Multi-Purpose Vegetable Fat Spreads and Liquid Vegetable Fat Based Emulsions with Added Plant Sterol Esters for Cooking and Baking Applications (*PS-Spreads/Liquids for CB)*

Submitted pursuant to
Regulation (EC) No 258/97 of the European Parliament
and of the Council of 27th January 1997 concerning
Novel Foods and Novel Food ingredients

NON- CONFIDENTIAL

Prepared by: Unilever Research and Development
Vlaardingen B.V.
Olivier van Noortlaan 120
3133 AT Vlaardingen
The Netherlands

16 May 2013

Multi-Purpose Vegetable Fat Spreads and Liquid Vegetable Fat Based Emulsions with Added Plant Sterol Esters for Cooking and Baking Applications (*PS-Spreads/Liquids for CB*)

Table of Contents

Page

List of Appendices

List of Figures and Tables

Multi-Purpose Vegetable Fat Spreads and Liquid Vegetable Fat Based Emulsions with Added Plant Sterol Esters for Cooking and Baking Applications (*PS-Spreads/Liquids for CB*)

EXECUTIVE SUMMARY

In 2000, Unilever launched a vegetable fat spread with added plant sterol (PS) esters for spreading application, which was the first functional food authorised by the European Union under the Novel Foods Regulation (EC) No 258/97 (Commission of the European Communities, 2000). In 2004, the regulation allowed for the extension of the range of products with added plant sterol esters to include salad dressings, milk type products, milk based fruit drinks, fermented milk type products, soya drinks, cheese type products, yogurt type products and spicy sauces. In 2006, rye bread was also included (European Parliament and Council of the European Union, 1997, amended 2009).

The original Novel Food Authorisation for yellow fat spreads, as defined by Council Regulation 2991/94/EC (Council of the European Union, 1994), did not explicitly exclude cooking and baking fats with added phytosterol esters from the authorisation. However, in the April 2000 opinion of the Scientific Committee on Food on the safety of the use of phytosterols esters in yellow fat spreads, it is indicated that the product is not intended for use in cooking (SCF, 2000). In addition, the Commission Decisions 2004/333/EC (ADM), 2004/334/EC (PharmaConsult Oy), and 2004/336/EC (Teriaka Ltd.) explicitly excluded cooking and frying fats with added phytosterols/ phytostanols.

Plant stanols are not subject of the Novel Foods legislation, as they were marketed prior to May 1997.

Since the launch of its spread with added plant sterol esters in 2000, Unilever has performed regular Post Launch Monitoring (PLM). These Unilever PLM data together with other intake data indicate that plant sterol and plant stanol intakes are below the recommended intake range for cholesterol lowering, *i.e.*, 1.5 to 3.0 g/day. Current mean plant sterol and plant stanol intake estimates in users of products with added plant sterols and plant stanols in Europe range from 0.4 to 2.5 g/day.

To provide consumers that need to lower their blood cholesterol with more consumption opportunities to reach the desirable intake, Unilever is applying for an extension to its current Novel Foods Authorisations for "multi-purpose vegetable fat spreads (*PS-Spreads for CB*) and liquid vegetable fat based emulsions (*PS-Liquids for CB*) with added plant sterol esters for home cooking and baking (CB) applications", referred to as *PS-Spreads/Liquids for CB*. These products will be placed on the market under the *pro.activ*® product range which is part of the heart health brands *Becel*, *Flora* and *Fruit d'Or* and will be targeted at consumers that need to lower their blood cholesterol. There are no changes in the product specifications for the Novel Food from those that are currently authorised for Yellow fat spreads with added plant sterol esters, and the production process is also the same.

Plant stanol containing products intended for cooking and baking are already available on the market as they are not subject to the Novel Foods legislation.

In the original Novel Food dossier for yellow fat spreads with added phytosterol esters, a no-observed-adverse-effect level (NOAEL) of 4,200 mg/kg body weight was established. This NOAEL is conservative since doses higher than 4,200 mg/kg body weight were not tested in animal studies. In their 2002 opinion, the Scientific Committee on Foods (SCF) did not derive a formal Acceptable Daily Intake from the NOAEL. However, the SCF noted that blood carotenoids, especially β-carotene can be moderately decreased after consumption of 3 g plant sterols or plant stanols, and that evidence indicating additional cholesterol lowering benefits at intakes over 3 g was not available. Therefore, the SCF advised that as a precaution/guidance, intake of plant sterols and plant stanols should not exceed 3 g/day, and that an ample intake of fruits and vegetables should be advised to counteract potential effects on blood carotenoid levels. This dietary advice and the 3 g/day upper consumption advice were subsequently taken up in labelling regulations. As a consequence the upper consumption advice of 3 g/day is at almost the same level as the recommended intake of 1.5 to 3.0 g/day. For the same reasons product labelling also indicates that the products are not suitable for children below 5 years and pregnant and lactating women.

The PLM data together with other intake assessment data also show that some consumers of plant sterol and plant stanol products moderately exceed 3 g/day. Current intakes at the 95^{th} percentile range from 1.1 to 4.2 g/day.

When we take the Commission Recommendation 97/618/EC decision trees (Commission of the European Communities, 1997) into account, we conclude that the issues for consideration to the Committee are:

1. Given the fact that the recommended intake for the health benefit of plant sterols (and plant stanols) is between 1.5 and 3.0 g/day it is unlikely that it can always be avoided that some users exceed the 3 g upper consumption advice. Whilst acknowledging that the 3 g/day upper consumption advice should clearly be maintained, is there a basis for toxicological concern for consumption over this level?

   - The products will contain comprehensive labelling to prevent over-consumption of plant sterols among target consumers, and to avoid consumption among non-target consumers of children under 5 years and pregnant and lactating women.
   - Recent clinical data continue to support the safe use of plant sterols up to at least 9 g/day

2. Will additional plant sterol intake from home cooking and baking result in any issues of toxicological concern in target group consumers?

- We present data that adults can increase their plant sterol intake with a mean of 0.4 g/day by home cooking and baking applications. This is a meaningful contribution to the current shortfall from recommended intakes in many target consumers.

- The 95th percentile for estimated plant sterol intake from home cooking and baking applications was 1.2 g/day. We performed worst case estimations showing a total plant sterol intake of 5.4 g/day for consumers age 19 to 30 years at the 95th percentile from the replacement of all margarine used in spreading, cooking and baking with the *PS-Spreads/Liquids for CB.*

3. Will potential plant sterol intakes from home cooking and baking in non-target groups result in any issues of toxicological concern?

    - With our pro.activ® products we clearly communicate that products are not appropriate for children below 5 years of age and for pregnant and lactating women. In addition, the 2008 European Food Safety Authority evaluation confirms that there is little consumption in families with children (EFSA, 2008a).

    - We have calculated worse case scenarios *i.e.,* in the extremely unlikely case that all fat used for spreading, cooking and baking would contain added plant sterols. These scenarios show that the 95th percentile for intake in children <5 years of age would be 1.5 to 2.0 g/day (86 to 107 mg/kg/day). For women of childbearing age the 95th percentile would be 2.9 to 3.5 g/day (44 to 47 mg/kg/day). This is not expected to raise a safety concern, as recent clinical data support safe use of plant sterols up to at least 9 g/day.

4. Is there a significant increase in exposure to heat degradation products (plant sterol oxides) as a result of the new proposed uses, and what are the toxicological implications of this?

    - We have demonstrated in baking and cooking trials that there are only small increases in the generation of plant sterol oxides compared to spread products without added plant sterols. These are not considered to be of biological significance.

    - Toxicology studies conducted on behalf of Unilever on a plant sterol oxide concentrate confirm that they do not demonstrate genotoxicity. In a sub-chronic rat study the no-observed-effect level (NOEL) for plant sterol oxides was determined to be approximately 128 and 144 mg/kg body weight/day for male and female rats, respectively. Exposure to plant sterol oxidation products from the use of *PS-Spreads/Liquids for CB* results in margins of safety of 400 and 131 in adults and children respectively, which is sufficiently large to further support the safety of *PS-Spreads/Liquids for CB*.

In summary, this dossier has demonstrated that:

a. There is a short-fall in plant sterol intake from the recommended range of intake for cholesterol lowering from all sources based on currently available product formats.

b. By extending the use of the vegetable fat products with added plant sterol esters for cooking and baking applications (PS-Spreads/Liquids for CB) this can contribute to achieving the desirable intake.

c. Appropriate risk management measures are in place to ensure that the consumer is advised to consume no more than 3 g of phytosterol per day.

d. Appropriate risk management measures are in place to ensure that consumption outside the target group (children <5 years of age and pregnant and lactating women) can be avoided.

e. The *PS-Spreads/Liquids for CB* will be presented in a format that allows accurate measurement of defined servings to prevent over-consumption.

f. Plant stanol esters are already widely available and presented for baking and cooking purposes in the EU.

g. The theoretical worst-case deterministic estimations for consumers could result in a total consumption from the *PS-Spreads/Liquids for CB* of about 5.4 g/day for adult consumers aged 19-30 years at the 95^{th} percentile. This is not expected to raise a safety concern, as recent clinical data support safe use of plant sterols up to at least 9 g/day.

h. Cooking and baking with *PS-Spreads/Liquids for CB* will expose the consumer to a small increase in plant sterol oxides. Toxicity studies on plant sterol oxides have been shown to raise no concerns for human safety.

INTRODUCTION

An elevated blood concentration of total and especially low-density lipoprotein (LDL)-cholesterol is an established risk factor for coronary heart disease (Expert Panel on Detection Evaluation and Treatment of High Blood Cholesterol in Adults, 2001; EFSA, 2008a, 2012a). Current studies in European populations reveal that 70 to 75% of the adults in the age group of 35 to 70 years have an unfavourable total blood cholesterol concentration exceeding the desirable level of 200 mg/dl or 5.2 mmol/l (Tolonen *et al.*, 2005; Bundesgesundheitsbericht, 2006).

Dietary and lifestyle recommendations are the cornerstone of coronary heart disease prevention. The inclusion of foods with added plant sterols and plant stanols (phytosterols) in their free or esterified form, to further enhance the cholesterol-lowering benefit of a healthy diet is part of the nutritional guidelines of several leading authorities [European Guidelines on cardiovascular disease prevention in clinical practice (Reiner *et al.*, 2011; Perk *et al.*, 2012)].

Phytosterols are known to reduce plasma LDL-cholesterol concentrations (Katan *et al.*, 2003; AbuMweis *et al.*, 2008; Demonty *et al.*, 2009; Musa-Veloso *et al.*, 2011). In 2008, the European Food Safety Authority (EFSA) published their positive opinion on a reduction of disease risk health claim related to the effects of plant sterols on blood cholesterol and the risk of coronary heart disease (EFSA, 2008b). This was followed in 2009 by another favourable opinion with respect to a quantified reduction of blood LDL-cholesterol by 7 to 10.5% with phytosterol intakes of 1.5 to 2.4 g/day (EFSA, 2009a). On May 15th 2012, EFSA published their scientific opinion on extending the conditions of use for a quantified reduction of blood cholesterol claim related to phytosterols to a daily intake of 1.5 to 3.0 g. EFSA also concluded that the quantified beneficial effect on blood LDL-cholesterol reduction for plant stanols is 7 to 11.4% (EFSA, 2012a) and for plant sterols is 7 to 11.3% (EFSA, 2012b). In addition, the panel concluded that phytosterols at daily intakes ranging from 1.5 to 3.0 g have a similar efficacy on blood LDL-cholesterol lowering. Therefore, phytosterols are recognised as equally effective ingredients for foods targeted at lowering blood LDL-cholesterol concentrations as part of a healthy diet and lifestyle, which can make a major contribution to public health.

Plant sterols are the plant counterparts of cholesterol and in plants they play a role in cell membrane function. They are present naturally in all plant foods such as vegetable oils, nuts, seeds, fruits, vegetables, grains and cereal products including bread (Weihrauch and Gardner, 1978; Moreau *et al.*, 2002; Katan *et al.*, 2003). The habitual daily intake of naturally occurring plant sterols, by a typical Western diet ranges between 200 to 300 mg per day. However, to fully benefit from the cholesterol lowering effects of plant-sterols higher intakes of approximately 1.5 to 3 g per day are needed.

In 2000, Unilever launched a vegetable fat spread with added plant sterol esters for spreading application, which was the first functional food authorised by the European Union under the Novel Foods Regulation (EC) No 258/97 (Commission of the European Communities, 2000). In 2004, the regulation allowed for the extension of the range of products with added plant sterol esters to include: salad dressings, milk type products, milk based fruit drinks, fermented milk type products, soya drinks, cheese type products, yogurt type products and spicy sauces. In 2006, rye bread was also included.

Rational for this dossier:

'In this dossier, Unilever is applying for an extension to its current Novel Foods Authorisations for "Multi-Purpose Vegetable Fat Spreads and Liquid Vegetable Fat Based Emulsions with added phytosterol esters for cooking and baking applications". This will be referred to collectively as '*PS-Spreads/Liquids for CB'* in this application.

[redacted] The availability of *PS-Spreads/Liquids for CB* in addition to vegetable fat spreads with added plant sterol esters for spreading purposes only will provide consumers who need to lower their cholesterol with more consumption opportunities during the day to reach the intake range of 1.5 to 3.0 g/day of phytosterols for a significant blood cholesterol lowering effect.

The original Novel Food Authorisation for yellow fat spreads, as defined by Council Regulation 2991/94/EC (Council of the European Union, 1994), did not explicitly exclude cooking and baking fats with added phytosterol esters from the authorisation. However, in the April 2000 opinion of the Scientific Committee on Food on the safety of the use of phytosterols esters in yellow fat spreads, it is indicated that the product is not intended for use in cooking (SCF, 2000). In addition, the Commission Decisions 2004/333/EC (ADM), 2004/334/EC (PharmaConsult Oy), and 2004/336/EC (Teriaka Ltd.) explicitly excluded cooking and frying fats with added phytosterols/ phytostanols.

These products will be placed on the market under the *pro.activ*® product range, which is part of the heart health brands Becel, Flora and Fruit d'Or and will be targeted at consumers that need to lower their cholesterol.

This application will allow for further equivalence between plant sterol esters and plant stanols as both are suitable for consumer usage in spreading, cooking and baking. Plant stanols and their esters can be applied in any food without specific Novel Food Authorisation, whereas plant sterol esters require Novel Food Authorisation when the use is extended to new product formats.

1. ADMINISTRATIVE DATA

The present petition is submitted by:

Unilever PLC and Unilever N.V.

Address of the applicant is as follows:

Unilever PLC
100 Victoria Embankment,
London, EC4Y 0DY, UK

Unilever N.V.
Weena 455,
Rotterdam,
3013 AL, NL

2. GENERAL DESCRIPTION

Unilever is applying for the use of multi-purpose vegetable fat spreads and liquid vegetable fat based emulsions with added plant sterol esters to include cooking and baking applications (‘*PS-Spreads/Liquids for CB’)*.

These products will be marketed under the well established *pro.activ*® range under Unilever’s heart health brands Becel, Flora, Fruit d’Or. Therefore the plant sterol esters will be added to products which already have a heart healthy nutritional profile.

In scope of this dossier are in-home cooking and baking applications (excluding deep-frying).

The product range extension encompasses:

1: A multi-purpose spreadable vegetable fat product (tub format) with added plant sterol esters that, next to the spreading application as authorised in 2000, will be communicated to consumers that it can be used in cooking and baking (‘*PS-Spreads for CB*’). Examples of the product in tub format are provided in Figure 1.

Figure 1 Examples of the *PS-Spreads for CB* in Tub Format

2: A liquid vegetable fat based emulsion with added plant sterol esters for cooking and baking applications (‘*PS-Liquids for CB*’). Examples of products currently on the market without added plant sterol esters are provided in Figure 2 below.

Figure 2 Examples of the Liquid Vegetable Fat-based Emulsions without Added Plant Sterol Esters Currently on the EU Market

When referring to the liquid and tub format of the products with added plant sterol esters subject of this application, these will be collectively referred to as '*PS-Spreads/Liquids for CB'*.

This dossier will address:

1. Scenarios of consumption of plant sterols from *PS-Spreads/Liquids for CB* in target and non-target populations.

2. The potential formation and safety assessment of plant sterol-oxides during cooking and baking processes.

Throughout this dossier, 2 population groups are frequently referred to in relation to their intake of the *PS-Spreads/Liquids for CB* – 'Target' and 'Non-Target' populations. The definitions for these are as follows:

Target population: People that need to lower their blood cholesterol

Non-target population: Children under 5 years and pregnant and lactating women as referred to under Article 2 point 5 of Commission Regulation (EC) Nº 608/2004 (Commission of the European Communities, 2004e).

In addition this dossier will provide evidence that the product extension and usage fits within a healthy diet.

3. IDENTIFICATION OF THE ESSENTIAL INFORMATION REQUIREMENTS

In accordance with the European (EU) guidelines, the requirements for the submission of a dossier for this class of Novel Food are as follows:

Dossier headings	Addressed in this dossier
I. Specification of the Novel Food	No change to specification to previous authorisation
II. Effect of the Production Process Applied to the Novel Food	No change to specification to previous authorisation
III. History of Source Organism	No change to specification to previous authorisation
IV. Effect of the Genetic Modification on the Properties of the Host Organism	Not applicable
V. Genetic Stability of the Genetically Modified Organisms Used as Novel Foods Source	Not applicable
VI. Specificity of Expression of Novel Genetic Material	Not applicable
VII. Transfer of Genetic Material from Genetically Modified Organisms (GMO)	Not applicable
VIII. Ability of the Genetically Modified Microorganism (GMM) to Survive in and Colonise the Human Gut	Not applicable
IX. Anticipated Intake/Extent of Use	Consumption in the target and non-target populations.
X. Information from Previous Human Exposure	Update on background exposure
XI. Nutritional Information	Comparison on fat composition with existing cooking and baking fats
XII. Microbiological Information	No change to specification to previous authorisation
XIII. Additional Toxicological and Human Safety Information	Plant sterol oxidation products (POPs) Safety of plant sterols with respect to potential higher intakes

I SPECIFICATION OF THE NOVEL FOOD

Unilever is applying for the use of multi-purpose vegetable fat spreads and liquid vegetable fat based emulsions with added plant sterol esters to include cooking and baking applications ('*PS-Spreads/Liquids for CB')*.

The composition of the plant sterols used in the *PS-Spreads/Liquids for CB* are identical to the ingredients used in Unilever's previously authorised products under *Commission Decision (EC) No 2000/500 of 24 July 2000 on authorising the placing on the market of 'yellow fat spreads with added phytosterol esters* and *Commission Decision (EC) 2004/335 of 24 March 2004 on authorising the placing on the market of milk type products and yoghurt type products with added phytosterol esters as a Novel Food or Novel Food ingredient under Regulation (EC) No 258/97 of the European Parliament and of the Council* (Commission of the European Communities, 2000, 2004c). A full description of the ingredient and the specification was provided in the original EU Novel Foods submission by Unilever in 1998 and as reviewed by the Netherlands Competent Authority (Netherlands Preliminary Advisory Committee on the Safety of Novel Foods, 1998) reproduced in Table I.1-1 below.

Table I.1-1 Current and Proposed Regulatory Specification for the Composition of Phytosterols

Component	Minimum	Maximum
Campesterol	10 %	40 %
Stigmasterol	6 %	30 %
β-Sitosterol	30 %	65 %
Other	0%	5%

As presented in Table I.1-2, the *PS-Spreads/Liquids for CB,* subject of this application, have a composition of approximately 25 to 95% fat, and are high in polyunsaturated fatty acids, relatively low in saturated fatty acids. Both the *PS-Spreads for CB* and the *PS-Liquids for CB* contain protein; varying levels of emulsifiers, stabilisers, salt and vitamins; with a plant sterol content expressed as free plant sterol equivalent, of 7.5 g/100 g product (based on 12.5 g plant sterol esters per 100 g). The key differences between the 2 products are their physical states and packaging.

