davidson *et al*., 2001; Judd *et al*., 2002) and lean ground beef has also been reported (Carr *et al.,* 2002; Matvienko *et al.,* 2002) with similar results to those obtained with other food matrices.

In summary, the cholesterol-lowering effect of phytosterol esters is not influenced by the food matrix.

6.2 Effect on carotenoids

Clinical studies using 1-4g day of phytosterols (given as phytosterol esters) have indicated a modest reduction (10-25%) in the absorption of the most lipophilic carotenoids (e.g. β-carotene). Comparable reductions in carotenoids have been reported during both short term (three weeks) and long term (52 weeks) trials (Hendriks *et al*., 1999 & 2003). At the recommended intakes of 2-3g free phytosterols/day of the spreads, 'milk' and 'yoghurt' type products, it is expected that any effects on carotenoid lowering will be within this range.

Carotenoids levels are influenced by various factors such as diet, person to person variation and seasonal variations (which are also linked to diet). The plasma level of carotenoids can vary from season to season by up to 30% depending on the main fruit and vegetables available at the time (van het Hof, 1999; Lux and Naidoo, 1994; Olmedilla *et al*., 1994; Saintot *et al*., 1995; Scott *et al*., 1996). Thus a reduction in carotenoids of 10-25% should be considered in the context of these other factors. Furthermore, phytosterol ester enriched spreads are recommended as part of a healthy diet rich in fruit and vegetables and the 'milk' and 'yoghurt' type products will be similarly labelled. As demonstrated by Judd *et al*.,(2002) and Noakes *et al*., (2002), this can have a significant influence on plasma carotenoid levels. For example in the study by Noakes *et al*., (2002) the addition of one extra serving of a high carotenoid fruit or vegetable per day, when consuming phytosterol ester containing spreads, maintained plasma carotenoid levels. In another recently published study, no changes in serum carotenoid concentrations were observed when spreads containing phytosterol and phytostanol-esters were taken as part of a controlled diet (Raeini-Sarjaz *et al.,* 2002).

7. MICROBIOLOGICAL ASPECTS

The microbiological stability of 'milk' and 'yoghurt' type products and spreads containing phytosterol esters is governed by the same principles as conventional products.
Formulation and process rules currently used to ensure design safety of conventional milk and yoghurt products, spreads and salad dressings, are equally applicable to the new products made with phytosterol esters and have been used in setting formulations.

The accepted principles of Good Manufacturing Practice (GMP) used for conventional milk and yoghurt products and spreads will be used to control quality and safety during manufacture.
'Milk' and 'yoghurt' type products containing phytosterol esters have been tested for their microbiological and chemical stability over time, including the impact of pasteurisation, and these have been found to be similar to standard products (Unilever 2002).

8. TOXICOLOGICAL INFORMATION

There is a history of safe consumption of phytosterols within the normal dietary intake of between 200-400mg/day. However, it was estimated that the use of phytosterol esters in yellow fat spreads would lead to a five to ten fold increase in the consumption of phytosterols. Based on that estimate, a thorough toxicological evaluation was required.

Hence, a comprehensive safety testing programme was carried out to address:

- mutagenicity,
- absorption,
- sub-chronic toxicity,
- reproductive toxicity (including oestrogenicity), and
- tolerability of high doses in humans.

The conclusions from these studies were as follows:

- No evidence of genotoxicity (Wolfreys and Hepburn, 2002)
- Absorption is very low (Sanders *et al*., 2000)
- No evidence of subchronic toxicity – NOAEL of 4.1g phytosterols/kg/bodyweight/day in a 90 day rat feeding study (Hepburn *et al.,* 1999)
- No effect on the reproductive system, and no oestrogenic activity (Baker *et al*., 1999; Waalkens-Berendsen., 1999)
- High doses produced no adverse physiological effects in humans (Weststrate *et al*., 1999; Ayesh *et al.,* 1999)

A full toxicological assessment of phytosterol esters using the above studies and data available from the literature was carried out as part of the previous Novel Foods submission (Unilever documents D97/042, D98/002 and D98/028).

