SUBSTANTIAL EQUIVALENCE NOTIFICATION:

FORBES CHOLESTEROL-LOWERING PHYTOSTEROL ESTERS.

12th January 2002

Forbes Medi-Tech Inc,

Suite 200, 750 West Pender Street,
Vancouver B.C.
Canada V6C 2T8.

Contents

Introduction
1. Administrative data
2. General description
3. Identification of essential information requirements.
4. Consultation of structured schemes:
    - I Specification of the novel food.
        - I.A. Chemical definition.
        - I.B. Minor sterols
        - I.C. Chemical contaminants
        - I.D. Food-Grade Specifications
        - I.E. Substantial equivalence
        - I.F. Esterification
    - II Effect of the production process applied to the novel food.
        - II.A. Extraction.
        - II.B. Complexation.
        - II.C. Crystallisation.
        - II.D. Esterification
        - II.E. Substantial equivalence
    - III History of the organism used as the source of the novel food.
        - III.A. Natural occurrence
        - III.B. Use as a food ingredient.
        - III.C. Substantial equivalence
    - IX Anticipated intake / extent of use of the novel food.
        - IX.A. Use levels
        - IX.B. Projected intakes
        - IX.C Substantial equivalence
    - X Information from previous human exposure to the novel food or its source.
        - X.A Clinical use.
    - XI Nutritional information on the novel food.
        - XI.A Effect on reducing blood cholesterol levels
        - XI.B Effect on blood phytosterol levels.
        - XI.C. Phytosterolemia in humans.
        - XI.D. Effect on vitamin and nutrient absorption.
        - XI.E. Substantial equivalence.
    - XII Microbiological information on the novel food.
    - XIII Toxicological information on the novel food.
        - XIII.A. Preclinical toxicology
            - XIII.A.a. Genotoxicity assays
            - XIII.A.b. *In Vitro* genotoxicity assays
            - XIII.A.c. *In Vivo* genotoxicity assays
        - XIII.B. Toxicological studies with FCP-3P7
            - XIII.B.a. Uterotrophic assay in immature female rats
            - XIII.B.b. Three-month dietary study with FCP-3P7 in Sprague-Dawley rats
        - XIII.C. Toxicology Studies with Cytellin (Positol)
        - XIII.D. Published toxicology studies with phytosterols

XIII.D.a. Genotoxicity
XIII.D.b. Subchronic toxicity
XIII.D.c. Reproductive toxicity
XIII.E. Clinical toxicology.
XIII.E.a. Clinical studies employing FCP-3P7
XIII.E.b. Literature review.
XIII.F. Substantial equivalence.
5. Evaluation and conclusion by the applicant.
6. Summary by the applicant

Annexes

Annex A. Structures of major phytosterols.
Annex B. Analytical studies.
Annex C. Clinical studies.
Annex D. Toxicological studies.
Volume 1. Genotoxicity reports.
Volume 2. Uterotrophic assay in immature female rats.
Volume 3. Three month dietary toxicity study in Sprague-Dawley rats – Volume 1.
Volume 4. Three month dietary toxicity study in Sprague-Dawley rats – Volume 2.
Volume 5. Three month dietary toxicity study in Sprague-Dawley rats – Anatomical pathology report.
Annex E. Sample label.
Annex F. Post-market monitoring.

Substantial Equivalence Notification:

Forbes Cholesterol-Lowering Phytosterol Esters.

Introduction

FCP-3P7, is the product code for a cholesterol-lowering food ingredient containing esters of plant sterols and plant stanols produced by Forbes Medi-Tech. FCP-3P7 has a composition intermediate between the active ingredients of two existing phytosterol containing yellow fat spread products available in EU markets: Benecol and Flora pro.activ. Forbes Medi-Tech wishes to market FCP-3P7 in EU countries as a yellow fat spread ingredient that could be substituted directly for either of the two existing products. However, before introducing any new food product to the European market it is necessary to consider its position under the provisions of Regulation (EC) No 258/97 concerning novel foods and novel food ingredients. The Novel Foods Regulations are designed to ensure that novel foods and novel food ingredients are subjected to a thorough assessment of wholesomeness to protect public health.

For three categories of novel foods, including foods and food ingredients derived from plants or animals obtained by traditional propagating or breeding and having a safe history of food use, a simplified procedure for pre-market approval can be applied provided that the food/ingredient is *substantially equivalent to existing foods or food ingredients as regards their composition, nutritional value, metabolism, intended use and level of undesirable substances contained therein* (Article 3.4. Regulation 258/97). In accordance with the provisions of Article 3.4 of Regulation 258/97 this submission sets out the case for proposing FCP- 3P7 as being a food ingredient derived from plants obtained by traditional propagating with a safe history of use that is substantially equivalent to the existing food ingredients contained in Benecol and Flora pro.activ

Commission Recommendation 29/07/97 provides guidance on the presentation of information necessary to support applications for placing on the market novel foods and novel food ingredients. To provide a comprehensive assessment of the substantial equivalence of FCP-3P7 to existing food ingredients, the structure provided in those Recommendations have been used to prepare this submission although it is not intended to provide a complete wholesomeness assessment as described in Regulation 258/97. Some new sub-headings have been introduced to improve clarity.

1. Administrative data

The applicant and manufacturer of FCP-3P7 is Forbes Medi-Tech Inc, Vancouver B.C. The person responsible for the dossier is:

Name: David Stewart, Ph.D. , Vice President, Regulatory Affairs
Address: Suite 200, 750 West Pender Street,
Vancouver B.C. Canada V6C 2T8.
Phone: +1 604 689 5899
e-mail: dstewart@forbesmedi.com

2. General description

FCP-3P7 is a cholesterol-lowering food ingredient containing esters of phytosterols and phytostanols. FCP-3P7 belongs in Class 1.1 as defined in chapter 4 of the Recommendations because the source of the novel food ingredient (wood sterols) has a history of food use in the community.

Wood sterols are a component of the range of cholesterol-lowering products marketed in Europe by McNeil Consumer Nutritionals under the trade name "Benecol". Yellow fat spreads containing phytostanol esters, have been on sale in Finland since before the EC Novel Food Regulation (258/97) came into effect. Benecol was launched in Finland in 1995 and has since become popular. When the EC Novel Foods Regulation was introduced in 1997, approval for Benecol had been sought through the Finnish Competent Authority and the European Commission. Both these bodies took the view that because there was a significant history of consumption of the product in Finland, this product could not be considered a novel food under the EC Novel Foods Regulation. In 1999 Benecol was on sale in Belgium, Holland, Luxembourg and the UK. A second cholesterol lowering phytosterol containing yellow fat spread ("Flora pro.activ") has subsequently been approved under the novel foods Regulations. FCP-3P7 has a composition that is intermediate between Benecol and Flora pro.activ.

Benecol and other phytostanol and phytosterol containing food products also have a history of food use in non-EU countries including the USA. Flora pro.activ is marketed as "Take Control" in the USA.

3. Identification of essential information requirements.

Schemes I, II, III IX, X, XI, XII and XIII laid down in Commission Recommendation 29/07/97 would apply to a novel food in category 1.1:

I Specification of the novel food.

II Effect of the production process applied to the novel food.

III History of the organism used as the source of the novel food.

IX Anticipated intake / extent of use of the novel food.

X Information from previous human exposure to the novel food or its source.

XI Nutritional information on the novel food.

XII Microbiological information on the novel food.

XIII Toxicological information on the novel food.

It should be noted that all of the data included in X, XI, XII, and XIII are based on studies with the non-esterified form of FCP-3P7. In this application it will be established that the non-esterified and esterified forms of phytosterols are substantially equivalent and such data are directly applicable to the esterified form.

4. Consultation of structured schemes (decision trees).

I Specification of the novel food

Scheme I requires information about the composition of the novel food, including analytical information about potentially toxic inherent constituents, contaminants and nutrients, which should be representative of the novel food when produced on a commercial scale including specifications to ensure that the novel food marketed is the same as that evaluated.

The tall oil phytosterols in FCP-3P7 are extracted from tall oil soap, a by-product of the pulping process for coniferous trees in North America and Europe. FCP-3P7 is intended for use in yellow fat spreads.

I.A. Chemical definition.

The FCP-3P7 product under consideration is predominantly a mixture of esters of four phytosterols: sitosterol, sitostanol, campesterol, and campestanol. A small percentage of minor phytosterols such as stigmasterol is also present as well as a fraction of a percentage of long chain aliphatic alcohols. The natural ratio of phytosterols varies from batch to batch and this is taken into account in the specification. The properties of the major phytosterol components are described below. (N.B. The term 'phytosterol' is frequently used as a generic to include both sterols and stanols.)

Sitosterol: (3β)-Stigmast-5-en-3ol; $C_{29}H_{50}O$;
Mol. wt. 414.72. Plates from alcohol.
Melting point 140 (138-142) degrees Celsius.
Soluble in acetone and ethyl acetate.

Sitostanol: (3β, 5α)-Stigmastan-3-ol; $C_{29}H_{52}O$;

Mol. wt. 416.73. Monohydrate, crystals, melting point 138-139 degrees Celsius.

(Melting point 144-145 degrees Celsius when dry.)

Soluble in acetone and ethyl acetate.

Campesterol: (3β,24R)-Ergost-5-en-3-ol; $C_{28}H_{48}O$;

Mol. wt. 400.69

Melting point 157-158 degrees Celsius.

Soluble in acetone and ethyl acetate.

Campestanol: (3β, 5α, 24R)-Ergostan-3-ol; $C_{28}H_{50}O$;

Mol. wt. 402.70

Melting point 146.5-147.8 degrees Celsius.

Soluble in acetone and ethyl acetate.

The chemical structures of the four major phytosterol constituents of FCP-3P7 are provided in Annex A.

I.B. Minor sterols

Table I-i lists minor sterol components that have been observed in batches of FCP-3P7 sterols. The minor sterol components primarily represent variation in the position and/or number of double bonds within sitosterol (C_{29}) and campesterol (C_{28}) structures. Also present in trace amounts are saturated long chain (C_{15} – C_{25}) aliphatic alcohols. These minor, long chain alcohol components are substances commonly found in the diet

Table I-i :FCP-3P7 minor components

α-sitosterol	24-Methyl diene isomers (C_{28} compounds)
Stigmasterol	24-Ethyl di- and tri- ene isomers (C_{29} compounds)
Ergosterol	Trace phytosterols and phytostanols
C_{15} - C_{25} Aliphatic alcohols (< 0.5%)	

I.C. Chemical contaminants.

To detect the presence of heavy metal contaminants, the following analytical methodologies are routinely employed (Table I-ii):

Table I-ii: Tests employed to detect the presence of heavy metals in FCP-3P7

Analyte	Test Method	Limits
Total heavy metals, including Pb, Hg, Cd and As	AOAC 986.15 and AOAC 971.21	NMT 10 ppm
Arsenic	AOAC 986.15	NMT 2 ppm
Lead	AOAC 986.15	NMT 0.25 ppm
Cadmium	AOAC 986.15	NMT 1 ppm
Mercury	AOAC 971.21	NMT 1 ppm

NMT - Not more than.

The results of the heavy metal testing for batches FM-PH-15 and FM-PH-52 and the range of values over the last six batches of the tall oil phytosterol product are shown below in Table I-iii. Analytical methods and typical analyses are provided in Annex B.

Table I-iii: Test results for heavy metals in batches of FCP-3P7.

Test Item	Limit	Results		
		FM-PH-15	FM-PH-52	Range previous 6 Batches
Total heavy metals, including Pb, Hg, Cd, and As.	NMT 10 ppm	Not Detected	< 1.07 ppm	0 - <1.06 ppm
As	NMT 2 ppm	Not Detected	< 2 ppm	0 - <2 ppm

Benzo[a]pyrene was not detected by GC/MS analysis of two batches of FCP-3P1 (FM-PH-15 and FM-PH-52), the mixture of phytosterols used to prepare the esterified FCP-3P7 food ingredient. Further, analysis of one batch of the FCP-3P1 (Batch E7-04-017) was conducted to confirm the absence of pentachlorophenols, dioxins, and furans. The results indicated no detectable levels of these compounds. These structures would not be expected to survive the alkaline digestion used to free wood fibres in the pulping process.

I.D. Food-Grade Specifications.

Table I-iv Proposed Food-Grade Specifications for FCP-3P7 Sterols.

Phytosterol content	> 95%
Sitosterol	36% to 79%*
Sitostanol	6% to 34%*
Campesterol	4% to 25%*
Campestanol	0% to 14%*
Total major sterols	> 86%*
Loss on drying (water)	< 5%
Solvents (isopropanol)	< 0.5%
Residue on ignition	< 0.1%
Heavy metals	< 10 ppm
Arsenic	< 2 ppm
Lead	< 0.25 ppm
Total aerobic count	< 10,000 CFU/g
Combined moulds & yeasts	< 1000 CFU/g
Coliformes	negative
Salmonella	negative

* Expected sterol profile based on the variability in sourcing/seasonal variation of the plant sterols.

Table I-v. Proposed Food Grade Specifications for FCP-3P7 sterol esters

Test Name	Method	Specification
Appearance	Visual	White to Off-white lipid
Sterol ester	GC-FID	> 97.0%
Free Sterols	GC-FID	< 3.0%
Acid Value	Titration	<2 mg KOH/gm sample
Water Content	Karl Fischer	<0.2%
Lead	ICP Scan	<0.25 ppm
Cadmium	ICP Scan	<1.0 ppm
Arsenic	ICP Scan	<2.0 ppm
Mercury	CVUV Scan	<1.0 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

Certificates of analysis for ten batches of FCP-3P7 sterols and three batches of FCP-3P7 sterol esters are provided at Annex B.

I.E. Substantial equivalence.

FCP-3P7 is a yellow fat spread ingredient based on a non-hydrogenated tall oil product containing esters of phytosterols and phytostanols that has cholesterol-

lowering properties. Currently, there are two vegetable oil spread products containing phytosterols on the European market which contain up to 14% by weight of added fatty acid esterified phytosterols and which make this claim: Benecol, which is marketed by McNeil Consumer Nutritionals and contains primarily hydrogenated tall oil blended with vegetable oil sterols and Flora pro.activ, manufactured by Unilever, which contains predominantly sterols derived from vegetable oil. FCP-3P7 has a composition that is intermediate between the active ingredients of Benecol and Flora pro.activ (Table I.vi). All phytosterol products contain variable ratios of phytosterols and phytostanols. This reflects natural variation in plant sources and also variation in the degree of hydrogenation applied in the production process. The specification for FCP-3P7 is intended to accommodate this natural variation in observed sterol/stanol profiles. FCP-3P7's upper limit for sitosterol is 71%. Section IX demonstrates that this does not result in increased intakes of sitosterol.

Benecol uses sterols from two sources, tall oil (from coniferous trees) and vegetable sterols obtained as a by-product of vitamin E production. These latter vegetable sterols originally derive primarily from soy and also contain variable amounts of rapeseed sterols. Flora pro.activ uses only vegetable sterols. The sterols isolated from vegetable and coniferous sources are complex mixtures of different sterol molecules. When these sterols are hydrogenated, nearly all of the unsaturated components are converted to either sitostanol or campestanol.

FCP-3P7 contains significant levels of sitosterol and campesterol, similar to those occurring in Flora pro.activ. Unlike Flora pro.activ, it contains only minor quantities of stigmasterol and other sterols but significant levels of the naturally occurring saturated (stanol) compounds sitostanol and campestanol as found in Benecol. Benecol employs a hydrogenation process to saturate most double bonds present in the sterol components, converting them to stanols, predominantly sitostanol and campestanol. The phytosterols in Flora pro.activ are not hydrogenated and contain up to 8% by weight of minor sterol and non-sterol components (see Table I-vi). Similarly, FCP-3P7 contains a number of the same minor components, primarily representing variation in the position and / or number of double bonds within sitosterol (C_{29}) and campesterol (C_{28}) structures. Also present are trace quantities of C_{15}-C_{25} saturated aliphatic alcohols. All minor components represent substances commonly found in the diet and in one or both of the current products. Thus FCP-3P7 has compositional elements that are common to one or both of the existing products and which, in aggregate, support its substantial equivalence.

It is intended that FCP-3P7 should be consumed in a manner identical to Benecol and Flora pro.activ to provide consumers with an additional product choice. The FCP-3P7 tall oil phytosterol esters product merely revises the ratio of major sterols to stanols to an intermediate composition when compared to the other two currently marketed yellow fat spreads. The nutritional and physiological properties, intended use and projected intakes of FCP-3P7 would therefore be expected to be equivalent to those of Benecol and Flora pro.activ. This will be confirmed in subsequent sections of this submission.

Table I-vi. Comparison of sterol compositions of sterol / stanol esters.

Sterol	Flora pro.activ (expected sterol profile)[1]	FCP-3P7[2]	Benecol[3]
Brassicasterol	0 - 9	n.d.	0 - 0.2
Campesterol	10 - 40	6 – 21	0 - 2
Campestanol	0 - 6	2 – 11	8 - 32
Stigmasterol	6 - 30	0 - 1	0 - 1
Sitosterol	30 - 65	40 – 71	0 - 7
Sitostanol	0 - 10	9 – 31	58 - 83
Minor sterols	0 - 13	5 to 15	0 - 9

1 Opinion of the Scientific Committee for Food on a request for the safety assessment of the use of phytosterol esters in yellow fat spreads. European Commission Health and Consumer Protection Directorate General, April 2000.
2 Data are based on GC-FID analyses of batches obtained between 1997 and 2000. Methods of analyses and reports are supplied in Annex B.
3 Benecol is a mixture of both wood and vegetable oil stanol esters. Benecol values were taken from publications. See Annex B.
n.d – Not detected.

I.F. Equivalence of esterified and free phytosterols

The phytosterols in FCP-3P7, Flora pro.activ and Benecol have been esterified with common vegetable oil fatty acids to enhance their solubility in a vegetable oil based product matrix. The degree of esterification may vary but does not materially affect the substantial equivalence of phytosterol products because the ester forms are de-esterified *in vivo*.

Dietary cholesterol esters are hydrolysed to free cholesterol and fatty acids by pancreatic carboxyl ester lipase (Homan and Krause, 1997). Likewise, phytosterol esters are hydrolysed to free sterols and fatty acids (Swell *et al*, 1954; Best and Duncan, 1958; Mattson, 1977) in the small intestine. It is generally recognized by FDA scientists and other investigators studying phytosterols that the active form of the phytosterol ester is the free phytosterol (FDA Interim Ruling on Health Claims for sterol and stanol esters, 2000).

Plant sterols and stanols are thought to decrease cholesterol absorption by competing with cholesterol for incorporation into mixed micelles (Cater and Grundy; 1998.). The mixed micelles are formed in the small intestine as mixtures of lipids, cholesterol and bile salts. The formation of these micelles is fundamental to the subsequent absorption of cholesterol. Thus, phytosterols regardless of the degree of esterification interact at this site. Hydrolysis of cholesterol esters by pancreatic lipases occurs in the same micellar structures. The pancreatic lipase enzymes have a unique requirement for a mixed lipid:water interface for activity (Verger, 1984).

The available human data on activity of non-esterified and esterified phytosterols indicate that on the basis of phytosterol content, there exists no clinically significant difference in the LDL cholesterol lowering activity between

these forms. Table I-vii presents dose response data for effects on LDL cholesterol of phytosterol esters and free phytosterols. The term phytosterols commonly encompasses both plant stanols and sterols. The dose response characteristics are similar between esterified and non-esterified forms of phytosterols. Some studies also presented data on apo lipoprotein B which is the protein component of LDL.

Table I-viii shows studies in which direct comparisons of sterols and stanols were made. There are no apparent differences in the activity of sterols and stanols in LDL cholesterol lowering activity. Furthermore, it has been established that none of the various forms of phytosterols have any clinically significant effects on either triglycerides or HDL cholesterol (Morrell, 2000. Jones *et al* 1997.).

Although there is considerable variation in the measured effects of sterols, stanols, and sterol esters on serum carotene, there is a similar action in lowering serum levels of carotenes by all forms of phytosterols. Table XI-v in section XI.D presents dose response data for the effects of phytosterol esters and free phytosterols on serum carotene serum and retinol levels. No significant changes in retinol levels were seen in any of the various studies on sterol esters, stanol esters and free sterols.