The composition of the *PS-Spreads/Liquids for CB* are similar (with the exception of the presence of plant sterol esters) to products already established on the market.

[redacted]				
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]

[redacted]

II EFFECT OF THE PRODUCTION PROCESS APPLIED TO THE NOVEL FOOD

The production process of the plant sterol esters themselves used in the *PS-Spreads/Liquids for CB* identical to the ingredient used in Unilever's previous authorisations *Commission Decision (EC) No 2000/500* and *Commission Decision (EC) 2004/335* (Commission of the European Communities, 2000, 2004c). A full description of the ingredient and the production process was provided in the original EU Novel Foods submission by Unilever in May 1998 as reviewed by the Netherlands Competent Authority (Netherlands Preliminary Advisory Committee on the Safety of Novel Foods, 1998).

The production process of the *PS-Spreads/Liquids for CB,* subject of this application, is identical to the production processes that are commonly used for vegetable fat products with or without added plant sterols (esters) already established.

III HISTORY OF SOURCE ORGANISM

The plant sterols in the Novel Food products are derived from plant based sources as previously approved under *Commission Decisions (EC) No 2000/500/EC, 2003/333/EC, 2003/334/EC, 20003/335/EC, 2003/336/EC, 2006/59/EC and relevant notifications of substantial equivalence* (Commission of the European Communities, 2000, 2004a-d).

IV to VIII

Sections IV to VIII of the EU recommendation are not applicable to the Novel Food since no GM technology is involved.

IX ANTICIPATED INTAKE/EXTENT OF USE

Thus far, in Sections I-VIII of this dossier the following points have been addressed:

1. Yellow fat spreads with added plant sterol esters are already approved as a Novel Food in the EU,

2. There are no changes in the product specifications for the Novel Food from those that are currently approved for yellow fat spreads with added plant sterol esters,

3. The production process for the *PS-Spreads/Liquids for CB* is the same as that for the currently approved vegetable fat spreads with added plant sterols.

European Commission Recommendation 97/618/EC (Commission of the European Communities, 1997) dictates that for the proposed extension to the *PS-Spreads/Liquids for CB*, the presentation of a complete evaluation of the potential additional exposure to plant sterol esters for both the target and non-target groups (that are representative of the whole EU is required. As part of this assessment, both additional intake and scenarios of replacement with existing foods must also be considered.

IX.1 Proposed Uses: Theoretical Scenarios of Intake

Plant sterol esters are proposed for use in vegetable fat products intended for cooking and baking applications (*PS-Spreads/Liquids for CB*). The uses cover vegetable fat products that are liquid emulsions (*PS-Liquids for CB*), as well as vegetable fat products (*PS-Spreads for CB*) that are similar in composition to the previously authorised vegetable fat spreads with added plant sterols, which will be presented as a multi-purpose tub.

The proposed plant sterol level to be added to the *PS-Spreads/Liquids for CB* is 7.5 g per 100 g/100 mL product, which is the same plant sterol level currently applied to spreads with added plant sterols on the market.

IX.2 Estimated Intakes based on Representative EU Food Consumption Surveys

This section will present an overview of the estimated intakes of plant sterols based on the potential intake of the *PS-Spreads/Liquids for CB*. Intakes are assessed using data from the following EU datasets:

1. The United Kingdom – survey data from the United Kingdom (UK) National Diet and Nutrition Survey (NDNS) programme are recognised as being amongst the most accurate and detailed datasets in the EU and are therefore widely used for risk assessment purposes in the EU.

2. The Netherlands – survey data from the Netherlands are also collected at a detailed and accurate level. The Dutch consumer is recognised as being amongst the highest

level consumers of margarine for spreading, cooking and baking in the EU. In addition consumer use of liquid emulsions is well established in The Netherlands.

3. The European Food Safety Authority (EFSA) Comprehensive database – this database was established to allow comparisons of intakes across the EU member states. These data permit further context to be drawn upon the results of the UK and the Netherlands. Detailed intake results are provided in Appendix G and a summary of results are presented in Section IX.2.3.

For the intake assessment, specific focus is on the target groups (*i.e.,* adults who need to reduce their cholesterol), and on non-target groups (*i.e.,* children <5 years and pregnant/ lactating women). With increasing age, blood cholesterol concentrations increase therefore, where possible adults >45 years were specifically considered as a proxy for the target group (as this was a target age group selected by EFSA (2008a). For the non-target groups, young toddlers and children were used to represent children <5 years of age. In addition, women of child-bearing age were investigated as the dietary surveys do not provide information on pregnant or lactating women.

Theoretical intakes of plant sterols from the proposed addition to margarines are only provided in this section. Intake assessments of plant sterols from the total diet, including background diet and from other foods with added plant sterols (such as dairy products) have been previously conducted in various EU countries and the summary of these intakes is presented in Section X. Furthermore, a practical insight into how products with added plant sterol are consumed over the longer term is provided in Section X.2.1 with the recent Unilever PLM data, which was conducted over a period of 1 year.

IX.2.1 Consumption Estimates Based on UK National Diet and Nutrition Survey Data (2008-2010 Rolling Survey dataset)

Estimates for the intake of plant sterols in the EU from the *PS-Spreads/Liquids for CB*, were based on the proposed use-levels and food consumption data collected as part of the UK NDNS programme (Department of Health, 2011; UKDA, 2012). A detailed intakes report for this analysis is provided in Appendix A, which provides information pertaining to the background and methodology of the UK NDNS programme along with the complete results. Calculations for the mean and high-level 95th percentile) all-person and all-user intakes, and percent consuming were performed for each of the individual proposed food-uses for the *PS-Spreads/Liquids for CB*. Similar calculations were used to determine the estimated total intake of plant sterols from all proposed *PS-Spreads/Liquids for CB* uses. The per person and per kilogram body weight intakes were reported for the following population groups:

toddlers, ages 1 to 3;
children, ages 4 to 10;
teenagers, ages 11 to 18;
adults, ages 19 to 44 (and split for females);
adults, ages 45 years+

Statistical analysis and data management were conducted in Creme software. For the assessment, estimates for the intake of plant sterols from the *PS-Spreads/Liquids for CB* by the UK population was generated by Creme software, using consumption data from individual dietary records, detailing food items ingested by each survey participant on each of the survey days. Estimates for the daily intake of plant sterols from the *PS-Spreads/ Liquids for CB* represent projected 4-day averages for each individual from days 1 to 4 of NDNS data. All-person intake refers to the estimated intake of plant sterols from the *PS-Spreads/Liquids for CB* averaged over all individuals surveyed regardless of whether they consumed food products in which plant sterols are currently proposed for use, and therefore includes "zero" consumers (those who reported no intake of food products for which plant sterols are proposed for use during the 4 survey days). All-user intake refers to the estimated intake of plant sterols by those individuals consuming the *PS-Spreads/Liquids for CB*, hence the 'all-user' designation. Individuals were considered users if they consumed a *PS-Spreads/Liquids for CB* on 1 of the 4 survey days. As total population and all-user intakes were found to be similar, only the all-user results are presented in this dossier. Detailed results and the total population intakes are provided in Appendix A.

IX.2.1.1 Food Usage Data

The level of plant sterols for the *PS-Spreads/Liquids for CB* employed in the intake analysis is 7.5 g plant sterol per 100 g. Two intake assessments were conducted. The first is an assessment of the plant sterol intake based on the direct replacement of fats and oils used in cooking and baking purposes in the UK (replacement with the *PS-Spreads/Liquids for CB*). The second assessment also includes the additional plant sterol intake from the replacement of all fats used for spreading purposes with the *PS-Spreads/Liquids for CB*. This assessment will be referred to as the 'Total Margarine' assessment. *PS-Spreads/Liquids for CB* are proposed for use in home cooking as a direct replacement of other fats and oils currently in use by the UK population. Applications include use in home-baking and preparation of home cooked foods (roasting and shallow-frying foods, in different dishes and in soups and sauces). Vegetable fat spreads with added plant sterols are currently on the market in the UK, but in the present analysis all fat spreads, including spreadable butter, are replaced with the *PS-Spreads/Liquids for CB* (in the Total Margarine assessment).

Food codes representative of each proposed food-use were chosen from the food code list associated with the food consumption survey and grouped according to the proposed uses for the plant sterol enrichment assessment. Some foods could be identified as being 'homemade' through the name and/or description of the food. The variable "location of consumption" per food-code was also examined where available, and foods consumed in restaurants, school and work canteens, coffee shops, child-nurseries, *etc*. were excluded where possible. Ingredient fractions based on recipe data (UK and Irish standard recipes) were used to identify the quantity of added fat used in each food.

IX.2.1.2 Food Survey Results

Estimates for the total daily intakes of plant sterols from all proposed food-uses in the *PS-Spreads/Liquids for CB* are provided in Tables IX.2.1-1 and IX.2.1-2. Estimates for the total daily intakes of plant sterols from all proposed food-uses in the 'Total Margarine' assessment are provided in Tables IX.2.1-3 and IX.2.1-4.

IX.2.1.2.1 Estimated Plant Sterol Intakes from Cooking and Baking Uses Only

Table IX.2.1-1 summarises the estimated total intake of plant sterols from the *PS-Spreads/Liquids for CB* (g/person/day) by UK population group. Table IX.2.1-2 presents these data on a per kilogram body weight basis (mg/kg body weight/day). As would be expected for a 4-day survey, the percentage of users was relatively high among all age groups evaluated in the current intake assessment. Greater than 76.7% of the population would be user of the *PS-Spreads/Liquids for CB* (Table IX.2.1-1). Adults aged ≥45 years would have the greatest percentage of users at 83.9%.

In relation to all-user intakes, adults aged ≥45 years were determined to have the greatest mean and 95th percentile all-user intakes of plant sterols from the *PS-Spreads/Liquids for CB* on an absolute basis, at 0.4 and 1.2 g/person/day, respectively. Toddlers also had the lowest mean and 95th percentile all-user intakes of 0.2 and 0.4 g/person/day, respectively (Table IX.2.1-1).

Table IX.2.1-1 Summary of the Estimated Daily Intake of Plant Sterols from the *PS-Spreads/Liquids for CB* in the UK by Population Group (NDNS Data)

Population Group	Age Group (Years)	Total n	All-Users Consumption			
			%	n	Mean (g)	P95 (g)
Toddlers	1-3	219	76.7	168	0.16	0.41
Children	4-10	423	80.1	339	0.24	0.62
Teenagers	11-18	453	80.1	363	0.31	0.82
Adults	19-44	452	82.7	374	0.37	0.99
Female Adults	19-44	256	80.1	205	0.31	0.93
Adults	45+	579	83.9	486	0.42	1.15

CB = Cooking and baking; P95 = 95th percentile; PS = Plant sterols

On a body weight basis, toddlers were identified as having the highest mean intake of any population group, of 11.0 mg/kg body weight/day, while children (aged 4 to 10 years) had the highest 95th percentile intake of 29.6 mg/kg body weight/day. Female adults aged 19 to 44 years also had the lowest mean all-user intake of 4.7 mg/kg body weight/day, while adults aged 19 to 44 had the lowest 95th percentile intake of 14.0 mg/kg body weight/day (Table IX.2.1-2).

Table IX.2.1-2 Summary of the Estimated Daily Per Kilogram Body Weight Intake of Plant Sterols from the *PS-Spreads/Liquids for CB* in the UK by Population Group (NDNS Data)

Population Group	Age Group (Years)	Total n	All-Users Consumption			
			%	n	Mean (mg)	P95 (mg)
Toddlers	1-3	219	76.7	168	11.01	28.54
Children	4-10	423	80.1	339	9.78	29.57
Teenagers	11-18	453	80.1	363	5.60	14.70
Adults	19-44	452	82.7	374	5.05	13.96
Female Adults	19-44	256	80.1	205	4.66	14.40
Adults	45+	579	83.9	486	5.47	14.53

CB = Cooking and baking; P95 = 95th percentile; PS = Plant sterols

IX.2.1.2.2 Estimated Plant Sterol Intakes from the Total Margarine Assessment i.e., Cooking, Baking and Spreading

Table IX.2.1-3 summarises the estimated total intake of plant sterols from the Total Margarine assessment *i.e.,* the use of the *PS-Spreads/Liquids for CB* for all uses including spreading (g/person/day) by UK population group. Table IX.2.1-4 presents this data on a per kilogram body weight basis (mg/kg body weight/day). The percentage of users was high among all age groups evaluated in the current intake assessment; greater than 96.9% of the population groups consisted of users of those food products in which plant sterols is proposed for use in the *PS-Spreads/Liquids for CB* (Table IX.2.1-3). Children aged 4 to 10 years and adults aged ≥45 years both had the greatest percentage of users at 98.6%.

In relation to all-user intakes, adults aged ≥45 years were determined to have the greatest mean and 95th percentile all-user intakes of plant sterols from the Total Margarine assessment on an absolute basis, at 1.4 and 3.2 g/person/day, respectively. Toddlers had the lowest mean and 95th percentile all-user intakes of 0.7 and 1.4 g/person/day, respectively (Table IX.2.1-3).

Table IX.2.1-3 Summary of the Estimated Daily Intake of Plant Sterols from the Total Margarine Assessment in the UK by Population Group (NDNS Data)

Population Group	Age Group (Years)	Total n	All-Users Consumption			
			%	n	Mean (g)	P95 (g)
Toddlers	1-3	219	97.7	214	0.66	1.42
Children	4-10	423	98.6	417	0.96	2.12
Teenagers	11-18	453	97.8	443	1.02	2.44
Adults	19-44	452	96.9	438	1.24	2.87
Female Adults	19-44	256	97.3	249	1.10	2.48
Adults	45+	579	98.6	571	1.38	3.23

P95 = 95th percentile

On a body weight basis, toddlers were identified as having the highest mean and 95th percentile all-user intakes of any population group, of 46.5 and 102.4 mg/kg body weight/ day, respectively. Female adults aged 19 to 44 years had the lowest mean all-user intake of 16.4 mg/kg body weight/day, while adults aged 19 to 44 years had the lowest 95th percentile intake of 37.9 mg/kg body weight/day (Table IX.2.1-4).

Table IX.2.1-4 Summary of the Estimated Daily Per Kilogram Body Weight Intake of Plant Sterols from the Total Margarine Assessment in the UK by Population Group (NDNS Data)

Population Group	Age Group (Years)	Total n	All-Users Consumption			
			%	n	Mean (mg)	P95 (mg)
Toddlers	1-3	219	97.7	214	46.51	102.42
Children	4-10	423	98.6	417	38.43	93.64
Teenagers	11-18	453	97.8	443	18.29	45.86
Adults	19-44	452	96.9	438	16.79	37.87
Female Adults	19-44	256	97.3	249	16.38	38.81
Adults	45+	579	98.6	571	18.20	43.07

P95 = 95th percentile

IX.2.1.3 Conclusions on Intakes Based on the UK NDNS Data

In summary, on an all-user basis, adults aged ≥45 years were determined to have the greatest mean and 95th percentile all-user intakes of plant sterols from the *PS-Spreads/ Liquids for CB* on an absolute basis, at 0.4 and 1.2 g/person/day, respectively. Toddlers had the lowest mean and 95th percentile all-user intakes of 0.2 and 0.4 g/person/day, respectively. In relation to body weight, for all-users, toddlers had the highest mean intake of any population group, of 11.0 mg/kg body weight/day, while children (aged 4 to 10 years) had the highest 95th percentile intake of 29.6 mg/kg body weight/day.

For the Total Margarine assessment, in relation to all-user intakes, adults aged ≥45 years were determined to have the greatest mean and 95th percentile all-user intakes of plant sterols on an absolute basis, at 1.4 and 3.2 g/person/day, respectively. Toddlers had the lowest mean and 95th percentile all-user intakes of 0.7 and 1.4 g/person/day, respectively. On a body weight basis, toddlers had the highest mean and 95th percentile all-user intakes of any population group, of 46.5 and 102.4 mg/kg body weight/day, respectively. Consumption data and information pertaining to the individual proposed use in the *PS-Spreads/Liquids for CB* and for the Total Margarine assessment were used to estimate plant sterol intakes of specific demographic groups in the UK population from this application alone. This type of intake methodology is generally considered to be a conservative estimate of consumption due to the assumptions made. For example, here it is assumed that all home-made foods and dishes would be prepared using the *PS-Spreads/Liquids for CB*. For the Total Margarine assessment it is also assumed that all fat spreads consumed would have added plant sterols [*i.e.*, 100% market share, while a more realistic estimate is probably closer to 10% (EFSA, 2008a)]. It is further assumed that all population groups could be consumers of the *PS-Spreads/Liquids for CB*, while in reality these products are intended for consumption by adults who need to reduce their cholesterol (in this case the group of adults aged greater than 45 years). In addition, it is well established that the length of a dietary survey affects the estimated consumption of individual users. Short-term surveys, such as a 4-day survey, perhaps overestimate consumption of food products that are consumed relatively infrequently.

IX.2.2 Consumption Estimates Based on Dutch National Food Consumption Data

To present an assessment of plant sterol intake for *PS-Spreads/Liquids for CB including spreading next to cooking and baking* data from the 2 Dutch National Food consumption surveys were used (DNFCS young children 2005-2006 and DNFCS older children and adults 2007-2010). These data for *PS-Spreads/Liquids for CB* intakes can be considered as 'worst-case' as total margarine intake in the Netherlands is one of the highest in Europe (refer to Section IX.2.3 for a description of margarine intake in Europe), and the use of vegetable fat liquid emulsions for cooking is a well-established practice. Appendix F provides a detailed description of the background and survey methodology for the Dutch Dietary Surveys, along with detailed results.

IX.2.2.1 Food Survey Results

<u>*DNFCS-Young Children 2005-2006*</u>

To examine the potential intake of plant sterols in younger children, food consumption data from The Netherlands National Food Consumption Survey - Young Children (DNFCS-YC) 2005/2006 (Ocké *et al.*, 2008), was considered. Intake for the food category 'Fats' was broken down further to present intake data for 'Margarine'. In this survey, the food category margarine includes both spreads and liquid margarine used for cooking/baking. Estimation of the potential intake of plant sterols from the total replacement of all margarines with added plant sterols was examined for the total population and for all-users (Table IX.2.2-1). As the results for both are similar, the results are presented here for all-users while the complete set of results is provided in Appendix F.

Table IX.2.2-1 Margarine Intake (g/day) and Plant Sterol (PS) Intake Based on Replacement with *PS-Spreads/Liquids for CB* Using Data from the DNFCS-YC 2005-2006^ in All-Users Only

	% consumers	Margarine intake (g/day)		PS intake from margarine (g/day)*		PS intake from margarine (mg/kg bw/day)**	
		Mean	P95^	Mean	P95	Mean	P95
Boys: 2-3 yrs	96	11	22.5	0.8	1.7	51.0	108.3
Girls: 2-3 yrs	93	10	19.9	0.8	1.5	53.0	99.3
Boys: 4-6 yrs	91	13	26.2	1.0	2.0	46.9	93.9
Girls: 4-6 yrs	94	11	24.2	0.8	1.8	37.9	85.3

*Assume all margarine replaced with plant sterol added margarine at 7.5 g/100 g
**Body weights were measured. Average body weights were as follows: Boys: 2-3 yrs 15.7 kg, 4-6 yrs 21.3 kg; Girls 2-3 yrs 15.1 kg, 4-6 yrs 21.1 kg
^P95 intakes were calculated as mean and standard deviation based on a normal distribution

Based on replacement of margarine with *PS-Spreads/Liquids for CB* in all users only, estimated plant sterol intake ranges from a mean of 0.8 g/day to 1.0 g/day in males and females 2 to 6 years old. Intakes at the 95th percentile ranged from 1.5 g/day to 2.0 g/day (Table IX.2.2-1). Expressed on a body weight basis, plant sterol intakes from *PS-Spreads/ Liquids for CB* were highest in 2- to 3-year-old boys with a mean and P95 intake of 51.0 and 108.3 mg/kg body weight (bw)/day, respectively.