Based on this assessment, the EC Scientific Committee on Foods concluded that the use of phytosterol esters in yellow fat spreads at a maximum level, corresponding to 8% free phytosterols, is safe for human use.

Further SCF assessments on phytosterols and their esters from ADM (SCF 2003), Multibene (SCF 2003a), Oy Karl Fazer AB, Pouttu Ltd. and Teriaka Ltd. (SCF 2003b) also considered the envisaged applications to be safe.

Since Cognis' phytosterols and their esters are identical in composition and manufacturing to those currently used, accordingly, the respective safety assessments are applicable.

On the toxicology of free phytosterols in comparison to phytosterol esters, the SCF stated in 2003 (SCF 2003 b):

"The toxicological information available on non-esterified phytosterols is less extensive than that on phytosterol esters with fatty acids evaluated for the use in yellow fat spreads (SCF, 2000) and on the phytostanol esters used in margarine and other types of products (SCF, 2002). Phytosterol esters, however, are hydrolysed by pancreatic cholesterol esterase (Swell *et al.*, 1954; SCF, 2000) and are similar to free phytosterols in the ability to lower cholesterol absorption in rats, suggesting that the liberated phytosterols are the active moieties of the esters (Best and Duncan, 1958; Mattson *et al.*, 1977). In the gut of rats, β-sitosterol linoleate has been shown to undergo extensive hydrolysis to liberate the free sterol and vice versa the free β-sitosterol to be esterified with fatty acids (Minter and Sanders, 1997, unpublished results but cited in SCF 2003b). Therefore, the available toxicological data on phytosterol esters are considered relevant for the evaluation of the free phytosterols as well. Thus, the safety assessment of the plant sterol-enriched foods can largely be based on the data reviewed and evaluated by the Committee in its opinions on phytosterol esters in yellow fat spreads (SCF, 2000) and on long-term effects of elevated levels of phytosterols from multiple dietary sources (SCF, 2002)."

The general innocuousness of phytosterols from various sources has been confirmed by a notification dossier filed by Forbes MediTec in 2002 (Forbes MediTec 2002). Forbes MediTec's phytosterol esters, different from any other phytosterol food product, have been isolated from tall oil. Comprehensive studies on the genotoxicity, endocrine modulation, toxicity to reproduction and subchronic toxicity have been performed on the ingredient, supported by a number of clinical trials that confirmed safety and efficacy. No adverse effects were reported. The FSA in 2003 expressed a favourable opinion on the substantial equivalence of these phytosterol esters compared to the Unilever phytosterol esters and phytostanols (which are no Novel Foods).

Other information on long term use of high doses of phytosterols

From the 1950's through to 1982, β-sitosterol (actually mixtures of phytosterols) was marketed in the USA as a treatment for hypercholesterolaemia under the trade name Cytellin by Eli Lilly. Its use was discontinued by the FDA at the request of the Company (Federal Register, 1985). The reason for withdrawal was lack of palatability (and hence compliance) and the availability of more effective hypocholesterolaemic drugs, especially statins. Cytellin preparations contained unesterified phytosterols suspended in a variety of vehicles, the main ones being methylcellulose and vegetable oils. The source of phytosterols also changed during the lifetime of the drug; initially soyabean and cottonseed oils were used but tall oil (derived from pine trees) was introduced later.

Initially a daily dose of 6-12g of phytosterols was recommended, but later the maximum effect in adults was achieved with 3g phytosterols per day. In clinical trials much higher doses had been used, for example in one study reported by Lees *et al.,* (1977) 9-

24g/day were given for between three months and three years. Between 1952 and 1954 the amount of sitosterol in preparations ranged from 0.3 to 53g/day, although doses between 1 and 10g/day were used in half the studies (Pollak and Kritchevsky, 1981).