Sterol esters, stanol esters, and sterol/stanol mixtures (e.g. FCP-3P7 sterols) have undergone toxicological testing under standardised conditions which meet the International Standard for Good Laboratory Practices. None of the above types of phytosterols have shown any evidence of significant toxicity. Studies have been done on genotoxicity and mutagenicity (stanol esters; Turnbull *et al*) (FCP-3P7 sterols; this application). Phytosterols show no estrogenic activity when administered orally (sterol esters; Baker *et al*, 1999), (stanol esters; Turnbull *et al*, 1999) and (FCP-3P7 sterols; this application). Furthermore, all have been tested in subchronic toxicity studies with top doses of 5% phytosterols in the diet in the rat (highest allowable dose under ICH Guidelines), (sterol esters; Hepburn et al, 1999); (stanol esters; Turnbull *et al*, 1999), and (FCP-3P7 sterols; this application). Reproduction toxicity studies have also been carried out (stanol esters; Slesinski et al, 1999), (sterol esters; Waalkens-Berensen et al, 1999) and (stanol esters; Whittaker et al, 1999).

The overall conclusion is that there are no significant differences between stanols and their corresponding sterols and esters thereof regarding their safety and efficacy. This is what would be expected from consideration of their structural similarities and identical routes of activation and absorption. Therefore, stanols, sterols, and their esterified forms should be considered as equivalent.

Toxicological and nutritional studies on FCP-3P7 were performed using the non-esterified form of FCP-3P7 phytosterols (referred to in the study reports as FCP-3P1). Since all sterols are activated and absorbed in the same way, the non-esterified form of FCP-3P7 can be considered to be physiologically equivalent to the esterified form This means that studies performed using FCP-3P1 are directly applicable to FCP-3P7.

Table I-vii: Dose response studies with phytosterols in food matrices

Reference	No. per group	Phytosterol	Food Matrix	Dosage g/day[1]	Duration (days)	Placebo LDL Cholesterol (mmol/L)	Change in LDL-C	Change in Apo B
Hendriks *et al*., 1999.	80	Sterol esters	"Margarine" suspension	0.00 0.83 1.61 3.24	25 25 25 25	3.05 - - -	- -6.2%* -9.2%* -9.8%*	N/a
Davidson *et al*, 2001	21 21 19 23	Sterol esters	"Margarine" or salad dressing	0.0 3.0 6.0 9.0	56 56 56 56	3.29 - - -	- -5.0%* -2.8%* -9.0%*	N/a
Maki *et al*, 2001	83 75 35	Sterol esters	"Margarine" suspension	0.0 1.1 2.2	35 35 35	4.08 - -	- -4.9%* -5.4%*	3.3%* -2.9%* -5.1%*
Christiansen *et al*, 2001	46 41	Sterols	"Margarine" suspension	0.0 1.5 3.0	180 180 180	4.29 - -	- -11.3%* -10.6%*	N/a
Study CLF9904	24 23 28 23	Sterol/stanol mixture	Milk Drink	0.0 0.9 1.8 3.6	28 28 28 28	4.19 - - -	- -7.4%* -8.6%* -13. 2%*	N/a
Hallikainen et al., 2000	22	Stanol esters	"Margarine" suspension	0.0 0.8 1.6 2.4 3.2	28 28 28 28 28	4.42 - - - -	- -1.7% -5.6%* -9.7%* -10.4%*	- -8.7%* -9.2%* -10.1%* -13.7%*

1. Calculated on basis of phytosterols content
* Difference from placebo is statistically significant

Table I-viii: Comparison of sterols and stanols for effectiveness in lowering LDL cholesterol

Reference	No. per group	Phytosterol	Matrix	Dosage g/day[1]	Duration (days)	Control LDL Cholesterol (mmol/L)	Change in LDL-C
Vanstone *et al.*, 2001	15	Sterols	Butter	1.8	21	4.30	-10.1%*
	15	Sterols/Stanols mixture	Butter	1.8	21	4.30	-15.4%*
	15	Stanols	Butter	1.8	21	4.30	-13.8%*
Jones, *et al.*, 2000.	15	Sterol esters	"Margarine " suspension	1.8	22	4.46	-13.2%*
	15	Stanol esters	"Margarine " suspension	1.8	22	4.46	-6.4%*
Weststrate and Meijer, 1998.	95	Sterol esters	"Margarine " suspension	3	24-25	3.36	-13.1%*
	95	Stanol esters	"Margarine " suspension	3	24-25	3.36	-11.9%*

1. Calculated on basis of phytosterols content
* Difference from placebo is statistically significant

II Effect of the production process applied to the novel food.

Scheme II requires information about whether the process results in a significant change in the composition or structure of the novel food compound compared to its traditional counterpart.

Tall oil phytosterols in FCP-3P7 are extracted from tall oil soap, a by-product of the pulping process used for coniferous trees in North America and Europe. The trees are reduced to fine wood chips and then digested at pH 14 for about 18 hours at 50 degrees to free the wood fibres. The lipid layer that forms at the top of the digestion is tall oil soap. It is skimmed off and used as a source of phytosterols. The phytosterols are then extracted directly from the tall oil soap using the Forbes Medi-Tech Inc. proprietary and patented extraction processes. The sterols are extracted and purified in a three-step process.

II.A. Extraction

Starting Material: Tall oil soap with >2% sterols

Product: Extract with 15 to 25% sterols

The first step is a solvent extraction (isopropanol) 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 aqueous phase contains residual solvents, residual tall oil soap, and water. The residual solvents are recovered, the water is removed from the tall oil soap

and the extracted tall oil soap is then sent to an acidulation plant for further processing.

The organic phase contains extracted organic materials, phytosterols and approximately 70% of the solvents. The organic solvents are recovered. The extract, largely free of solvents, contains 15 to 25% sterols and is then used in the next step of the process.

II.B. Complexation

Starting Material: Extract from Step 1 containing 15 – 25% sterols.

Product: Crude sterols with 60 –75% purity

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 solvent, and complexing agent in a stainless steel reactor. The sterols rapidly bind to the agent. The complexed sterols are 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, which contains 60-75% sterols, is called crude sterols and is used in the next step of the process.

II.C. Crystallisation

Starting Material: Crude sterols from step 2 with 60-75% purity

Product: Purified sterols with >95% purity

The crude sterols are dissolved in alcohol (isopropanol) at elevated temperature. The temperature of the mixture is reduced to allow crystallisation of the sterols. The crystals are recovered and then dried. The mixture is tested for content of sterols. If the desired purity is not achieved, the mixture is re-crystallised a second time.

II.D. Esterification

Sterol esters are produced by reacting the purified sterols with fatty acid methyl esters in the presence of sodium methoxide. The fatty acids are from soy, canola, sunflower, or other food grade vegetable oils. Methanol is produced as a by-product of the esterification and is removed during the process. The sterols, fatty acid methyl esters are mixed in a reactor and dried under vacuum at an elevated temperature to remove water. The esterification reaction is initiated and catalysed by the addition of sodium methoxide. The reaction is carried out at >100° C under vacuum. Food grade anti-foaming agent is added as needed to control problem foaming caused by the evolution of methanol. Under these conditions nearly all the methanol is removed during the reaction. When the reaction is complete, the product is cooled to < 100° C and water is added to deactivate the catalyst, which is converted irreversibly to sodium hydroxide and methanol. The water contains most of the sodium hydroxide, methanol, and fatty acid soaps and is separated from the product by centrifugation.

After the reaction is complete and the catalyst removed, multiple adsorbents are used to remove trace components.

II.F. Substantial equivalence

The processes of extraction, complexation and crystallisation produce purified sterols the composition of which is dependent on the sterol composition of the starting product. The method of production and the product composition are equivalent to those phytosterol products already used in Europe as cholesterol-lowering ingredients in yellow-fat spreads.

III History of the organism used as the source of the NF.

Scheme III requires information about the source organism of the novel food and whether it or substances in it are detrimental to human health.

III.A. Natural occurrence

Phytosterols or plant sterols occur naturally in the non-saponifiable material of plant oils. The most abundant phytosterol in nature is ß-sitosterol. Campesterol, stigmasterol and dihydrobrassicasterol exist at much lower concentrations. Sitostanol, the saturated derivative of ß-sitosterol and stigmasterol, is found in negligible concentrations in plant sources and, hence, is almost absent from typical Western diets. Most phytosterols are similar to cholesterol in their basic skeleton structure except that they contain methyl, ethyl, di-methyl, di-ethyl or other groups next to their C_{24} position on the aliphatic side chain of the compound (Pollak and Kritchevsky, 1981). When phytosterols are saturated at the 5 α-position using commercial processes, compounds such as sitostanol are formed. Saturated phytosterol derivatives are not abundant in nature (Weihrauch and Gardner, 1978; Dutta and Appelqvist, 1996). This difference in the chemical structure between cholesterol and phytosterol results in significant biological and physiological variations. For instance, phytosterols are not synthesized in humans (Salen et al., 1970) and their absorption rate in the intestine is about 0 to 5% (Grundy et al., 1969), unlike cholesterol which is produced in humans and exhibits an absorption rate of 40 to 50 % in normal subjects (Grundy et al., 1969).

The presence and distribution of phytosterols across plant species have been extensively described by Pollak and Kritchevsky (1981). Phytosterols are found in plants that include ornamental, edible types as well as herbs, shrubs and trees (Pollak and Kritchevsky, 1981). At least 44 sterols from seven different plant classes have been identified (Bean, 1973). The list of phytosterols, their sources and botanical functions has grown. Crombie (1961), Shoppee (1964) and Bean (1973) listed a large number of sterols and their sources in plants. The greatest number of phytosterols, naturally present in pure or esterified form, or conjugated as glycosides, were found in the angiosperms and the most dominant were ß-sitosterol, campesterol and stigmasterol.

There are several factors that affect the distribution of phytosterols in plants. Among other factors, the phytosterol content of any given plant depends on the length of daylight, degree of soil alkalinity, and time of plant harvest. For example, light exposure or photoperiod has been shown to lower ß-sitosterol in leaves of *Solanum audigena* (Bae and Mercer, 1970). Soil alkalinity, seasons and leaves shedding have been also reported to alter the concentrations of ß-sitosterol and campesterol in the plants (Misra et al., 1961; Davis, 1971).

Dietary phytosterol levels among different populations vary greatly depending primarily on the type and amount of plant foods consumed. Western diets, for example, typically contain lower levels of phytosterols than diets of many other parts of the world. In 1991, the British consumed 104, 49, 10, and 4 mg per day of β-sitosterol, campesterol, stigmasterol and stigmastanol [sitostanol], respectively, representing a total phytosterol intake of 167 mg per day (Morton et al., 1995). The primary sources of phytosterols in the British diet are fats and oils, although breads and other cereals were also important sources (Morton et al., 1995). A trend toward increased phytosterol intakes was observed between 1987 and 1991 in Britain, possibly due to increased use of vegetable oils for cooking

The Tarahumara Indians of Mexico who consume a diet containing unusually high amounts of beans and corn reportedly ingest over 400 mg of phytosterols per day (Cerqueira et al., 1979). In Japan, phytosterol intakes remained at about 373 mg per day from 1957 to 1982, while cholesterol consumption simultaneously increased over twofold (Hirai et al., 1986). The most commonly ingested phytosterol is β-sitosterol (54%), while significant levels of campesterol (14%), brassicasterol (10%), and stigmasterol (7.5%) are also consumed (Hirai et al., 1986).

III.B. Use as a food ingredient

Yellow fat spreads containing tall oil phytostanol esters, have been on sale in Finland since before the EC Novel Food Regulation (258/97) came into effect. Benecol was launched in Finland in 1995 and has since become very popular. When the EC Novel Foods Regulation was introduced in 1997, approval for Benecol had been sought through the Finnish Competent Authority and the European Commission. Both these bodies took the view that because there was a significant history of consumption of the product in Finland, this product could not be considered a novel food under the EC Novel Foods Regulation. In 1999 Benecol was on sale in Belgium, Holland, Luxembourg and the UK. A second cholesterol lowering phytosterol containing yellow fat spread (Flora pro.activ) has now been approved as a food under the novel foods regulations and has been on sale since June 2000 in the UK.

Benecol and other phytostanol and phytosterol containing food products also have a history of food use in non-EU countries including the USA. Flora pro.activ is marketed by Lipton as “Take Control” in the USA. In addition, Forbes product, Phytrol (also termed FCP-3P1, the non-esterified version of FCP-3P7) has successfully undergone the FDA GRAS (Generally Recognized as Safe) procedure. The phytosterols used in FCP-3P7 also have a history of clinical use (see Scheme III). The safety of ingested phytosterols has been

thoroughly reviewed and discussed in the process of establishing the respective FDA GRAS status of the Take Control, Benecol and Phytrol vegetable oil spreads. The development of the Lipton product, Take Control, has yielded substantial research into the safety of phytosterols, particularly sitosterol, campesterol, and stigmasterol [Baker et al, 1999; Hepburn et al, 1999; Jones et al, 1999; Wallkens-Berendsen et al, 1999]. Similarly, the development of the McNeil product, Benecol, has also yielded substantial research into the safety of phytosterols, particularly sitostanol and campestanol [Slesinski et al, 1999; Turnbull et al, 1999; Turnbull et al, 1999; Turnbull et al, 1999;Whittaker et al, 1999].

III.C. Substantial equivalence

When McNeil's Benecol and Unilever's Flora pro.activ were marketed, phytosterols became available in the EU for every day consumer use incorporated into yellow fat spreads with the aim of achieving a healthy cholesterol level. The phytosterols employed in Benecol are sourced from a blend of vegetable oil and tall oil. The resultant phytosterol blend is then hydrogenated to convert the plant sterols to stanols; principally sitostanol and campestanol. The remaining fraction is comprised largely of unsaturated sterols. The phytosterols employed in Flora pro.activ are obtained from vegetable oil (e.g., soybean, canola, corn) distillate. The major sterol components show some variation from batch to batch depending upon which form of vegetable oil is employed in the production of the phytosterol product. However, the predominant phytosterols in Flora pro.activ, by weight, are sitosterol, campesterol, and stigmasterol. Up to 8% of Flora pro.activ may be comprised of 20 to 30 different minor sterol components. The phytosterols in each Benecol and Flora pro.activ are esterified with vegetable oil fatty acids.

The phytosterol ester composition of FCP-3P7 is intermediate between those of Benecol and Flora pro.activ and is thus equivalent to these two products in terms of their safe history of food use. Benecol and Flora pro.activ have undergone extensive safety testing. Similarly, safety testing carried out on FCP-3P7 sterols (see Sections XI and XIII below) demonstrates that it is equivalent in terms of its lack of potential for detriment to human health.

IX Anticipated intake / extent of use of the novel food.

Section IX requires information about anticipated uses of the novel food, intakes for groups at special risk, geographical limits and the effects of substitution for existing foods in the diet.

IX.A. Use levels.

The intended use of FCP-3P7 is to incorporate it into a vegetable oil based spread product at a concentration of up to 12.5% by weight in the esterified form. This represents an application and a phytosterol content which is slightly

lower that of Flora pro.activ and Benecol which have an incorporation rate up to 14% by weight of esterified phytosterols.

The intended consumer daily consumption of FCP-3P7 in yellow fat spread provides 1.5 grams of phytosterols (Table IX-i). This intake rate is slightly less than that of Flora pro.activ or Benecol yellow fat spread products based on free phytosterol content. The recommended usage is based on efficacy studies carried out using FCP-3P7 phytosterols.(see section XI.A).

Table IX-i. Intended use conditions for phytosterol yellow fat spreads

	Flora pro.activ	**FCP 3P7**	**Benecol**
Incorporation rate (%)	13.80*	12.50	14.00*
Serving size (g)	10.00*	10.00	12.00*
Amount per serving (as esters, g):	1.38	1.25	1.68
Amount per serving (as sterols, g):	0.83	0.75	1.01
Servings per day	2*	2	2 – 3*
Daily intake (sterols, g/day)	1.66	1.50	2.02 – 3.02

* Information taken from Flora pro.activ and Benecol product labels.

IX.B. Projected intakes of individual phytosterols.

Comparative projected daily intakes of individual phytosterols are summarised in Table IX-ii. Although FCP-3P7 differs slightly in sterol composition (Table I-v) and use levels (Table IX-i) compared with Flora pro.activ and Benecol, FCP-3P7 is substantially equivalent to the two products because the resulting intakes of the individual sterols in FCP-3P7 will always be equal to or lower than current levels derived from Flora pro.activ and Benecol.

Table IX-ii. Range of projected intakes of FCP-3P7 phytosterols.

	Flora pro.activ		**FCP 3P7**		**Benecol**	
Sterol	**lower**	**upper**	**lower**	**upper**	**lower**	**upper**
Brassicasterol	0.00	0.07	0.00	0.00	0.00	0.01
Campesterol	0.08	0.33	0.05	0.16	0.00	0.02
Campestanol	0.00	0.05	0.02	0.08	0.07	0.27
Stigmasterol	0.05	0.25	0.00	0.01	0.00	0.01
Sitosterol	0.25	0.54	0.30	0.53	0.00	0.06
Sitostanol	0.00	0.08	0.07	0.23	0.49	0.70
Minor sterols	0.00	0.11	0.04	0.11	0.00	0.08

FCP-3P7 is intended for use by adults as part of a cholesterol-lowering diet. Packaging for the ingredient FCP 3P7 will bear a label containing the warnings set out at point 6 of Annex E. In order to ensure that these warning also appear on retail product packaging a clause will be included in any contract with prospective manufacturers. This wording is designed to ensure that any subsequent advice on labelling from any European authority is effectively transmitted via manufacturers and retailer to consumers.

Contract clause:

> The manufacturer shall include warnings on all labelling and packaging of the product, with such prominence, wording and positioning as shall from time to time be required by Forbes Medi-Tech. For illustration only, an example of such warning could read: "Pregnant women, lactating women and children under five have special dietary needs and should talk to their doctors before using any product to manage cholesterol".

It is intended that yellow fat spread products that contain FCP-3P7 will provide an alternative to existing phytosterol containing yellow fat spreads and not be consumed in conjunction with them. In order to ensure that consumption remains within recommended levels and that the product is being consumed by targeted individuals whilst being avoided by inappropriate consumers a post market monitoring programme will be instigated. The details of the planned programme are provided at Annex F.

It is anticipated that the novel food will be marketed in the UK and Irish Republic initially but this will not affect projected intakes.

IX.C Substantial equivalence.

FCP-3P7 is intended to replace equivalent yellow fat spreads on a gram per gram basis and so the level of substitution is expected to have no nutritional significance for any consumer group. Intakes of total sterols/stanols and of individual phytosterols will be equal to or lower for FCP-3P7 than for existing phytosterols ingredients and consequently intakes are substantially equivalent. The introduction of FCP-3P7 into the European market will have no expected impact on total phytosterol intakes because the product is intended only to substitute for yellow fat spreads already marketed in European countries.

X Information from previous human exposure to the novel food or its source.

Scheme X requires information about previous direct, indirect, intended or unintended exposure to the novel food or its source, which is relevant to the Community situation with respect to production, preparation, population, lifestyles and intakes.

Over the past fifty years, clinical research has indicated that increased phytosterol intake can have an effect on lowering blood cholesterol levels in humans. Human studies with dose levels of up to 25,000 mg per day (i.e., about 100 times the normal dietary intake) and lasting up to several years, have been performed (Pollak and Kritchevesky, 1981; Pollak, 1985). Some of these studies, which have been conducted as early as the 1950s, have involved over 1800 men, women, adolescents, and children. Within the context of these studies, repeated observation of no adverse side effects has occurred (Lees et al., 1977; Oster et al., 1977).

Attempts to capitalize upon the perceived benefits of increased phytosterol intake are not new to the market place. A drug product marketed by Eli Lilly between the 1950s into the 1980s, named Cytellin, contained phytosterols and was available in the United States to treat hypercholesterolemia. The same product was also available in Canada and sold under the brand name Positol. The phytosterol composition of Cytellin was also derived from tall oil phytosterols. It was composed of approximately 80% sitosterol and 10% campesterol with another 9% of the product composed of stanols. Therapeutic levels ranged from 9000 to 30,000 mg/day. Repeated clinical investigation of Cytellin reported no contraindications or side-effects (Lesesne et al., 1955; Joyner and Kuo, 1955; Kuo, 1956; Best et al., 1955; Duncan and Best, 1963; Farquhar et al., 1956).

XI Nutritional information on the novel food.

Scheme XI requires information to show that the novel food is nutritionally equivalent to existing foods that it might replace, to show that the novel food does not affect the bioavailability of nutrients or have any adverse physiological effects.

