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GRAS Notice (GRN) No. 438
http://www.fda.gov/Food/FoodIngredientsPackaging/GenerallyRecognizedasSafeGRAS/GRASListings/default.htm

ORIGINAL SUBMISSION

RAISIO

SENT *VIA* FEDEX
June 27, 2012
Mary Ditto, Ph.D.
Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
Food And Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740-3835

RECEIVED
JUL 11 2012
Division of
Biotechnology and
GRAS Notice Review

Re: GRAS Notification for Plant Stanol Ester

Dear Dr. Ditto,

In accordance with 21 CFR §170.36 [Notice of a claim for exemption based on a Generally Recognized As Safe (GRAS) determination] published in the *Federal Register* [62 FR 18938 (17 April 1997)], I am submitting in triplicate, as the Notifier [Raisio Nutrition Ltd., Benecol Division, P.O. Box 101 (Raisionkaari 55), FI21201 Raisio, Finland], a Notice of the determination, on the basis of scientific procedures, that plant stanol ester distributed by Raisio, as defined in the enclosed documents, is GRAS under specific conditions of use as an ingredient in multiple food categories, and therefore, is exempt from the premarket approval requirements of the *Federal, Food, Drug and Cosmetic Act*. Information setting forth the basis for the GRAS determination, includes a comprehensive summary of the data available that has been reviewed by an independent panel of experts (the Expert Panel) qualified by scientific training and experience to evaluate the safety of plant stanol ester in traditional food products.

Should you have any questions or concerns regarding this GRAS Notice, please do not hesitate to contact me at any point during the review process so that we may provide a response in a timely manner.

Sincerely,

(b) (6)

Ingmar Wester
R&D Director
Raisio Nutrition Ltd.

000009

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GRAS Exemption Claim for Plant Stanol Ester

Submitted to:

Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD
U.S.A. 20740-3835

Submitted by:

Raisio Nutrition Ltd., Benecol Division
P.O. Box 101 (Raisionkaari 55)
FI-21201 Raisio
Finland

June 18, 2012

GRAS Exemption Claim for Plant Stanol Ester

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I GRAS EXEMPTION CLAIM

A. Claim of Exemption From the Requirement for Premarket Approval Pursuant to Proposed 21 CFR §170.36(c)(1) [62 FR 18938 (17 April 1997)]

Raisio Nutrition Ltd. (Raisio) has determined that plant stanol ester is Generally Recognized as Safe (GRAS) under the conditions of intended use in food as described in Section I.D. below, consistent with Section 201(s) of the *Federal Food, Drug, and Cosmetic Act* (FFDCA); therefore, the use of plant stanol ester in food and beverages as described herein is exempt from the requirement of premarket approval.

Signed,

(b) (6)

June 18, 2012

Ingmar Wester
R&D Director
Raisio Nutrition Ltd.
Finland

June 18, 2012

B. Name and Address of Notifier

Ingmar Wester
R&D Director
Raisio Nutrition Ltd.
Benecol Division
P.O. Box 101 (Raisionkaari 55)
FI-21201 Raisio
Finland

C. Common Name of the Notified Substance

Plant stanol ester

D. Conditions of Intended Use in Food

Plant stanol ester manufactured by Raisio is GRAS for addition to spreads, dressings, and snack bars at a use level of 1.0 g of plant stanols (or 1.7 g of plant stanol ester) per reference amount customarily consumed (RACC) (U.S. FDA, 1999a,b,c). Raisio intends to expand the use of its plant stanol ester to include the following additional food categories: baked goods and baking mixes; beverages and beverage bases; breakfast cereals; cheeses; coffee and tea; dairy product analogs; fats and oils; grain products and pastas; gravies and sauces; milk; milk products; processed fruits and fruit juices; processed vegetables and vegetable juices; soft candy; and soups and soup mixes. Use levels of plant stanols in these products will range

candy; and soups and soup mixes. Use levels of plant stanols in these products will range between 0.5 to 2.0 g of plant stanols, provided as a plant stanol ester, per RACC as described in Table I-1. The minimum use-level of 0.5 g of plant stanols (delivered as plant stanol ester) per RACC was chosen based on the Agency's Proposed Rule for the authorized health claim related to "phytosterols"[1] and reduced risk of coronary heart disease (CHD), wherein it is explicitly stated that "*...the agency is proposing to amend 21 CFR § 101.83(c)(2)(iii)(A) to permit health claims on foods that contain at least 0.5 g per RACC of phytosterols, expressed as the weight of nonesterified phytosterols*" (U.S. FDA, 2012a).