In their review of the vast number of clinical studies conducted on β-sitosterol, Pollak and Kritchevsky (1981) concluded that there were no adverse effects or side effects of sitosterol, even when taken for a long time.

The study by Riley and Steiner (1957) is an example of one of the studies where very high doses of β-sitosterol were given. Patients were treated with a 20% liquid suspension of Cytellin. The substance was administered orally before each of the three daily meals in divided dosages, totalling 19 to 52g per day to 13 patients with coronary atherosclerosis. The period of sitosterol administration varied between one to six months per patient. There were no effects on liver function parameters and there was no mention of any adverse effects.

In summary, a thorough toxicological evaluation of free phytosterols or their esters has not identified any adverse health effects up to the maximum dose levels that it is possible to test. Also, human trials involving large daily intakes of phytosterols have not reported any adverse health effects.

9. RISK ASSESSMENT

9.1 Determination of the no-observed adverse effect level

The acceptable daily intake (ADI) is set on the basis of the highest no-observed adverse effect level (NOAEL) in animal studies (JECFA, 1987). The NOAEL for phytosterol esters was established by the 13-week rat feeding study. It is the study of longest duration with phytosterol esters and was conducted according to OECD guidelines that require intensive and extensive examination of the animals. In this study, rats received phytosterols (as phytosterol esters) in their diet at levels of 0, 0.1, 1, 2, or 5%. A dietary level of 5% is generally recognised as the maximum level required for the testing of non nutritive components, without having to modify the diet to maintain nutritional balance (FDA, 2002). There were no adverse effects at the highest concentration and therefore it can be used as the NOAEL. Mean daily intake of phytosterols over the 13 weeks was 3900mg/kg body weight/day in males and 4200mg/kg body weight/day in females. The mean value (4.1g/kg body weight/day) will be used in the risk assessment.

This NOAEL is supported by the lack of adverse effects in the two-generation rat reproduction study when phytosterols comprised 5% of the diet (1.54-5.62g phytosterols/kg body weight/day).

9.2 Determination of the safety factor

In the traditional assessment of safety of micro-food additives, an appropriate safety factor is applied to the NOAEL in the study, using the most sensitive animal species and/or the study giving the lowest NOAEL. A 100-fold safety factor is typically applied to micro-food ingredients, to take into account possible differences in susceptibility between humans and the test species and possible individual susceptibilities within the human population, although higher and lower values may be used depending on the specific material in question (Renwick, 1991). However, for macro-ingredients, where the human intakes are considerably larger than what is usually encountered for micro-food additives and where animal studies cannot create exposures high enough to elicit toxicity, it may not be possible to apply a safety factor of 100. If such substances are free from toxicity when tested in animals at the maximum inclusion levels, without causing nutritional or physiological disturbances, then smaller safety factors may be appropriate, especially if additional data are available to support safety. Such data include chemical structure suggesting low probable toxicity, adequate and reliable human clinical data, a history of safe intake of the ingredient from traditional foods, and studies that indicate that additional exposures are unlikely to result in adverse effects (Rubery *et al*., 1990; Borzelleca, 1992).

A safety factor of less than 100 is appropriate for phytosterol esters because phytosterols are poorly absorbed from the gastrointestinal tract (generally <10%), are rapidly eliminated, and do not accumulate in the mammalian body. There is an absence of structural alerts for toxicity. The material was not genotoxic in a series of *in vitro* mutagenicity and cytogenetic studies. There was no indication of toxicity or any