The consumption of phytosterols has been linked to three potential nutritional effects: reduction in circulating cholesterol, increase in circulating phytosterols and reduction of vitamin and nutrient absorption. This section will provide information based on studies using FCP-3P7 phytosterols (Table XI-i) and studies reported in the literature that will support the substantial equivalence of FCP-3P7 to the two existing products.

Table XI-i. Studies undertaken using FCP-3P7 sterols in nutritional analysis.

Study	Species	Treatment time)	Number of subjects
CLF9601	Human volunteer	10 days	11
CLF9602	Human volunteer	10 days	12
CLF9701 (Jones *et al* 1999)	Human volunteer	30 days	32
CLF 9904	Human volunteer	28 days	66
115-003	Sprague-Dawley rats	3 months	192

Studies were performed using the non-esterified form of FCP-3P7 phytosterols (referred to in the reports as FCP-3P1) These are physiologically equivalent to their esterified counterparts so that studies performed using FCP-3P1 are directly applicable to FCP-3P7 (for more information see Section I.F.)

XI.A. Effect on reducing blood cholesterol levels.

It is well established that phytosterols are effective in lowering blood cholesterol levels when administered orally in animals and humans. Maximum lowering of LDL cholesterol was observed in human studies with plant-derived sterols in the range of 13-15%. A study by Weststrate *et al* 1998 directly compared a Flora pro.activ product with that of a Benecol product in humans over a 25-day treatment period. Data from this study are presented in Table XI-ii. For purposes of comparison, 30-day data from a FCP-3P7 sterols (yellow fat spread) study by Jones *et al* 1999 are also presented in Table XI-i.

Table XI-ii: Comparative effectiveness of sterol products in a margarine matrix

	Flora pro.activ	**FCP-3P7**	**Benecol**
Dosage	3 g per day	1.5 g/70kg/day	3 g per day
Total Cholesterol	-8.3	-8.3	-7.3
LDL Cholesterol	-13.0	-14.3	-13.0
HDL Cholesterol	+0.6*	-1.5*	+0.1*

Values are percentage changes compared to placebo group. Dosages are calculated on basis of phytosterol content.

* Differences are not statistically significant.

In this comparison, FCP-3P7 sterols at a the proposed dosage appear to be equally effective and thus substantially equivalent to the other two products. More recent studies have shown that other phytosterols may be effective at lower doses as well (Hendricks *et al* 1999).

XI.B. Effect on circulating phytosterols.

Only 1 to 42 μmol/L of phytosterols are found in human serum under normal conditions with dietary intakes of 160-360 mg/day, however plasma levels have been shown to increase up to two-fold by dietary supplementation (Connor, 1968; Cerquira *et al.*, 1979; Salen *et al.*, 1970). The effect of orally administered phytosterols is dependent on the sterol composition administered. Where the sterols have a high sitosterol content, plasma levels of campesterol are depressed and plasma levels of sitosterol are raised. (Tables XI-iii and XI-iv). Phytosterol esters of phytosterols from soy, which have a high content of sitosterol and campesterol, raised the concentration of both phytosterols in the plasma (Westrate & Meijer, 1998). Hydrogenated phytosterols, which contain primarily sitostanol, when administered orally, consistently depress plasma levels of both campesterol and sitosterol. For example, 3 g per day of sitostanol ester depresses campesterol and sitosterol levels by 44% and 43%, respectively (Gylling and Mietinen, 1994). It was concluded that phytostanols not only interfere with cholesterol uptake but also interfere with the uptake of other phytosterols. Administration of sitosterol, while reducing uptake of campesterol, increases the blood levels of sitosterol because of the excess

sitosterol available for uptake from the gut. A preparation with a high content of sitosterol and campesterol raises plasma levels of both components. Administered sitostanol blocks the uptake of sitosterol and campesterol in addition to blocking cholesterol uptake. The tall oil phytosterol blend FCP-3P7 contains enough sitostanol to partially block the uptake of both sitosterol and campesterol and thus offsets the increased load of phytosterols presented to the uptake mechanism in the intestine.

Table XI-iii. Effect of oral phytosterols on plasma levels of sitosterol and campesterol in adults.

Dose of Plant Sterols g/day	Plasma Levels		Reference
	Sitosterol (μmol/L)	Campesterol (μmol/L)	
0.22 g/d sitosterol	13.7	No data	Salen *et al.*, 1970
5.65 g/d sitosterol	26.5	No data	
Control	8.0	17.5	Weststrate and Meijer, 1998
3 g/d soy phytosterol esters	11.1	30.2	
3 g/d stanol esters	5.1	14.5	
Control	No data	18.6	Miettinen *et al.*, 1995.
2.6 g/d stanol esters	No data	10.7	

Table XI-iv Effect of oral phytosterols on plasma levels of sitosterol and campesterol in children.

Dose of Plant Sterols (g/day)	Plasma Levels		Reference
	Sitosterol (μmol/L)	Campesterol (μmol/L)	
Control	37.8 ± 26.2	36.7 ± 26.8	Becker, 1993
1.5 (Stanol)	18.6 ± 7.5	18.9 ± 8.4	
6 (Sterol)	43.0 ± 19.4	26.5 ± 12.0	
Control	1.56 ± 1.12	1.45 ± 1.26	Becker, 1992
6.0 (Sterols)	1.90 ± 0.98	1.11 ± 0.59	
Control	0.88 ± 0.24	No data	Schlierf *et al.*, 1978
12.0 (Sterols)	1.48 ± 0.62	No data	
Control	13.3 ± 1.0	30.1 ± 2.4	Gylling *et al.*., 1995.
3.0 (Stanol esters)	8.5 ± 0.6	15.6 ± 1.4	

Note: Becker 1992, 1993: appears to be a phytosterol preparation with a high content of sitosterol similar to FCP-3P7.

The effects of FCP-3P7 sterols intake on plasma levels of sitosterol and campesterol in human studies are shown in Table XI-v. At a dosage of 1.5g /70 kg per day, there was no consistent effect on plasma levels of sitosterol or campesterol. Reports of clinical studies are provided in Annex C.

Table XI-v. Phytosterol plasma concentration in humans after treatment with 1.5g / 70kg / day of FCP-3P7 sterols*

Study	Treatment Time (days)	Sitosterol (μmol/ L)		Campesterol (μmol/ L)	
		Control	Treated	Control	Treated
CLF9601	10	1.58 ± 0.30	3.04 ± 1.1	19.6 ± 3.7	13.4 ± 4.0
CLF9602	10	9.6 ± 3.0	9.2 ± 3.3	12.3 ± 2.8	18.1 ± 6.0
CLF9701	30	6.1 ± 0.5	4.4 ± 1.7	26.4 ± 12.0	27.5 ± 11.7

*dosage is calculated on basis of phytosterol content

XI.C. Phytosterolemia in Humans

Phytosterolemia (Sitosterolemia), a very rare lipid storage disease characterized chemically by increased plant sterol levels and 5α-saturated stanols in plasma and tissue, is associated with premature atherosclerosis. As of 1992, 27 individuals with phytosterolemia had been detected (Bhattacharyya *et al.*, 1991; Salen *et al.*, 1992). Table XI-vi lists plasma levels of phytosterols that occur in this disease. Phytosterols account for an average of 13% of the total sterols present in plasma in phytosterolemics, compared to about 0.4% in normal subjects. Clinical studies have shown that consumption of plant sterols does not exacerbate hyperphytosterolemia in such subjects.

Table XI-vi Plasma Levels of phytosterols in Phytosterolemia

Sterol	Average Plasma Level (μmol/L)	Range (μmol/L)	References
Sitosterol	850	340 - 1570	Salen *et al.*, 1985
Campesterol	425	186 - 596	
Sitostanol	100	46 - 144	Bhattacharyya *et al.*, 1974
Campestanol	70	20 - 99	
Cholesterol	6300	3300 - 12000	Miettinen, 1980

Phytosterolemia is inherited as a recessive trait. Heterozygotes are clinically and biochemically normal although plasma phytosterol levels of some heterozygotes may be slightly increased over control levels (Salen *et al.*, 1992). The absorption rate of phytosterols is very high in phytosterolemics. The sterol uptake mechanism in the intestine does not distinguish between cholesterol and phytosterols, thus approximately equal proportions of sitosterol and cholesterol

are absorbed (Salen *et al.*, 1992). As diet contains only trace amounts of 5α-saturated stanols, it is thought that the stanols are produced endogenously in large amounts. In normal subjects, the liver secretes sitosterol into the bile so there is a three-fold enrichment of sitosterol to cholesterol as compared to blood (Salen *et al.*, 1970). In phytosterolemic subjects, sitosterol appears in the same or lower proportions relative to cholesterol in the bile as compared to blood. In addition, less cholesterol is secreted into the bile (Bhattacharyya & Connor, 1974). The large quantities of sitosterol and cholestanol in the liver of phytosterolemic subjects competitively inhibits cholesterol 7α-hydroxylase mediated bile acid synthesis (Shefer *et al.*, 1994).

Two studies have examined the effects of phytosterols on subjects that had either homozygous or heterozygous forms of this disease. Lutjohann et al., 1995 showed that administration of stanols in a dose of 0.5g three times daily to phytosterolemic subjects was followed by reduced cholesterol absorption, increased faecal output of cholesterol and plant sterols, and a marked reduction in serum phytosterols. The effect of sterols in homozygous subjects has not been studied. Heterzygotes have a higher than normal absorption of phytosterols. Stalenhoef et al., 2001 conducted an open feeding study to determine whether plant sterol-enriched margarine can be used safely in heterozygotes. An intake of around 3g/d of plant sterols (Becel pro-aktiv, Unilever), increased campesterol or sitosterol levels in blood similar to those found in normal subjects. In addition, plasma cholesterol levels were reduced to the same extent as in normal or hypercholesterolemic individuals. It appears that in subjects heterozygous for phytosterolemia plant sterols might be efficacious in reducing cholesterol, without significant accumulation of plant sterols in their plasma (Stalenhof et al., 2001).

XI.D. Phytosterol Effects on Vitamin and Nutrient Levels

The metabolism of ingested phytosterols and the influence of phytosterols on absorption of fat soluble nutrients, particularly the fat soluble Vitamins A, D, E and K and beta carotene, has been thoroughly reviewed and discussed in the evaluation of Flora pro.activ (SCF, 2000). Similarly, the sterols and stanols in FCP 3P7 have been evaluated for their effects on absorption of fat soluble vitamins.

FCP-3P7 sterols were evaluated in a 3-month dietary toxicity study involving Sprague-Dawley rats (Study 115-003, Redfield Laboratories, Arizona, report dated August 31, 2000). The study was performed using FCP-3P1 , the non-esterified form of FCP-3P7, which is physiologically equivalent to FCP-3P7 (see Section I.F.). Three FCP-3P7 sterol dose levels in diet were evaluated for 90 days versus a control for treatment effects on serum levels of Vitamins A and E. Results of this study are summarized in Tables XI-vii and XI-viii below.

The results demonstrate no treatment-related differences when group mean serum concentrations of vitamins A or E levels were compared to controls.

Table XI-vii: Serum Vitamin A (t-retinol, ng/ml) concentrations (mean ± standard deviation) in rats after 90-days of treatment.

Group*	Control	1.25% FCP-3P7#	2.5% FCP-3P7#	5.0% FCP-3P7#
Male	100.27 ± 48.54	95.77 ± 56.87	97.85 ± 60.47	112.35 ± 60.11
Female	47.52 ± 31.75	47.09 ± 23.90	62.70 ± 35.10	58.18 ± 26.81

as free sterols.

* = 20 rats/sex/group except Group 1 male had 19, Group 3 had 18, and Group 4 male had 19 rats/sex/group. The FCP-3P7 sterols were included in the diet at the indicated concentrations. The phytosterol ±intake at each dosage level is estimated to be 0, 999, 2021, 4160 mg/kg/day in males and 0, 1163, 2358, 4839 mg/kg/day in females based on dietary intake and phytosterol analysis in the feed.

Table XI-viii: Serum Vitamin E (tocopherol, ng/ml) concentrations (mean ± standard deviation) in rats after 90-days of treatment

Group*	Control	1.25% FCP-3P7#	2.5% FCP-3P7#	5.0% FCP-3P7#
Male	387.4 ± 185.8	402.1 ± 161.8	424.3 ± 198.0	397.6 ± 226.2
Female	509.6 ± 266.1	630.7 ± 395.1	522.4 ± 210.6	521.2 ± 237.1

as free sterols.

* = 20 rats/sex/group except Group 1 male had 19, Group 3 had 18, and Group 4 male had 19 rats/sex/group. The FCP-3P7 sterols were included in the diet at the indicated concentrations. The phytosterol ±intake at each dosage level is estimated to be 0, 999, 2021, 4160 mg/kg/day in males and 0, 1163, 2358, 4839 mg/kg/day in females based on dietary intake and phytosterol analysis in the feed.

The mechanism of absorption is similar for fat-soluble vitamins A, D, E, K, and beta-carotene. Since FCP-3P1 sterols did not inhibit the absorption of vitamins A and E, it is reasonable to conclude that absorption of Vitamins D, K, and beta-carotene were likewise not impaired.

In study CLF 9904[1] 123 moderately hypercholesterolemic subjects consumed three milk drinks containing either 0.0, 0.9, 1.8 or 3.6 g of FCP-3P7 in its non-esterified form per day for four weeks. There were highly significant differences between treatment groups with respect to total cholesterol, LDL cholesterol and LDL:HDL ratios. The groups given 1.8 g/day and 3.6 g/day free sterols experienced significant decreases in total cholesterol during the treatment period.

[1] Note: The report refers to a trademarked product (Phytrol) which is the non-esterified form of FCP-3P7.

Table XI-ix presents Vitamin A, Vitamin E and β-carotene plasma values at baseline and after treatment with FCP-3P7 phytosterol enriched milk-based beverage. There were no significant differences in Vitamins A and E plasma values between treatment groups in either the absolute values at the end of the study or in the relative change from baseline.

Alpha-carotene levels at baseline and after treatment are also summarized in Table XI-ix. There was no significant difference between treatment groups either at baseline or after treatment. Subjects randomised to 3.6g/day FCP-3P7 phytosterols experienced a small decrease in alpha-carotene levels after treatment and this difference was significantly different ($p<0.05$) from the small increase observed in the placebo group. Beta carotene is recognized as the precursor for the synthesis of Vitamin A - alpha carotene is considered less significant. This study demonstrated no effect of FCP-3P7 phytosterols on fat soluble Vitamins A and E and β-carotene absorption or plasma levels. Reports of clinical studies are provided in Annex C.

Table XI-ix: Effect of FCP-3P7 sterols in a milk-based beverage on serum Vitamin A, Vitamin E and carotenoid levels.

Parameter/group	Mean	Mean		Difference from placebo (change from baseline)		
	Baseline	**After treatment**	**N**	**Mean**	**95% C.I.**	**Significance**
Vitamin A µmol/l						
Placebo	2.32	2.39	31			
0.9 g./day	2.14	2.14	28	-0.08	(-0.25, 0.09)	NS
1.8 g/day	2.06	2.09	31	-0.06	(-0.22, 0.11)	NS
3.6 g/day	2.15	2.20	32	-0.05	(-0.22, 0.11)	NS
Vitamin E µmol/l						
Placebo	37.6	38.7	31			
0.9 g./day	38.5	36.2	28	-3.5	(-8.0, 1.1)	NS
1.8 g/day	37.7	38.0	31	-0.8	(-5.3, 3.6)	NS
3.6 g/day	35.4	35.2	32	-1.2	(-5.6, 3.2)	NS
Parameter/group	**Median[1]**	**Median[1]**	**N**	**Significance of difference**		
	Baseline	**After treatment**				
α-Carotene µmol/l						
Placebo	0.21	0.33	31	NS		
0.9 g./day	0.23	0.25	32	NS		
1.8 g/day	0.31	0.30	33	NS		
3.6 g/day	0.34	0.28	33	p<0.01		
β-Carotene µmol/l						
Placebo	0.55	0.66	31	NS		
0.9 g./day	0.68	0.82	32	NS		
1.8 g/day	1.13	0.93	33	NS		
3.6 g/day	0.81	0.77	33	NS		

1 Non-Gaussian distribution

XI.E. Substantial equivalence.

The above studies were performed with the FCP-3P7 sterol component of the ester and not the ester itself. At the time of the report of the Scientific Committee on Food in April 2000, virtually all of the data available for effects of phytosterols on fat soluble vitamins and nutrients was from studies on phytosterol esters and not free phytosterols. New studies have become available since that report. We have attempted to make a complete review of studies in which the effects of phytosterols on carotenoids have been reported. The purpose of this review was to examine whether any obvious differences exist between stanol esters, sterol esters, free phytosterols with respect to effects on carotenoids.

A total of 18 studies on phytosterols and carotenoids were available for evaluation. Two studies used the free phytosterol form, Christiansen et al, 2001

and CLF9904 which used the free phytosterol form of FCP-3P7. One study by Relas *et al*, 2001 looked at the effects of stanol esters on post-prandial serum levels of carotenes. No significant effects were seen at this short time interval. This data was omitted from the compilations which are presented in Data Tables XI-xiii to XI-xvi. There are no standardised units for measuring carotenes. Some investigators report only the total of alpha and beta carotene, others segregated the measurements. The units used varied from umol/L to ug/mL, and various combinations. In the data tables XI-xiii to XI-xiv, the most common units, umol/L were used. Where other units were used, the data were converted to the same units. In one study, only ratios of β carotenes to cholesterol were presented (Nguyen *et al*,1999). To provide a comparison, this study was compared with the same data from other studies where available (see Data Table XI-xv). The rationale for making this calculation is that it corrects for the drop in LDL which carries most of the carotene. However, the decline in β carotene when expressed in this form is very similar to that of the absolute measurements (see Data Tables XI-xiii to xv).

Tables XI-xiii, XI-xiv, and XI-vi include data from study CLF9904. The values from Table XI-ix were used when making comparisons with published studies. To keep the comparison consistent with the published studies, only the values at the end of the treatment interval were used for calculating percentage changes due to treatment. This is a different treatment of the data from that in the CLF9904 report. The statistical analysis of the study took into account the non-Gaussian distribution of the alpha and beta carotene data and compared the data against baseline.

In summarizing the data from Data Tables XI-xiii to XI-xvi, only the data presented as the sum of α+β carotene was used because this allowed the most complete data set. Some investigators presented data only as the sum of the two forms. Where separate alpha plus beta carotene measurements were provided but no sum of alpha + beta carotene was calculated, the separate measurements were added together to obtain the α+β total. It is also worth noting that α+β carotene are both considered to be precursors of retinol (vitamin A). The trends observed for α+β carotene are the same as for either beta carotene, the major component in serum or for the beta carotene/cholesterol ratio and would not alter the conclusions drawn.

Although study CLF9904 with FCP-3P7 sterols when expressed as the sum of alpha+beta carotenes showed no effect on carotene levels, another study with free sterols, Christiansen et al 2001, reported some reduction in carotene levels. The difference between the two studies could be due to a number of factors including variability or perhaps study duration. The Christiansen study interval was 6 months as compared to 4 weeks. At this point, there is not enough data available to conclude that there is any difference between the free phytosterol form and the esterified form.

To obtain the overall dose response characteristics, data on the percentage changes in α+β carotene from Data Table XI-xiv were sorted according to the dose ranges indicated in Table XI-x. The data from 15 of the 18 studies could be compared directly. The data from Nguyen *et al*, 1998 which was presented only as ratios and Hallikainen *et al* 1999 which measured only beta-carotene were omitted. Only one study included dosages at 4 g per day and above

(Davidson et al, 2001). Most of the studies were less than 3 months. Two studies of one year duration (Gylling *et al*, 1999) and one study of 6 months duration (Christiansen *et al*, 2001) have been completed. Not enough data are available to compare dose response characteristics at longer time intervals.

Inspection of the available data indicated that all types of phytosterols including free sterols, sterol esters and stanol esters had some effect on carotene levels. It can be noted from Table XI-x that when the means of percentage changes in $\alpha+\beta$ carotene are compared, there is a dose response trend in which the reduction in $\alpha+\beta$ carotene increases with dosage.