Table I-1 Summary of the Individual Proposed Food-Uses and Use-Levels for Plant Stanols Provided as Plant Stanol Ester) in the United States (2003-2004, 2005-2006 NHANES Data)

Food Category	Proposed Food-Uses	Serving Size (g or mL)[a]	Plant Stanols Use Level[b]	
			mg/Serving[c]	%
Baked Goods and Baking Mixes	Bagels	55 g	500	0.91
	Biscuits	55 g	500	0.91
	Breads and Rolls	50 g	500	1.0
	Low-Fat or Calorie Reduced Cookies and Brownies	30 g (cookies) 40 g (brownies)	1,000 500	3.34 1.25
	Cornbread, Corn Muffins, and Tortillas	55 g	500	0.91
	Crackers	30 g	500	1.67
	English Muffins	55 g	500	0.91
	Muffins	55 g	500	0.91
	Quick Breads	55 g	500	0.91
Beverages and Beverage Bases	Sports, Energy, and Isotonic Drinks (including enhanced waters)	240 mL	500	0.21
	Meal Replacement Beverages (Slimming-Type)	240 mL	1,000	0.42
Breakfast Cereals	Instant and Regular Hot Breakfast Cereals	240 mL	2,000	0.80
	Ready-to-Eat Breakfast Cereals (Adult-oriented)	15 g 30 g 55 g	1,000	6.67 3.33 1.82
Cheeses	Imitation Cheese	30 g	1,000	3.33
	Low-Fat or Calorie Reduced Natural Cheese and Cream Cheese	5 to 110 g	500	0.45 to 10.0
	Processed Cheese and Spreads	30 g	1,000	3.33
Coffee and Tea	Instant Coffee	240 mL	1,000	0.42
	Instant Tea	240 mL	1,000	0.42
Dairy Product Analogs	Coffee Whiteners	2 g (powder) or 15 mL (liquid)	500	25.0 or 3.33
	Non-Dairy Milk and Imitation Cream	240 mL	2,000	0.83

[1] The term "phytosterols" was used by the Agency to refer collectively to plant stanols and plant sterols.

Table I-1 Summary of the Individual Proposed Food-Uses and Use-Levels for Plant Stanols Provided as Plant Stanol Ester) in the United States (2003-2004, 2005-2006 NHANES Data)

Food Category	Proposed Food-Uses	Serving Size (g or mL)[a]	Plant Stanols Use Level[b]	
			mg/Serving[c]	%
Fats and Oils	Fat-Based Sauces	55 g	500	0.91
	Margarine, and Margarine-Like Spreads	15 mL	1,000	6.67
	Mayonnaise and Mayonnaise-Type Dressings	15 g	1,000	6.67
	Salad Dressings	30 g	1,000	3.34
	Vegetable Oil	15 mL	500	3.33
Grain Products and Pastas	Cereal and Grain-Based Bars	40 g	2,000	5.00
	Meal Replacement Bars	40 g	1,000	2.5
	Pasta and Noodle Products	140 g (prepared)	1,000	0.71
Gravies and Sauces	Tomato-Based Sauces	125 g	500	0.40
	White Sauces and Milk Gravies	60 mL	500	0.83
Milk	Reduced Fat Milks	240 mL	1,000	0.42
Milk Products	Cream	15 mL	500	3.33
	Flavored Milk and Milk-Type Drinks	240 mL	1,000	0.42
	Sour Cream	30 g	500	1.67
	Yogurt	225 g	2,000	0.89
	Yogurt Drinks[d]	240 g	2,000	0.83
	Yogurt Shot[e]	65 mL - 150 mL	2,000	1.33
Processed Fruits and Fruit Juices	Fruit-Flavored Drinks and Ades	240 mL	1,000	0.42
	Fruit Juices	240 mL	1,000	0.42
	Fruit Smoothies	240 mL	2,000	0.83
	Juice Shot[e]	65 mL - 150 mL	2,000	1.33
	Nectars	240 mL	2,000	0.83
Processed Vegetables and Vegetable Juices	Vegetable Juices	240 mL	2,000	0.83
Soft Candy	Low-Fat or Calorie Reduced Chocolate Bars	40 g	500	1.25
	Soft Candy	5[f] g	500	10.0
Soups and Soup Mixes	Prepared Soups	245 g	1,000	0.41