<u>*DNFCS 2007-2010*</u>

Estimated intakes of plant sterols from *PS-Spreads/Liquids for CB* related to margarine intakes reported in the DNFCS 2007-2010 (van Rossum *et al.*, 2011) were examined. The food category 'Margarine' includes margarines used in cooking and baking applications (including liquid margarine), along with that used in spreading. Results are presented for all-users (Table IX.2.2-2), while results for the total population are provided in Appendix F.

Table IX.2.2-2 Margarine Intake (g/day) and Plant Sterol (PS) Intake Based on Replacement with *PS-Spreads/Liquids for CB* Using Data from the DNFCS 2007-2010^ for All-Users only

	Margarine intake (g/day)		PS intake from margarine (g/day)*		PS intake from margarine (mg/kg bw/day)**	
	Median	P95	Median	P95	Median	P95
7-8 years	15	40	1.1	3.0	38.8	103.4
Male						
9-13	18	49	1.4	3.7	32.1	87.5
14-18	18	63	1.4	4.7	20.1	70.5
19-30	22	72	1.7	5.4	20.4	66.7
31-50	24	67	1.8	5.0	20.5	57.1
51-69	24	60	1.8	4.5	20.7	51.7
Female						
9-13	13	38	1.0	2.9	22.2	64.8
14-18	12	38	0.9	2.9	14.8	46.7
19-30	15	45	1.1	3.4	15.4	46.2
31-50	15	45	1.1	3.4	14.8	44.4
51-69	17	46	1.3	3.5	17.2	46.6

*Assume all margarine replaced with plant sterol added margarine at 7.5g/100g
^Weighted for socio-demographic factors, season and day of the week
**Body weights were reported by participants. Average body weights were as follows: 7-8 y 29kg; Males: 9-13y 42kg, 14-18y 67kg, 19-30y 81kg, 31-50y 88kg, 51-69y 87kg; Females: 9-13y 44kg, 14-18y 61kg, 19-30y 73kg, 31-50y 76kg, 51-69y 74kg

For all-users, plant sterol intake, based on replacement of all margarine with *PS-Spreads/ Liquids for CB*, ranges from a median of 0.9 g/day to 1.8 g/day in males and females 7 years old and older. Intakes at the 95th percentile ranged from 2.9 g/day to 5.4 g/day (Table IX.2.2-2). Expressed on a body weight basis, the plant sterol intakes from *PS-Spreads/ Liquids for CB* were highest in the youngest age group, 7 to 8 year-olds with a median and P95 intake of 38.8 and 103.4 mg/kg bw/day, respectively.

IX.2.2.2 Conclusions on Intakes Based on Dutch National Food Consumption Data

In summary, on an all-user basis, intakes in the non-target groups of children based on replacement of margarine with *PS-Spreads/Liquids for CB*, estimated plant sterol intake ranges from a mean of 0.8 g/day to 1.0 g/day in males and females 2 to 6 years old. Intakes at the 95th percentile ranged from 1.5 g/day to 2.0 g/day. Expressed on a body weight basis, plant sterol intakes were highest in 2- to 3-year-old boys with a mean and P95 intake of 51.0 and 108.3 mg/kg bw/day, respectively. In adults aged 19 to 69 years, plant sterol intakes in margarine users only ranged from a mean of 1.1 to 1.3 g/day in females and 1.7 to 1.8 g/day in males. Intakes at the 95th percentile ranged from 3.4 to 3.5 g/day in females and from 4.5 to 5.4 g/day in males. Taking body weight into account, plant sterol intakes in all-users ranged from a mean of 14.8 to 17.2 mg/kg bw/day in females and 20.4 to 20.7 mg/kg bw/day in males. Intakes at the 95th percentile ranged from 44.4 to 46.2 mg/kg bw/day in females and from 51.7 to 66.7 mg/kg bw/day in males.

Margarine consumption data from both the DNFCS-Young Children 2005-2006 and the DNFCS 2007-2010 were used to estimate plant sterol intakes of specific demographic groups in the Dutch population. This type of intake methodology is generally considered to be a worst-case assumption, as firstly margarine intakes are one of the highest in Europe in the Netherlands, and also as a result of several conservative assumptions made. For example, here it is assumed that all margarine that were consumed would be replaced by the *PS-Spreads/Liquids for CB* [100% market share while a more realistic estimate is probably closer to 10% (EFSA, 2008a)], and that these products would be consumed by all population groups. In addition, it is well established that the length of a dietary survey affects the estimated consumption of individual users. Short-term surveys, such as a 2-day survey, may overestimate consumption of many foodstuffs. Margarine intakes amongst the total population and users-only are quite similar, indicating that margarine is consumed by the majority of the Dutch population on at least 1 of the 2 survey days.

IX.2.3 EFSA Comprehensive Database

Margarine consumption data from the EFSA Comprehensive dataset were used to estimate the total population and all-user plant sterol intakes of specific demographic groups in different member states in the EU. Detailed data are provided in Appendix G.

In summary, intakes in the non-target groups of *toddlers* (1 to 3 years), for the total population, based on margarine use, estimated mean plant sterol intakes ranged from 0.1 to 0.7 g/day (3.8 to 47.5 mg/kg bw/day) with a 95th percentile range of 0.4 to 1.6 g/day (31.3 to 111.6 mg/kg bw/day). For users, estimated mean plant sterol intakes ranged from 0.2 to 0.7 g/day (25.0 to 50.6 mg/kg bw/day), with a 95th percentile range of 0.6 to 1.6 g/day (36.5 to 112.1 mg/kg bw/day).

In children (3 to 9 years) for the total population, based on margarine use, estimated mean plant sterol intakes ranged from <0.01 to 0.9 g/day (0.1 to 40.3 mg/kg bw/day), with a 95th percentile range of 0.3 to 1.9 g/day (14.0 to 110.6 mg/kg bw/day). For users, estimated mean plant sterol intakes ranged from 0.2 to 1.0 g/day (10.0 to 44.7 mg/kg bw/day) with a 95th percentile range of 0.5 to 3.4 g/day (22.5 to 111.9 mg/kg bw/day).

In *adults* (18 to 64 years), for the total population, based on margarine use, estimated mean plant sterol intakes ranged from <0.01 to 1.5 g/day (0.01 to 20.5 mg/kg bw/day), with a 95th percentile range of 0.6 to 5.4 g/day (9.0 to 74.7 mg/kg bw/day). For users, estimated mean plant sterol intakes ranged from 0.6 to 1.7 g/day (8.0 to 23.9 mg/kg bw/day), with a 95th percentile range of 1.4 to 6.4 g/day (15.5 to 92.0 mg/kg bw/day). In the *elderly* (65 to 74 years), for the total population, based on margarine use, estimated mean plant sterol intakes ranged from <0.01 to 2.0 g/day (8.5 to 27.5 mg/kg bw/day), with a 95th percentile range of 2.7 to 6.7 g/day (27.3 to 93.5 mg/kg bw/day). For users, estimated mean plant sterol intakes ranged from 0.3 to 2.5 g/day (12.7 to 35.2 mg/kg bw/day), with a 95th percentile range of 0.5 to 7.1 g/day (30.7 to 100.5 mg/kg bw/day).

IX.2.4 Summary of Theoretical Plant Sterol Intakes

A summary of the estimated intake of plant sterols in margarine for use in spreading and in cooking and baking is provided in Table IX.2.4-1. In general, estimated plant sterol intakes across the 3 datasets were similar. Intakes calculated using the UK NDNS survey were slightly lower at the upper end of the range than were observed for the Dutch surveys and for the EFSA Comprehensive database. This is because the exposure assessment conducted in the UK NDNS was at a food-code level. However, it is important to note that different methodologies were employed in the calculations for the three datasets due to the way the data were presented. Despite the different survey methodologies, the plant sterol intake estimations based on each survey are similar (Table IX.2.4-1). However, caution is needed when conducting direct comparisons. Results from intake estimates based on data from the UK NDNS survey demonstrate that the additional impact on plant sterol intakes from using *PS-Spreads/Liquids* for cooking and baking is 0.4 g/day (mean for all users) in adults aged ≥45 years. For all-user data, plant sterols intakes at the 95th percentile based on total margarine intake for most population groups exceeded 3 g/day (Commission of the European Communities, 2004 – 608/2004e)[1] at the upper end of the range for both the Netherlands and EFSA Comprehensive Database data (EFSA, 2011a). However, these intake estimates are very conservative as they assume that all margarine consumed had added plant sterols. Intakes in the target and non-target populations are summarised in Section IX.4.

[1] According to Commission Regulation 608/2004/EC, it is a regulatory requirement to inform consumers that the consumption of more than 3 g/day of added plant sterols/plant stanols should be avoided.

Table IX.2.4-1 Summary of Estimated Plant Sterol Intakes from Total Margarine Intakes used in Spreading, Cooking and Baking in All-Users

	Range of Plant Sterol Intake based on Total Margarine Intake (g/day) in All-Users								
	Netherlands			UK			EFSA Comprehensive Database		
	Age	**Median**	**P95**	***Age***	**Mean**	**P95**	***Age***	**Mean**	**P95**
Infants	*na*	na	na	na	na	na	*0-11mths*	0-0.2	0-0.4
Toddlers	*2-6*	0.8-1.0*	1.5-2.0	*1-3*	0.7	1.4	*1-3*	0.2-0.7	0.6-1.6
Children	*7-13*	1.0-1.4	2.9-3.7	*4-10*	1.0	2.1	*3-9*	0.2-1.0	0.5-3.4
Adolescents	*14-18*	0.9-1.4	2.9-4.7	*11-18*	1.0	2.4	*10-17*	0.4-1.1	0.9-3.4
Adults	*19-30*	1.1-1.7	3.4-5.4	*19-44*	1.2	2.9	na	na	na
Female adults	*19-50*	1.1	3.4	*19-44*	1.1	2.5	na	na	na
Adults	*31-69*	1.1-1.8	3.4-5.0	*45+*	1.4	3.2	*18-64*	0.6-1.7	1.4-6.4
Elderly	*na*	na	na	na	na	na	*65-74*	0.3-2.5	0.5-7.1

na = data not available for this population group ; nc = not calculated due to insufficient data; P95 = 95th percentile intakes
*Data on The Netherlands toddlers aged 2-6 are presented as mean intakes rather than median intakes. Therefore, the Netherlands 'toddlers' include children aged 2-6 to keep these data separate from other the Netherlands data which are presented as medians.

IX.3 Labelling Foodstuffs to Inform Consumers

There are mandatory labelling requirements in place for foods and food ingredients with added plant sterols, plant sterol esters, plant stanols and/or plant stanol esters. The conditions laid down for all sources of added phytosterols (Novel Food or otherwise) are described under *Commission Regulation (EC) Nº 608/2004 concerning the labelling of foods and food ingredients with added plant-sterols, plant-sterol esters, plant-stanols and/or plant-stanol esters* (Commission of the European Communities, 2004e) and they provide the appropriate risk management measures. [redacted]

IX.4 Conclusions

The proposed extension of Unilever's heart healthy pro.activ® range of Becel, Flora, Fruit d'Or brands to include *PS-Spreads/Liquids for CB* will result in a greater probability that mean plant sterol intakes from additional sources will help reach the desired consumption level of 1.5 to 3.0 g/day of phytosterols. Whilst theoretical deterministic estimations may result in plant sterol intakes of 5.4 g/day at the 95th percentile of margarine intake in adults 19 to 30 years old in the Netherlands (Table IX.4-1), all consumers of products with added plant sterols are provided with comprehensive labelling to educate them on the amount of servings required to achieve a plant sterols intake for significant cholesterol lowering. Such labelling requirements further inform consumers that these products are not intended for non-target groups of children under 5 years and pregnant and lactating women. The proposed Unilever *PS-Spreads/Liquids for CB* will be specifically packaged and labelled to enable optimal consumer use of the products.

Table IX.4-1 provides a summary of estimated plant sterol intakes in the Dutch population based on intakes of margarine for users only. These data will be used to represent potential worst-case estimates of plant sterol intakes from *PS-Spreads/Liquids for CB* in the following sections on toxicology (Section XIII). These data will be used in the toxicology section discussions as they are robust data based on detailed dietary assessments and they are based on total margarine intakes for nationally representative samples of Dutch children and adults who are recognised as having relatively high margarine intakes in Europe. The highest absolute plant sterol intakes based on margarine intake was observed in adults aged 31 to 69 years, with an all-user median intake of 1.1 to 1.8 g/day and an intake of 3.4 to 5.0 g/day at the 95th percentile. The highest plant sterol intakes in relation to body weight were observed in the non-target group of children (aged 2 to 6 years in the Dutch dataset), with a mean intake in all-users of 37.9 to 53.0 mg/kg bw/day and an intake of 85.3 to 108.3 mg/kg bw/day at the 95th percentile.

Table IX.4-1	**Summary of Estimated Intakes of Plant Sterols Based on Margarine Intake in the Dutch Population in Non-Target and Target Groups**				
	Age (yrs)	**All-Users (g/d)**		**All-Users (mg/kg bw/d)**	
		Median	**P95**	**Median**	**P95**
Non-Target groups					
Children	2-6	0.8-1.0^	1.5-2.0	37.9-53.0^	85.3-108.3
Women*	19-50	1.1	3.4	14.8-15.4	44.4-46.2
Target groups					
Adults	19-30	1.1-1.7	3.4-5.4	11.8-18.1	36.5-48.3
Adults	31-69	1.1-1.8	3.4-5.0	14.8-20.7	44.4-57.1

^Mean intakes are presented for children aged 2-6 years rather than median intakes.
*Women of child-bearing age are included as a non-target group to represent the potential intakes in pregnant and lactating women, as this specific population group was not included in the Dutch dietary survey.

X INFORMATION FROM PREVIOUS HUMAN EXPOSURE

Thus far, in Sections I-IX of this dossier the following points have been concluded:

1. Yellow fat spreads with added phytosterols are already approved as a Novel Food in the EU.
2. There are no changes in the product specifications for the Novel Food from those that are currently approved for yellow fat spreads with added phytosterols.
3. The production process for the plant sterol-added vegetable fat products intended for cooking and baking (*PS-Spreads/Liquids for CB*) is the same as that for the currently available liquid vegetable fat emulsions for cooking and baking without plant sterols and the approved plant sterol-added spreads.
4. The results of the intake assessment conclude that:
    a. There is a short-fall in the optimal level of plant sterol intake from all sources based on existing approved enrichment categories.
    b. By adding *PS-Spreads/Liquids for CB* this shortfall can contribute to achieving the optimal level.
    c. In line with Commission Regulation 608/2004 appropriate risk management measures are in place to ensure that the consumer is advised to avoid intakes of more than 3 g of plant sterols per day (Commission of the European Communities, 2004e)[2]. The theoretical worst-case deterministic estimations amongst consumers with the highest intake of plant sterols could result in a total consumption from the *PS-Spreads/Liquids for CB* of about 5.4 g/day at the 95th percentile amongst consumers aged 19 to 30 years.
    d. In the Netherlands, adults aged 45+ years had an all-user plant sterol median intake of 1.1 to 1.8 g/day and an intake of 3.4 to 5.0 g/day at the 95th percentile based on the scenario of margarine intake. Children aged 2-6 years had an all-user plant sterol mean intake of 0.8 to 1.0 g/day and an intake of 1.5 to 2.0 g/day at the 95th percentile based on the scenario of margarine intake.
    e. *PS-Spreads/Liquids for CB* will be presented in a format that allows accurate measurement of servings to prevent over consumption.

The European Commission Recommendation 97/618/EC lays down the requirement to provide information from previous direct, indirect, intended, or unintended human exposure to the Novel Food or its source which is relevant to the EU situation with respect to production, preparation, population, lifestyles and intakes be provided (Commission of the European Communities, 1997).

[2] According to Commission Regulation 608/2004/EC, it is a regulatory requirement to inform consumers that the consumption of more than 3 g/day of added plant sterols/plant stanols should be avoided.

Each will be considered in turn followed by an assessment of the total current exposure from all sources as assessed by both classic deterministic and post launch monitoring methodology:

Since the market authorisation for foods with added plant sterols in the EU from 2000 onwards, a variety of products with added plant sterol and plant stanols were launched in all or most European countries. Consumers may choose from different food categories and brands to meet the current recommended daily intake of 1.5 to 3.0 g plant-sterols and plant stanols per day in order to achieve a significant cholesterol lowering effect.

Plant sterols and plant stanols are currently consumed in the European Union from 4 food sources:

1. Previously approved and notified Novel Foods with added plant sterol esters specific to Unilever.
2. Previously approved and notified Novel Foods with added plant sterols and plant sterol esters specific to other manufacturers.
3. Foods with added plant stanols and stanol esters.
4. Plant sterols and plant stanols naturally present in the diet.

X.1 Contributors to Plant Sterol Intake

X.1.1 Previously Authorised and Notified Foods with Added Plant Sterol Esters Specific to Unilever

In Europe Unilever has previously gained authorisation for the following additional uses of plant sterol esters

Spreads

As discussed earlier in 2000 Unilever gained authorisation for the use of plant sterol esters in yellow fat spreads under Commission Decision 2000/500/EC (Commission of the European Communities, 2000).

Milk and Yoghurt Type Products

In 2004, Unilever gained subsequent authorisation for the use of plant sterol esters in milk and yoghurt type products under Commission Decision 2004/335/EC (Commission of the European Communities, 2004c).

Soya Drinks

In 2010 Unilever successfully notified the use of plant sterol esters as substantially equivalent in Europe to products already authorised in the EU (European Commission, 2012a).

X.1.2 Previously Authorised Novel Foods with Added Plant Sterols Specific to Other Manufacturers in the European Union

In addition to the uses approved for Unilever, a number of other Novel Food Decisions have granted authorisation for other food categories as laid down in Table X.1.2-1 below. Further details of these authorisations can be found on the European Commission's website[3].

[3] http://ec.europa.eu/food/food/biotechnology/novelfood/authorisations_en.htm

Table X.1.2-1 Categories of Products Authorised for Other Manufacturers in EU	
Food Category	**Conditions of Use**
Spreads	Yellow fat spreads excluding cooking and frying fats and spreads based on butter or other animal fat with added plant-sterols. A portion will not contain more than 3 g (in case of 1 portion per day) or more than 1 g (in case of 3 portions per day) of added plant-sterol esters.
Milk Type Products	A portion will not contain more than 3 g (in case of 1 portion per day) or more than 1 g (in case of 3 portions per day) of added plant-sterols/plant-stanols; a container of beverages will not contain more than 3 g of plant-sterols/plant-stanols.
Soya Drinks	
Fermented Milk Products	
Milk Based Fruit Drinks	
Rice Based Drinks	
Milk Based Products	
Salad Dressings	Salad dressings shall be packed as single portions.
Spicy Sauces	Spicy sauces shall be packed as single portions.
Cheese Type Products	Fat content ≤ 12 g per 100 g, where the milk fat and/or protein has been partly or fully replaced by vegetable fat or protein.
Yoghurt Type Products	Milk type products, such as semi-skimmed and skimmed milk type products and yoghurt type products, where the milk fat has been reduced or partly or fully replaced by vegetable fat
Rye Bread Containing ≥50% Rye	No specific conditions of use

X.1.3 Foods with Added Plant Stanols Currently on the Market

The brand leader in plant stanol/stanol ester added foods is Benecol®. They have been marketing a range of products containing stanols prior to 1997. The current range of products largely reflects the same categories as approved for Novel Food products with added plant sterols, including vegetable fat spreads, yoghurts, yoghurt drinks and dairy-free drinks (McNeil Nutritionals Ltd., 2012a).