Table XI-x : Relation between dose of phytosterols and $\alpha+\beta$ carotene levels in all studies

	Dose: g per day of phytosterols						
Dose Range	**below 0.99**	**1 to 1.99**	**2 to 2.99**	**3 to 3.99**	**4.0**	**6.0**	**9.0**
Minimum	-12.3	-25.0	-41.6	-32.5	-19.6	-29.2	-30.0
Average % of Control	-3.2	-5.6	-21.2	-17.2	-19.6	-29.2	-30.0
Maximum	8.1	24.2	-8.6	6.1	-19.6	-29.2	-30.0
Number of studies	3	6	9	10	1	1	1

Six of the studies were in the form of a dose response design, these are summarised in Table XI-xi. Most of these studies showed a dose/response trend. The study of longest duration was by Christiansen et al, 2001, which examined the effects of dosages of free sterols at dosages of 1.5 and 3 g per day for 6 months. At both doses, the reduction in $\alpha+\beta$ carotene was 14.9%. The study by Davidson et al, 2001, examined the effects of dosages as high 9g per day for 8 weeks. At doses of 6g and 9g per day $\alpha+\beta$ carotene decreased by 29% and 30% respectively. Dose response data is also presented for retinol. None of the changes in retinol reported were statistically significant.

Table XI-xi. Dose response studies with phytosterols in food matrices; effect on retinol and carotene precursors.

Reference	No. per Group	Phytosterol	Food Matrix	Dosage g/day[1]	Duration (days)	α+β Carotene % of control	Change in Retinol
Hendriks *et al*., 1999.	60 60 60 60	Sterol esters	"Margarine" suspension	0.00 0.83 1.61 3.24	25 25 25 25	0.00% -12.28% -8.77% -29.82%	N/a
Davidson *et al*, 2001	21 21 19 23	Sterol esters	"Margarine" or salad dressing	0.00 3.0 6.0 9.0	56 56 56 56	0.0% 4.0% -29.2% -30.0%	0.00% -11.3% -13.9% -9.57%
Maki *et al*, 2001	0 92 40	Sterol esters	"Margarine" suspension	0.00 1.1 2.2	35 35 35	0.0% -5.9% -21.6%	0.00% 4.55% -4.55%
Christiansen *et al*, 2001	46 46 41	Sterols	"Margarine" suspension	0.00 1.5 3.0	180 180 180	0.0% -14.9% -14.9%	0.00% -3.16% -2.11%
Study CLF 9904	24 23 28 23	Sterol/stanol mixture	Milk Drink	0.00 0.9 1.8 3.6	28 28 28 28	0.0% 8.08% 24.24% 6.06%	0.00% -10.5% -12.6% -7.9%
Hallikainen *et al*., 2000	22	Stanol esters	"Margarine" suspension	0.0 0.8 1.6 2.4 3.2	28 28 28 28 28	0.0% -5.4% -3.2% -8.6% -9.7%	0.00% 1.03% -2.74% -0.34% -1.03%

1. Calculated on basis of phytosterols content

Most of the clinical studies on the effect of phytosterols on serum α+β carotene levels showed some evidence of suppression, particularly at higher doses. However, the dose-response relationship appears to be inconsistent and subject to a large degree of variability.

To obtain an estimate of the endogenous variability of the α+β carotene measurement in untreated subjects, the changes reported for untreated groups of subjects are summarized in Table XI-xii. Six studies were available in which carotenes were measured at the beginning and end of the clinical studies in untreated subjects. The reported changes varied from -7.27% to 30.3%. These changes are similar in magnitude to those reported when subjects are exposed to phytosterols.

Table XI-xii Variability in baseline alpha & beta carotenes in untreated subjects

Reference	Group Size	Duration	Baseline	Endpoint	Percent Change
Christiansen *et al*, 2001	46	180 days	0.74	0.87	17.6
Davidson *et al*, 2001	84	56 days	0.40	0.47	17.5
Gyling *et al*, 1999	49	365 days	1.38	1.42	2.90
Maki *et al*, 2001	83	35 days	0.55	0.51	-7.27
Plat *et al*, 2001	42	56 days	1.66	1.57	-5.42
Study CLF9904	31	28 days	0.76	0.99	30.3
Average	56	125 days	0.92	0.97	9.26

Natural levels of serum carotenes vary widely and this variability obscures any clear relationship between dose, duration of exposure and effect. At the proposed dose of 1.5 g per day for FCP-3P7, any reductions in carotenes would be minimal (see Table XI-x). The variation in serum levels of carotenes measured in subjects not exposed to elevated phytosterol intakes is larger than the expected reduction at this dose level and suggests that factors such as the consumption of fruits and vegetables or the use of fortification or supplements will be much more significant in determining serum levels of carotenes. At present, there is not adequate data available to suggest that any significant differences exist between sterols, sterol esters, stanol esters or mixtures thereof with respect to effects on serum carotene levels. FCP-3P7 which is a mixture of sterol and stanol esters is therefore substantially equivalent to both of the marketed sterol ester and stanol ester products.

Data Table XI-xiii: Listing of serum alpha-carotene and beta-carotene levels in various human clinical studies on the effects of phytosterols

Reference	Phytosterol	Test Period	Dose total phyto-sterols - g/day	Number of Subjects	Baseline a-carotene umol/L	After Tx a-carotene umol/L	% of control	Baseline b-carotene umol/L	After Tx b-carotene umol/L	% of control*
Christiansen et al., 2001	Wood sterol	6 months	0.00	46M/F	0.15	0.23	0.00	0.59	0.64	0.00
			1.50	46M/F	0.15	0.19	-17.39	0.61	0.55	-14.06
			3.00	41M/F	0.14	0.21	-8.70	0.58	0.53	-17.19
Davidson et al., 2001	Sterol ester	8 weeks	0.00	21M/F	0.08	0.08	0.00	0.32	0.38	0.00
			3.00	21M/F	0.08	0.11	33.33	0.32	0.37	-2.44
			6.00	19M/F	0.08	0.07	-22.22	0.29	0.26	-30.73
			9.00	23M/F	0.08	0.07	-22.22	0.33	0.26	-31.71
Gylling et al., 1996	Stanol ester	12 months	3.20	51M/F	0.46	0.32	-30.43	1.63	1.10	-32.52
Gylling et al., 1999a	Stanol ester	12 months	0.00	49M/F	0.28	0.27	0.00	1.10	1.15	0.00
			3.00	102M/F	0.26	0.18	-33.33	1.00	0.65	-43.48
Gylling et al., 1999b	Stanol ester	6 weeks	3.50	23F	0.46	0.32	-30.43	1.63	1.10	-32.52
	Stanol ester		3.40	23F	0.46	0.31	-32.61	1.63	1.10	-32.52
	Stanol ester		2.50	21F	0.46	0.32	-30.43	1.63	1.19	-26.99
Hallikainen et al., 1999	Control	8 weeks	0.00	6M/11F				1.00	1.06	0.00
	Stanol ester		2.31	8M/10F				1.66	1.22	15.09
	Stanol ester		2.16	6M/14F				1.47	1.07	0.94
Hallikainen et al., 2000a	Stanol ester	4 weeks	2.02	34M/F	0.66	0.64	-3.03	1.39	1.23	-11.51
	Sterol ester		2.06	34M/F	0.66	0.61	-7.58	1.39	1.16	-16.55
Hallikainen et al., 2000b	Stanol Ester	4 weeks	0.00	8M/14F	0.28	0.28	0.00	0.64	0.64	0.00
			0.80	8M/14F	0.28	0.28	0.00	0.64	0.61	-4.69
			1.60	8M/14F	0.28	0.30	7.14	0.64	0.60	-6.25
			2.40	8M/14F	0.28	0.29	3.57	0.64	0.55	-14.06
			3.20	8M/14F	0.28	0.29	3.57	0.64	0.55	-14.06
Maki et al., 2001	Sterol ester	5 weeks	Control	83M/F	0.12	0.09	0.00	0.43	0.42	0.00
			1.10	74M/F	0.11	0.09	0.00	0.49	0.39	-7.14
			2.20	33M/F	0.10	0.08	-11.11	0.37	0.32	-23.81
Plat et al., 2000	Stanol ester	4 weeks	2.50	39M/F	0.05	0.04	-20.00	0.32	0.26	-18.75
Tammi et al., 2000	Stanol ester	3 months	0.00	74M/F				0.82	0.84	0.00
			1.50	74M/F				0.86	0.63	-25.00
Vuorio et al., 2000	Stanol ester	3 months	2.24	24M/F	0.37	0.20	-45.95	1.34	0.91	-32.09
Study #CLF 9904	Wood sterols	4 weeks	Placebo	31	0.21	0.33	0.00	0.55	0.66	0.00
			0.90	32	0.23	0.25	-24.24	0.68	0.82	24.24
			1.80	33	0.31	0.30	-9.09	1.13	0.93	40.91
			3.60	33	0.34	0.28	-15.15	0.81	0.77	16.67

*Values were calculated as percent of placebo/control value at the end of the experimental interval.
M = male, F = Female

Data Table XI-xiv: Listing of serum alpha+ beta carotene levels in various human clinical studies on the effects of phytosterols

Reference	Phytosterol	Test Period	Dose total phytosterols - g/day	Number of Subjects	Baseline a + b-carotene umol/L	After Tx a + b-carotene umol/L	% of Control*
Christiansen et al., 2001	Wood sterol	6 months	0	46M/F	0.74	0.87	0.00
			1.50	46M/F	0.76	0.74	-14.94
			3.00	41M/F	0.72	0.74	-14.94
Davidson et al., 2001	Sterol ester	8 weeks	0.00	21M/F	0.40	0.47	0.00
			3.00	21M/F	0.40	0.48	4.00
			6.00	19M/F	0.37	0.33	-29.20
			9.00	23M/F	0.41	0.33	-30.00
Gylling et al., 1996	Stanol ester	1 year	3.20	51M/F	2.09	1.42	-32.10
Gylling et al., 1999a	Stanol ester	1 year	0.00	49M/F	1.38	1.42	0.00
			3.00	102M/F	1.26	0.83	-41.55
Gylling et al., 1999b	Stanol ester	6 weeks	3.50	23F	2.09	1.42	-32.10
	Stanol ester	6 weeks	3.40	23F	2.09	1.41	-32.50
	Stanol ester	5 weeks	2.50	21F	2.09	1.51	-27.80
Hallikainen et al., 2000a	Stanol ester	4 weeks	2.02	34M/F	2.05	1.87	-8.78
	Sterol ester		2.06	43M/F	2.05	1.77	-13.66
Hallikainen et al., 2000b	Stanol Ester	4 weeks	0.80	8M, 14F	0.93	0.88	-5.38
			1.60	8M, 14F	0.93	0.90	-3.23
			2.40	8M, 14F	0.93	0.85	-8.60
			3.20	8M, 14F	0.93	0.84	-9.68
Hendriks et al., 1999	Sterol ester	8 weeks	0.83	60M/F	0.57	0.50	-12.28
			1.61	60M/F	0.57	0.52	-8.77
			3.24	60M/F	0.57	0.40	-29.82
Maki et al., 2001	Sterol ester	5 weeks	0.00	83M/F	0.55	0.51	0.00
			1.10	74M/F	0.60	0.48	-5.88
			2.20	33M/F	0.47	0.40	-21.57
Plat et al., 2000	Stanol ester	4 weeks	2.50	39M/F	0.37	0.30	-18.90
Plat et al., 2001	Control	8 weeks	0.00	42M/F	1.66	1.57	0.00
	Stanol ester		3.80	36M/F	1.62	1.33	-15.11
	Stanol ester		4.00	34M/F	1.67	1.26	-19.58
Tammi et al., 2000	Stanol ester	3 months	0.00	74M/F	0.82	0.84	0.00
			1.50	74M/F	0.86	0.63	-25.00
Vuorio et al., 2000	Stanol ester	3 months	2.24	24M/F	1.71	1.11	-35.10
Weststrate and Meijer, 1998	Sterol ester	14 weeks	3.00	No data	0.453	0.346	-23.46
	Stanol ester		3.00	No data	0.453	0.352	-22.22
CLF9904	Wood sterol	4 weeks	0.00	31	0.76	0.99	0.00
			0.90	32	0.91	1.07	8.08
			1.80	33	1.44	1.23	24.24
			3.60	33	1.15	1.05	6.06

M = male, F = Female

* Values were calculated as percent of control/placebo values at the end of the experimental period

Data Table XI-xv: Listing of ratio of serum beta-carotene to cholesterol levels in various human clinical studies on the effects of phytosterols

Reference	Phytosterol	Test Period	Dose phytosterols - g/day	Number of Subjects	Baseline: b-carotene /cholesterol umol/mmol	After Tx: b-carotene /cholesterol umol/mmol	% of Control*
Davidson et al., 2001	Sterol ester	8 weeks	0.00	21M/F	0.066	0.081	0.0
			3.00	21M/F	0.069	0.067	-17.0
			6.00	19M/F	0.069	0.057	-29.5
			9.00	23M/F	0.064	0.055	-32.1
Gylling et al., 1999a	Stanol ester	1 year	0.00	49M/F	0.170	0.180	0.0
			3.00	102M/F	0.160	0.120	-33.3
Gylling et al., 1999b	Stanol ester	6 weeks	3.50	23F	0.270	0.190	-29.6
	Stanol ester		3.40	23F	0.270	0.190	-29.6
	Stanol ester		2.50	21F	0.270	0.210	-22.2
Hallikainen et al., 1999	Control	8 weeks	0.00	6M, 11F	0.170	0.190	0.0
	Stanol ester		2.31	8M, 10F	0.270	0.240	26.3
	Stanol ester		2.16	6M, 14F	0.240	0.210	10.5
Hallikainen et al., 2000a	Stanol ester	4 weeks	2.02	34M/F	0.230	0.230	0.0
	Sterol ester		2.06	34M/F	0.230	0.210	-8.7
Maki et al., 2001	Sterol ester	5 weeks	0.00	83M/F	0.075	0.068	0.0
			1.10	74M/F	0.077	0.066	-2.9
			2.20	33M/F	0.058	0.050	-26.5
Nguyen et al., 1999	Stanol ester	8 weeks	0.00	19M/F	0.015	0.015	0.0
			3.00g Euro. Formula	17M/F	0.012	0.007	-50.4
			3.00g U.S. Formula	19/M/F	0.021	0.013	-15.7
			2.01g U.S. Formula	18M/F	0.013	0.010	-36.2
Vuorio et al., 2000	Stanol ester	3 months	2.24	24M/F	0.180	0.140	-22.2

*Values were calculated as percent of placebo/control value at the end of the experimental interval.

M = Male, F = Female

Data Table XI-xvi: Listing of serum retinol levels in various human clinical studies on the effects of phytosterols

Reference	Phyto-sterol	Test Period	Dose total phyto-sterols - g/day	Number of Subjects	Baseline Retinol umol/L	After Tx Retinol umol/L	% of Control*
Christiansen et al., 2001	Wood sterol	6 months	0	46M/F	2.08	1.90	0.00
			1.50	46M/F	2.05	1.84	-3.16
			3.00	41M/F	2.07	1.86	-2.11
Davidson et al., 2001	Sterol ester	8 weeks	0.00	84M/F	1.75	2.01	0.00
			3.00		1.83	1.78	-11.30
			6.00		1.80	1.73	-13.91
			9.00		1.87	1.81	-9.57
Gylling et al., 1999a	Stanol ester	1 year	0.00	49M/F	2.68	2.65	0.00
			3.00	102M/F	2.71	2.74	3.40
Gylling et al., 1999b	Stanol ester	6 weeks	3.20	23F	2.25	2.27	0.00
	Stanol ester		3.20	23F	2.25	2.33	3.56
	Stanol ester		2.43	21F	2.25	2.33	3.56
Hallikainen et al., 1999	Control	8 weeks	0.00	55M/F	2.30	2.21	0.00
	Wood stanol ester		2.31		2.50	2.36	6.79
	Veg. oil stanol ester		2.16		2.21	2.12	-4.07
Hallikainen et al., 2000a	Stanol ester	4 weeks	2.02	34M/F	2.80	2.71	-3.21
	Sterol ester		2.06		2.80	2.70	-3.57
Hallikainen et al., 2000b	Stanol Ester	4 weeks	0.00	22M/F	2.92	2.92	0.00
			0.80		2.92	2.95	1.03
			1.60		2.92	2.84	-2.74
			2.40		2.92	2.91	-0.34
			3.20		2.92	2.89	-1.03
Maki et al., 2001	Control	5 weeks	0.00	83M/F	2.20	2.20	0.00
	Sterol ester		1.10	74M/F	2.30	2.30	4.55
			2.20	33M/F	2.10	2.10	-4.55
Nguyen et al., 1999	Stanol ester	8 weeks	0.00g - Control	19M/F	27.94	27.79	0.00
			3.00g Euro.Formula	17M/F	26.54	27.40	-1.42
			3.00g U.S. Formula	19/M/F	26.84	27.06	-2.62
			2.01g U.S. Formula	18M/F	28.90	28.91	4.04
Plat et al., 2000	Stanol ester	4 weeks	2.50	39M/F	2.12	2.10	-0.94
Plat et al., 2001	Control	8 weeks	0.00	42M/F	1.90	1.91	0.00
	Veg. oil stanol		3.80	36M/F	1.98	1.97	3.14
	Wood stanol		4.00	34M/F	2.13	2.10	9.85
Tammi et al., 2000	Stanol ester	3 months	0.00	74M/F	1.29	1.32	0.00
			1.50	74M/F	1.29	1.26	-4.55
Vuorio et al., 2000	Stanol ester	3 months	2.24	24M/F	1.52	1.45	-4.61
Study #CLF 9904	Wood sterol	28 days	Placebo	31	2.32	2.39	0.00
			0.90	32	2.14	2.14	-10.46
			1.80	33	2.06	2.09	-12.55
			3.60	33	2.15	2.20	-7.95

*Values were calculated as percent of placebo/control value at the end of the experimental interval.

"Number of Subjects" Key: M - male, F - Female

XII Microbiological information on the novel food.

Scheme XII requires a characterisation of any micro organisms present and an analysis of any metabolites.

The food grade specification (see Section ID) for FCP-3P7 limits microbiological properties to:

Total aerobic count	< 10,000 CFU/g
Combined moulds & yeasts	< 1000 CFU/g
Coliformes	negative
E. Coli	negative
Salmonella	negative

XIII Toxicological information on the novel food.

Scheme XIII requires information about any traditional counterpart to the novel food that can be used as a baseline to facilitate the toxicological assessment and when compared to the traditional counterpart, whether the novel food contains any new toxicants or changed levels of existing toxicants. Alternatively information is required from a range of toxicological studies appropriate to the novel food to show that the novel food is safe under anticipated conditions of preparation and use and information which suggests that the novel food might pose an allergenic risk to humans.

In the case of FCP-3P7, although the novel food has traditional counterparts (i.e. existing phytosterol containing yellow fat spreads), toxicological studies are available to demonstrate that the novel food is safe under anticipated conditions of use. This section will provide information based on studies using FCP-3P7 phytosterols (Table XIII-i) and studies reported in the literature that will support the substantial equivalence of FCP-3P7 to the two existing products.

Studies were performed using non-esterified forms of FCP-3P7 phytosterols (referred to in the reports as FCP-3P1) These are physiologically equivalent to their esterified counterparts so that toxicological studies performed using FCP-3P1 are directly applicable to FCP-3P7 (see Section I.F.)

Table XIII-i. Studies undertaken using FCP-3P7 sterols in toxicological assessment.