[a] Serving sizes based on the US FDA Reference amounts customarily consumed per eating occasion (RACCs), April 1, 2011, 21 CFR §101.12 (U.S. FDA, 2012b). When a range of values is reported for a proposed food-use, particular foods within that food-use may differ with respect to their RACC.
[b] Use levels represent maximum/up to amounts.
[c] Plant stanols per serving, resulting from the addition of plant stanol ester to the food.
[d] No food codes were identified for yogurt drinks in the NHANES 2003-2004, 2005-2006 databases; therefore, food codes of dairy-based fruit smoothie drinks were selected as surrogates for yogurt drinks.
[e] No food codes for juice shots and yogurt shots were available in the NHANES 2003-2004, 2005-2006 databases.
[f] No RACC exists for these products; thus, the serving size was based on commercially available chews.

E. Basis for the GRAS Determination

Pursuant to Title 21, Section 170.30 of the *Code of Federal Regulations* (CFR) (U.S. FDA, 2012c), the proposed food and beverage uses of plant stanol ester as described in Table I-1, have been determined to be GRAS on the basis of scientific procedures. This GRAS determination is based on data generally available in the public domain pertaining to the safety of plant stanols and plant stanol ester, as discussed herein, and on consensus among a panel of experts (the Expert Panel) who are qualified by scientific training and experience to evaluate the safety of plant stanol ester as a component of food [see Appendix A, entitled "Expert Panel Report on the Generally Recognized as Safe (GRAS) Use of Plant Stanol Ester as a Direct Food Ingredient in Multiple Food and Beverage Products"].

The Expert Panel consisted of the following qualified scientific experts: G. Harvey Anderson Ph.D. (University of Toronto); Samuel M. Cohen, M.D., Ph.D. (University of Nebraska Medical Center); Stephen L. Taylor Ph.D. (University of Nebraska); and Gilbert R. Thompson M.D., FRCP (Imperial College, London). The Expert Panel, convened on behalf of Raisio, independently and collectively, critically evaluated all data and information presented herein and concluded that the proposed food uses of plant stanol ester described in Table I-1, meeting appropriate food-grade specifications and manufactured according to current Good Manufacturing Practice (cGMP), are GRAS based on scientific procedures.

Food uses of plant stanol ester as described herein are therefore excluded from the definition of a food additive, and thus, may be marketed and sold for its intended purpose in the U.S. without the promulgation of a food additive regulation under Title 21 of the CFR.

F. Availability of Information

The data and information that serve as the basis for this GRAS Notification will be sent to the U.S. Food and Drug Administration (FDA) upon request, or will be available for review and copying at reasonable times at the offices of:

Raisio Nutrition Ltd., Benecol Division
P.O. Box 101 (Raisionkaari 55)
FI-21201 Raisio
Finland

Should the FDA have any questions or additional information requests regarding this notification, Raisio will supply these data and information.

II. DETAILED INFORMATION ON CHEMISTRY AND MANUFACTURING OF PLANT STANOL ESTER

Detailed information on the chemical identity, structure and physical characteristics, manufacturing, product specifications, purity and stability of Raisio's plant stanol ester have been previously reviewed (U.S. FDA, 1999a). All raw materials and processing-aids used in the manufacture of Raisio's plant stanol ester are suitable food grade materials that are GRAS or permitted by U.S. regulation for use in the manufacture of plant stanol ester.

A. Identity

In the academic community and the fats and oils industry, plant stanol ester is also commonly referred to as "plant stanol fatty acid ester", "stanol ester", "phytostanol fatty acid ester" or "phytostanol ester". Plant sterols are naturally occurring compounds that are analogous to cholesterol in animals. More than 40 plant sterols have been identified; sitosterol, campesterol and stigmasterol are the most abundant and food products containing these compounds have also been marketed. Plant stanols are saturated sterols, with sitostanol and campestanol being the primary forms. Plant stanols are typically esterified with fatty acids that range in chain length from C12 to C28 to enhance their solubility in foods and dispersion in the intestines.