However, within their spreads portfolio Benecol® is selling high fat “Olive Spread’ and ‘Buttery Spread’ which are been advertised for use in cooking and baking applications, such as in sauces, baking and frying (McNeil Nutritionals Ltd., 2012b). They provide recipe ideas that include spreads with added plant stanols, which are similar to the proposed new uses of Unilever’s *PS-Spreads/Liquids for CB* (McNeil Nutritionals Ltd., 2012c). Unilever products will simply replace and/or provide an alternative to products like Benecol® Olive Spread and Benecol® Buttery Spread.

X.1.4 Plant Sterols and Stanols Naturally Present In the Diet

Plant sterols naturally occur in all foods of plant origin such as vegetable oils, nuts, seeds, grain products, fruits and vegetables. The average daily intake of plant sterols and stanols, of which the majority is plant sterols, from a typical Western European diet ranges from approximately 200 to 300 mg/day based on data from cohorts in several European countries with higher intakes for men than for women (Table X.1.4-1). For vegetarians, the daily intake of plant sterols and stanols is generally higher and in the range of 500 mg to 1 g (Vuoristo and Miettinen, 1994; Jenkins *et al.*, 2001).

The daily intake of plant stanols has been less well studied, but available data report an intake in the range of approximately 10 to 25 mg/day (Normén *et al.*, 2001; Andersson *et al.*, 2004; Valsta *et al.*, 2004; Jiménez-Escrig *et al.*, 2006; Klingberg *et al.*, 2008a). The dietary intake of plant stanols comes mainly from the consumption of fibre-rich breads and cereal-based products.

Table X.1.4-1 Total Phytosterol Intake from Natural Sources for Men and Women in Several European Countries

Country & population	Total plant-sterol intake (mg/day) (mean ± SD)		Reference
	men	women	
Netherlands Netherlands Cohort Study on Diet and Cancer	307 ± 104	263 ± 84	Normén *et al.*, 2001
United Kingdom EPIC-Norfolk cohort EPIC-Norfolk cohort	310 ± 108 300 ± 108	303 ± 100 293 ± 100	Andersson *et al.*, 2004 Klingberg *et al.*, 2008a
Finland National FINDIET survey	305	237	Valsta *et al.*, 2004
Spain Spanish National Food Consumption survey Spanish EPIC cohort	276* 338 ± 116	276* 250 ± 92	Jiménez-Escrig *et al.*, 2006 Escurriol *et al.*, 2009
Sweden Västerbotten Intervention Program	252 ± 95	212 ± 74	Klingberg *et al.*, 2008b
Ireland North/South Ireland Food Consumption Survey	283 ± 98	228 ± 69	Hearty *et al.*, 2009
Belgium Belgian National Food Consumption survey	301 ± 131	229 ± 98	Sioen *et al.*, 2011a

EPIC: European Prospective Investigation into Cancer
* Not specified for men and women separately

X.2 Estimated Exposure to Plant Sterols from Foods with Added Plant Sterols

Although it is important to be aware of the intake of plant sterols from the background diet, when considering the recommended phytosterol intake for achieving a cholesterol lowering effect (*i.e.*, 1.5 to 3.0 g/day) (EFSA, 2009a), intakes from products with added plant sterols should only be taken into account. Intake assessments of plant sterols from foods with added plant sterols (such as vegetable fat spreads and dairy products) have been conducted in various EU countries. It is important that the intake of plant sterols when added to foods on the market are monitored through periodic intake assessments and PLM initiatives, particularly when new products are launched onto the market.

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]				
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]		[redacted]	
[redacted]	[redacted]		[redacted]	
			[redacted]	
[redacted]				[redacted]
[redacted]				[redacted]
[redacted]				
	[redacted]	[redacted]		[redacted]
		[redacted]		
[redacted]	[redacted]		[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]

[redacted]

[redacted]

[redacted]

[redacted]			
	[redacted]		
	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]

[redacted]

[redacted]

[redacted]					
	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]

[redacted]

[redacted]

[redacted]

X.2.2 EFSA Summary Reports on Plant Sterol Intakes

EFSA provided its most recent deterministic assessment of total intakes for existing uses of plant sterols and stanols within its *Scientific Opinion on the safety of stigmasterol-rich plant sterols as food additive* (EFSA, 2012c). This review also took account of all the previous exposure estimates and risk assessment conducted by the Scientific Committee on Food and EFSA. Following their assessment of plant sterol and plant stanol intakes from all sources (*i.e.*, habitual intake from the background diet plus foods with added plant sterols or plant stanols plus the proposed addition of stigmasterol to alcoholic beverages as a food additive), the Panel noted that:

"Based on data reported in the literature, total exposure to plant sterols from all sources was estimated at an average daily intake of approximately 2770 mg/day (46 mg/kg bw/day) for both adults and the elderly"

In addition, EFSA had previously evaluated the consumption of foods with added plant sterols in 2008 (EFSA, 2008a). For their evaluation, they used several published and unpublished consumer and market research information and a meta-analysis of information published in literature. EFSA reported that although estimates of plant sterol intake were still difficult to obtain from the available data, in general there was little overconsumption as only a small subgroup of 1 to 4% of the population was identified with intakes greater than 3 g/day. EFSA reported that more than half of consumers of the plant sterol products belong to the intended target group, particularly at sustained levels of intake. Thus more than 60%

of the consumers of food products with added plant sterols had high blood cholesterol levels and a large majority belonged to the over 45 age group. Consumption among children was low, (8% children less than 5 years) and less than 1% of these children having occasional or regular use. In this report, EFSA also conclude that the market share for products with added plant sterols "is likely less or much less than 10%", which demonstrates the very conservative approach of assuming 100% market share that most studies use (including the estimates presented in this report).

Table X.2.2-1 provides a summary of phytosterol intakes in adults as assessed through various recent published reports. In summary, mean phytosterol intakes in Europe from products with added phytosterols range from 0.35 to 2.5 g/day, with intakes at the 95th percentile ranging from 1.06 to 4.20 g/day.

Table X.2.2-1 Summary of Phytosterol Intakes (g/day) Based on Products with Added Phytosterol in Adults from Recent Published Reports

Report/Country	Reference	Average (g/day)	P95 (g/day)	P97.5 (g/day)
EFSA summary report*	EFSA 2008a	Mean: 0.9-2.5 Median: 1.0-1.9	2.2-3.6	3.0-6.6
Belgium^	Sioen *et al.*, 2011b	Mean: 1.51	4.20	5.07
Post launch monitoring (PLM)§	Europanel, 2013	Mean: 0.35-0.86 Median: 0.12-0.26	1.06-3.70	nc

nc = not calculated due to insufficient data
*The EFSA 2008 summary report provides phytosterol intakes from four studies: PLM, Ireland, Germany and the UK.
^Phytosterol intakes are provided for consumers of products with added phytosterols in Belgium
§The PLM study is described in detail in Section X.2.1.

Plant sterol intakes in Belgian children were assessed by Sioen *et al.* (2011b). They found that 21% of pre-school children (2.5 to 7 years) included in the survey were consumers of products with added plant sterols (n=29), and in these consumers, mean plant sterol intake was 0.7 g/day (median of 0.5 g/day) with a minimum-maximum range intake of 0.01 to 2.1 g/day.

X.3 Conclusions

[redacted] In summary, mean phytosterol intakes in European adults from products with added phytosterols based on published reports range from 0.35 to 2.5 g/day.

[4] According to Commission Regulation 608/2004/EC, it is a regulatory requirement to inform consumers that the consumption of more than 3 g/day of added plant sterols/plant stanols should be avoided.

XI NUTRITIONAL INFORMATION ON THE NOVEL FOOD

Thus far, in Sections I-X of this dossier the following points have been concluded:

1. Yellow fat spreads with added phytosterols are already approved as a Novel Food in the EU.
2. There are no changes in the product specifications for the Novel Food from those that are currently approved for yellow fat spreads with added phytosterols.
3. The production process for the plant sterol-added vegetable fat products intended for cooking and baking (*PS-Spreads/Liquids for CB*) is the same as that for the currently available liquid vegetable fat emulsions for cooking and baking without plant sterols and the approved plant sterol-added spreads.
4. The results of the intake assessment conclude that:
    a. There is a short-fall in the optimal level of plant sterol intake from all sources based on existing approved enrichment categories.
    b. By adding *PS-Spreads/Liquids for CB* this shortfall can contribute to achieving the optimal level.
    c. In line with Commission Regulation 608/2004 appropriate risk management measures are in place to ensure that the consumer is advised to avoid intakes of more than 3 g of plant sterols per day (Commission of the European Communities, 2004e)[5]. The theoretical worst-case deterministic estimations amongst consumers with the highest intake of plant sterols could result in a total consumption from the *PS-Spreads/Liquids for CB* of about 5.4 g/day at the 95th percentile amongst consumers aged 19 to 30 years.
    d. In the Netherlands, adults aged 45+ years had an all-user plant sterol median intake of 1.1 to 1.8 g/day and an intake of 3.4 to 5.0 g/day at the 95th percentile based on the scenario of margarine intake. Children aged 2 to 6 years had an all-user plant sterol mean intake of 0.8 to 1.0 g/day and an intake of 1.5 to 2.0 g/day at the 95th percentile based on the scenario of margarine intake.
    e. *PS-Spreads/Liquids for CB* will be presented in a format that allows accurate measurement of servings to prevent over consumption.
5. Food products containing plant stanol esters are already widely available and presented for baking and cooking purposes in the EU.
6. Mean phytosterol intakes in Europe from products with added phytosterols based on recent published reports range from 0.35 to 2.5 g/day.

[5] According to Commission Regulation 608/2004/EC, it is a regulatory requirement to inform consumers that the consumption of more than 3 g/day of added plant sterols/plant stanols should be avoided.

The Commission Recommendations require demonstration that the Novel Food is nutritionally equivalent to existing foods that it might replace in the diet. We provide a discussion on this point below.

XI.1 Nutritional Equivalency

As discussed in Section X, the proposed extension of use for plant sterol-esters into *PS-Spreads/Liquids for CB* will provide consumers with an alternative to existing products on the market. Specifically:

1. Replacing fats currently used in cooking and baking (*e.g.* butter).

2. An alternative to vegetable fat spreads with added plant stanols (*e.g.,* Benecol™) (see Section X.1.3), which can already be used in cooking and baking applications.

These products will be marketed under the pro.activ® range within Unilever's heart health brands BECEL/FLORA/Fruit d'Or. The products under these brands already have a heart healthy nutritional profile and those in the *pro.activ®* product range contain 12.5 g/100 g added plant sterol-esters (7.5 g/100 g free plant sterol equivalent) which provide additional nutritional benefits. The LDL-cholesterol lowering efficacy of plant sterols is well-established and has been summarised in several meta-analyses (Katan *et al.,* 2003; AbuMweiss *et al.,* 2008; Demonty *et al.,* 2009; Musa-Veloso *et al.,* 2011). Key experts in the field of blood lipid metabolism and on plant sterol and plant stanols recently reviewed published data in this field from the past decade and concluded that, based on the wealth of current clinical evidence demonstrating LDL-cholesterol lowering with plant sterol and plant stanol consumption, the use of plant sterols and plant stanols are recommended for use as dietary options to lower LDL-cholesterol for people who are at increased risk of cardiovascular disease (Plat *et al.*, 2012). In addition, the European Commission, have authorised health claims for the use of products with added plant sterols and plant stanols as part of a healthy diet and lifestyle to lower serum LDL-cholesterol (Table X1.1-1).

Table XI.1-1 Summary of the Approved Health Claims in the European Union						
Claim type	**Nutrient, substance, food or food category**	**Claim**	**Conditions of use of the claim / Restrictions of use / Reasons for non-authorisation**	**Health relationship**	**EFSA opinion reference / Journal reference**	**Commission Regulation**
Art.13(1)	Plant sterols and plant stanols	Plant sterols/stanols contribute to the maintenance of normal blood cholesterol levels	In order to bear the claim information shall be given to the consumer that the beneficial effect is obtained with a daily intake of at least 0.8 g of plant sterols/stanols.	maintenance of normal blood cholesterol concentrations	2010;8(10):1813, 2011;9(6):2203 (EFSA, 2010, 2011b)	Commission Regulation (EU) 432/2012 of 16/05/2012 (European Commission, 2012b)
Art.14(1)(a)	Plant sterols/Plant stanol esters	Plant sterols and plant stanol esters have been shown to lower/reduce blood cholesterol. High cholesterol is a risk factor in the development of coronary heart disease.	Information to the consumer that the beneficial effect is obtained with a daily intake of 1,5-2,4 g plant sterols/stanols. Reference to the magnitude of the effect may only be made for foods within the following categories: yellow fat spreads, dairy products, mayonnaise and salad dressings. When referring to the magnitude of the effect, the entire range '7 to 10%' and the duration to obtain the effect 'in 2 to 3 weeks' must be communicated to the consumer		Q-2008-779 (EFSA, 2009b)	Commission Regulation (EU) 384/2010 of 05/05/2010 (European Commission, 2010a)
Art.14(1)(a)	Plant sterols: Sterols extracted from plants, free or esterified with food grade fatty acids.	Plant sterols have been shown to lower/reduce blood cholesterol. High cholesterol is a risk factor in the development of coronary heart disease.	Information to the consumer that the beneficial effect is obtained with a daily intake of 1,5-2,4 g plant sterols. Reference to the magnitude of the effect may only be made for foods within the following categories: yellow fat spreads, dairy products, mayonnaise and salad dressings. When referring to the magnitude of the effect, the entire range '7 to 10%' and the duration to obtain the effect 'in 2 to 3 weeks' must be communicated to the consumer.		Q-2008-085 (EFSA, 2008b)	Commission Regulation (EC) 983/2009 of 21/10/2009, Amended by Commission Regulation (EC) 376/2010 of 03/05/2010 (European Commission, 2010b)

Table XI.1-1 Summary of the Approved Health Claims in the European Union

Claim type	Nutrient, substance, food or food category	Claim	Conditions of use of the claim / Restrictions of use / Reasons for non-authorisation	Health relationship	EFSA opinion reference / Journal reference	Commission Regulation
Art.14(1)(a)	Plant stanol esters	Plant stanol esters have been shown to lower/reduce blood cholesterol. High cholesterol is a risk factor in the development of coronary heart disease.	Information to the consumer that the beneficial effect is obtained with a daily intake of 1,5-2,4 g plant stanols. Reference to the magnitude of the effect may only be made for foods within the following categories: yellow fat spreads, dairy products, mayonnaise and salad dressings. When referring to the magnitude of the effect, the entire range '7 to 10%' and the duration to obtain the effect 'in 2 to 3 weeks' must be communicated to the consumer.		Q-2008-118 (EFSA, 2008c)	Commission Regulation (EC) 983/2009 of 21/10/2009, Amended by Commission Regulation (EC) 376/2010 of 03/05/2010 (European Commission, 2010b)

XI.2 Role of Cooking and Baking Fats in the Diet

The use of *PS-Spreads/Liquids for CB* could lead to the replacement of other fats in the diet currently used to cook and bake. The most commonly used fats for these purposes include: butter, liquid oils or vegetable fat products (with or without added plant sterols or plant stanols) in a variety of formats. Each of these fat sources contributes to an individual's dietary fatty acid intake.

Dietary fatty acids play an important role in blood lipid metabolism and research indicates that not only the fat quantity, but primarily the fat quality (or type of fat) plays a role in cardiovascular health (Jakobsen *et al.*, 2009; Mozaffarian *et al.*, 2010). It has been well established that saturated fatty acids raise blood cholesterol concentration while replacing saturated fatty acids with polyunsaturated fatty acids lowers blood total and especially LDL cholesterol concentrations (Keys *et al.*, 1957; FAO, 2010). Plant-based vegetable fat products are typically high in unsaturated fatty acids, especially polyunsaturated fatty acids, and low in saturated and trans fatty acids. Butter on the contrary, is high in saturated fatty acids, low in unsaturated fatty acids and contains relatively high amounts of trans fatty acids (Table XI.2-1). Therefore, the replacement of other fats, such as butter, with the proposed *PS-Spreads/Liquids for CB*, could positively impact the dietary fatty acid intake of consumers. In addition, due to the favourable fatty acid composition of soft and liquid vegetable fats they are frequently part of health recommendations as they provide a healthier alternative to other fats. The *PS-Spreads/Liquids for CB* as proposed in this dossier would enter the market in the form of a tub and/or liquid emulsion (*PS-Spreads for CB and PS-Liquids for CB*).

Table XI.2-1 Typical Fat Composition of Fats Used for Cooking & Baking

Fat Composition	Fat Products (g/100g)*			Current Becel Products without added Plant Sterols (g/100g)	
	Butter	Olive Oil	Sunflower oil	Becel Original (tub format)	Becel Liquid
Total Fat	81.1	100	99.5	60	74
Saturated Fat	53.4	14.3	11.7	12	9
Trans Fat	3.5	trace	0	0.5	<1
Monounsaturated Fats	19.8	73.8	21.4	17	27
Polyunsaturated Fats	2.4	7.9	62.8	31	38

* NEVO (Dutch food composition database) 2011. http://nevo-online.rivm.nl (RIVM, 2011)

XI.3 Replacement of Other Fats Commonly Used for Cooking & Baking by a Multi-purpose Tub or a Liquid with Added Plant Sterols

In order to assess the nutritional impact in a scenario whereby fats currently used for cooking and baking would be replaced with the proposed *PS-Spreads/Liquids for CB*, the percentage of saturated fatty acids of the proposed *PS-Spreads/Liquids for CB* compared to other fats used for cooking and baking are presented in Table XI.3-1.

Table X1.3-1 Saturated Fatty Acid Content as % of Total Fat Content of Various Fat Products Used for Cooking & Baking	
Product	**Saturated Fatty acids as % of Total Fat Content**
Proposed *PS-Spreads/Liquids for CB*	
Tub format	20 - 25%
Liquid emulsion format	10 – 15%
Fats currently used for cooking and baking	
Butter	65.8%
Olive Oil	14.3%
Sunflower Oil	11.8%
Becel Original (Tub)*	20.0%
Becel Liquid*	12.2%

*products without added plant sterols currently on the market

Based on these data, we have simulated the impact of changing from a currently used fat for cooking and baking to the *PS-Spreads/Liquids for CB*. Butter consumers would decrease their saturated fatty acid intake based on this replacement. For those who would switch from using products such as the Becel Original tub to a *PS-Spreads for CB* in tub format there would be no change in saturated fatty acid intake, because of similar fatty acids compositions. For consumers who currently use products such as Becel Liquid or vegetable oils, such as olive and sunflower oil, there could be a negligible increase in their saturated fatty acid intake upon switching to a *PS-Spreads for CB* in tub format. However, this replacement is considered not likely as those who currently use liquid oils, or products such as Becel Liquid are accustomed to using a liquid format for cooking and baking. Therefore, it is assumed that the liquid fat they currently use would be replaced by the *PS-Liquids for CB*, which has a saturated fatty acid content which is similar to these oils.