toxicological or histopathological changes in the 13-week feeding study in rats, or in the two generation reproduction study. Carcinogenicity studies on a structurally related compound, γ-oryzanol (a mixture of ferulic acid esters of phytosterols including β-sitosterol), in both rats (Tamagawa *et al*., 1992a) and mice (Tamagawa *et al*., 1992b) at doses of up to 2000mg/kg body weight/day, failed to elicit any treatment-related adverse effects or increase in tumour incidence. Chronic studies conducted with sitosterols in rats, rabbits and dogs, suggested no adverse effects (Shipley *et al*., 1958). Phytosterols inhibit the carcinogenicity of known carcinogens (Raicht *et al*., 1980; Yusakawa *et al*., 1991; Janizec and Rao, 1992) and have been associated epidemiologically with decreases in colon cancer (Nair *et al*., 1984; Hirai *et al*., 1986). Extended human testing of phytosterols involving high doses has been conducted for decades without any reported adverse health effects. This body of research is supported by recent clinical trials on phytosterol esters showing a lack of adverse effects (see Unilever document D01-033).

Renwick (1991) provides a technique to estimate a safety factor using pharmacokinetic and mechanistic data for an ingredient. He subdivides each of the two main parts of the general safety factor (a factor of ten for extrapolation from animal data to man, and a factor of ten for human heterogeneity) into kinetic and dynamic (mechanism of action), and then provides a way to estimate how each contributes to the total safety factor. Using this method, a safety factor well below 100 is appropriate. Renwick also shows that safety factors less than 100 are not uncommon for food constituents.

For these reasons, based on the totality of analytical, animal, and human data summarised in this document (structural analysis, the numerous well conducted clinical trials in a wide variety of subjects, pharmacokinetic information and the lack of observed toxicity in any species), and using the approaches to estimating a safety factor outlined by Borzelleca (1992) and Renwick (1991), a safety factor of 30, a half-log unit between 10 and 100, was used to calculate the acceptable daily intake (ADI).

9.3 Calculation of the acceptable daily intake

The ADI is calculated as the NOAEL/safety factor (JECFA, 1987). For free phytosterols this would be:

4.1g/kg/day / 30 = 137mg/kg/day.

For a 70kg adult this is 9.6g/person/day. For a 20kg child this is 2.7g/person/day.

These are considered to be conservative estimates as there were no adverse toxicological findings at the highest dose level tested (equivalent to 5% phytosterols) and an effect level could not be determined due to the nutritional limitations of testing higher doses. The NOAEL could be significantly higher than the level established here.

9.4 Determination of the estimated daily intake

If the use of phytosterol esters in the products is as follows:

- 1g (free) phytosterols in12.5g spread
- 1g (free) phytosterols in 125g/150g yoghurt
- 1g (free) phytosterols in 250ml milk

and the products are used as recommended on the labelling then the intake will be 2 to 3g (free) phytosterols/day.

If the labelling recommendations are not followed and it is assumed that the intakes of phytosterol ester products will be equivalent to that of unfortified products, then the potential intakes of free phytosterols has been estimated based on dietary survey and consumer purchase data. From these estimations, the highest potential daily intake of free phytosterols (g/person/day) for each country (irrespective of the source of the data) is estimated to be as follows:

Table 4. Highest potential daily phytosterol intakes (g/person/day) estimated from the dietary survey and consumer purchase data (from Unilever 2002)

	Consumers of at least one product type		***Consumers of all three products***	
	Median (age group)	95th percentile (age group	Median (age group)	95th percentile (age group)
Uk	1.6 (65+)	5.0 (65+)	2.7 (45-64)	5.8 (45-64)
Finland	3.0 (50-64)	6.8 (50-64)	3.5 (50-64)	7.7 (65+)
Germany	1.7 (60+)	4.9 (60+)	2.3 (60+)	7.2 (60+)
France	1.5 (60-64)	4.5 (60-64)	2.1 (60-64)	5.6 (60-64)
Netherlands			4.6 (46-65)	8.2 (6-16)

These intakes are considered to be a worst case situation as:

- The Post Launch Monitoring data has shown that the individual median and 95th percentile intakes of phytosterols are less than might be predicted by comparisons with unfortified products.