Study	Description
0521-2140	Evaluation in the Salmonella typhimurium / escherichia coli plate incorporation/preincubation mutation assay
0521-2400	*In vitro* mouse lymphoma mutagenesis assay.
0521-3300	*In vitro* test for chemical induction of chromosome aberrations in cultured human peripheral lymphocytes
0521-1521	*In vivo* test for chemical induction of micronucleated polychromatic erythrocytes in mouse bone marrow cells
115-003	Three-month dietary study in Sprague-Dawley rats Sprague-Dawley rats
706-001	Uterotrophic assay in immature female rats
CLF9601	Human volunteer study
CLF9602	Human volunteer study
CLF9701	Human volunteer study
CLF 9904	Human volunteer study

XIII.A. Preclinical toxicology

XIII.A.a. *In Vitro* Genotoxicity Assays

A genotoxic evaluation of FCP-3P7 sterols was conducted in the *Salmonella typhimurium* / *Escherichia coli* plate incorporation / pre-incubation mutation assay in the presence and absence of induced rat liver S-9 microsomal fraction. FCP-3P7 sterols were tested in *Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537 (104, 208, 417, 834 and 1667 μg/plate) and *Escherichia coli strain WP2UvrA* (104, 208, 417, 834 and 1667 μg/plate), for the potential to cause mutation both in the presence and absence of metabolic activation (SITEK Study 0521-2140). The plate incorporation method was employed in the definitive assay, as well as, the confirmatory assay. Results of both mutation assays indicated that the test article did not induce a significant increase in the number of revertant colonies for any of the strains tested in the presence or absence of the S-9 fraction. Therefore, under the conditions of this study, FCP-3P7 sterols were reported to be negative for mutagenic potential in *Salmonella typhimurium* and *Escherichia coli.*

An *in vitro* evaluation of FCP-3P7 sterols in the L5178Y TK +/- mouse lymphoma mutagenesis assay with colony size evaluation in the presence and absence of induced rat liver S-9 microsomal fraction was conducted along with a confirmatory study (SITEK Study 0521-2400). This is an *in vitro* mammalian cell mutation assay based on the detection and quantitation of forward mutation in a sub-line of mouse lymphoma L5178Y cells at the thymidine kinase locus. It was used to test the mutagenic potential of FCP-3P7 sterols at levels of 5.0, 10, 20, 40, 60, 80, 100 and 167 μg/ml. Following a 4-hour treatment period, all responses were negative, both in the presence and absence of metabolic

activation. Relative total growth (RTG) for the non-activated cultures was greater than 100%, and the RTG for S-9 activated cultures ranged from 54-110%. A confirmatory assay was subsequently performed without S-9 activation. Following a 24-hour treatment period, all responses were also negative in this assay. The RTG for treated cultures ranged from 71% to 133%. The solvent controls (DMSO and acetone) and positive controls (hycanthone methane sulphonate without activation, and 7,12-dimethylbenz(α)anthracene with activation) all produced acceptable colony size distributions. Based on these results, it was concluded that FCP-3P1 was not considered mutagenic under the conditions tested.

In an *in vitro* test for chemical induction of chromosome aberrations in cultured human peripheral lymphocytes, with and without metabolic activation, the mutagenic potential of FCP-3P7 sterols was investigated (SITEK Study 0521-3300). Using the chromosome aberration assay in cultured human peripheral blood lymphocytes, the mutagenic potential of FCP-3P7 was investigated at 100, 150, 300, 600, 750, 900 and 1200 μg/ml, with and without rat liver S-9 fraction. The test article was prepared in acetone, and duplicate cultures of each dose were established. In addition, solvent and positive controls (mitomycin at 0.1 and 0.2 μg/ml, and cyclophosphamide at 10 and 20 μg/ml, in non-activated and activated systems, respectively) were used to verify testing conditions. Cells were harvested 21 hours after treatment initiation in both systems, with 0.1 μg/ml colcemid present during the final two hours. Toxicity was measured by determining the Relative Mitotic Index (RMI), and the percentage of polyploid and endoreduplicated cells was determined at each concentration level. Data showed that FCP-3P7 sterols did not induce a statistically significant increase in the percentage of cells with aberrations, as compared to solvent controls, at any of the concentrations tested with and without metabolic activation. Results were subsequently confirmed by a confirmatory assay performed without S-9 activation. Given the results of the definitive and confirmatory assays, FCP-3P7 sterols were reported to have no effect on the frequency of chromosome aberration in peripheral blood lymphocytes, both in the presence and absence of S-9 metabolic activation. Reports of toxicological studies are provided in Annex D.

XIII.A.b. *In Vivo* Genotoxicity Assays

FCP-3P7 sterols evaluated at levels of 500, 1000 and 2000 mg/kg for the potential to induce micronucleated polychromatic erythrocytes (MPCE) in the bone marrow cells of male and female CD-1 mice (SITEK Study 0521-1521). A single dose of the test article was administered *via* oral gavage, and the percentage of polychromatic erythrocytes (PCE) and micronucleated polychromatic erythrocytes (MPCE) frequency was determined at approximately 24, 48 and 72 hours after dose administration. Two thousand PCEs per animal were analysed for the frequency of micronuclei, and cytotoxicity was assessed by scoring the number of PCEs and normochromatic erythrocytes (NCEs) in the first 200 erythrocytes for each animal. Results indicated there was no statistically significant increase in the number of MPCE in the FCP-3P7 sterols treated groups relative to control. In addition, there were no reductions (more than 20% of vehicle) in the percentage of PCE in other test groups receiving

FCP-3P7 sterols. Based on the results summarized above, it was concluded that under the current test conditions, FCP-3P7 sterols did not cause chromosome damage *in vivo*, nor was it a clastogenic agent. Reports of toxicological studies are provided in Annex D.

XIII.B. Toxicological Studies with FCP-3P7 sterols.

XIII.B.a. Uterotrophic assay in immature female rats

FCP-3P7 sterols were evaluated at levels of 1000, 2500 and 5000 mg/kg/day for the potential uterotrophic potential by measuring uterine weights at sacrifice of immature Crl:CD® (SD) IGS BR VAF/Plus® rats treated with the test article for four consecutive days (Argus Research Laboratories Study number 706-001A). Suspensions of FCP-3P7 sterols or vehicle were administered via gavage twice daily (separated by approximately 6 hours), beginning on day 19 postpartum. Results indicated that absolute and relative (to body weight) uterine weights were unaffected by dosages of FCP-3P7 sterols as high as 5000 mg/kg/day. The values were comparable, without dose-dependency and did not significantly differ from the control group value. It was concluded that dosages of FCP-3P7 sterols up to 5000 mg/kg/day administered for four consecutive days to immature female rats did not affect absolute or relative uterine weights and thus were without uterotrophic potential. Reports of toxicological studies are provided in Annex D.

XIII.B.b. Three-month dietary study with FCP-3P7 in Sprague-Dawley rats.

One hundred and ninety-two Sprague-Dawley rats (96 males and 96 females), 5 to 6 weeks of age, were divided into four groups, each consisting of 24 males and 24 females) (Primedica Study 115003). FCP-3P7 sterols was fed *ad libitum* for 91 days to male and female rats at concentrations of 1.25%, 2.5% and 5% in the diet. The observed intakes of FCP-3P7 sterols at these dietary concentrations were for male rats 999, 2021, and 4160 mg/kg/day respectively. The observed intakes of FCP-3P7 sterols for female rats at these dietary concentrations were 1163, 2358, and 4839 mg/kg/day respectively.

No consistent dose related changes that were considered a meaningful toxicological response were observed. It was concluded that the dietary administration of up to 5.0% FCP-3P7 sterols to male and female rats for 91 consecutive days did not result in any mortality or observable adverse effects. There were no significant changes in body weight; feed consumption or efficiency. No physical or ophtalmological changes were observed. Also examined were sperm concentrations, sperm motility, sperm morphology, haematology, serum chemistry, urinalysis parameters; organ weights and microscopic evaluations. The no effect level for FCP-3P7 sterols in the diet was considered to be 5.0%. Reports of toxicological studies are provided in Annex D.

XIII.C. Toxicology Studies with Cytellin (Positol)

Between 1954 and 1982, Eli Lilly Research Laboratories marketed a mixture of

phytosterols extracted from tall oil in the United States (Cytellin) and in Canada (Positol). Cytellin / Positol, marketed as an anti-hypercholesterolemic agent, was available either as a powder or liquid suspension, and the reported composition was sitosterol, sitostanol, campesterol, campestanol; 80:10:7:2. FCP-3P7 sterols are also extracted from tall oil and composed of the same four major constituent sterols. Although Cytellin / Positol was eventually withdrawn from the market due to business considerations, several toxicology studies had been conducted with the product. Refer to Table XIII-ii for a tabulated summary of these studies.

Table XIII-ii Toxicology Studies with Cytellin

Species and Number	DOSAGE	DURATION	RESULTS
Acute Studies			
Albino mice 565	5g/kg sitosterol triturated in sesame oil by stomach tube	Single dose	Sitosterol from tall oil show little or no toxicity following administration of large single oral doses to mice.
Subchronic Studies			
Rats 30 female	1% and 5% sitosterol in diet	18 months	Rats fed doses containing 5% sitosterol from tall oil for 18 months survived, gained weight comparable to controls, and upon sacrifice showed no visceral or hematopoetic damage and no alterations in serum cholesterol, lipid phosphorus or blood protein fractions.
Rats 20 female	Diet containing 5% Formula 226	8 months	*Rats fed diets containing 5% formula 226 for 8 months responded in similar manner*
Dogs 8 female mongrel dogs	Capsules: 4 dogs 500 mg/kg/d 4 dogs 1000 mg/kg/d	18 months	Dogs that received daily doses of 1000 mg/kg for 18 months survived, gained weight and had no haematological or visceral damage. Serum cholesterol, calcium and phosphorus, total lipids, lipid phosphorus, vitamin A and blood protein fractions were unaltered. The ultracentrifugal pattern was similar for treated and control dogs. Total lipid and free and total cholesterol values of the livers were also unchanged.
Dogs 3 dogs	1000 mg/kg/d of Formula 226*	8 months	Dogs that received daily doses of 1000 mg/kg of Formula 226 for 8 months were also normal.

* Formula 226, Each 100 cc. Contains:

Tall oil sterols 20g
Benzoic acid 0.1g
Sodium Carboxymethylcellulose 3.0g
Saccharin Soluble 10mg, Raspberry Flavour 0.0015 cc.
Sodium Lauryl Sulphate Purified 50 mg

The above information was obtained under “Freedom of Information” from the FDA in the United States.

XIII.D. Published Toxicology Studies with Phytosterols

Phytosterols have been extensively documented in many readily available scientific publications. This section seeks to document the general safety of phytosterols by reviewing scientific publications which discuss the safety of phytosterols in general. The results of this review are documented below and summarized in Table XIII-iii.

XIII.D.a. Genotoxicity

The results of a panel of genotoxicity tests with vegetable and tall oil stanol esters was reported by Turnbull *et al*., (1999). The study was in compliance with OECD Guideline 473. All tests gave negative results.

XIII.D.b. Subchronic Toxicity

Shipley *et al*., (1958) reported that no evidence of toxicity was observed in rabbits and dogs given large daily oral dietary supplements of sitosterol (mostly of tall oil origin), for periods of up to 2 years. Gross or microscopic alterations were not observed in any tissue, and there was no histological evidence of disposition of the plant sterols. In addition, chemical analysis of the aorta and liver showed no increase in sterol content.

An abstract by Robinson *et al*., (1998) describes a 90 day subchronic feeding study conducted in 160 Sprague-Dawley rats (80 male/80 female) to investigate the safety of phytostanols. Stanols (61, 305 and 915 mg stanol/kg bw/day) were administered *via* oral gavage in a cottonseed/soybean oil mixture, consisting of 65% sitostanol, 30% campestanol, 2.5% campesterol and 2.5% other sterols. Following the 13-week treatment period, no significant toxicological effects were reported.

A second study investigated the safety of stanol esters in male and female Wistar rats. Animals received either a wood-derived stanol ester preparation or a vegetable oil-derived stanol ester preparation, at dietary concentrations of 0, 0.2, 1 and 5% total stanols (174-5509 mg stanol esters/kg bw/day). Approximately 0.5 g total stanols/kg bw/day was provided at this dietary level. Following a 13-week treatment period, slightly decreased levels of plasma cholesterol and phospholipids were reported in stanol-treated males. Decreased levels of plant sterols and increased levels of stanols were observed in both males and females. A marked increase in the faecal excretion of sterols, including cholesterol and stanols, was reported in the stanol ester groups. Animals treated with the high-dose diets experienced a decrease in plasma levels of vitamin E, vitamin K, and to a lesser extent, vitamin D. Similar changes were also observed in hepatic levels of vitamins E and D. Based on these results, and the absence of any significant adverse clinical, pathological or histopathological effects, both preparations were considered well tolerated. The no observable adverse effect level (NOAEL) was reported to be the mid-dose level of 1% total dietary stanols. (Turnbull *et al*., 1999).

Malini and Vanithakumari (1990) described a study in which rats were administered sitosterol by subcutaneous injection at doses of 2.5, 5.0 and 10.0

mg/kg/day for 60 days. The sitosterol was well tolerated and no evidence of gross microscopic lesions either in the liver or kidney was observed. Furthermore, liver and kidney function tests were assessed by determining blood/serum parameters such as haemoglobin, blood glucose, serum protein, serum bilirubin, serum GPT and GOT. All clinical biochemical parameters were in the normal range with the exception of serum cholesterol, which was reduced at all doses of sitosterol.

The effect of tall oil phytosterols administered in the diet was investigated in the apo-E-KO-deficient mouse. Histological, haematological, and biochemical characteristics were examined. No toxicity was observed in the phytosterol treated group. Both treated and untreated mice exhibited arrested spermatogenesis and atrophy of the seminiferous tubules to a variable extent. This effect may be related to the difficulty of breeding this particular strain. The apo-E-KO-deficient mouse exhibits a number of abnormalities related to the genetic defect including xanthamatous skin lesions and oil red O-negative vacuolation in the liver and kidney parenchymal cells. The phytosterol treatment prevented these lesions (Moghadasian *et al.*, 1999).

Daily injections of soy phytosterols for three weeks resulted in a progressive accumulation in the serum, liver, and bile of exposed neonatal piglets. Serum bile acid levels were significantly higher in the sterol-treated piglets. In addition, a significant inhibition of secretory function in isolated rat hepatocyte couplets was observed (Clayton *et al*., 1998). Furthermore, neonatal piglets receiving daily injections of phytosterols in the absence of other parenteral nutrition components, experienced reduced bile flow (Iyer *et al*., 1998).

XIII.D.c. Reproductive Toxicity

Two tests of potential estrogenic activity were reported for plant stanols (soy or tall oil) and plant stanol esters by Turnbull *et al*., (1999). These were the E-screen test, which measures the ability of a substance to induce proliferation of oestrogen-responsive human breast adenocarcinoma (MCF-7) cells in culture, and an *in vivo* test, which measures uterotrophic activity in immature female rats fed the test substance. In the E-screen test, none of the stanol preparations produced any increase in cell proliferation when tested at 1,10, and 100 μM. In the *in vivo* test, neither stanol ester preparation caused any significant change in uterine weight when fed at a concentration of 8.3% in the diet for 4 days. Similarly, in vitro and in vivo tests for potential oestrogenic activity were reported for plant sterols and sterol esters by Baker et al., (1999). Competitive binding with immature rat uterine oestrogen receptor was used to measure the ability of phytosterols to bind to oestrogen receptors while the transcriptional activation of oestrogen responsive genes has been examined in an oestrogen-inducible yeast strain. Phytosterols did not display any activity in these in vitro assays. Sterol esters showed no uterotrophic activity when tested in immature female rats at oral doses of up to 500 mg/kg/day for 3 days.

Whittaker *et al.*, (1999) reported the results of a two-generation reproductive toxicity study performed according to OECD Guideline 414, and in compliance with the OECD principles of GLP. The test article was vegetable oil stanol esters at doses of up to 5% stanols in the diet. No adverse treatment related

effects were noted on reproductive performance of male or female rats in any dose group. In addition, no adverse developmental effects were noted in F_1 or F_2 pups of the low and mid-dose groups. A treatment related effect on body weight and body weight change was observed in both the F_1 and F_2 male and female pups of the high-dose group, particularly during the later stages of lactation. However, the lower body weight in the high-dose group pups was attributed to a reduction in the caloric intake of the test diet compared to the control.

Another two generation reproductive study investigated the effects of soy phytosterol esters in the rat was reported in the form of an abstract (Waaleknes-Berendsen *et al*., 1999). Soy phytosterol esters of up to 5000 mg/kg/day of phytosterols were tested. No effect on the reproduction of parental F_0- and F_1-generation Wistar rats or the development of F_1 and F_2 pups was reported.

A developmental toxicity study in rats was performed according to OECD Guideline 414 and was in compliance with OECD Principles of GLP. The test article was vegetable oil stanol esters administered in doses up to 5% stanols in the diet from days 0 to 21 of gestation. No adverse effects on reproduction or development were observed (Slesinski *et al*., 1999).

Malini et al (1991; 1993) investigated effects on male and female rat reproductive tissues. The investigators, using nonpurified sitosterol plant extracts reported various effects in both males and females which are at variance with findings reported by other investigators using purified sitosterol.

Burck *et al*., (1982) reported that introduction of 0.5 mg sitosterol sulphate into the vagina of female belted rabbits reduced the number of pregnancies. The number of embryos per pregnant rabbit was not affected. Sitosterol sulphate, but not sitosterol, has an acrosin inhibitory activity, which would reduce the efficiency of sperm in fertilizing the ova. Implantation of silicone rods containing sitosterol sulphate into uterine horns of rabbits for 16 days, significantly reduced the number of embryos present in those horns. No birth defects were reported. The release rate of sitosterol sulphate from the silicone rods was 1-2 μg per day. Neither treatment affected the number of corpora lutea.

In conclusion, the only evidence of toxicity to animals reported in the literature is for injected phytosterols. The blood levels of phytosterols achieved by this route of administration would be much higher than could be obtained by oral administration, where absorption is quite low.

Table XIII-iii Published studies with toxicology findings

Reference	Species/Strain, Sex, No/Group	Phytosterol	Source	Route of Administration	Dosage mg/kg/day	Duration	Tissues / Parameters Examined	Findings
Robinson *et al*., 1998.	Sprague-Dawley Rat 20M+20F per groups; 4 groups	Hydrogenated soy phytosterols	Soy	Oral , cotton-seed/soy oil mixture by gavage	Control 61 305 915	90 days	Standard tissue screen for GLP study	No toxicological effects
Turnbull *et al*., 1999.	Wistar rats (M&F) 20 rats/sex/group	Plant stanol esters	Tall oil (3 groups) Vegetable oil (3 groups)	Oral in diet	0.2%; 1%; 5% stanols in diet (0.34%; 1.68%; 8.39% stanol esters from tall oil 0.2%, 1%, 5% stanol esters from vegetable)	13 weeks	GLP study standard for US and EU requirements	No toxicity was associated with the subchronic ingestion of wood or vegetable oil derived stanol esters at dietary concentrations up to 1% (as free stanol; equivalent to about 0.5g total stanols/kg bw/d). At dietary levels of 5% (as free stanol), subchronic ingestion of these substances resulted in decreased plasma levels of the fat soluble vitamins E and K1 (~50%), and, to a lesser extent, vitamin D (-15%). Hepatic levels of vitamins E and D showed similar changes. *Both wood and vegetable oil derived stanol esters were well tolerated, as evidenced by the absence of clinical changes or major abnormalities in growth, food and water consumption, ophthalmoscopic findings, routine hematological and clinical chemistry values, renal concentrating ability, composition of the urine, appearance of the faeces, oestrus cycle length, organ weights, gross necropsy findings, and histopathological findings.* Females of the wood-derived stanol 5% dose group showed a statistically significant increase in thrombocyte count, and females of the vegetable derived stanol 5% dose group had an increased percentage of neutrophils and decreased percentage of lymphocytes (not ascribed to treatment because

Reference	Species/Strain, Sex, No/Group	Phytosterol	Source	Route of Administration	Dosage mg/kg/day	Duration	Tissues / Parameters Examined	Findings
								there was no clear dose- response relationship and no significant changes in absolute numbers of these cell types). Plasma sitostanol was increased in males of the 1 and 5% dose groups and in females in all treatment groups. Campestanol was increased in all groups fed vegetable oil-derived stanols. Uterine luminal dilatation was observed more frequently in females fed vegetable oil-derived stanols (5%) than in controls (not significant) and it was not accompanied by any histopathological urine changes, nor by treatment related changes in oestrous cycle length or other reproductive organs.
Turnbull *et al*., 1999.	E-screen test human breast carcinoma (MCF-7) cells in culture	Stanols (88-99% stanols)	Four samples of vegetable oil-derived stanols (88-99% stanols)	Cell culture	0,1,10, and 100μM stanols	6 days	GLP study standard for US and EU requirements	None of the stanol preparations produced any increase in cell proliferation when tested at 1,10, and 100μM. The highest dose of each stanol sample was associated with microscopic evidence of cytotoxicity and crystalline precipitation in the culture dishes. Slight to moderate cytotoxicity was seen with all four stanol samples at the highest dose tested. This was accompanied by crystals at the bottom of the culture wells at this dose level.
	In vivo test (immature 15 day old female Wistar rats)	Stanol fatty acid esters	One sample of tall-oil and one of vegetable oil-derived stanol fatty acid esters	Oral in diet	8.3% stanol esters(w/w) diet	4 days		In *in vivo* test, neither of two stanol ester preparations caused any significant change in absolute or relative uterus weight when fed at a concentration of 8.3% in the diet for 4 days. Thus, under the conditions of testing used, neither the free stanols nor the stanol fatty acid ester preparations showed evidence of estrogenic or uterotrophic activity. Animals fed stanol esters showed a slightly reduced body weight gain over the 4-day treatment period significant in the wood stanol ester group only). This was associated with a slightly reduced food consumption in these animals.