If consumers replace butter with the *PS-Liquids for CB*, their saturated fatty acid intake would decrease. Those who switch from products such as Becel Original tub would also decrease their saturated fatty acid intake. Consumers who switch from vegetable oils, such as olive or sunflower oil, or from products such as Becel Liquid will have no meaningful change in their saturated fatty acid intake.

XI.4 Conclusion

In conclusion, if consumers switch from other fats currently used for cooking and baking to the proposed *PS-Spreads/Liquids for CB*, there would be a nutritional benefit by increasing plant sterol intake and thereby contributing to the lowering of blood LDL-cholesterol concentrations. Consumers would also get an additional benefit from replacing some of their saturated fatty acid intake with unsaturated fatty acids. As the *PS-Spreads/Liquids for CB* provide a healthier alternative to other fats they can be considered to fit in a healthy and balanced diet.

XII MICROBIOLOGICAL INFORMATION

The microbiological specification and control of the plant sterol esters used in the *PS-Spreads/Liquids for CB* is identical to the ingredient used in Unilever's Yellow Fat Spreads that were previously approved under Commission Decision of 24 July 2000 on authorising the placing on the market of 'yellow fat spreads with added phytosterol esters' as a Novel Food or Novel Food ingredient under Regulation (EC) No 258/97 of the European Parliament and of the Council (Commission of the European Communities, 2000). .

XIII ADDITIONAL TOXICOLOGICAL AND HUMAN SAFETY INFORMATION

Thus far, in Sections I-XII of this dossier the following points have been concluded:

1. Yellow fat spreads with added phytosterols are already approved as a Novel Food in the EU.
2. There are no changes in the product specifications for the Novel Food from those that are currently approved for yellow fat spreads with added phytosterols.
3. The production process for the plant sterol-added vegetable fat products intended for cooking and baking (*PS-Spreads/Liquids for CB*) is the same as that for the currently available liquid vegetable fat emulsions for cooking and baking without plant sterols and the approved plant sterol-added spreads.
4. The results of the intake assessment conclude that:
    a. There is a short-fall in the optimal level of plant sterol intake from all sources based on existing approved enrichment categories.
    b. By adding *PS-Spreads/Liquids for CB* this shortfall can contribute to achieving the optimal level.
    c. In line with Commission Regulation 608/2004 appropriate risk management measures are in place to ensure that the consumer is advised to avoid intakes of more than 3 g of plant sterols per day (Commission of the European Communities, 2004e)[6]. The theoretical worst-case deterministic estimations amongst consumers with the highest intake of plant sterols could result in a total consumption from the *PS-Spreads/Liquids for CB* of about 5.4 g/day at the 95th percentile amongst consumers aged 19 to 30 years.
    d. In the Netherlands, adults aged 45+ years had an all-user plant sterol median intake of 1.1 to 1.8 g/day and an intake of 3.4 to 5.0 g/day at the 95th percentile based on the scenario of margarine intake. Children aged 2 to 6 years had an all-user plant sterol mean intake of 0.8 to 1.0 g/day and an intake of 1.5 to 2.0 g/day at the 95th percentile based on the scenario of margarine intake.
    e. *PS-Spreads/Liquids for CB* will be presented in a format that allows accurate measurement of servings to prevent over consumption.
5. Food products containing plant stanol esters are already widely available and presented for baking and cooking purposes in the EU.
6. Mean phytosterol intakes in Europe from products with added phytosterols based on recent published reports range from 0.35 to 2.5 g/day.

[6] According to Commission Regulation 608/2004/EC, it is a regulatory requirement to inform consumers that the consumption of more than 3 g/day of added plant sterols/plant stanols should be avoided.

7. The *PS-Spreads/Liquids for CB* proposed can be considered as a part of a healthy and balanced diet as they would provide a healthier alternative to other fats used for this purpose.
8. The microbiological specification is identical to the previously approved for yellow fat spreads with added phytosterol esters and to yellow fat spreads without plant sterols.

Taking the Commission Recommendation 97/618/EC decision trees (Commission of the European Communities, 1997) into account, we conclude that the issues for consideration to the Committee are:

1. Is there a significant increase in exposure to heat degradation products (plant sterol oxides) as a result of the new proposed uses, and what are the toxicological implications of this?

2. Will the potential increase in plant sterol intakes result in any additional issues of toxicological concern?

Each point will be addressed in the following sections.

XIII.1 Plant Sterol Oxidation Products (POPs)

The chemical structures of plant sterols are amenable to oxidative changes (Derewiaka and Obiedzinski, 2012). POPs products may be formed through enzymatic or non-enzymatic oxidation and can be initiated by factors such as heat, light, air, water, and transition metals (reviewed in Hovenkamp *et al.*, 2008; García-Llatas and Rodriguez-Estrada, 2011).

Considering that Unilever intends to expand the use of their vegetable fat products with added plant sterols to include cooking and baking applications, exposure to potential POPs formed due to oxidation of plant sterols at high temperatures during cooking and baking cannot be ruled out. As such, in the following the stability of *PS-Spreads/Liquids for CB* and the safety and toxicological implications of exposure to POPs is discussed.

XIII.2 Stability of *PS-Spreads/Liquids for CB* [redacted]

Several stability studies were conducted with Unilever's *PS-Spreads/Liquids for CB* under various cooking and baking conditions as described below.

XIII.2.1 Stability of Plant Sterol-Added Spread and Liquid Margarine under Various Cooking and Baking Conditions

The stability of a 70% fat *PS-Spread for CB* in tub format (10% as plant sterols, corresponding to 16% as plant sterol-esters) was assessed under various cooking and baking conditions. Briefly, 25 g of spread was heated to 180°C until all the water had evaporated (referred to as SV1). A sample was taken, and subsequently 10 g water was added to simulate addition of a product to be fried. Once the water was again completely evaporated (referred to as SV2) a sample was taken and the SV1 and SV2 fat residues were analysed for their sterol composition. An additional experiment was performed in which an egg was shallow fried in the spread and the fat residue was analysed. As demonstrated in Table XIII.2.1-1, the plant sterol content and profile of the *PS-Spread for CB* remained similar before and after shallow frying, and no significant decomposition or oxidation of the plant sterols was noted. Any deterioration observed was considered to be within the variation of the analytical method. It is therefore, concluded that the *PS-Spread for CB* in tub format is stable during use in shallow frying at high temperatures.

Table XIII.2.1-1 Sterol content and profile of a *PS-Spread for CB* in tub format before frying, after two stages of simulated shallow frying (SV1 and SV2) and after shallow frying an egg. Contents are calculated on the basis of the original spread as water will evaporate during frying.

	Fresh (n=2)		**SV1**		**SV2**		**Egg**	
Component	**[g/100 g]**	**[%]**	**[g/100 g]**	**[%]**	**[g/100g]**	**[%]**	**[g/100 g]**	**[%]**
Cholesterol	0	0	0.07	0.7	0.09	0.9	0.08	0.7
Brassicasterol	0.3	2.7	0.27	2.5	0.26	2.5	0.27	2.6
Campesterol	2.6	25.0	2.77	26.4	2.69	26.2	2.74	26.4
Campestanol	0.1	0.6	0	0	0	0	0	0
Stigmasterol	1.8	17.7	1.9	18.1	1.87	18.3	1.88	18.1
β-Sitosterol	4.9	47.6	5.15	49.0	5.03	49.0	5.08	48.9
Sitostanol	0.2	2.0	0	0	0	0	0	0
D5-Avenasterol	0.2	1.5	0.16	1.5	0.14	1.4	0.14	1.4
D7-Stigmastenol	0.1	0.5	0.04	0.4	0.04	0.4	0.04	0.4
Others	0.2	2.3	0.14	1.4	0.13	1.3	0.15	1.5
Total	10.2	100.0	10.5	100.0	10.26	100.0	10.38	100.0

XIII.2.2 Analysis of Plant Sterol Oxidation Products (POPs) under Practical Baking and Frying Conditions [redacted]

In order to evaluate the effect of frying and baking on the oxidation of plant sterols, the plant sterol oxide content of the above-mentioned samples was analysed before frying, after 2 stages of shallow frying (SV1 and SV2), and after baking a cake. The study was conducted using the *PS-Spread for CB* in tub format, a regular vegetable fat spread (Flora), and 2 vegetable oils (sunflower and maize). In addition, a beef steak was fried in the *PS-Spread for CB*, and also the 2 vegetable oils were analysed after being used for deep frying.

The analytical method employed (Louter *et al*., 2002) was developed at Unilever Research & Development (Vlaardingen, The Netherlands), which is similar to the method published by Dutta (Dutta and Appelqvist, 1997). As demonstrated in Table XIII.2.2-1, after 2 stages of shallow frying with the *PS-Spread for CB*, the level of POPs formed reached 76 mg/100 g, compared to 26 and 29 mg/100 g formed after shallow frying with sunflower and maize oils, respectively. Despite the fact that the level of plant sterols in the *PS-Spread for CB* is about 10 to 30 times higher, the amount of POP followed the same order as observed for regular vegetable fat spreads and ordinary vegetable oils.

After deep frying using maize oil, the amount of POPs was consistent with the levels formed after shallow frying with a *PS-Spread for CB* in tub format, suggesting that the levels of plant sterol oxides found in the plant sterol-added vegetable spread after frying are in the same order of magnitude to those already observed in other vegetable oil products. The level of POPs after baking cakes with the *PS-Spread for CB* was similar to those before frying, suggesting that under the conditions used for baking, plant sterols do not undergo oxidation and remain stable.

Table XIII.2.2-1 Total of POPs[a] [mg/100 g product] Before and After Different Cooking Conditions

Sample	Untreated	SV1	SV2	Baking	Steak	Deep frying
PS-Spread for CB in tub format (fresh)	14	22	42	12	58	
PS-Spread for CB in tub format (end of shelf life)	27	34	76	21		
vegetable fat spread without plant sterols (fresh)	4	4	7	4		
vegetable fat spread without plant sterols (end of shelf life)	9	13	25	7		
Sunflower oil	-	9	26			12
Maize oil	-	9	29			43

[a] Levels are reported as the polar fraction of the POPs, reflecting analytical methodology available at the time of testing in different vegetable fats before frying, after 2 stages of shallow frying (SV1 and SV2) and after baking a cake. Additionally a beef steak was fried in the *PS-Spread for CB* and 2 vegetable oils were analysed after being used for deep frying. Contents are calculated on the basis of the original spread as water will evaporate during frying.
-: data not available

XIII.2.2.1 Plant Sterol Oxidation after Heating PS-Spread for CB for an Extended Period of Time [redacted]

In another series of frying experiments, the *PS-Spread for CB* in tub format was heated for an extended period of time without adding water to simulate the use of food. These conditions are considered to represent a worst-case situation, since these temperature and time combinations rarely occur.

The experimental setting was similar to that described in Section XIII.1.1, except that instead of adding water after the evaporation of intrinsic moisture, the dish was kept at 180°C for another 15 minutes. Samples were taken after evaporation of the intrinsic water was completed (SV1) and at the end of the additional heating step. Analytical results demonstrated that after prolonged heating, the amount of POPs ranged from 34.3 to 48.5 mg/100 g product, which is comparable to contents observed in many other non-added products (see Table XIII.2.2-1).

XIII.2.2.2 Stability of a PS-Liquid for CB under Heat Stressing

A study was performed to evaluate the oxidation behaviour of the *PS-Liquid for CB* under heating stress. Shallow frying the plant sterols-added fat at about 205°C for 30 minutes is not a true representation of typical shallow frying conditions because normally, after adding the foods to be prepared to the heated pan, the temperature of the fat drops immediately due to the moisture in foods. The experimental conditions described herein simulate a worst-case scenario of not using the product according to its intended use.

Two samples of the *PS-Liquid for CB* (70% fat), with 12.5% plant sterol esters (equivalent to 7.5% plant sterols) were heated at a constant temperature of 205°C ± 5°C for 30 minutes, and the amount of POPs formed was determined after the heat processing, as presented in Table XIII.2.2.2-1.

Table XIII.2.2.2-1 Concentration of Plant Sterol Oxidation Products (POP) after Extended Heating of the *PS-Liquid for CB*

Sample	Amount of liquid fat processed [mg]	Total POP [mg/100 g]	Average POP of A & B [mg/100 g]
SCD201206-534 A	519.7	74.1	
SCD201206-534 B	504.5	72.2	73.2
SCD201206-535 A	506.0	80.0	
SCD201206-535 B	506.1	70.4	75.2

XIII.2.3 Conclusions

Several stability testing studies were conducted with Unilever's *PS-Spreads/Liquids for CB* under various cooking and baking conditions. The results of these studies indicate that the *PS-Spreads/Liquids for CB* that are the subject of this dossier are very stable. The levels of POPs in *PS-Spreads/Liquids for CB* are small and are of the same order of magnitude as existing food products both in the fresh product, after use in cooking and baking, and at the end of shelf-life. The profile of POPs produced under normal usage conditions (spreading, shallow frying and baking) is similar to existing foods, particularly vegetable oils. Even under extended heat stress, only small amounts of POPs are formed (up to 75.2 mg POPs/100 g serving or 22.56 mg POPs/day based on a daily intake of 30 g *PS-Spreads/Liquids for CB*).

XIII.3 Safety of Plant Sterol Oxidation Products

The safety of POPs was evaluated in a subchronic dietary toxicity study in rats and a battery of *in vitro* genotoxicity assays (bacterial mutation, chromosome aberration and micronucleus) as described below. The plant sterol oxide concentrate (POC) used in the aforementioned studies was prepared as described below.

XIII.3.1 Preparation of the Plant Sterol Oxide Concentrate Used In the Toxicity Studies

Plant sterols from a variety of common vegetable distillates were processed into plant sterol esters as described by Hepburn *et al*. (1999) and Wolfreys and Hepburn (2002). The composition of the plant sterol blend (see Table XIII.3.1-1) was similar to the composition used for the general toxicity assessment of plant sterols, performed by Unilever and evaluated by the European Commission during the original Novel Food assessment procedure (Hepburn *et al*., 1999).

Table XIII.3.1-1 Composition of the Plant Sterol Blend Used for the Manufacturing of the Plant Sterol Oxide Concentrate (POC) (Lea *et al*., 2004)

Type of Plant Sterol	Concentration (%)
Brassicasterol	3
Campesterol	25
Stigmasterol	19
Sitosterol	47
Minor plant sterols and stanols	1

As plant sterol oxides are only present at low levels in plant sterol esters, a POC was produced to provide sufficient quantities for toxicity testing. POC was generated under stress conditions by heating the blend under conditions mimicking severe cooking and frying (*i.e.,* 150 to 200°C for several hours in the presence of air). The oxidised material was hydrolyzed to obtain the unsaponifiable fraction, and was further purified by recrystallisation.

The composition of POC used for the toxicity studies is summarised in Table XIII.3.1-2. The concentrate contained about 30% of oxidised plant sterols, the remainder was primarily unoxidised plant sterols. Such a concentration by far exceeds the concentration one may expect in commercial products even after extended cooking, baking or frying.

Table XIII.3.1-2 Composition of the Plant Sterol Oxide Concentrate (POC) Used in the Toxicity Studies (Lea *et al*., 2004)

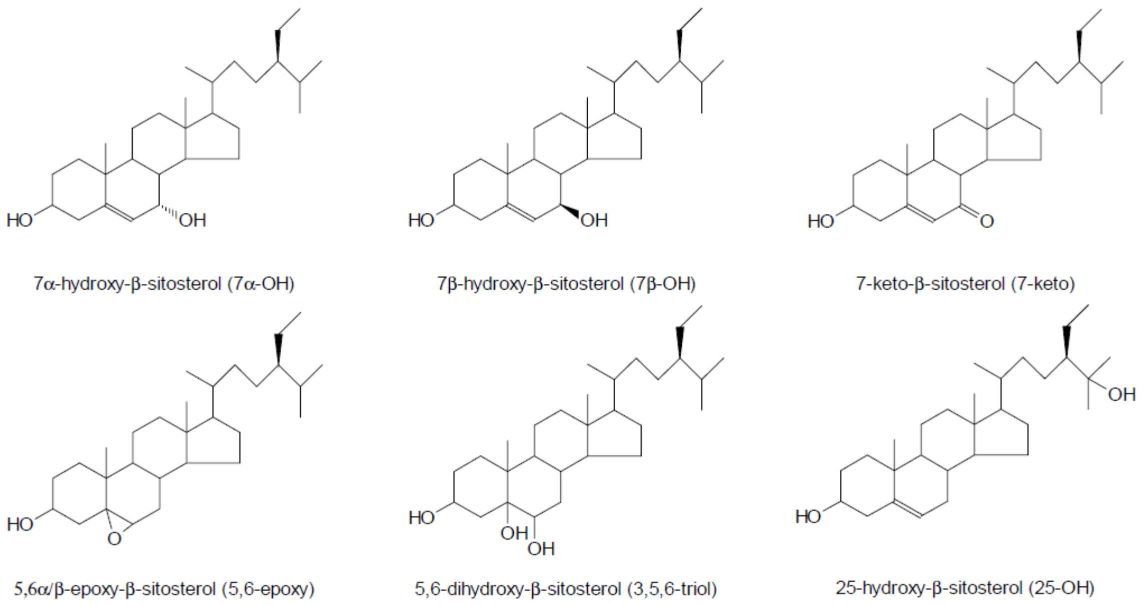
Composition	Level (%)	Method
Polar plant sterol oxides	26.2 ± 1.8 27.2 ± 0.5	HPLC Off-line LC-GC
Apolar plant sterol oxides	4.3 ± 0.7	Off-line LC-GC
Free plant sterols	19.1 ± 0.4	GC
Unknown*	Rest	GC + GPC

GPC: gel permeation chromatography; HPLC: high-performance liquid chromatography; LC-GC: liquid chromatography-gas chromatography
*The unknown fraction corresponds to sterol fragments.

Analysis of POC revealed 6 major oxidation products. Due to the dominance of sitosterol in the original plant sterol blend, sitosterol oxides were the most abundant oxides in the concentrate but similar oxides were also present for the other plant sterols. Typical oxides of sitosterol detected in POC are 7α-hydroxy-β-sitosterol (7α-OH), 7β-hydroxy-β-sitosterol

(7β-OH), 7-keto-sitosterol (7-keto-β–sitosterol (7-keto), 5,6α/β-epoxy-β-sitosterol (5,6-epoxy), 5,6-dihydroxy-β-sitosterol (3,5,6-triol) and 25-hydroxy-β-sitosterol (25-OH) as in Figure 3.

Figure 3 Major Oxidation Products of β-Sitosterol (Smith *et al.*, 1981; Smith, 1987, 1996)



XIII.3.2 *In Vitro* Genotoxicity Assays

XIII.3.2.1 Bacterial Mutation Assay

The study was commissioned by Unilever, and performed at Covance Laboratories Ltd. (Harrogate, UK) in compliance with the Organization for Economic Cooperation and Development (OECD) Guidelines for the Testing of Chemicals No. 471, and principles of Good Laboratory Practice (GLP) (OECD, 1997a).

Histidine-requiring strains of *Salmonella typhimurium* (TA98, TA100, TA102, TA1535, and TA1537) were incubated with POC up to precipitation level in the absence and presence of metabolic activation (an Aroclor1254-induced rat liver mitochondrial fraction (10% S9). The test article was used at concentrations ranging from 1.6 to 5,000 µg/plate and 20.48 to 2,000 µg/plate in the experiments without and with metabolic activation, respectively. The study was validated using positive controls such as 2-nitrofluorene, 9-aminoacridine, glutaraldehyde, and sodium azide in the absence of S9, and benzo[a]pyrene and 2-aminoanthracene in the presence of S9. No biologically or statistically significant increases in revertant colony numbers were noted in any of the bacterial strains tested (Lea *et al*., 2004).