- Consumer purchase data from the UK and Finland, where a range of comparable phytostanol-ester products are available, also indicates that intakes are not as high as might be predicted from intakes of unfortified products.

- Consumer purchase data for the UK and Finland also suggest that few households buy more than one product type from the range of phytostanol-ester products available.

9.5 Safety assessment

If the products are used as recommended, the intake will be in the range of 2-3g free phytosterols/day. This level of intake does not exceed that originally expected from the use of yellow fat spreads containing 8% phytosterol esters approved by the EC.

There is no evidence to suggest that consumers will not follow the advice provided on the labelling and supporting literature regarding recommended intakes, particularly as this states that there is no additional cholesterol-lowering benefit from eating more than the recommended amount of 2-3g phytosterols/day.

However, if the products are not used as recommended, the potential intakes of free phytosterols has been estimated based on consumption patterns of unfortifed foods. In reality, it is unlikely that all spread, 'milk' and 'yoghurt' type product consumption would be replaced by products containing phytosterol esters . Even if this were the case, then the data indicate that all intakes, including the 95th percentile intakes, would be below the acceptable daily intake of 9.6g free phytosterols/day.

9.6 Conclusion from the risk assessment

There is no evidence to suggest that an alternative to the range of existing cholesterol lowering products containing phytosterol esters available in the EU but not expanding the range of product types, will lead to an over consumption of phytosterol esters that would result in adverse public health effects.

10. OVERALL CONCLUSIONS

Cognis' phytosterol esters have been on the European market in significant amounts since the year 2000, via approved Novel Foods containing phytosterol esters, according to Commission Decision 2000/500/EC.

The innocuousness of plant phytosterols has been shown by numerous toxicological and clinical investigations. Cognis has proved compliance with the new phytosterol (ester) profile and purity criteria as required by Decision 2004/335/EC of the European Commission. Contaminants are specified according to current food grade requirements.

No additional source of phytosterols or their esters is added to the food chain by allowing the marketing of said Cognis ingredients for the use in milk and yoghurt type products and yellow fat spreads. The overall intake of phytosterols will not be affected, since there will be only alternative not cumulative consumption of multiple foods within one food category. Consequently, it can be guaranteed that the safe daily dose of 3 g of phytosterols is not exceeded by introducing said products to the market.

Suitable labelling according to Regulation 608/2004/EC for products containing Cognis' phytosterols will ensure that the intake of phytosterols and their esters remains within the range required to provide optimal cholesterol reduction.

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APPENDIX I:

- Purity specification of Cognis phytosterol/phytostanol esters

Appearance Visual	White to Off-white lipid
Sterol ester GC-FID	> 95.0%
Free Sterols GC-FID	< 5.0%
Acid Value Titration	<2 mg KOH/g sample
Water Content Karl Fischer	< 0.2%
Lead ICP Scan	< 0.1 ppm
Cadmium ICP Scan	< 0.1 ppm
Arsenic ICP Scan	< 0.1 ppm
Mercury CVUV Scan	< 0.1 ppm
Standard Plate Count BAM 8th Edition	<10,000 CFU/g
Total Yeasts and Moulds BAM 8th Edition	<1000 CFU/g
Total Coliforms BAM 8th Edition	Negative
Salmonella BAM 8th Edition	Negative

APPENDIX II

- Summary results from GC-FID analysis of four independent batches of Cognis' phytosterol esters

Batch No	2/2003-002	2/2003-003	2/2003-004	2/2003-007
Brassicasterol	2.5	2.4	2.3	2.9
Campesterol	26.5	27.3	23.4	28.6
Campestanol	0.7	0.7	0.7	0.6
Stigmasterol	17.0	19.0	14.4	16.6
β-Sitosterol	47.8	45.5	52.7	46.1
β-Sitostanol	2.6	2.1	3.5	2.1
Other sterols/stanols	2.9	3.0	3.0	3.1