Reference	Species/Strain, Sex, No/Group	Phytosterol	Source	Route of Administration	Dosage mg/kg/day	Duration	Tissues / Parameters Examined	Findings
Whittaker *et al*., 1999.	Wistar rats (M&F) 28 rats/group/ generation	Plant stanol esters	Tall oil and vegetable	Oral in diet	1%; 2.5% and 5% stanols in diet (1.75%; 4.38%; 8.76% total stanol esters)	10-13 weeks	GLP study standard for US and EU requirements	No effects on reproduction of parental F0- and F-1 generation Wistar rats. Consumption of plant stanol esters at dietary percentages up to 4.76% (equivalent to 2.5% total stanols) was not associated with adverse effects upon the reproduction or development of male or female rats over two generations. At dietary concentrations of 8.76% stanol esters (equivalent to 5.0% total stanols), ingestion of plant stanol esters was associated with increases in food consumption in male and female F0 and F1 generation rats, as well as decreases in body weight in male and female F1 and F2 pups (attributable to consumption of test substance, which is not absorbed and reduces the caloric value of the test diet compared to control). In the F1 generation both absolute and relative weights of the testes were increased in the 4.38% dose only. Furthermore, the relative weight of the epididymides of the F1 males of the 4.38% dose group was statistically significantly increased. These statistically significant effects on organ weights were not observed in the high-dose group and were not considered treatment related.
Waalkens-Berendsen *et al*., 1999.	Wistar Rats, 28 rats/group/ generation	Phytosterol esters	Soy	Oral	Max 8.1% PE in diet 5000 mg/kg/day sterols	NA	GLP study standard for US and EU requirements	No effects on reproduction of parental F0- and F1-generation Wistar rats and the development of F1- and F2 pups.
Slesinski *et al*., 1999.	28 Wistar rats per dose group	Stanol esters	Vegetable oil (Sito – 70) (68% sitostanol, 30% campestanol, 2% unsaturated sterol)	Oral in diet	0, 1, 2.5, 5% total stanols (equivalent to 0, 1.75, 4.38, 8.76% plant stanol esters)	21 days	GLP study standard for US and EU requirements	No adverse treatment-related maternal or foetal developmental effects were produced following ingestion of a diet containing up to 8.76% plant stanol fatty acid esters. This diet provided up to 5% of total dietary stanols equivalent to 2.4-3.5g stanols/kg bwt/d. No significant differences were seen in reproductive performance, maternal and foetal body weights, sex distribution, or visceral or skeletal malformations, anomalies, and variations. Vegetable oil-derived stanol fatty acid esters are concluded not to be developmental toxicants and did not produce any embryotoxic, fetotoxic, or teratogenic effects in Wistar rats under the conditions of this study. Statistically significant differences were noted in mean body weight relative to controls at the 0-7-day and 7- to 14-day period and in body weight gains during 0-7 days for the high dose group (attributable to decrease in caloric content of the diet from the levels of unabsorbable stanols at the highest dose). These changes were

Reference	Species/Strain, Sex, No/Group	Phytosterol	Source	Route of Administration	Dosage mg/kg/day	Duration	Tissues / Parameters Examined	Findings
								relatively small, transient in nature, and were not considered biologically meaningful as they were not seen in the 14- to 21-day terminal portion of the study.
Turnbull *et al*., 1999.	Ames assay (s. typhimurium) bacterial cell genotoxicity test L5178Y assay (mammalian cell) gene mutation assays Mammalian cell chromosome aberration assay (CHO cells)	Plant stanol fatty acid esters	Tall Oil and vegetable-derived plant stanol fatty acid esters Tall Oil Vegetable Tall Oil Vegetable	Cell culture Cell culture Cell culture	0, 62, 185, 556, 1667, 5000 μg/plate 20-500μg/ml 250-3000μg/ml 125-500μg/ml 500-2000μg/ml	4hrs 18 or 32 h without S9 rat liver microsome metabolic) and 3h with S9	GLP study standard for US and EU requirements	All tests gave negative results for both wood and vegetable oil stanol ester formulations. Thus, plant stanol esters are not genotoxic under the conditions of exposure tested.

Reference	Species/Strain, Sex, No/Group	Phytosterol	Source	Route of Administration	Dosage mg/kg/day	Duration	Tissues / Parameters Examined	Findings
Shipley *et al*., 1958.	Dogs, 13	Sitosterol in diet	Cytellin™, derived from Tall Oil, study from Eli Lilly Laboratories	Oral in diet	1000 mg/kg/day	8 to 22 months	Blood haematology, biochemistry, aorta, heart, lungs, liver, spleen kidneys, stomach, intestine, thymus, thyroid, adrenal glands, bone marrow	No gross or microscopic pathological changes; biochemistry and haematology normal. No evidence of phytosterol accumulation in any tissues. Vitamin A levels unchanged in blood.
	New Zealand White Rabbits, 6 M, 6F	Sitosterol in diet	Derived from either tall oil or cottonseed oil	Oral in diet	4000 mg/per rabbit per day	348 to 842 days	Heart, blood vessels, thyroid spleen, liver, intestine	No gross or microscopic pathological changes; biochemistry and haematology normal. No evidence of phytosterol accumulation in any tissues.
Malini *et al*., 1990.	Wistar albino rats 10 M &10 F	Sitosterol	Anacardium occidentale	Subcutaneously	2.5 mg/kg/D 5.0 10.0	60 days	liver kidney	There was no clear cut evidence of any gross or microscopic lesions in the liver or kidney. A marked fall in serum protein level only at dose of 1000μg of sitosterol. All parameters (blood/serum) were in normal range.
Moghadasian *et al*., 1999.	Apo-E-KO mice 6M Control 6M Treated	Phytosterols	Tall Oil	Oral in diet	3.34g/kg/d	18 weeks	Haematology, urinalysis, heart, lung, brain, kidney, skeletal muscle, skin, oesophagus, stomach, small & large intestine, liver, adrenal gland, spleen, pancreas, bladder	Haematology: Haemoglobin concentration, red cell counts, and haematocrit were comparable between groups; but there was a statistically significant reduction in platelet counts. Leukocyte counts showed a large but not significant variation between the two groups. Urinalysis: No significant differences were observed in the urine parameters. Macroscopic Organ Examination: No abnormalities except for skin lesions (thickened, red, alopecia) in two control mice. Histological Examination: The affected skin revealed numerous cholesterol crystals, cholesterol granulomas along with cellular reaction with eosinophils and histocytes. Routine histochemical staining revealed no histological abnormalities in the tissues examined except for slight histological changes in liver and kidney which were reduced in extent in the FCP treated group. Arrested spermatogenesis and atrophy in the seminiferous tubules was observed to a variable extent in both

Reference	Species/Strain, Sex, No/Group	Phytosterol	Source	Route of Administration	Dosage mg/kg/day	Duration	Tissues / Parameters Examined	Findings
								treated and untreated groups.
Iyer et al., 1998.	Neonatal Piglets	Soy phytosterols	Soy	Intravenous	18 nM per kg/ per day	14 days	Bile, liver, serum	Serum bile acid levels increased. Reduction in bile acid-stimulated bile flow. Normal liver function tests, liver histology remained normal.
Malini *et al*., 1993.	Wistar albino rats 10 F	Sitosterol	Anacardium occidentale	Subcutaneously	0.5 mg/kg/D 2.5 5.0	10 days	Uterus RNA, DNA, protein concentrations	Uterine weight and RNA concentrations increased in a dose dependent manner indicating that sitosterol has some intrinsic estrogenic property.
Burck *et al*., 1982.	Dutch-belted rabbits 20 F	Sitosterol Sulphate	Not identified	Intravaginal Intrauterine	0.5 1-2μg	16 days	pregnancy rate corpora lutea number of embryos	Introduction of 0.5 mg sitosterol sulphate into the vagina of rabbits before coitus lowered the pregnancy rate, but did not significantly reduce the number of embryos produced per pregnant animal. Sitosterol sulphate but not sitosterol is a potent acrosin inhibitor which would reduce the efficiency of fertilization. Implantation of silicone rods containing sitosterol sulphate into the uterine horns of rabbits significantly reduced the number of embryos present in those horns. Neither treatment affected the number of corpora lutea.
Malini *et al*., 1991.	Wistar albino rats 10 M	Sitosterol	Anacardium occidentale	Subcutaneously	0.5 mg/kg/D 5.0	16 days 32 days 48 days	testes	A significant decrease in testicular weight and sperm concentrations after long-term treatment with low dose of sitosterol. The weights of all accessory sex tissues except the epididymis increased following low dose sitosterol treatment. High dose treatment reduced the sperm concentrations as well as the weights of testis and accessory sex tissues to near normal conditions.

XIII.E. Clinical toxicology

XIII.E.a. Clinical Studies Employing FCP-3P7 Sterols

Table XIII-iv summarizes the clinical studies conducted to date with FCP-3P7 in human subjects. All of the studies were performed using FCP-3P1, the non-esterified form of FCP-3P7, which is physiologically equivalent (see Section I.F.) A total of 55 subjects were exposed to FCP-3P7 sterols in their diet at a dose of 1.5g per 70 kg body weight per day. No clinically significant adverse events were observed in these studies. Reports of clinical studies are provided in Annex C.

Table XIII-iv Clinical Studies on Dietary Administration of FCP-3P7 sterols

Study Number	Cholesterol Levels	Number & Sex	Food Matrix	Dosage g/70kg/day	Duration (days)
CLF9601	Normal	6M 5F	Vegetable Oil	1.5	10
CLF9602	Elevated	12M	Vegetable Oil	1.5	10
CLF9701*	Elevated	32M	Margarine	1.5	30
CLF9904	Elevated	78 M 98 F	Milk drink	0.9 / 1.8 / 3.6 / g/day	28

* Jones et al (1999).

In study CLF9601, FCP-3P7 sterols were incorporated into the standard diet of 11 healthy male and female volunteer test subjects at a dose level of 1.5 g phytosterol per 70 kg body weight. This was conducted over the course of 10 days, followed by a 14-day washout period, followed again by a second 10-day administration. When compared to the control group, results indicate that at relatively low doses, the phytosterol mixture effectively impeded cholesterol absorption, thus improving the plasma lipid profile through decreasing total and LDL-cholesterol levels as well as increasing the HDL/LDL ratio. No adverse effects were reported.

In study CLF9602, FCP-3P7 sterols were incorporated into the standard diet of 12 healthy male volunteer test subjects at a dose level of 1.5 g phytosterol per 70 kg body weight. This study was also conducted over the course of 10 days, followed by a 14-day washout period, followed again by a second 10-day administration period. Post treatment plasma LDL cholesterol level (4.1 ± 0.2 mmol/l) was lower ($p<0.05$) than that of post placebo treatment (4.3 ± 0.1 mmol/l). The treatment had no effect on plasma HDL and triglycerides versus placebo. No adverse effects were reported.

In study CLF9701, published by Jones et al (1999), FCP-3P7 sterols were incorporated into a double-blind, randomised, placebo controlled diet. A standard test diet consisting of 15% protein, 50% carbohydrates, and 35% fat was administered to 32 healthy volunteer test subjects for a period of 30 days. Treated subjects received a dose level of 1.5 g FCP-3P7 sterols per 70 kg body

weight per day, incorporated into margarine at a ratio of 1:20 (w/w). Another 16 volunteers received a placebo. Both the placebo and FCP-3P7 sterols containing diets were well tolerated with no reported discomfort and no significant adverse events. No change in body weight was noted for each of the study groups. The most significant dietary effect noted was the mean decline in total and LDL cholesterol. The difference between placebo and treated groups at day 30 for total and LDL cholesterol was 9.1% and 15.5% respectively. A small decrease in HDL occurred in both the control and treated groups. The mean decrease in the treated group was slightly greater than that of the control group but the difference was not clinically significant, was well within the variability of measurement, and was not statistically significant, as indicated in Section 1.3.3.3.

XIII.E.b. Literature Review

The safety of tall oil phytosterols in general, is further supported by the extensive history of human exposure to the constituent phytosterols, as documented in the published literature cited below.

Humans are continually exposed to phytosterols in the diet. The average dietary phytosterol intake is about 250 mg per day, with perhaps double that amount consumed by vegetarians. The scientific literature on the effects of human exposure to elevated intakes of phytosterols is extensive and dates from the early 1950's. Pollak and Kritchevsky (1981) reviewed published studies on the clinical use of phytosterols up to 1981. The authors estimate that clinical data on the cholesterol-lowering action of phytosterols in about 1800 subjects was available at the time of their review.

Table XIII-v summarizes clinical studies of phytosterols published since the review by Pollak & Kritchevsky (1981), as well as some earlier studies. Most of the recent studies have been conducted using sitostanol ester. As reflected in Table XIII-v, the occurrence of adverse effects associated with the use of phytosterols is rare. Prior to 1981, reports of adverse events consisted primarily of gastrointestinal disturbances. In more recent studies, reported adverse effects were mild and presented no consistent pattern that might suggest a relation to the use of phytosterols. Furthermore, to our knowledge, there has not been a single report of a serious adverse event associated with the use of phytosterols.

XIII.F Substantial equivalence.

FCP-3P7 has undergone extensive *in vitro* and *in vivo* testing followed by clinical studies. These studies have revealed no adverse toxicological consequences associated with FCP-3P7 intake. The scientific literature has been searched for additional information and no significant adverse toxicological effects have been observed to be associated with phytosterol intake. In particular, reports of toxicological studies performed using Benecol and Flora pro.activ phytosterols have shown no adverse effects and thus FCP-3P7 is substantially equivalent to those two yellow fat spreads in this regard.

Table XIII-v Summary of Safety of Orally Administered Phytosterols in Human Subjects from Published Sources

Reference	Population			Study Material			Exposure			Safety
	Disease State	Number & Sex	Age Range or Mean (yr)	Phytosterol	Plant Source	Dosage Form	Dosage g/day[2]	Duration (days)	Extent g/d x days x subjects	Adverse Events Reported
Hallikainen *et al*., 2000	HC	22 M&F	50.5 ± 11.7	Esterified stanols	NS	Margarine suspension	0.8 – 3.2	4 x 28	4928	No significant changes in retinal, α- and β-carotene or tocopherols. Lower lycopene in females – not dose-related.
Hendricks *et al*., 1999	NC and mildly HC	80 M&F	NS	Esterified sterols	Vegetable (predominantly soybean) distillates.	Margarine suspension.	0.85, 1.62 and 3.26 g/day (target)	3 x 24 or 25	10756.8	Some reduction in serum α- and β-carotene and lycopene. Not dose-related.
Jones, *et al*., 2000.	HL	15 M	37 - 61	Esterified sterols and stanols	Vegetable oil	Margarine suspension	1.81	2 x 21	1140.3	None
Ayesh *et al*., 1999	NC	12 M & 12 F	36 ± 2.7	Esterified sterols	NS	Margarine suspension	8.64	21 (M) 28 (F)	5080.3	No significant treatment-related changes in faecal short-chain fatty acids, bacterial enzyme activity or micro-organism counts. Serum progesterone levels in females reduced – not biologically significant.
Gylling & Miettinen, 1999.	Moderately HC	24 F	50 - 55	Sitostanol-rich ester; Campesterol-rich ester.	Tall oil and vegetable oil	Margarine and butter	4.9 to 6.7 g/d	35 or 42	8482.6	No significant effect on serum vitamin D or α-tocopherol. Serum α- and β-carotene reduced but retinal unaffected.
Wessttrate *et al*., 1999.	NC	6 M & 6 F	30 - 40	Esterified sterols	NS	Margarine	8.6	21 or 28	1083.6 – 1444.8	None

Reference	Population			Study Material			Exposure			Safety
	Disease State	Number & Sex	Age Range or Mean (yr)	Phytosterol	Plant Source	Dosage Form	Dosage g/day[2]	Duration (days)	Extent g/d x days x subjects	Adverse Events Reported
Weststrate JA and Meijer GW, 1998.	NC and mildly HC	95 M&F	48±12.8	Sitostanol ester (Benecol™) Soy PS ester	Tall oil Soy	Margarine suspension	2.74 3.24	24-25	6377 7541	None except effects on vitamin and nutrient levels in plasma (See Table 10-1).
Plat J and Mensink R, 1998.	Healthy volunteers	112		SITO 70 SITO 90	Vegetable Tall oil	Margarine suspension	3.8 4.9	56	23833.6 25088	Haematology and blood chemistry parameters remain within normal range.
Kris-Etherton PM *et al*., 1998.	HC	35M & 23F		Sitostanol mixture	Vegetable	Margarine suspension	3	28	4872	none
Cobb MM *et al*., 1997.	Sitosterolemic homozygote	1F	9	Sitosterol	Soybean oil Sesame oil	Oil suspension	0.06 0.09 0.122	56	34-67	none
Gylling *et al*., 1997.	Woman with angiographically documented CAD Women treated with simvastatin for more than 1 year	22F 10F		Sitostanol ester (Benecol™)	Tall oil	Margarine suspension	3 3	49 90	3234 2700	None
Gylling *et al*, 1996	NIDDM with HC	8 M	60.2±1.6	Sitostanol Ester	Tall Oil	Margarine suspension	3.0	42	1008	None

Reference	Population			Study Material			Exposure			Safety
	Disease State	Number & Sex	Age Range or Mean (yr)	Phytosterol	Plant Source	Dosage Form	Dosage g/day[2]	Duration (days)	Extent g/d x days x subjects	Adverse Events Reported
Gylling *et al*, 1995	FH	7 M 7 F	9.1±1.1	Sitostanol ester	Tall Oil	Margarine suspension	3.0	42	1764	none
Gylling *et al*, 1995	NIDDM with HC	6 M	63.2±1.2	Sitostanol ester	Tall Oil	Margarine suspension	3.0	28	504	none
Pelletier *et al*, 1995	Healthy volunteers	12M	22.7±2.6	Sitostanol ester	Soybean Phytosterol	Margarine suspension	0.740	28	249	none
Miettinen *et al*, 1995	HC	64 M 89 F	25-64	Sitostanol Ester	Tall Oil	Margarine suspension	2.6 (n=51) 2.6 (n=51) 1.8 (n=51)	365 180 180	48399 23868 16254	none
Denke *et al*, 1995	HC	33 M	31-70	Sitostanol	Tall Oil	Margarine suspension	3.0	30	2970	none
Gylling *et al*, 1994	NIDDM with HC	11 M	57.8±1.9	Sitostanol Ester	Tall Oil	Margarine suspension	3.0	42	1386	none
Miettinen & Vanhanen, 1994	HC	22 M 9F	45±3	Sitosterol Sitostanol Sitostanol ester	Tall Oil	Margarine suspension	0.7 (n=9) 0.7 (n=7) 0.8 (n=7)	63 63 63	1367	none
Vanhanen *et al*, 1994	HC	11M 4F	33-60 M 37-55 F	Sitostanol ester Sitosterol	No data	Margarine suspension	0.8 (n=7) 2.0 (n=7)	63 42	352 588	none
Vanhanen *et al*, 1993	HC	47M 20F	25-60	Sitostanol Ester	No data	Margarine suspension	3.4 (n=34)	42	9568	none

Reference	Population			Study Material			Exposure			Safety
	Disease State	Number & Sex	Age Range or Mean (yr)	Phytosterol	Plant Source	Dosage Form	Dosage g/day[2]	Duration (days)	Extent g/d x days x subjects	Adverse Events Reported
Becker *et al*, 1993	FH	6 M 3 F	10-14	Sitosterol Sitostanol	No data	Pastil	6.0 1.5	84 196	4536 2646	none
Becker *et al*, 1992	FH	7 M&F	5-10	Sitosterol	No data	Pastil	6.0	84	3528	Slight, but significant decrease in haemoglobin concentration (-5%), decrease alkaline phosphatase activity (-19%), decrease in appetite in 2 children for about 2 weeks.
Vanhanen & Mietinen, 1992	HC	24 M&F	25-45	Sitosterol Sitostanol	No data	Margarine suspension	0.625 (n=8) 0.630 (n=8)	54 54	270 272	none
Heinemann *et al*, 1986	HC and FH	3 M 3 F	27-59	Sitostanol	No data	Capsule	1.5	28	252	none
Weisweiler *et al*, 1984	FH (type IIa)	6M 4F	29-67	Sitosterol	No data	Capsule ?	6.0	56	3360	none
Mattson *et al*, 1982	Unknown cholesterol status	9 M&F	adults	Sitosterol	No data	Aqueous Suspension (Cytellin™)	1.0	30	270	none
Schlierf *et al*, 1978	FH (type II)	12 M&F	8-20	Sitosterol	No data	Granule	12.0	56	8064	none

Reference	Population			Study Material			Exposure			Safety
	Disease State	Number & Sex	Age Range or Mean (yr)	Phytosterol	Plant Source	Dosage Form	Dosage g/day[2]	Duration (days)	Extent g/d x days x subjects	Adverse Events Reported
Lees *et al*, 1977	FH (type II)	9M 3F	Adults	Sitosterol Campesterol	Soybean	Capsule	18.0	280 (average) (364-728)	60480	none
Lees *et al*, 1977	FH (type II)	6 M	Adults	Sitosterol Campesterol	Soybean	Capsule	18.0	Ave 280	30240	none
Lees *et al*, 1977	FH (type II)	9 M	adults	Campesterol	Tall Oil	Capsule	3.0	Ave 196	5292	none
Lees *et al*, 1977	FH (type II)	14 M 17 F	adults and children	Phytosterol mixture	Tall Oil	Capsule	3.0	Ave 168	15624	mild constipation in a few patients
Lees *et al*, 1977	FH (type II)	5 M 13 F	adults and children	Phytosterol mixture	Tall Oil	Capsule	6.0	Ave 140	15120	
Duncan *et al*., 1963	HC	1M 1F	58 69	Sitosterol	Unknown	unknown	18-20	2190 240	43800 4800	none
Reeves, 1959	Healthy volunteers	7M 1F	31-61	20% Sitosterol suspension	Tall Oil	Cytellin™	6-18	30 (5 patients) 60 (3 patients)	2700-3240	The only side effect was a slight to moderate increase in the number of daily bowel movements but no actual diarrhoea occurred.
Cooper, 1958	Atherosclerotic patients	25	unknown	Sitosterol	Tall Oil	Cytellin™	12	140	42000	Three patients reported constipation, the rest thought their stools were bulkier and looser.