XIII.3.2.2 In Vitro Chromosome Aberration Assay

The study was commissioned by Unilever, and performed at Covance Laboratories Ltd. (Harrogate, UK) according to the OECD Guidelines for the Testing of Chemicals No. 473, and principles of GLP (OECD, 1997b).

The potential of POC to induce chromosome aberrations was investigated in 2 independent experiments using human peripheral blood lymphocytes *in vitro*. Whole blood cultures (duplicate, pooled from 3 donors) were used without and with metabolic activation (10% S9) (Lea *et al*., 2004).

In the first experiment, the concentrations tested were 131.1, 256, and 500 µg/mL in the absence of S9 and 256, 400, and 500 µg/mL in the presence of S9, with treatment for 3 hours followed by a 17-hour recovery period. In the second experiment, concentrations of 67.11, 131.1, and 204.8 µ/mL were tested in the absence of S9 with continuous treatment for 20 hours, and in the presence of S9, concentrations of 320, 500, and 625 µg/mL were tested with treatment for 3 hours followed by a 17-hour recovery period. The mitotic index was calculated (number of dividing cells per 1,000 cells), and the maximum concentration that resulted in about 50% reduction of mitotic cells was determined. The study was validated using positive controls such as 4-nitroquinoline 1-oxide in the absence of S9 and cyclophosphamide in the presence of S9.

The frequency of cells with structural aberrations was similar to those in the concurrent negative controls. No increase in the number of aberrant cells was observed. The increase in polyploidy and endoreduplicated cells that was noted with the highest concentration tested in experiment 1 following 3-hour treatment in the absence and presence of S9 was not reproduced in experiment 2 neither with 20-hour incubation or 3-hour treatment in the presence of metabolic activation (Lea *et al*., 2004). Nonetheless, an *in vitro* micronucleus assay was performed to clarify the discrepancies.

XIII.3.2.3 In Vitro Micronucleous Assay

An *in vitro* micronucleus assay was performed to directly investigate the potential of POC to induce aneuploidy (abnormal number of chromosomes). Human lymphocytes were treated with 3 different concentrations under 2 treatment regimens: 0, 163.8, 320, and 400 µg/mL in the absence of S9 for 3 hours followed by a 17-hour recovery period; 0, 320, 500, 625 µg/mL in the presence of S9 for 3 hours followed by a 17-hour recovery period; and 0, 42.95, 67.11, and 104.9 µg/mL continuously for 20 hours. Cell division was arrested with cytochalasin B, allowing micronuclei to be formed if chromosome breakage or duplication took place. The study was validated using positive controls such as 4-nitroquinoline 1-oxide in the absence of S9 and cyclophosphamide in the presence of S9. No statistically significant increases in the frequency of micronuclei in bi-nucleated cells were detected. Thus, there is no evidence that POC under the conditions of this study causes aneuploidy (Lea *et al*., 2004).

XIII.3.3 Subchronic Dietary Toxicity Study in Rats (Lea *et al*., 2004)

The 90-day subchronic toxicity study was conducted at TNO Nutrition and Food Research (Zeist, The Netherlands) sponsored by Unilever and in compliance with the OECD Guidelines for the Testing of Chemicals No. 408, and principles of GLP (OECD, 1998). The same POC used in the above-described genotoxicity assays also was used in this study.

Groups of 20 male and 20 female Wistar rats were fed a control diet without added plant sterols or diets with either plant sterol esters alone (5.67%) or plant sterol esters supplemented with 0.2, 0.6, or 1.6% POC (Lea *et al*., 2004). The composition of the diets used in the 90-day rat feeding study is summarised in Table XIII.3.3-1.

Table XIII.3.3-1 Composition and Analysis of Diets Used in the 90-Day Rat Feeding Study (Lea *et al*., 2004)

Test group	Nominal dietary concentration of plant sterol esters + plant sterol oxides (% w/w)	Nominal dietary concentration of plant sterol oxides (% w/w)	Expected level of dietary plant sterol oxides (% w/w)	Mean dietary concentration of plant sterol oxides (% w/w)	
				Study Batch 1	Study Batch 4
Control diet	0	0	0	0.002	0.002
0.2% POC	5.87	0.2	0.054	0.055	0.068
0.6% POC	6.27	0.6	0.163	0.161	0.205
1.6% POC	7.27	1.6	0.435	0.45	0.443
Plant sterol ester diet	5.67	0	-	0.006	0.006

POC: Plant sterol oxide concentrate

Measured parameters comprised clinical observation, food and water consumption, neurobehavioral testing, ophthalmoscopic examination, clinical pathology and a renal concentrating test, gross necropsy, selected organ weights and histopathology of specified organs or tissues. The calculated mean intake of POC over the course of the study was 139, 425, and 1,133 mg/kg bw/day in males and 160, 481, and 1,269 mg/kg bw/day in females in the groups with 0.2, 0.6, and 1.6% POC in the diet, respectively. Administration of POC did not have an effect on survival. No treatment-related effects on clinical observations were seen, and no ocular changes were observed. At the highest-dose tested, female rats showed a slight decrease (13%) in growth as compared with the control and the plant sterol ester groups, whereas no effect on body weight was noted in male rats.

Neurobehavioral testing did not reveal any signs of neurotoxicity for the groups that received the plant sterol esters, or combined plant sterol ester and POC-added diets. The dietary administration of POC had no major effect on haematological parameters compared with plant sterol esters or control diet groups. Only in the high-dose groups, slight increases in thrombocyte counts (males) and decreases in haemoglobin, packed cell volume and mean corpuscular volume (females) were observed as compared with animals receiving the plant sterol ester or control diets.

Clinical chemistry parameters showed a similar trend. No major effects of POC treatment were observed for the lower dose groups, but in the high-dose groups receiving 1.6% POC, slight decreases in glucose concentrations and increases in albumin as well as in the albumin:globulin ratio were observed in males. Gamma-glutamyl transferase levels increased in females and triglycerides reduced in both sexes. Increased alkaline phosphatase and cholesterol levels were observed in all groups receiving plant sterols as compared with the control groups.

All other minor statistical changes in clinical parameters were considered not to be of biological or toxicological relevance. Urine parameters and renal concentration function were not affected by the enriched diets. Organ weights were not affected by the plant sterol esters or POC-added diets, except for a slight increase in liver weight in females in the 1.6% POC group as compared with the lower dose groups or controls.

No treatment-related macroscopic findings were observed at necropsy. An increased incidence of necrotic hepatocytes in the liver of males in the 0.2% POC group as compared with the controls was considered not to be treatment-related because the observations were not dose-dependent, and not observed in the higher dose groups.

Based on the results of this study the no-observed-effect level (NOEL) was 0.6% POC, equivalent to mean intakes of 425 and 481 mg/kg bw/day for male and female rats, respectively. As demonstrated in Table XIII.3.3-1, POC-containing diets include approximately 30% plant sterol oxides, as such, the NOEL for POC corresponds to intakes of approximately 128 and 144 mg plant sterol oxides/kg bw/day for male and female rats, respectively.

XIII.3.4 Human Studies

The effects of plant sterol and stanol consumption on fasting plasma POP was evaluated in a recent randomised, placebo-controlled, double blind, cross-over study (Baumgartner *et al*., 2013a). Forty-three healthy subjects (17 males and 26 females; age: 18 to 70 years) were randomly allocated to 3 intervention periods of consuming a vegetable fat spread with added plant sterols (3 g/day of plant sterols), a vegetable fat spread with added plant stanols (3 g/day of plant stanols), or a control vegetable fat spread for 4 weeks separated by washout periods of 4 weeks. The results of the study demonstrated that daily consumption of a vegetable fat spread containing added plant sterols does not increase plasma POP concentrations.

In a follow-up assessment, it was demonstrated that plasma POP concentrations remained relatively stable during the 20-week study period (Baumgartner *et al*., 2013b).

XIII.3.5 Conclusions and Risk Assessment

Overall, the results of a battery of *in vitro* genotoxicity studies demonstrated that a POC containing approximately 30% plant sterol oxides did not exhibit mutagenic activity in *S. typhimurium* in the presence or absence of metabolic activation, and did not display clastogenic properties or chromosomal aberrations in human peripheral blood lymphocytes in the presence or absence of metabolic activation.

In a 13-week subchronic toxicity study in rats, administration of a POC containing approximately 30% plant sterol oxides at doses of 0, 0.2, 0.6, or 1.6% in the diet (corresponding to 0, 139, 425, and 1,133 mg/kg bw/day in males and 0, 160, 481, and 1,269 mg/kg bw/day in females, respectively) did not result in any treatment-related clinical signs or toxicity, changes in body weight gain, food consumption, ophthalmology, neurobehavioral abnormalities, haematology, clinical chemistry, urinalysis, organ weights, or in any gross and microscopic findings. The NOEL was 0.6% POC based on small changes in clinical pathology parameters at the high dose, the toxicological significance of which is questionable. Based on the results of this study, and considering that POC contains approximately 30% plant sterol oxides, the NOEL for plant sterol oxides was determined to be approximately 128 and 144 mg/kg bw/day for male and female rats, respectively. This corresponds to intakes of about 8.9 to 10 g/day for a 70 kg adult. In addition, in a recent randomised, placebo-controlled, double blind, cross-over study it was demonstrated that daily consumption of a vegetable fat spread with added plant sterols (3 g/day of plant sterols) by healthy subjects does not increase plasma POP concentrations (Baumgartner *et al*., 2013a).

The material used in the toxicity study described above is considered representative of the oxidation products (POPs) produced from the exposure to *PS-Spreads/Liquids for CB* under extreme cooking and baking conditions. Therefore, for the purpose of assessing the safety of POPs from exposure to *PS-Spreads/Liquids for CB*, the margin of safety (MOS) is calculated. The MOS is the ratio of the NOEL to the estimated human exposure to a substance. As such, the MOS for POPs can be calculated as follows:

MOS_{POPs} = Systemic NOEL/Estimated human exposure

where,

Systemic NOEL = 128 mg/kg bw/day (lower value derived from a subchronic toxicity study in rats)

Estimated human exposure = 22.56 mg POPs/day equivalent to 0.32 mg/kg bw/day for a 70 kg adult or 0.98 mg/kg bw/day for a 23 kg child of 3 to 10 years (EFSA, 2012d).

MOS_{POPs} (Adults) = 128 mg/kg bw/day/0.32 mg/kg bw/day = 400

MOS_{POPs} (Children) = 128 mg/kg bw/day/0.98 mg/kg bw/day = 131

Exposure to POPs from the use of *PS-Spreads/Liquids for CB* results in margins of safety of 400 and 131 in adults and children, respectively, which are large enough to account for the inherent uncertainty/variability and inter and intra-species extrapolations, and therefore, further support the safety of *PS-Spreads/Liquids for CB*. It should be noted that this is considered conservative as the lower value for NOEL derived from the subchronic toxicity study has been used.

XIII.4 Safety of Plant Sterols with Respect to Potential Higher Intakes

Prior to launching yellow fat spreads and dairy type foods with added plant sterols, Unilever performed a number of toxicological and human studies to determine the safety of the plant sterol ingredients that were used in those products. Based on the results of these studies, along with the body of evidence supplied by the other applicants and the critical evaluation by EFSA, the European Commission authorised the commercialisation of plant sterol-added foods within the European Community (*e.g.*, 2000/500/EC and 2004/335/EC) (Commission of the European Communities, 2000, 2004c).

Considering that the current application of *PS-Spreads/Liquids for CB* is extending the use to cooking and baking, a higher plant sterol intake may be anticipated. The theoretical worst-case deterministic estimations for consumers with high intakes could result in a total consumption from approved and proposed spread and fat products of about 5.4 g/day for adult consumers (based on the 95th percentile consumption of Dutch adult consumers between the ages of 19 and 30 years (refer to Table IX.2.2-4)

In order to set the stage for the potential increase of intakes in consumers with high intakes from *PS-Spreads/Liquids for CB*, it is prudent to review the latest safety evaluations of plant sterols/stanols and their esters from EFSA and the Joint FAO/WHO Expert Committee on Food Additives (JECFA).

XIII.4.1 Current Position of EFSA and JECFA on the Safety of Plant Sterols

The safety of plant sterols, stanols, and their esters has been extensively evaluated by several scientific authorities, including EFSA and JECFA (EFSA, 2012e; JECFA, 2009). In the most recent of these reviews, EFSA released its most recent evaluation of all the relevant data publically available up to the beginning of 2011 [including the previous opinions of the Scientific Committee on Food (SCF) and EFSA] on plant sterols and stanols that was presented in "*Scientific Opinion on the safety of stigmasterol-rich plant sterols as food additive* (EFSA, 2012c)*.* In this assessment, the Panel reviewed the study by Kelly *et al*. (2011) that evaluated the effects of long-term plant sterol and stanol consumption on retinal vasculature. The Panel concluded that there is not sufficient evidence for the atherogenic effects due to increased phytosterol concentrations as a result of the intake of foods with added plant sterols. This conclusion was on the basis that the effects noted in the study were marginal, and the subjects in the study were undergoing statin treatment that could have rendered them more susceptible to venular changes due to vascular pre-damage. This conclusion echoes that of Food Safety Australia New Zealand (FSANZ), also in 2012 who also concluded that this study "does not warrant cause for concern with regard to the safety of phytosterol/stanol-enriched foods" (FSANZ, 2012a,b).

In 2002, the SCF issued an opinion on the long-term effects of the intake of elevated levels of phytosterols from multiple dietary sources (SCF, 2002). In this opinion, the SCF also considered the potential interference of dietary phytosterols with the absorption of carotenoids, noting that decreases in blood carotenoids may amount to 33% after 1-year consumption of vegetable fat spreads providing 3 g phytosterols per day. As such, it was recommended that diets with natural sources of β-carotene be used to counterbalance the potential reduction of blood β-carotene and other fat-soluble nutrients (*i.e.*, vitamin E and tocopherols) following long-term consumption of foods with added phytosterols. No other health concerns were reported in this opinion. While an upper level of total daily intake of phytosterols was not established, the Committee concluded that in the absence of evidence indicating additional benefits at higher intakes, the intake of phytosterols should not exceed 1 to 3 g/day.

The available scientific data to support the safety of phytosterols, and their corresponding esters were also reviewed by JECFA. At their 69th meeting, JECFA established a group Acceptable Daily Intake (ADI) of 0 to 40 mg free phytosterols/kg body weight. This corresponds to a daily intake of 2.9 g plant sterols/day for a 70 kg individual.

XIII.4.2 Relevant Clinical Study Data

A number of clinical studies have been published, supporting the safe use of plant sterol and stanol intakes of greater than 3 g/day. A summary of these studies is presented in Table XIII.4.2-1. No adverse effects have been attributed to plant sterol and stanol intake in these studies at levels up to 9 g/day.

Table XIII.4.2-1 Summary of Human Studies on Plant Sterols and Stanols at Levels Greater than 3 g/day				
Study Design and Treatment Duration	**Study Population (Sex, Age, BMI or Body Weight, Health Status)**	**Dose of Plant Sterols/Stanols and Delivery Matrix**	**Safety-Related Results**	**Reference**
Studies Conducted in Normocholesterolemic Subjects				
Randomised, double-blind, placebo-controlled, parallel study 3 weeks for M; 4 weeks for F	24 healthy adults (12 M, 12 F; mean age of 36 years; mean BMI of 24 kg/m^2)	0 g/d from control spread (n=12) 8.6 g/d from PE-enriched margarine (n=12)	• NSD in health of the subjects • No clinically significant changes in haematology, clinical chemistry, and urinalysis parameters • NSD in serum estradiol and estrone levels (F) ↓ serum progesterone levels (F); authors did not consider this to be biologically significant	Ayesh *et al*., 1999; Weststrate *et al*., 1999
Randomised, double-blind, placebo-controlled, parallel study 8 weeks	84 healthy adults (46 M, 38 F; mean age of 46 years; mean BW of 79 kg)	0 g/d (n=21) 3 g/d from PE-enriched spread and control salad dressing (n=21) 6 g/d from control spread and PE-enriched salad dressing (n=19) 9 g/d from PE-enriched spread and salad dressing (n=23)	• 7 of 84 subjects dropped out; 6 withdrew consent; 1 reported serious adverse event unrelated to treatment • 62% of subjects reported adverse events; complaints primarily in GI (dyspepsia, diarrhoea, constipation) and respiratory systems • 20 of 52 adverse events related to treatment (flatulence, discoloration of faeces, gastroesophageal reflux, appetite changes, leg cramps, low WBC, skin rash) • No significant between-group differences in number of subjects reporting adverse events • 1 body system (body as a whole) showed significant difference among dose groups in number of adverse events; authors considered unrelated to treatment • No serious adverse events related to treatment • No significant between-group differences in body weight, vital signs, haematology, urinalysis, and fat-soluble vitamins • Significant ↓ in % Δ ALT in 6 g/d compared to control 9 g/d • Significant ↑ in % Δ CK; authors attributed	Davidson *et al*., 2001

Table XIII.4.2-1 Summary of Human Studies on Plant Sterols and Stanols at Levels Greater than 3 g/day				
Study Design and Treatment Duration	**Study Population (Sex, Age, BMI or Body Weight, Health Status)**	**Dose of Plant Sterols/Stanols and Delivery Matrix**	**Safety-Related Results**	**Reference**
			to chance • Significant ↓ in carotenoids; levels remained within normal ranges; authors expected these changes given high lipophilicity of carotenoids • Authors concluded consumption up to 9 g/d is safe and well-tolerated	
Randomised, single-blind, placebo-controlled, parallel study 2 months	108 subjects with metabolic syndrome (60 M, 48 F; 30 to 65 years old; mean age 45 years)	0 or 4 g/day In yogurt beverage (2 g/serving); 2 times/day	• NSD in plasma total antioxidant capacity (as measured by the oxygen radical absorbance capacity and the ferric reducing antioxidant power)	Sialvera *et al.*, 2013
Studies Conducted in Subjects with Hypercholesterolemia				
Randomised controlled study 4 weeks	93 healthy male and female free-living adults (24/group, 12 males and 12 females) with mild hypercholesterolemia, age 18-70 years	0, 3, 6, and 9 g/day of phytostanols (79% sitostanol/16% campestanol) in ester form In margarine or yogurt formulation. Group 1 consumed non PS enriched margarine and yogurt, Group 2 consumed PS enriched margarine. Group 3 consumed PS enriched yogurt. Group 4 consumed both.	• NSD in food intake and body weights between groups • No changes in indicators of kidney or liver function • Dose-dependent decreases in total cholesterol and LDL-cholesterol (17.9% decrease at highest dose) but no changes in HDL-cholesterol or triglycerides. • Effect of diet for α-tocopherol and β-carotene but changes statistically insignificant as where lutein, zeaxanthin and canthaxanthin. Serum sitosterol and campesterol decreased, while sitostanol and campestanol increased slightly.	Mensink *et al.*, 2010
Randomised, placebo-controlled, double-blind study 4 weeks	47 subjects male and female subjects: subjects with borderline-high cholesterol or mild hypercholesterolemia, (n=24) and subjects with normal cholesterol (n=23), age not reported.	Placebo chocolate or chocolates containing plant sterols at 3.6 mg/day	• NSD differences in the analysis of the parameters in blood, urine, physical examinations • No adverse events reported	Hoshino *et al.*, 2012

Table XIII.4.2-1 Summary of Human Studies on Plant Sterols and Stanols at Levels Greater than 3 g/day				
Study Design and Treatment Duration	**Study Population (Sex, Age, BMI or Body Weight, Health Status)**	**Dose of Plant Sterols/Stanols and Delivery Matrix**	**Safety-Related Results**	**Reference**
Randomised, double blind, placebo controlled, parallel study 10 weeks	49 male and female free-living adults (25/group, 6 males, 19 females) with mild to moderate hypercholesterolemia, aged 18 to 75 years	8.8 g of phytostanols per day as ester in spread and oat based drink	• NSD in body weight, blood pressure, diet and haematology parameters, • ↑ alanine aminotransferase (not relevant). • Reduction in total serum cholesterol and LDL-cholesterol by 12.8% and 17.3% from baseline and 12.0 and 17.1% from controls. • Serum carotenoids and fat soluble vitamins were within normal reference values. Serum vitamin A, 25-OH vitamin D and δ-tocopherol levels were unchanged. α and β-carotene were decreased by 40% while α-tocopherol was decreased by 15%.	Gylling *et al.*, 2010

XIII.4.3 Conclusions

The proposed extension of use of Unilever's vegetable fat products with added plant sterols to include cooking and baking applications (*PS-Spreads/Liquids for CB)* will result in a greater probability that median intakes will help reach the desired objective consumption level of 1.5 to 3 g/day of phytosterols. The theoretical worst-case deterministic estimations for consumers with high intakes (95th percentile) could result in a total consumption of about 5.4 g/day Even though the assumed maximum consumption in some population groups of consumers with high intakes could exceed the upper consumption advice of 3 g phytosterols per day; this is not expected to raise a safety concern due to the following points:

1) In practice post-market and consumer data indicate that this is not likely to occur across the population for a sustained period.
2) The products will contain comprehensive labelling to prevent over-consumption of plant sterols among target consumers, and to avoid consumption among non-target consumers of children under 5 years and pregnant and lactating women.
3) The NOAEL in all dietary safety studies has always been the highest dose tested and in most cases the highest dose possible to test without nutritional imbalance in the diet).
4) Recent clinical data continue to support the safe use of plant sterols up to at least 9 g/day.