Reference	Population			Study Material			Exposure			Safety
	Disease State	Number & Sex	Age Range or Mean (yr)	Phytosterol	Plant Source	Dosage Form	Dosage g/day[2]	Duration (days)	Extent g/d x days x subjects	Adverse Events Reported
Lehmann, 1957.	MI (6) Angina (6) Familial tuberous xanthomatosis (1) HC (1)	9M 6F	adults	Sitosterol	Tall Oil	Cytellin™	20	30-150	9000-45000	none
Farquhar *et al*, 1956	Patients with myocardial infarction	15 M	26-45	Sitosterol	No data	Capsule (Cytellin™)	12.0-18.0	84-168	15120-45360	none
Sachs and Weston, 1956	5 healthy subjects; 1 FH	6	Unknown	Sitosterol and sitostanol	Tall Oil	Cytellin™	9-12	56	3024-4032	none
	4 healthy subjects; 2 CAD; 3HC	9					9-45	90-180	7290-72900	none
	1 biliary cirrhosis	1	73				18	28	504	none
Lesesne *et al*., 1955	6 with HC 5 with atherosclerotic and/or hypertensive heart disease	4M 3F	33-55	Mixtures of phytosterols, primarily Sitosterol	Soybean (n=3) Tall Oil (n=4)	Powder	9 plus extra 3 g with extra meals	84-224 Ave 192	14112 Ave 12096	1 Subject: Fatigue and unexplained weight loss of 10 lb.; 1 Subject: on weight reduction diet for 2 months prior to treatment, continued to lose weight. No other events reported.
Best *et al*., 1955	12 HC 2 volunteers	10M 4F	33-77	Sitosterol	Tall Oil	Cytellin™	20-25 on occasion 50	91-448 Ave 280	98000	none

Reference	Population			Study Material			Exposure			Safety
	Disease State	Number & Sex	Age Range or Mean (yr)	Phytosterol	Plant Source	Dosage Form	Dosage g/day[2]	Duration (days)	Extent g/d x days x subjects	Adverse Events Reported
Barber *et al*., 1955	Coronary artery disease	18M 8F	unknown	Sitosterol	Unknown	palatable biscuit	9	147	34398	none
Joyner *et al*., 1955	4 hypertension, the other angina pectoris, 1 HC	I part: 4F&3M II part: 2 HC	39-50 F 34-62 M	13% Sitosterol 85% Sitosterol	Tall-Oil	Cytellin™	6-15	28	3780	none
Best *et al*., 1954	2 Volunteers 7 HC	9	unknown	Sitosterol	Tall Oil	Cytellin™	5-6	91-203 Ave 154	8316	none

1 NC = Normocholesterolemic; FH = Familial Hypercholesterolemia; NIDDM = Non-insulin dependent diabetes mellitus; HC = Hypercholesterolemia

2 Total combined dose phytosterols where phytosterols are a mixture.

5. Evaluation and conclusion by the applicant.

FCP-3P7 is a cholesterol-lowering food ingredient containing esters of phytosterols and phytostanols, produced by Forbes Medi-Tech. FCP-3P7 has a composition intermediate between the active ingredients of two existing phytosterol containing yellow fat spread products available in EU markets: Benecol and Flora pro.activ. For three categories of novel foods, including foods and food ingredients derived from plants or animals obtained by traditional propagating or breeding and having a safe history of food use, a simplified procedure for pre-market approval can be applied provided that the food/ingredient is *substantially equivalent to existing foods or food ingredients as regards their composition, nutritional value, metabolism, intended use and level of undesirable substances contained therein* (Article 3.4. Regulation 258/97). In accordance with the provisions of Article 3.4 of Regulation 258/97 this submission sets out the case for proposing FCP- 3P7 as being a food ingredient derived from plants obtained by traditional propagating with a safe history of use that is substantially equivalent to the existing food ingredients contained in Benecol and Flora pro.activ

All phytosterol products contain variable ratios of phytosterols and phytostanols. This reflects natural variation in plant sources and also variation in the degree of hydrogenation applied in the production process. It is intended that FCP-3P7 should be consumed in a manner identical to Benecol and Flora pro.activ to provide consumers with an additional product choice. The FCP-3P7 tall oil phytosterol product merely revises the ratio of major sterols to stanols to an intermediate composition when compared to the other two currently marketed yellow fat spreads. The nutritional and physiological properties, intended use and projected intakes of FCP-3P7 would therefore be expected to be equivalent to those of Benecol and Flora pro.activ.

The method of production and the product composition and specification are essentially equivalent to those phytosterol products already used in Europe as cholesterol-lowering ingredients in yellow-fat spreads.

The intended use of FCP-3P7 is to incorporate it into a vegetable oil based spread product at a concentration of up to 12.5% by weight in the esterified form. This represents an application and a phytosterol content which is slightly lower that of Flora pro.activ and Benecol which have an incorporation rate up to 14% by weight of esterified phytosterols. The intended consumer daily consumption of FCP-3P7 in yellow fat spread provides 1.5 grams of phytosterols. This intake rate is substantially equivalent to those of Flora pro.activ or Benecol yellow fat spread products. Intakes of individual phytosterols will be equal to or lower than those resulting from equivalent products currently available on European markets. The introduction of FCP-3P7 into the European market will have no impact on individual total phytosterol intakes because the product is intended to substitute for products already marketed in European countries.

Testing for contaminants and undesirable substances has revealed none other than some minor phytosterols that are also present in the other cholesterol-lowering phytosterols. Nutritional and toxicological testing in animal models

and human volunteer studies, using the physiologically-equivalent non-esterified form of FCP-3P7, have revealed no adverse effects other than a small effect on serum α-carotene levels which is well within the limits of normal fluctuations. FCP-3P7 is thus equivalent to the other two products and all other cholesterol-lowering phytosterols in this regard.

FCP-3P7 is intended for use by adults as part of a cholesterol-lowering diet. Packaging for the ingredient FCP 3P7 will bear a label containing the warnings and this will be enforced with manufacturers and retailer through contract law. In order to ensure that consumption remains within recommended levels and that the product is being consumed by targeted individuals whilst being avoided by inappropriate consumers a post market monitoring programme will be instigated.

Literature reports on the effects of phytosterols indicate that they are largely independent of the specific phytosterol/stanol composition of any given product. Comparative data presented herein confirm that the cholesterol lowering effects of FCP-3P7 sterols are equivalent to those of Benecol and Flora pro.activ at doses used in each of these products. There have been a number of reports which indicate that phytosterols esterified with fatty acids may interfere with the uptake of fat soluble vitamins and nutrients, primarily carotenoids, from the intestine. *In vivo* and human clinical studies using the physiologically-equivalent non-esterified form of FCP-3P7 have shown no significant effect on vitamin A, vitamin E, α carotene or β-carotene plasma values outside of the normal range of fluctuation. These studies confirm that FCP-3P7 is substantially equivalent to existing phytosterol ester containing yellow-fat spread ingredients with regard to its cholesterol lowering effects, effects on circulating phytosterol levels and effects on vitamin and nutrient absorption.

FCP-3P7 has undergone extensive *in vitro* and *in vivo* testing followed by clinical studies using the physiologically-equivalent non-esterified form of FCP-3P7. These studies have revealed no adverse toxicological consequences associated with FCP-3P7 intake. The scientific literature has been searched for additional information and no significant adverse toxicological effects have been observed to be associated with phytosterol intake. In particular, reports of toxicological studies performed using Benecol and Flora pro.activ phytosterols have shown no adverse effects and thus FCP-3P7 is substantially equivalent to those two yellow fat spreads in this regard.

Forbes' FCP-3P7 is substantially equivalent to existing cholesterol-lowering phytosterol ester ingredients with regard to its composition, nutritional value, metabolism, intended use, history of use, beneficial effects, and toxicology and level of undesirable substances contained therein. The simplified procedure for pre-market approval, as set out in Article 3.4. of Regulation 258/97, should therefore be applied.

6. Summary by the applicant

FCP-3P7 is a cholesterol-lowering food ingredient containing esters of phytosterols and phytostanols produced by Forbes Medi-Tech intended for use in yellow fat spreads. FCP-3P7 has a composition intermediate between the active ingredients of two existing phytosterol products available in EU markets: Benecol and Flora pro.activ. For three categories of novel foods, including foods and food ingredients derived from plants or animals obtained by traditional propagating or breeding and having a safe history of food use, a simplified procedure for pre-market approval can be applied provided that the food/ingredient is *substantially equivalent to existing foods or food ingredients as regards their composition, nutritional value, metabolism, intended use and level of undesirable substances contained therein* (Article 3.4. Regulation 258/97). In accordance with the provisions of Article 3.4 of Regulation 258/97 this submission sets out the case for proposing FCP- 3P7 as being a food ingredient derived from plants obtained by traditional propagating with a safe history of use that is substantially equivalent to the existing food ingredients contained in Benecol and Flora pro.activ

The tall oil phytosterols in FCP-3P7 are extracted from tall oil soap, a by-product of the pulping process for coniferous trees in North America and Europe. The FCP-3P7 product under consideration is predominantly a mixture of four phytosterols: sitosterol, sitostanol, campesterol, and campestanol. A small percentage of minor phytosterols such as stigmasterol is also present as well as a fraction of a percentage of long chain aliphatic alcohols. The natural ratio of phytosterols varies from batch to batch and this is taken into account in the specification.

Currently, there are two vegetable oil spread products containing phytosterols on the European market which contain up to 14% by weight of added fatty acid esterified phytosterols. All phytosterol products contain variable ratios of phytosterols and phytostanols. This reflects natural variation in plant sources and also variation in the degree of hydrogenation applied in the production process. It is intended that FCP-3P7 should be consumed in a manner identical to Benecol and Flora pro.activ to provide consumers with an additional product choice. The FCP-3P7 tall oil phytosterol product merely revises the ratio of major sterols to stanols to an intermediate composition when compared to the other two currently marketed yellow fat spreads. The nutritional and physiological properties, intended use and projected intakes of FCP-3P7 would therefore be expected to be equivalent to those of Benecol and Flora pro.activ.

The processes of extraction, complexation and crystallisation produce purified sterols the composition of which is dependent on the sterol composition of the starting product. The method of production and the product composition are equivalent to those phytosterol products already used in Europe as cholesterol-lowering ingredients in yellow-fat spreads.

The intended use of FCP-3P7 is to incorporate it into a vegetable oil based spread product at a concentration of up to 12.5% by weight in the esterified form. This represents an application and a phytosterol content which is slightly lower that of Flora pro.activ and Benecol which have an incorporation rate up to

14% by weight of esterified phytosterols. The intended consumer daily consumption of FCP-3P7 in yellow fat spread provides 1.5 grams of phytosterols. This intake rate is substantially equivalent to those of Flora pro.activ or Benecol yellow fat spread products. Intakes of individual phytosterols will be equal to or lower than those resulting from equivalent products currently available on European markets. The introduction of FCP-3P7 into the European market will have minimal impact on total phytosterol intakes because the product is intended to substitute for products already marketed in European countries.

Intended use conditions for phytosterol products

	Flora pro.activ	**FCP 3P7**	**Benecol**
Incorporation rate (%)	13.80*	12.50	14.00*
Serving size (g)	10.00*	10.00	12.00*
Amount per serving (as esters, g):	1.38	1.25	1.68
Amount per serving (as sterols, g):	0.83	0.75	1.01
Servings per day	2*	2	2 – 3*
Daily intake (sterols, g/day)	1.66	1.50	2.02 – 3.02

* Information taken from Flora pro.activ and Benecol product labels.

FCP-3P7 is intended for use by adults as part of a cholesterol-lowering diet. Packaging for the ingredient FCP 3P7 will bear a label containing the warnings and this will be enforced with manufacturers and retailer through contract law. In order to ensure that consumption remains within recommended levels and that the product is being consumed by targeted individuals whilst being avoided by inappropriate consumers a post market monitoring programme will be instigated.

Literature reports on the effects of phytosterols indicate that they are largely independent of the specific phytosterol/stanol composition of any given product. Comparative data presented herein confirm that the cholesterol lowering effects of FCP-3P7 sterols are equivalent to those of Benecol and Flora pro.activ at doses used in each of these products. There have been a number of reports which indicate that phytosterols esterified with fatty acids may interfere with the uptake of fat soluble vitamins and nutrients, primarily carotenoids, from the intestine. *In vivo* and human clinical studies using the physiologically-equivalent non-esterified form of FCP-3P7 have shown no significant effect on vitamin A, vitamin E, α carotene or β-carotene plasma values outside of the normal range of fluctuation. These studies confirm that FCP-3P7 is substantially equivalent to existing phytosterol ester containing yellow-fat spread ingredients with regard to its cholesterol lowering effects, effects on circulating phytosterol levels and effects on vitamin and nutrient absorption.

FCP-3P7 has undergone extensive *in vitro* and *in vivo* testing followed by clinical studies using the physiologically-equivalent non-esterified form of FCP-3P7. These studies have revealed no adverse toxicological consequences associated with FCP-3P7 intake. The scientific literature has been searched for additional information and no significant adverse toxicological effects have been observed to be associated with phytosterol intake. In particular, reports of

toxicological studies performed using Benecol and Flora pro.activ phytosterols have shown no adverse effects and thus FCP-3P7 is substantially equivalent to those two yellow fat spreads in this regard.

Forbes' FCP-3P7 is substantially equivalent to existing cholesterol-lowering phytosterol ester ingredients with regard to its composition, nutritional value, metabolism, intended use, history of use, beneficial effects, and toxicology and level of undesirable substances contained therein. The simplified procedure for pre-market approval, as set out in Article 3.4. of Regulation 258/97, should therefore be applied.

References

Advisory Committee on Novel Foods and Processes. 1999. ACNFP/MIN/42. Minutes of the 42nd Meeting held 8 July 1999.

Ayesh R, Weststrate JA, Drewit PN and Hepburn PA. 1999. Safety evaluation of phytosterol esters. Part 5. Faecal short-chain fatty acid and microflora content, faecal bacterial enzyme activity and serum female sex hormones in healthy normolipidaemic volunteers consuming a controlled diet either with or without a phytosterol ester-enriched margarine. Food and Chemical Toxicology: 37, 1127 – 1138.

Bae, M. and Mercer, E.I. 1970. The effect of long- and short-day photoperiods on the sterol levels in the leaves of *salanum andigena*. Phytochemistry 9, 65-68.

Baker VA, Hepburn PA, Kennedy SJ, Jones PA, Lea LJ, Sumpter JP, and Ashby J. 1999. Safety evaluation of phytosterol esters: Part 1. Assessment of oestrogenicity using a compination of in vivo and in vitro assays. Food Chem Toxicol 37(1):13-22.

Barber JM, Grant AP. 1955. The serum cholesterol and other lipids after administration of sitosterol. Br. Heart J. 17: 296-298.

Bean GA. 1973. Phytosterols. Adv. Lipid Res. 11:193-218.

Becker M, Staab D, von Bergmann K. 1992. Long-term treatment of severe familial hypercholesterolemia in children: effect of sitosterol and bezafibrate. Pediatr 89:138-42.

Becker M, Staab D, von Bergmann K. 1993. Treatment of severe familial hypercholesterolemia in childhood with sitosterol an sitostanol. J Pediatr 122:292-6.

Beer MU, Pritchard H, Belsey, EM, Davidson, M, 2000. Effect of a milk drink enriched with increasing doses of free tall oil phytosterols on plasma lipid levels of mildly hypercholesteroleamic subjects. Novartis Consumer Health unpublished. Clinical Study report #CLF9904.

Best, MM and Duncan, CH 1958. Effects of the esterification of supplemental cholesterol and sitosterol in the diet. J. Nutrition 65:169-181.

Best MM, Duncan CH, Van Loon EJ, Wathen JD. 1954. Lowering of serum cholesterol by the administration of a plant sterol. Circulation 10: 201-206.

Best MM, Duncan CH, Van Loon EJ, Wathen JD. 1955. The effects of sitosterol on serum lipids. Am. J. Med. 19: 61-70.

Bhattacharyya A, Connor WE, Lin DS. 1991. Sluggish sitosterol turnover and hepatic failure to excrete sitosterol into bile cause expansion of body pool of sitosterol in patients with sitosterolemia and xanthomatosis. Arteriocsler. Thromb. Vol 11. 5:1287-1294

Bhattacharyya A, Connor WE. 1974. β-sitosterolemia and xanthomatosis: A newly described lipid storage disease in two sisters. J Clin Invest. 53: 1033-1043.

Burck PJ, Thakkar AL, Zimmerman RE. 1982. Antifertility action of a sterol sulphate in the rabbit. J Reprod Fert 66:109-12.

Cater, NB and Grundy, SM; Lowering serum cholesterol with plant sterols and stanols. Managing High Cholesterol. A postgraduate medicine special report, pp.6-14, 1998.

Cerqueira, M.T., Fry, M.M., and Connor, W.E. 1979. The food and nutrient intakes of the Tarahumara Indians of Mexico. Am.J.Clin.Nutr. 32, 905-915.

Christiansen L.I. et al. 2001. Cholesterol-lowering effect of spreads enriched with microcrystalline plant sterols in hypercholesterolemic subjects. European Journal of Nutrition 40: 66-73,.

Clayton PT, Whitfield P, and Iyer, K. 1998. The role of phytosterols in the pathogenesis of liver complications of pediatric parenteral nutrition. Nutrition. 14, 158-164.

Cobb MM, Salen G, Tint GS. 1997. Comparative effect of dietary sitosterol on plasma sterols and cholesterol and bile acid synthesis in a sitosterolemic homozygote and heterozygote subjects. J Am College Nutr. 16 (6): 605-613.

Commission Recommendation 97/618/EC of 29 July 1997 concerning the scientific aspects and the presentation of information necessary to support applications for the placing on the market of novel foods and novel food ingredients and the preparation of initial assessment reports under Regulation (EC) No 259/97 of the European Parliament and of the Council. OJ No 253, 16.9.1997, p1.

Connor, W.E. 1968. Dietary sterols: Their relationship to ahterosclerosis. J. Am. Dietetic Assoc. 52: 202-208.