OVERALL CONCLUSIONS

Unilever is applying for an extension to its current Novel Foods Authorisations for "multi-purpose vegetable fat spreads and liquid vegetable fat based emulsions with added plant sterol esters for cooking and baking applications", referred to as *PS-Spreads/Liquids for CB*. Considering that the intakes of phytosterols are currently below the desirable intake of 1.5 to 3.0 g/day, many consumers may not be achieving a significant cholesterol lowering benefit. Extending the applications of vegetable fat products in the form of a multi-purpose vegetable fat spread (*PS-Spreads for CB*) and a vegetable fat based liquid emulsion (*PS-Liquids for CB*) to cooking and baking is expected to provide the target consumers with more consumption opportunities during the day to reach the desirable intake levels.

The proposed use of Unilever's *PS-Spreads/Liquids for CB* to include cooking and baking applications will result in a greater probability that median intakes will help reach the desired objective consumption level of 1.5 to 3 g/day of phytosterols. The theoretical worst-case deterministic estimations for consumers with high intakes (95th percentile) could result in a total consumption of about 5.4 g/day. Even though the assumed maximum consumption in some population groups of consumers with high intakes could exceed the upper consumption advice of 3 g phytosterols per day; this is not expected to raise a safety concern due to the following points:

1) In practice post-market and consumer data indicate that this is not likely to occur across the population for a sustained period,
2) The products will contain comprehensive labelling to prevent over-consumption of plant sterols among target consumers, and to avoid consumption among non-target consumers of children under 5 years and pregnant and lactating women,
3) The NOAEL in all dietary safety studies has always been the highest dose tested (and in most cases the highest dose that it is possible to test without dietary adjustment), and
4) Recent clinical data support the safety of plant sterols up to at least 9 g/day.

We have demonstrated in normal baking and cooking trials that there are only small increases in the generation of plant sterol oxides (POP) compared to spread products without added plant sterols. These are not considered to be of biological significance.

Toxicology studies conducted on behalf of Unilever with a POC confirm that they do not demonstrate genotoxicity. In a sub-chronic rat study the NOEL for plant sterol oxides was determined to be approximately 128 and 144 mg/kg bw/day for male and female rats, respectively. Exposure to POPs from the use of *PS-Spreads/Liquids for CB* results in margins of safety of 400 and 131 in adults and children respectively, which is sufficiently large to further support the safety of *PS-Spreads/Liquids for CB*.

Although the worse case potential exposure to plant sterols may exceed the precautionary upper consumption advice established by the SCF and EFSA of 3 g/day (Commission of the

European Communities, 2004e)[7], this level represents an extremely conservative approach. Whilst we continue to advocate in line with the current recommendations that intakes of phytosterols should not exceed 3 g/day evidence from toxicological studies and especially clinical data demonstrates that exposure beyond this level does not represent a safety concern.

In conclusion, the data provided show that average phytosterol intakes amongst consumers are often below the desired 1.5 to 3.0 g/day to achieve a significant cholesterol-lowering effect. The authorisation of multi-purpose vegetable fat spreads and liquid vegetable fat based emulsions with added plant sterol esters for cooking and baking applications (referred to as *PS-Spreads/Liquids for CB* in this dossier) will provide consumers who need to lower their cholesterol with more opportunities to achieve the desirable phytosterol intake.

[7] According to Commission Regulation 608/2004/EC, it is a regulatory requirement to inform consumers that the consumption of more than 3 g/day of added plant sterols/plant stanols should be avoided.

GLOSSARY

ADI	Acceptable Daily Intake
bw	Body Weight
CB	Cooking and baking
DH	Department of Health
DNFCS	Netherlands National Food Consumption Survey
EFSA	European Food Safety Authority
EPIC	European Prospective Investigation into Cancer
EU	European Union
FAO	Food and Agriculture Organization
FSA	Food Standards Agency
FSANZ	Food Safety Australia New Zealand
GLP	Good Laboratory Practice
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LDL	Low-density Lipoprotein
MOS	Margin of Safety
NDNS	National Diet and Nutrition Survey
NEVO	Dutch Food Composition Database
NOAEL	No-Observed-Adverse-Effect Level
NOEL	No-Observed-Effect Level
OECD	Organization for Economic Cooperation and Development
PLM	Post Launch Monitoring
POC	Plant Sterol Oxide Concentrate
POPs	Plant Sterol Oxidation Products
PS	Plant Sterol
RIVM	Dutch National Institute for Public Health and the Environment
SCF	Scientific Committee for Food
U.S.	United States
UK	United Kingdom
WHO	World Health Organization
YC	Young Children

REFERENCES

AbuMweis SS, Barake R, Jones PJ. Plant sterols/stanols as cholesterol lowering agents: a meta-analysis of randomized controlled trials. *Food & Nutrition Research* 2008, 52: doi 10.3402/fnr.v3452i3400.1811.

Andersson SW, Skinner J, Ellegård L, Welch AA, Bingham S, Mulligan A, Andersson H, Khaw KT. Intake of dietary plant sterols is inversely related to serum cholesterol concentration in men and women in the EPIC Norfolk population: a cross-sectional study. *European Journal of Clinical Nutrition* 2004, 58: 1378-1385.

Ayesh R, Weststrate JA, Drewitt PN, Hepburn PA. 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* 1999, 37: 1127-1138.

Baumgartner S, Mensink RP, Husche C, Lütjohann D, Plat J. Effects of plant sterol- or stanol-enriched margarine on fasting plasma oxyphytosterol concentrations in healthy subjects. *Atherosclerosis* 2013a, 227: 414-49.

Baumgartner S, Mensink RP, Hartog GD, Bast A, Bekers O, Husche C, Lütjohann D, Plat J. Oxyphytosterol formation in humans; identification of high vs. low oxidizers. *Biochemical Pharmacology* 2013b, Mar 12. doi:pii: S0006-2952(13)00162-7. 10.1016/j.bcp.2013.02.035. [accepted manuscript].

Bundesgesundheitsbericht. Gesundheitsberichterstattung des Bundes. Gesundheit in Deutschland; 2006. Available from: http://www.gbe-bund.de/.

Commission of the European Communities. Commission Recommendation 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 258/97 of the European Parliament and of the Council (97/618/EC) [L253]. *Official Journal of the European Communities* 1997, 40: 1-36. Available from: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CELEX:31997H0618:EN:HTML.

Commission of the European Communities. Commission Decision of 24 July 2000 on authorising the placing on the market of 'yellow fat spreads with added phytosterol esters' as a novel food or novel food ingredient under Regulation (EC) No 258/97 of the European Parliament and of the Council (notified under document number C(2000) 2121) (2000/500/EC) [L200]. *Official Journal of the European Communities* 2000, 43: 59-60. Available from: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2000:200:0059:0060:EN:PDF.

Commission of the European Communities. Commission Decision of 31 March 2004 authorising the placing on the market of yellow fat spreads, salad dressings, milk type products, fermented milk type products, soya drinks and cheese type products with added phytosterols/phytostanols as novel foods or novel food ingredients under Regulation (EC) No 258/97 of the European Parliament and of the Council (notified under document number C(2004) 1243) (2004/333/EC) [L105]. *Official Journal of the European Union* 2004a, 47: 40-42. Available from: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2004:105:0040:0042:EN:PDF.

Commission of the European Communities. Commission Decision of 31 March 2004 authorising the placing on the market of yellow fat spreads, milk type products, yoghurt type products, and spicy sauces with added phytosterols/phytostanols as novel foods or novel food ingredients under Regulation (EC) No 258/97 of the European Parliament and of the Council (notified under document number C(2004) 1244) (2004/334/EC) [L105]. *Official Journal of the European Union* 2004b, 47: 43-45. Available from: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2004:105:0043:0045:EN:PDF.

Commission of the European Communities. Commission Decision of 31 March 2004 authorising the placing on the market of milk type products and yoghurt type products with added phytosterol esters as novel food ingredients under Regulation (EC) No 258/97 of the European Parliament and of the Council (notified under document number C(2004) 1245) (2004/335/EC) [L105]. *Official Journal of the European Union* 2004c, 47: 46-48. Available from: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2004:105:0046:0048:EN:PDF.

Commission of the European Communities. Commission Decision of 31 March 2004 authorising the placing on the market of yellow fat spreads, milk based fruit drinks, yoghurt type products and cheese type products with added phytosterols/phytostanols as novel foods or novel food ingredients under Regulation (EC) No 258/97 of the European Parliament and of the Council (notified under document number C(2004) (2004/336/EC) [L105]. *Official Journal of the European Union* 2004d, 47: 49-51. Available from: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2004:105:0049:0051:EN:PDF.

Commission of the European Communities. Commission Regulation (EC) No 608/2004 of 31 March 2004 concerning the labelling of foods and food ingredients with added phytosterols, phytosterol esters, phytostanols and/or phytostanol esters [L97]. *Official Journal of the European Union* 2004e, 47: 44-45. Available from: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2004:097:0044:0045:EN:PDF.

Council of the European Union. Council Regulation (EC) No 2991/94 of 5 December 1994 laying down standards for spreadable fats [L316]. *Official Journal of the European Communities* 37: 2–7. Available from: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:1994:316:0002:0007:EN:PDF.

Davidson MH, Maki KC, Umporowicz DM, Ingram KA, Dicklin MR, Schaefer E, Lane RW, McNamara JR, Ribaya-Mercado JD, Perrone G, Robins SJ, Franke WC. Safety and tolerability of esterified phytosterols administered in reduced-fat spread and salad dressing to healthy adult men and women. *Journal of the American College of Nutrition* 2001, 20: 307-319.

Demonty I, Ras RT, van der Knaap HC, Duchateau GS, Meijer L, Zock PL, Geleijnse JM, Trautwein EA. Continuous dose-response relationship of the LDL-cholesterol-lowering effect of phytosterol intake [plus supplemental data]. *Journal of Nutrition* 2009, 139: 271-284.

Department of Health. *National Diet and Nutrition Survey Rolling Programme*. Department of Health / Food Standards Agency (FSA), London, UK; 2011. Available from: http://www.dh.gov.uk/en/Publicationsandstatistics/PublishedSurvey/ListOfSurveySince1990/Surveylistlifestyle/DH_128165 [Last modified date: 21 July 2011].

Derewiaka D, Obiedzinski M. Phytosterol oxides content in selected thermally processed products. *European Food Research and Technology = Zeitschrift für Lebensmittel-Untersuchung und -Forschung. A* 2012, 234: 703-712.

Dutta PC, Appelqvist L-A. Studies on phytosterol oxides. I: Effect of storage on the content in potato chips prepared in different vegetable oils. *Journal of the American Oil Chemists' Society* 1997, 74: 647-657.

EFSA. Consumption of Food and Beverages With Added Plant Sterols in the European Union (EFSA/DATEX 03): A Report from the Data Collection and Exposure Unit in Response to a Request from the European Commission [European Food Safety Authority]. *EFSA Journal* 2008a, 133: 1-21. Available from: http://www.efsa.europa.eu/en/scdocs/scdoc/133r.htm.

EFSA. Scientific Opinion of the Panel on Dietetic Products Nutrition and Allergies on a request from Unilever PLC/NV on Plant Sterols and lower/reduced blood cholesterol, reduced the risk of (coronary) heart disease. (Question no EFSA-Q-2008-085, adopted on 11 July 2008 by European Food Safety Authority). *EFSA Journal* 2008b, 781: 1-12. Available from: http://www.efsa.europa.eu/en/efsajournal/pub/781.htm.

EFSA. Scientific Opinion of the Panel on Dietetic Products, Nutrition and Allergies on a request from McNeil Nutritionals Ltd. related to the scientific substantiation of a health claim on plant stanol esters and lower/reduced blood cholesterol and reduced risk of (coronary) heart disease. (Question number: EFSA-Q-2008-118, Adopted: 2 October 2008 by European Food Safety Authority). *EFSA Journal* 2008c, 825, 1-13. Available from: http://www.efsa.europa.eu/en/efsajournal/pub/825.htm.

EFSA. Scientific Opinion of the Panel on Dietetic Products Nutrition and Allergies on a request from the European Commission and a similar request from France in relation to the authorisation procedure for health claims on plant sterols/stanols and lowering/reducing blood LDL-cholesterol pursuant to Article 14 of Regulation (EC) No 1924/2006. (Question no EFSA-Q-2009-00530 and EFSA-Q-2009-00718, adopted on 30 June 2009 by European Food Safety Authority). *EFSA Journal* 2009a, 1175: 1-9. Available from: http://www.efsa.europa.eu/en/efsajournal/pub/1175.htm.

EFSA. Scientific Opinion of the Panel on Dietetic Products, Nutrition and Allergies on a request from Danone France related to the scientific substantiation of a health claim on phytosterols and lowering/reducing blood cholesterol and reduced risk of (coronary) heart disease. (Question no EFSA-Q-2008-779, adopted on 02 July 2009 by European Food Safety Authority). *EFSA Journal* 2009b, 1177: 1-12. Available from: http://www.efsa.europa.eu/en/efsajournal/pub/1177.htm.

EFSA. EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA); Scientific Opinion on the substantiation of health claims related to plant sterols and plant stanols and maintenance of normal blood cholesterol concentrations (ID 549, 550, 567, 713, 1234, 1235, 1466, 1634, 1984, 2909, 3140), and maintenance of normal prostate size and normal urination (ID 714, 1467, 1635) pursuant to Article 13(1) of Regulation (EC) No 1924/2006. (Question no EFSA-Q-2008-1337, EFSA-Q-2008-1500, EFSA-Q-2008-2203, EFSA-Q-2008-2370, EFSA-Q-2008-3642, … *[etc.]*, adopted on 10 September 2010 by European Food Safety Authority). *EFSA Journal* 2010, 8: 1813. [22 pp.]. doi:10.2903/j.efsa.2010.1813. Available from: http://www.efsa.europa.eu/en/scdocs/scdoc/1813.htm.

EFSA. *The EFSA Comprehensive European Food Consumption Database*. European Food Safety Authority (EFSA), Parma, Italy; 2011a. Available from: http://www.efsa.europa.eu/en/datexfoodcdb/datexfooddb.htm.

EFSA. EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA); Scientific Opinion on health claims already evaluated (ID 215, 568, 674, 712, 1398, 1633, 1974, 4191, 4192, 4193, 4236, 4335, 4698, 4704) pursuant to Article 13(1) of Regulation (EC) No 1924/2006. (Question no EFSA-Q-2008-1002, EFSA-Q-2008-1355, EFSA-Q-2008-1461, EFSA-Q-2008-1499, EFSA-Q-2008-2135, … *[etc.]*, adopted on 08 April 2011 by European Food Safety Authority). *EFSA Journal* 2011b, 9: 2203. [22 pp.]. doi:10.2903/j.efsa.2011.2203. Available from: http://www.efsa.europa.eu/en/efsajournal/pub/2203.htm.

EFSA. EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA); Scientific Opinion on the substantiation of a health claim related to 3 g/day plant sterols/stanols and lowering blood LDL-cholesterol and reduced risk of (coronary) heart disease pursuant to Article 19 of Regulation (EC) No 1924/2006. (Question no EFSA-Q-2011-01241, adopted on 26 April 2012 by European Food Safety Authority). *EFSA Journal* 2012a, 10: 2693. [13 pp.]. doi:10.2903/j.efsa.2012.2693. Available from: http://www.efsa.europa.eu/en/efsajournal/pub/2693.htm.

EFSA. EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA); Scientific Opinion on the substantiation of a health claim related to 3 g/day plant stanols as plant stanol esters and lowering blood LDL-cholesterol and reduced risk of (coronary) heart disease pursuant to Article 14 of Regulation (EC) No 1924/2006. (Question no EFSA-Q-2011-00851, adopted on 26 April 2012 by European Food Safety Authority). *EFSA Journal* 2012b 10: 2692. [18 pp.]. doi:10.2903/j.efsa.2012.2692. Available from: http://www.efsa.europa.eu/en/efsajournal/pub/2692.htm.

EFSA. EFSA Panel on Food additives and Nutrient Sources added to Food (ANS); Scientific Opinion on the safety of stigmasterol-rich plant sterols as food additive. (Question no EFSA-Q-2011-00428, adopted on 19 April 2012 by European Food Safety Authority). *EFSA Journal* 2012c, 10: 2659. [39 pp.] doi:10.2903/j.efsa.2012.2659. Available from: http://www.efsa.europa.eu/en/efsajournal/doc/2659.pdf.

EFSA. EFSA Scientific Committee; Guidance on selected default values to be used by the EFSA Scientific Committee, Scientific Panels and Units in the absence of actual measured data. (Question no EFSA-Q-2010-00221, adopted on 8 February 2012 by European Food Safety Authority). *EFSA Journal* 2012d, 10: 2579. [32 pp.] doi:10.2903/j.efsa.2012.2579. Available from: http://www.efsa.europa.eu/en/efsajournal/pub/2579.htm.

EFSA. EFSA Panel on Food additives and Nutrient Sources added to Food (ANS); Scientific Opinion on the safety of stigmasterol-rich plant sterols as food additive. (Question no EFSA-Q-2011-00428, adopted on 19 April 2012 by European Food Safety Authority). *EFSA Journal* 2012e, 10: 2659. [38 pp.] doi:10.2903/j.efsa.2012.2659. Available from: http://www.efsa.europa.eu/fr/efsajournal/pub/2659.htm.

Escurriol V, Marí-Dell'Olmo M, Rohlfs I, Borrell C, Chirlaque MD, Buckland G, Rodriguez L, Sánchez MJ, Amiano P, Egüés N, Ros E. Plant sterol intake and education level in the Spanish EPIC cohort. *Nutrition* 2009, 25: 769-773.

[redacted]

European Commission. Commission Regulation (EU) No 384/2010 of 5 May 2010 on the authorisation and refusal of authorisation of certain health claims made on foods and referring to the reduction of disease risk and to children's development and health [L113]. *Official Journal of the European Union* 2010a, 53: 6-10. Available from: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2010:113:0006:0010:EN:PDF.

European Commission. Commission Regulation (EU) No 376/2010 of 3 May 2010 amending Regulation (EC) No 983/2009 on the authorisation and refusal of authorisation of certain health claims made on food and referring to the reduction of disease risk and to children's development and health [L111]. *Official Journal of the European Union* 2010b, 53: 3-4. Available from: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2010:111:0003:0004:EN:PDF.

European Commission. No. 152. Unilever PLC [Soya drinks with added phytosterols, 2010]. In: Notifications Pursuant to Article 5 of Regulation (EC) N° 258/97 of the European Parliament and of the Council. European Commission (EC), Brussels, Belgium; 2012a. Available from: http://ec.europa.eu/food/food/biotechnology/novelfood/notif_list_en.pdf#page=71.

European Commission. Commission Regulation (EU) No 432/2012 of 16 May 2012 establishing a list of permitted health claims made on foods, other than those referring to the reduction of disease risk and to children's development and health [L136]. *Official Journal of the European Union* 2012, 55: 1-40. Available from: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2012:136:0001:0040:EN:PDF.

European Parliament and the Council of the European Union. Regulation (EC) No 258/97 of the European Parliament and of the Council of 27 January 1997 concerning novel foods and novel food ingredients [L43]. *Official Journal of the European Communities* 1997, 40: 1–6. http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CONSLEG:1997R0258:20090807:EN:PDF [Consolidated version: 2009-08-07].

Expert Panel on Detection Evaluation and Treatment of High Blood Cholesterol in Adults. National Cholesterol Education Program (NCEP): Executive summary of the Third Report of the Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). *Journal of the American Medical Association* 2001, 285: 2486-2497.

FAO. *Fats and Fatty Acids in Human Nutrition. Report of an Expert Consultation*, Nov. 10-14, 2008, Geneva, Switz. (FAO Food and Nutrition Paper no. 91). Food and Agriculture Organization of the United Nations (FAO), Rome, Italy; 2010. Available from: http://www.fao.org/docrep/013/i1953e/i1953e00.pdf.

FSANZ. *Application A1065. Packaging Size for Phytosterol-enriched Milk*. Foods Standards Australia New Zealand (FSANZ), Canberra, Australia / Wellington, NZ; 2012a. Available from: http://www.foodstandards.gov.au/foodstandards/applications/applicationa1065pack5285.cfm.

FSANZ. *Supporting Document 1 - Risk Assessment Report: Application A1065. Packaging Size for Phytosterol-enriched Milk*. Foods Standards Australia New Zealand (FSANZ), Canberra, Australia / Wellington, NZ; 2012b. Available from: http://www.foodstandards.gov.au/_srcfiles/A1065_SD1.pdf.

García-Llatas G, Rodríguez-Estrada MT. Current and new insights on phytosterol oxides in plant sterol-enriched food. *Chemistry and Physics of Lipids* 2011, 164: 607-624.

Gylling H, Hallikainen M, Nissinen MJ, Miettinen TA. The effect of a very high daily plant stanol ester intake on serum lipids, carotenoids, and fat-soluble vitamins. *Clinical Nutrition* 2010, 29: 112-118.

Hearty AP, Duffy E, Gibney MJ. Intake estimates of naturally occurring phytosterols using deterministic and probabilistic methods in a representative Irish population. *International Journal of Food Sciences and Nutrition* 2009, 60: 533-546.

Hepburn, P., Howlett, J., Boeing, H., Cockburn, A., Constable, A., Davi, A., de Jong, N., Moseley, B., Oberdorfer, R., Robertson, C., Wal, J.M., Samuels, F. Review: The application of post-market monitoring to novel foods. *Food and Chemical Toxicology* 2008, 6 9-33.

Hepburn PA, Homer SA, Smith M. Safety evaluation of phytosterol esters: Part 2. Subchronic 90-day oral toxicity study on phytosterol esters: a novel functional food. *Food and Chemical Toxicology* 1999, 37: 521-532.

Hoshino M, Soejima Y, Maeda Y, Ogata T, Shimura S, Kajimoto K, Inoue S. Reduction of serum total cholesterol and LDL-cholesterol by chocolate containing plant sterol in subjects with borderline-high cholesterol or mild hypercholesterolemia —efficacy and safety of long-term intake in a randomized double-blind, placebo-controlled trial. *[Nippon Eiyo Shokuryo Gakkaishi] Journal of Japanese Society of Nutrition and Food Science* 2012, 65: 261-270.

Hovenkamp E, Demonty I, Plat J, Lütjohann D, Mensink RP, Trautwein EA. Biological effects of oxidized phytosterols: a review of the current knowledge. *Progress in Lipid Research* 2008, 47: 37-49.

Jakobsen MU, O'Reilly EJ, Heitmann BL, Pereira MA, Bälter K, Fraser GE, Goldbourt U, Hallmans G, Knekt P, Liu S, Pietinen P, Spiegelman D, Stevens J, Virtamo J, Willett WC, Ascherio A. Major types of dietary fat and risk of coronary heart disease: a pooled analysis of 11 cohort studies. *American Journal of Clinical Nutrition* 2009, 89: 1425-1432.

JECFA. *Toxicological Evaluation of Certain Food Additives*. Prepared by the Sixty-ninth meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), June 17-26-29, 2008, Rome, Italy. (WHO Food Additives Series, no 60). World Health Organization (WHO), Geneva, Switz.; 2009. Available from: http://whqlibdoc.who.int/publications/2009/9789241660600_eng.pdf.

Jenkins DJ, Kendall CW, Popovich DG, Vidgen E, Mehling CC, Vuksan V, Ransom TP, Rao AV, Rosenberg-Zand R, Tariq N, Corey P, Jones PJ, Raeini M, Story JA, Furumoto EJ, Illingworth DR, Pappu AS, Connelly PW. Effect of a very-high-fiber vegetable, fruit, and nut diet on serum lipids and colonic function. *Metabolism* 2001, 50: 494-503.

Jiménez-Escrig A, Santos-Hidalgo AB, Saura-Calixto F. Common sources and estimated intake of plant sterols in the Spanish diet. *Journal of Agricultural and Food Chemistry* 2006, 54: 3462-3471.

Katan MB, Grundy SM, Jones P, Law M, Miettinen T, Paoletti R. Efficacy and safety of plant stanols and sterols in the management of blood cholesterol levels. *Mayo Clinic Proceedings* 2003, 78: 965-978.

Kelly ER, Plat J, Mensink RP, Berendschot TT. Effects of long term plant sterol and -stanol consumption on the retinal vasculature: a randomized controlled trial in statin users. *Atherosclerosis* 2011, 214: 225-230.

Keys A, Anderson JT, Grande F. Prediction of serum-cholesterol responses of man to changes in fats in the diet. *Lancet* 1957, 270: 959-966.

Klingberg S, Andersson H, Mulligan A, Bhaniani A, Welch A, Bingham S, Khaw KT, Andersson S, Ellegård L. Food sources of plant sterols in the EPIC Norfolk population. *European Journal of Clinical Nutrition* 2008a, 62: 695-703.

Klingberg S, Ellegård L, Johansson I, Hallmans G, Weinehall L, Andersson H, Winkvist A. Inverse relation between dietary intake of naturally occurring plant sterols and serum cholesterol in northern Sweden. *American Journal of Clinical Nutrition* 2008b, 87: 993-1001.

Lea LJ, Hepburn PA. Safety evaluation of phytosterol-esters. Part 9: results of a European post-launch monitoring programme. *Food and Chemical Toxicology* 2006, 44: 1213-1222.

Lea LJ, Hepburn PA, Wolfreys AM, Baldrick P. Safety evaluation of phytosterol esters. Part 8. Lack of genotoxicity and subchronic toxicity with phytosterol oxides. *Food and Chemical Toxicology* 2004, 42: 771-783.

Louter A, Bauer-Plank C, de Groot W. Characterisation of phytosterol oxidation products in functional foods and functional food ingredients. Presented at: *The 93rd American Oil Chemists' Society Annual Meeting and Expo*, May 5-8, Montreal, Canada. Book of abstracts. American Oil Chemists' Society (AOCS), Champaign (IL); 2002, p. 11. Cited In: Lea et al., 2004.

McNeil Nutritionals Ltd. *Benecol® – The Range*. McNeil Nutritionals Ltd., Buckingham, Engl.; 2012a. Available from: http://www.benecol.co.uk/our-food-range/category [© McNeil Nutritionals Ltd. 2002 – 2012].

McNeil Nutritionals Ltd. *Benecol® – Tasty Recipes, Baking*. McNeil Nutritionals Ltd., Buckingham, Engl.; 2012b. Available from: http://www.benecol.co.uk/tasty-recipes/baking [© McNeil Nutritionals Ltd. 2002 – 2012].

McNeil Nutritionals Ltd. *Benecol® – Tasty Recipes, Light Bites*. McNeil Nutritionals Ltd., Buckingham, Engl.; 2012c. Available from: http://www.benecol.co.uk/tasty-recipes/light-bites [© McNeil Nutritionals Ltd. 2002 – 2012].

Mensink RP, de Jong A, Lütjohann D, Haenen GRMM, Plat J. Plant stanols dose-dependently decrease LDL-cholesterol concentrations, but not cholesterol-standardized fat-soluble antioxidant concentrations, at intakes up to 9 g/d. *The American Journal of Clinical Nutrition* 2010, 92: 24-33.

Moreau RA, Whitaker BD, Hicks KB. Phytosterols, phytostanols, and their conjugates in foods: structural diversity, quantitative analysis, and health-promoting uses. *Progress in Lipid Research* 2002, 41: 457-500.

Mozaffarian D, Micha R, Wallace S. Effects on coronary heart disease of increasing polyunsaturated fat in place of saturated fat: a systematic review and meta-analysis of randomized controlled trials. *PLoS Medicine* 2010, 7, e1000252. doi: 10.1371/journal.pmed.1000252.

Musa-Veloso K, Poon TH, Elliot JA, Chung C. A comparison of the LDL-cholesterol lowering efficacy of plant stanols and plant sterols over a continuous dose range: Results of a meta-analysis of randomized, placebo-controlled trials. *Prostaglandins, Leukotrienes and Essential Fatty Acids* 2011, 85: 9-28.

Netherlands Preliminary Advisory Committee on the Safety of Novel Foods. *Initial assessment of an application according to article 4 of regulation 258/97 of January 27th 1997, of the European Parliament and Council- Unilever: use of phytosterol-esters in yellow fat spreads.* (No GZB/VVB-985999). Provisional Committee for the Safety Evaluation of Novel Foods (VCVNV) [for the Minister of Health, Welfare and Sport], The Hague, The Netherlands; 1998.

Normén AL, Brants HA, Voorrips LE, Andersson HA, van den Brandt PA, Goldbohm RA. Plant sterol intakes and colorectal cancer risk in the Netherlands Cohort Study on Diet and Cancer. *American Journal of Clinical Nutrition* 2001, 74: 141-148.

Ocké MC, van Rossum CTM, Fransen HP, Buurma EM, de Boer EJ, Brants HAM, Niekerk EM, van der Laan JD, Drijvers JJMM, Ghameshlou Z. *Dutch National Food Consumption Survey Young Children 2005/2006. [Voedselconsumptiepeiling bij peuters en kleuters 2005/2006*]. (RIVM Report 350070001/2008). Rijksinstiuut Voor Volksgezondheid en Milieu (RIVM), Bilthoven, The Netherlands; 2008. Available from: http://www.rivm.nl/bibliotheek/rapporten/350070001.html.

OECD. Bacterial reverse mutation test. In: *OECD Guidelines for the Testing of Chemicals*. (OECD Guideline no 471) [Updated & Adopted 21st July 1997]. Organisation for Economic Co-Operation and Development (OECD), Paris, France; 1997a. Available from: http://www.oecd-ilibrary.org/environment/test-no-471-bacterial-reverse-mutation-test_9789264071247-en;jsessionid=1ijgh8o34k0qd.delta.\

OECD. *In vitro* mammalian chromosome aberration test. In: *OECD Guidelines for the Testing of Chemicals*. (OECD Guideline no 473) [Updated & Adopted 21st July 1997]. Organisation for Economic Co-operation and Development (OECD), Paris, France; 1997b. Available from: http://www.oecd-ilibrary.org/environment/test-no-473-in-vitro-mammalian-chromosome-aberration-test_9789264071261-en.

OECD. Repeated dose 90-day oral toxicity study in rodents. In: *OECD Guidelines for the Testing of Chemicals*. (OECD Guideline no 408) [Updated & Adopted 21st September 1998]. Organisation for Economic Co-Operation and Development (OECD), Paris, France; 1998. Available from: http://www.oecd-ilibrary.org/environment/test-no-408-repeated-dose-90-day-oral-toxicity-study-in-rodents_9789264070707-en;jsessionid=1m6bvwspup322.delta.

Perk J, De Backer G, Gohlke H, Graham I, Reiner Z, Verschuren M, Albus C, Benlian P, Boysen G, Cifkova R, Deaton C, Ebrahim S, Fisher M, Germano G, Hobbs R, Hoes A, Karadeniz S, Mezzani A, Prescott E, Ryden L, Scherer M, Syvänne M, Scholte op Reimer WJ, Vrints C, Wood D, Zamorano JL, Zannad F. European Guidelines on cardiovascular disease prevention in clinical practice (version 2012). The Fifth Joint Task Force of the European Society of Cardiology and Other Societies on Cardiovascular Disease Prevention in Clinical Practice (constituted by representatives of nine societies and by invited experts). Developed with the special contribution of the European Association for Cardiovascular Prevention & Rehabilitation (EACPR). *European Heart Journal* 2012, 33: 1635-1701.

Plat J, Mackay D, Baumgartner S, Clifton PM, Gylling H, Jones PJ. Progress and prospective of plant sterol and plant stanol research: report of the Maastricht meeting. *Atherosclerosis* 2012, 225: 521-533.

Reiner Z, Catapano AL, De Backer G, Graham I, Taskinen MR, Wiklund O, Agewall S, Alegria E, Chapman MJ, Durrington P, Erdine S, Halcox J, Hobbs R, Kjekshus J, Filardi PP, Riccardi G, Storey RF, Wood D; ESC Committee for Practice Guidelines (CPG) 2008-2010 and 2010-2012 Committees. ESC/EAS Guidelines for the management of dyslipidaemias: the Task Force for the management of dyslipidaemias of the European Society of Cardiology (ESC) and the European Atherosclerosis Society (EAS). *European Heart Journal* 2011, 32: 1769-1818.

RIVM. *Nederlands Voedingsstoffenbestand (NEVO) [Dutch Food Composition Database].* Rijksinstiuut Voor Volksgezondheid en Milieu (RIVM), Bilthoven, The Netherlands; 2011. Available from: http://nevo-online.rivm.nl/.

SCF. *Opinion of the Scientific Committee on Food on a Request for the Safety Assessment of the use of Phytosterol Esters in Yellow Fat Spreads (Expressed on 6 April 2000)*. (SCF/CS/NF/DOS/1 Final). Brussels Belgium: European Commission, Health & Consumer Protection Directorate-General, Scientific Committee on Food (SCF), Brussels, Belgium; 2000. Available at: http://ec.europa.eu/food/fs/sc/scf/out56_en.pdf.

SCF. *General View on the Long-Term Effects of the Intake of Elevated Levels of Phytosterols from Multiple Dietary Sources, With Particular Attention to the Effects on β-Carotene. (expressed on 26 September 2002)*. (SCF/CS/NF/DOS/20 ADD 1 Final). European Commission, Health & Consumer Protection Directorate-General, Scientific Committee on Food (SCF), Brussels, Belgium; 2002. Available from: http://ec.europa.eu/food/fs/sc/scf/out144_en.pdf

Sialvera TE, Koutelidakis AE, Richter DJ, Yfanti G, Kapsokefalou M, Micha R, Goumas G, Diamantopoulos E, Zampelas A. Phytosterol supplementation does not affect plasma antioxidant capacity in patients with metabolic syndrome. *International Journal of Food Sciences and Nutrition* 2013, 64: 21-27.

Sioen I, Matthys C, Huybrechts I, Van Camp J, De Henauw S. Consumption of plant sterols in Belgium: estimated intakes and sources of naturally occurring plant sterols and β-carotene. *British Journal of Nutrition* 2011a, 105: 960-966.

Sioen I, Matthys C, Huybrechts I, Van Camp J, De Henauw S. Consumption of plant sterols in Belgium: consumption patterns of plant sterol-enriched foods in Flanders, Belgium. *British Journal of Nutrition* 2011b, 105: 911-918.

Smith LL. Cholesterol autoxidation 1981-1986. *Chemistry and Physics of Lipids* 1987, 44: 87-125.

Smith LL. Review of progress in sterol oxidations: 1987-1995. *Lipids* 1996, 31: 453-487.

Smith LL, Teng JI, Lin YY, Seitz PK, McGehee MF. Sterol metabolism--XLVII. Oxidized cholesterol esters in human tissues. *Journal of Steroid Biochemistry* 1981, 14: 889-900.

Tolonen H, Keil U, Ferrario M, Evans A; WHO MONICA Project. Prevalence, awareness and treatment of hypercholesterolaemia in 32 populations: results from the WHO MONICA Project. *International Journal of Epidemiology* 2005, 34: 181-192.

UKDA. *National Diet and Nutrition Survey, 2008-2010 [computer file]*, 3rd ed. (SN: 6533). UK Data Archive (UKDA) [distributor], Colchester, Essex; 2012. Available from: http://dx.doi.org/10.5255/UKDA-SN-6533-2.

Valsta LM, Lemström A, Ovaskainen ML, Lampi AM, Toivo J, Korhonen T, et al. Estimation of plant sterol and cholesterol intake in Finland: quality of new values and their effect on intake. *British Journal of Nutrition* 2004, 92: 671-678.

van Rossum CTM, Fransen HP, Verkaik-Kloosterman J, Buurma-Rethans EJM, Ocké MC. *Dutch National Food Consumption Survey 2007-2010: Diet of children and adults aged 7 to 69 years. [Nederlandse voedselconsumptiepeiling 2007-2010 : Voeding van kinderen en volwassenen van 7 tot 69 jaar]*. (RIVM rapport 350050006). Rijksinstiuut Voor Volksgezondheid en Milieu (RIVM), Bilthoven, The Netherlands; 2011. Available from: http://www.rivm.nl/bibliotheek/rapporten/350050006.html.

Vuoristo M, Miettinen TA. Absorption, metabolism, and serum concentrations of cholesterol in vegetarians: Effects of cholesterol feeding. *American Journal of Clinical Nutrition* 1994, 59: 1325-1331.

Weihrauch JL, Gardner JM. Sterol content of foods of plant origin. *Journal of the American Dietetic Association* 1978, 73: 39-47.

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

Wolfreys AM, Hepburn PA. Safety evaluation of phytosterol esters. Part 7. Assessment of mutagenic activity of phytosterols, phytosterol esters and the cholesterol derivative, 4-cholesten-3-one. *Food and Chemical Toxicology* 2002, 40: 461-470.