Cooper EE. 1958. Dietary and pharmaceutical approaches to atherosclerosis; special reference to beta-sitosterol. Texas St. J. Med. 54:29-36.

Crombie, W.M.L. 1961. Chemical composition of plant tissues and related data. In Long Biochemist handbook. Spon, ed. (pp. 937-1053).

Davidson M.H. et al. 2001. Safety and tolerability of esterified phytosterols administered in reduced-fat spread and salad dressing to healthy adult men and women. J. Am. Col. Nut. 20(4): 307-319.

Davis, D.L. 1971. Sterol distribution within green air-cured tobacco. Phytochemistry 11, 489-494.

Denke M.A. 1995. Lack of efficacy of low-dose sitostanol therapy as an adjunct to a cholesterol-lowering diet in men with moderate hypercholesterolemia. Am J Clin Nutr 61:392-6.

Duncan C.H, Best M.M. 1963. Long-term use of sitosterol as a hypocholesterolemic agent. J Kentucky med. Ass. 61: 45-47.

Dutta, P.C. and Appelqvist, L.A. 1996. Saturated sterols (stanols) in unhydrogenated and hydrogenated edible vegetable oils and in cereal lipids. J.Sci.Food Agricul. 71, 383-391.

Farquhar JW, Smith RE, Dempsey ME. 1956. The effect of beta sitosterol on the serum lipids of young men with arteriosclerotic heart disease. Circulation 14:77-82.

Grundy, S.M., Ahrens, E.H., Jr., and Davignon, J. 1969. The interaction of cholesterol absorption and cholesterol synthesis in man. J.Lipid Res. 10, 304-315.

Gylling H and Miettinen TA. 1999. Cholesterol reduction by different plant stanol mixtures and with variable fat intake. Metabolism, 48: 575-580.

Gylling H, Miettinen TA. 1994. Serum cholesterol lipoprotein metabolism in hypercholesterolemic NIDDM patients before and during sitostanol ester-margarine treatment. Diabetologia 37:773-80.

Gylling H, Miettinen TA. 1995. The effect of cholesterol absorption inhibition on low density lipoprotein cholesterol level. Atherosclerosis 117:305-8.

Gylling H, Miettinen TA. 1996. Effects of inhibiting cholesterol absorption and synthesis on cholesterol and lipoprotein metabolism in hypercholesterolemic non-insulin-dependent diabetic men. J. Lipid Research 37:1776-85.

Gylling H, Radhakrishnan R, Miettinen TA. 1997. Reduction of serum cholesterol in postmenopausal women with previous myocardial infarction and cholesterol malabsorption induced by dietary sitostanol ester margarine. Circulation. No 12. 96: 42264231.

Gylling H, Siimes MA, Miettinen TA. 1995. Sitostanol ester margarine in dietary treatment of children with familial hypercholesterolemia. J. Lipid Research 36:1807-12.

Gylling H. et al. 1999. Retinol, vitamin D, carotenes and α-tocopherol in serum of a moderately hypercholesterolemic population consuming sitostanol ester margarine. Atherosclerosis 145: 279-285,.

Gylling HK, Puska P, Vartiainen E, et al. 1996. Serum retinol, beta-tocopherol, carotenes, and lipid peroxide production during serum cholesterol lowering by sitostanol ester margarine in a mildly hypercholesterolemic population (abstract 3379). Circulation; 94 (Suppl 1): 1-578.

Hallikainen M.A. and Uusitupa M.I.J. 1999. Effects of 2 low-fat stanol ester-containing margarines on serum cholesterol concentrations as part of a low-fat diet in hypercholesterolemic subjects. Am. J. Clin. Nutr. 69: 403-410.

Hallikainen M.A. et al. 1999. Plant stanol esters affect serum cholesterol concentrations of hypercholesterolemic men and women in a dose-dependant manner. Am. Soc. Nutr. Sci.: 767-776, Nov. 1999.

Hallikainen MA, Sarkkinen ES, Gylling H, Erkkila AT, Uusitupa MI, 2000. Comparison of the effects of plant sterol ester and plant stanol ester-enriched margarines in lowering serum cholesterol concentrations in hypercholesterolaemic subjects on a low-fat diet. Eur J Clin Nutr 54:715-25.

Hallikainen, M.A., E.S. Sarkkinen, and M.I.J. Uusitupa. 2000. Plant stanol esters affect serum cholesteerol concentrations of hypercholesterolemic men and women in a dose-dependent manner. J. Nutrition 130:767-776.

Heinemann T, Leiss O, von Bergmann K. 1986. Effect of low-dose sitostanol on serum cholesterol in patients with hypercholesterolemia. Atheroscler 61:219-23.

Hellman L, et al. 1953. Metabolism of cholesterol-4-C14 in hypercholesterolemia. Circulation 8:434-435.

Hendricks HFJ, Weststrate JA, van Vleit t and Meijer GW. 1999. Spreads enriched with three different levels of vegetable oil sterols and the degree of cholesterol lowering in normocholesterolaemic and mildly hypercholeststolaemic subjects. European Journal of Clinical Nutrition, 53: 319-327.

Hepburn PA, Homer SA, and Smith M. 1999. Safety evaluation of phytosterol esters: Part 2. Subchronic 90-day oral toxicity study on phytosterol esters: A novel functional food. Food Chem Toxicol 37(5);521-532.

Hirai, K., Shimazu, C., Takezoe, R., and Ozeki, Y. 1986. Cholesterol, phytosterol and polyunsaturated fatty acid levels in 1982 and 1957 Japanese diets. J.Nutr.Sci.Vitaminol. 32, 363-372.

Homan, R and Krause, BR 1997. Established and emerging strategies for the inhibition of cholesterol absorption . Curr. Pharmaceut. Design, 3:29-44.

Iyer KR, Spitz L, Clayton P. 1998. New insight into mechanisms of parenteral nutrition-associated cholestasis: role of plant sterols. J. Ped. Surg. 33 (1) 1-6.

Jones et al 1997. Dietary phytosterols as cholesterol lowering agents in humans Can. J. Physiol. Pharmacol. 75:217-227..

Jones PJH, Ntanios FY, Raeini-Sarjaz M, Vanstone CA. 1999. Cholesterol-lowering efficacy of a sitostanol-containing phytosterol mixture with a prudent diet in hyperlipidemic men. Am J. Clin Nutr 69: 1140-50.

Jones, PJH, M. Raeini-Sarjaz, F.Y. Ntanios, C.A. Vanstone, J.Y.Feng, and W.E. Parsons. 2000. Modulation of plasma lipid levels and cholesterol kinetics by phytosterol versus phytostanol esters. J. Lipid Res. 41:697-705.

Joyner C, Kuo PT. 1955. The effect of sitosterol administration upon the serum cholesterol level and lipoprotein pattern. Am. J. med. Sci. 230: 636-647.

Kris-Etherton PM, Etherton TD, Pearson TA, Phyllips K, Reed R, Windhouser M, Champagne C, Lefevre M. 1998. Stanol supplemented margarine (SM) loweres LDL-C in moderately hypercholesterolemic subjects fed an average American diet (AAD). Experimental Biology Meeting, San Francisco.

Kritchevsky DL, Davidson LM, Mosbach EH, Cohen BI. 1981 Identification of acid steroids in feces of monkeys fed β-sitosterol. Lipids 16: 77-78.

Kuo PT. 1956. Effects of lipemia on tissue oxygenation in arteriosclerosis patients. Circulation 14:946.

Lees AM, Mok HY, Lees RS, McCluskey MA, Grundy SM. 1977. Plant sterols as cholesterol-lowering agents: clinical trials in patients with hypercholesterolemia and studies of sterol balance. Atheroscler 28:325-38.

Lehmann JH. 1957. Clinical experiences with beta-sitosterol, a new anti-cholesterolemic agent. NW. Med., Seattle 56: 43-46.

Lesesne JM, Castor CW, Hoobler SW. 1955. Prolonged reduction in human blood cholesterol levels induced by plant sterols. Univ. Mich. Med. Bull. 21: 13-17.

Lutjohann, D., I. Bjorkhem, U.F. Beil, and K. von Bergmann. 1995. Sterol absorption and sterol balance in phytosterolemia evaluated by deuterium-labeled sterols: effect of sitostanol treatment. J. Lipid Res. 36:1763-1773.

Maki K.C. et al. 2001. Lipid responses to plant-sterol-enriched reduced-fat spreads incorporated into a National Cholesterol Education Program Step 1 – Diet. Am. J. Clin. Nutr. 74: 33-43.

Malini T, Vanithakumari G. 1990. Rat toxicity studies with beta-sitosterol. J. Ethnopharma 28:221-34.

Malini T, Vanithakumari G. 1991. Antifertility effects of beta-sitosterol in male albino rats. J. Ethnopharm 35:149-53.

Malini T, Vanithakumari G. 1993. Effect of beta-sitosterol on uterine biochemistry: a comparative study with estradiol and progesterone. Biochem Molecular Biol Int 31:659-68.

Mattson FH, Volpenhein RA, Erickson BA. 1977. Effect of plant sterol esters on the absorption of dietary cholesterol. J. Nutr. 107:1139-1146.

Mattson FH, Grundy SM, Crouse JR. 1982. Optimizing the effect of plant sterols on cholesterol absorption in man. Am J. Clin Nutr 35:697-700.

Mensink PR, Plat J. October 1998. Studies of effects of plant stanol ester on lipid parameters, vitamin levels, and thrombogenic factors in a normocholesterolemic population. Dallas conference: Plant sterol ester: a new tool in dietary management of cholesterol.

Miettinen TA, Puska P, Gylling H, Vanhanen H, Vartiainen E. 1995. Reduction of serum cholesterol with sitostanol-ester margarine in a mildly hypercholesterolemic population. New Engl J Med 333:1308-12.

Miettinen TA, Vanhanen H. 1994. Dietary sitostanol related to absorption, synthesis and serum level of cholesterol in different apolipoprotein E phenotypes. Atheroscler 105:217-26.

Miettinen TA. 1980. Phytosterolemia, xanthomatosis and premature atherosclerotic arterial disease: a case with high plant sterol absorption, impaired sterol elimination and low cholesterol synthesis. Eur J Clin Invest. 10: 27-35.

Misra, P.S., Bhakuni, D.S., Sharma, V.N., and Kaul, K.N. 1961. Utilization of argenome mexicana in lowering alaklinity of soil. Vigyan Praguti 10, 215-217.

Moghadasian MH, et al. 1999. Histologic, hematologic, and biochemical characteristics of Apo-E-KO mice E-deficient mice: Effects of dietary cholesterol and phytosterols. Laboratory Investigation 79:355-364.

Morrell, J. 2000. Dietary intervention with plant stanols and sterols – an opportunity to reduce heart disease risk in the UK. British J. Cardiology, , vol 7, Issue 8 pp. 486-494.

Morton, G.M., Lee, S.M., Buss, D.H., and Lawrence, P. 1995. Intakes and major dietary sources of chlesterol and phytosterols in the British diet. J.Hum.Nutr.Diet. 8, 429-440.

Nguyen T.T. et al. 1999. Cholesterol-lowering effect of stanol ester in a U.S. population of mildly hypercholesterolemic men and women: A randomized controlled trial. Mayo. Clin. Proc. 74: 1198-1206.

Oster P, Schlierf G, Huek C et al. 1977. Sitosterol treatment of familial type II hyperlipoproteinemia in children and adults. In: Schettler H, Goto Y, Hata Y and Klose G (Eds). Atherosclerosis IV. Berlin: Springer-Verlag, p552.

Pelletier, X, S. Belbraouet, D. Mirabel, F. Mordret, J. Luc Perrin, X. Pages, G. Debry. 1995. A diet moderately enriched in phytosterols lowers plasma cholesterol concentrations in normocholesterolemic humans. Ann. Nutr. Metab. 39:291-295,.

Plat J and Mensink R. 1998. Safety aspects of dietary plant sterols and stanols. Postgraduate Medicine -A Special Report: New developments in the dietary management of high cholesterol, November 1998.

Plat J. and Mensink K. 2001. Effects of diets enriched with two different plant stanol ester mixtures on plasma Ubiquinol-10 and fat-soluble antioxidant concentrations. Metabolsim 50(5): 520-529.

Plat J. et al., 2000. Effects on serum lipids, lipoproteins and fat soluble antioxidant concentrations of consumption frequency of margarines and shortenings enriched with plant stanol esters. Euro. J. Clin. Nutr. 54: 671-677.

Pollak OJ, Kritchevsky D. 1981. Sitosterol. Monographs on atherosclerosis. ISBN 3-8055-0568-X.

Pollak OJ. 1985. Effect of plant sterols on serum lipids and atherosclerosis. Pharmac Ther 31:177-208.

Reeves JE. 1959. Hypercholesterolemia: treatment with sitosterol and a low cholesterol diet. Am. Practit. 10: 1193-1197.

Regulation (EC) No 258/97 of the European Parliament and of the Council of 27 January 1997 concerning novel foods and novel food ingredients. OJ No L43, 14.2.1997, p1.

Relas H. et al. Acute effect of dietary stanyl ester dose on post-absorptive α-tocopherol, β-carotene, retinol and retinyl palmitate concentrations. Brit. J. Nutr. 85: 141-147, 2001.

Robinson M, Wnorowski G, Dreher M. 1998. Dietary stanols as anti-hypercholesterolemic agents: a 90-day sub-chronic feeding trial as a safety assessment in the rat. #1202 Abstract from F.A.S.B. meeting in San Francisco. (Nabisco Inc, East Hanover, NJ 07936 and Product Safety Labs, East Brunswick, NJ 08816).

Sachs BA and Weston RE. 1956. Sitosterol administration in normal and hypercholesterolemic subjects; the effect in man of sitosterol therapy on serum lipids and lipoproteins. Archs intern. Med. 97: 738-752.

Salen G, Ahrens EH, Grundy SM. 1970. Metabolism of beta-sitosterol in man. J. Clin Invest. 49:952-967.

Salen G, Kwiterovich PO, Shefer S, Tint GS, Horak I, Shore V, Dayal B, Horak E. 1985. Increased plasma cholestanol and 5α-saturated plant sterol derivatives in subjects with sitosterolemia and xanthomatosis. J. Lipid Res 26: 203-209.

Salen G, Shefer L, Nguyen L, Ness GC, Tint GS, Shore V. 1992. J. Lipid Res 33:945-55.

Salen, G., Ahrens, E.H., Jr., and Grundy, S.M. 1970. Metabolism of beta-sitosterol in man. J.Clin.Invest. 49, 952-967.

Schlierf G, Oster P. Heuck CC, Raetzer H, Schellenberg B. 1978. Sitosterol in juvenile type II hyperlipoproteinemia. Atheroscler 30:245-48.

Scientific Committee on Food, 2000. Opinion of the Scientific Committee on Food on a request for the safety assessment of the use of phytosterol esters in yellow fat spreads; 6 April 2000. SCF/CS/NF/DOS/1 FINAL. European Commission; Health and Consumer Protection Directorate General.

Shefer S, Salen G, Bullock J. 1994. Hepatology 20:213-19.

Shipley RE, Pfeiffer RR, Marsh MM, Anderson RC. 1958. Sitosterol feeding: Chronic animal and clinical toxicology and tissue analysis. Circulation Research, 6: 373-382.

Shoppee, C.W. 1964. Chemistry of steroids (London: Butterworth).

Simell O, Tammi A. Gylling H, Pulkki K, Ronnemaa T, and the STRIP Study Group. October 1998. Studies with plant stanol ester in children. Dallas conference: Plant sterol ester: a new tool in dietary management of cholesterol.

Slesinski RS, Turnbull D, Frankos VH, Wolterbeek APM and Waalkens-Berendsen DH. 1999. Developmental toxicity study in vegetable-oil derived stanol fatty acid esters. Regulatory Toxicology and Pharmacology 29: 227-233.

Stalenhoef AFH, Hectors M, Demacker PNM. 2001. Effect of plant sterol-enriched margarine on plasma lipids and sterols in subjects heterozygous for phytosterolaemia. Journal of Internal Medicine 249: 163-166.

Swell, et al. 1954. Sterol specificity of pancreatic cholesterol esterase. . Proc. Soc. Exp Biol. Med. 87:21-218.

Tammi A. et al. 2000. Plant stanol ester margarine lowers serum total and low-density lipoprotein cholesterol concentrations of healthy children: The Strip Project. The Journal of Pediatrics 136(4): 503-510.

Turnbull D, Frankos VH, Leeman WR, and Jonker D. 1999. Short-term tests of estrogenic potential of plant stanols and plant stanol esters. Regulatory Toxicology and Pharmacology 29:211-215.

Turnbull D, Frankos VH, van Delft JHM, and deVogel N. 1999. Genotoxicity evaluation of wood-derived and vegetable-oil derived stanol esters. Regulatory Toxicology and Pharmacology 29: 205-210.

Turnbull D, Whittaker MH, Frankos VH, and Jonker D. 1999. 13-week oral toxicity study with stanol esters in rats. Regulatory Toxicology and Pharmacology 29:216-226.

US FDA Interim Ruling on Health Claims for sterol and stanol esters, 2000

US FDA. 21 CFR Part 101. Food labeling: Health claims; plant sterol/stanol esters and coronary heart disease; Interim Final Rule. September 8, 2000, pages 54690 and 54691.

Uusitupa M. October 1998. Study of efficacy of plant stanol ester in subjects consuming NCEP diets. Dallas conference: Plant sterol ester: a new tool in dietary management of cholesterol.

Vanhanen HT and Mietinen TA. 1992. Effects of unsaturated and saturated dietary plant sterols on their serum contents. Clin Chim Acta 205:97-107.

Vanhanen HT, Blomqvist S, Ehnholm C, Hyvonen M, Jauhiainen M, Torstila I, and Miettinen TA. 1993. Serum cholesterol, cholesterol precursors, and plant sterols in hypercholesterolemic subjects with different apoE phenotypes during dietary sitostanol ester treatment. J. Lipid Res 34:1535-44.

Vanhanen HT, Kajander J, Lehtovitra H, and Miettinen TA. 1994. Serum levels, absorption efficiency, fecal elimination and synthesis of cholesterol during increasing doses of dietary sitostanol esters in hypercholesterolemic subjects. Clin Sci 87:61-7.

Vanstone, CA, Raeini-Sarjaz, M, Parson, WE, Jones, PJH, 2001. Unesterified plant sterols and stanols lower low-density (LDL) cholesterol independent of the degree of hydrogenation in hypercholesterolemics. Accepted for publication in 2002 by the American Journal of Clinical Nutrition.

Verger, R. Pancreatic Lipases. In: Lipases. Eds. B. Borgstrom and H.L.Brockman, 1984, Elsevier, Oxford, pp. 84-141

Vuorio A.F. et al. Feb. 2000. Stanol ester margarine alone and with Simvastatin lowers serum cholesterol in families with familial hypercholesterolemia caused by the FH-North Karelia Mutation. Arterioscler. Thromb. Vasc. Biol.: 500-506.

Waalkens-Brendsen DH, Wolterbeek APM, Wijnands MVW, Richold M, Hepburn PA. 1999. Safety evaluation of phytosterol esters. Part 3. Two-generation reproduction study of phytosterol esters in rats. A novel functional food. Food Chem Toxicol 37(7);683-696.

Weihrauch, J.L. and Gardner, J.M. 1978. Sterol content of foods of plant origin. J.Am.Diet.Assoc. 73 , 39-47.

Weisweiler P, Heinemann V, Schwandt P. 1984. Serum lipoproteins and lecithin: cholesterol Acyltransferase (LCAT) activity in hypercholesterolemic subjects given ∃-sitosterol. Int J. Clin Pharmacol Therapy Tox 22:204-6.

Weststrate JA and Meijer GW. 1998. Plant sterol-enriched margarines and reduction of plasma total- and LDL cholesterol concentrations in normocholesterolemic and mildly hypercholesterolemic subjects. Eur J. Clin Nutr 52: 334-343.

Weststrate JA, Ayesh R, Bauer-plank C and Drewitt PN. 1999. Safety evaluation of phytosterol esters. Part 4. faecal concentrations of bile acids and neutral sterols in healthy normolipidaemic volunteers consuming a controlled diet either with or without a phytosterol ester-enriched margerine. 1999. Food and Chemical Toxicology: 37: 1063-1071.

Whittaker MH, Frankos VH, Wolterbeek APM, and Waalkens-Berendsen DH. 1999. Two-generation reproductive toxicity study of plant stanol esters in rats. Regulatory Toxicology and Pharmacology 29:196-204.