The physical characteristics of any batch of plant stanol ester are determined primarily by the fatty acid profile of a given batch, which, in turn, is dependent on the fatty acid source used for esterification. Raisio's plant stanol ester exhibits a polymorphic behavior similar to that of normal crystallizing triglyceride fats, and the basic procedures used for handling and producing products made from plant stanol ester are those used for normal fats. Plant stanol esters manufactured by Raisio are esterified with fatty acids derived from any food grade and permitted vegetable oil or vegetable oil blend. Fatty acid blends with reduced content of saturated fatty acids, *e.g.* sunflower oil fatty acids with reduced content of saturated fatty acids and with high polyunsaturated fatty acid content, resulting in a plant stanol ester that is a creamy white solid or a pale yellow liquid at room temperature. The plant stanol/plant sterol moiety of the plant stanol fatty acid ester blend has a composition of >94.0% by weight of total stanols (*i.e.,* sitostanol and campestanol) and <6.0% by weight unsaturated sterols. Campestanol does not exceed 32%, by weight, of the total plant stanol content.

B. Method of Manufacture

Plant stanols are prepared by common hydrogenation methods employed by the food industry using commercially available plant sterols, derived from vegetable oil or wood sources (also called tall oil sterols), or blends thereof, using a palladium catalyst. These plant stanols are then esterified with fatty acid alcohol ester isolated from vegetable oils, fats or their blends and added to various food matrices.

Raisio's plant stanol ester ingredient is manufactured using cGMP, using suitable food grade ingredients and processing-aids that have previously been determined to be GRAS, or are permitted by U.S. regulation for their use in the manufacture of plant stanol ester in the U.S. The current manufacturing process for Raisio's plant stanol ester is consistent with that previously described (U.S. FDA, 1999a). Two additional processing-aids (activated carbon and sodium acetate) have been incorporated into the saturation step for optimization of the manufacturing process. Activated carbon is used as an adsorbent to remove trace impurities from plant sterols prior to the saturation process. The used activated carbon is effectively removed by filtration and thus is not present in the finished plant stanol ester product. Sodium acetate is used as a buffering agent to adjust the saturation reaction mixture pH and is removed during the palladium catalyst filtration and esterification washing step, and thus is not present in the finished plant stanol ester product. Overall, no changes to the manufacturing process have been applied since the original GRAS determination that would result in significant material changes to Raisio's plant stanol ester ingredient.

Finally, Raisio may produce a powdered plant stanol ester by conventional spray-drying techniques, using food grade carriers (*e.g.,* maltodextrin) and food-grade proteins (*e.g.,* sodium caseinate) permitted for use in food in the United States.

C. Specifications for Plant Stanol Ester

Current product specifications and recent batch analyses for 3 non-consecutive lots of plant stanol ester demonstrating compliance with the product specifications are presented below in Tables II-1 and II-2, respectively.

Table II-1 Product Specifications for Raisio's Plant Stanol Ester		
Parameter	**Specification**	**Method**
Physical Properties		
Appearance	Pale Yellow (liquid)/creamy white (solid)	Visual
Flavor	Bland	Organoleptic
Chemical Composition		
Free sterols	≤ 3%	Raisio BD 1100 or equal SPE-method, GC
Total stanols[a]	≥ 56%	Raisio BD 1121 or equal Method modified from ISO6799, IUPAC 2.401 and IUPAC 2.403, GC
Campestanol	Max 32% (of total stanols)	Raisio BD 1121 or equal
Sitostanol	Min 68% (of total stanols)	Raisio BD 1121 or equal
Free fatty acids[b]	≤ 0.1 %	AOCS Ca 5a-40
Peroxide value	max 0.5 meq/kg	AOCS Cd 8b-90
Moisture	max 0.1 weight-%	AOCS Ca 2e-84
Residual Solvents (*e.g.*, 1-propanol)[c]	≤50 ppm	Internal method, GC-MS

AOCS = American Oil Chemists' Society; GC = Gas Chromatography; ISO = International Standards Organization; IUPAC = International Union of Pure and Applied Chemistry; MS= mass spectrometry; SPE = Solid Phase Extraction. Impurities are measured on a periodic basis according to an internal monitoring program
[a] Derived from both free and esterified sitostanol and campestanol.
[b] Equivalent to acid value of max. 0.2 mg KOH/g.
[c] ≤50 ppm either singly or in combination.

Table II-2 Batch Analyses of Raisio's Plant Stanol Ester				
Parameter	**Specification**	**Plant Stanol Ester Lot No.**		
		EP054B11	**EP073B11**	**EP018B11**
Physical Properties				
Appearance	Pale Yellow (liquid)/ creamy white (solid)	Complies	Complies	Complies
Flavor	Bland	Complies	Complies	Complies
Chemical Composition[a]				
Free sterols	≤ 3 %	0.5	0.6	0.6
Total stanols[b]	≥ 56 %	56.5	56.7	57.5
Campestanol	Max 32 % (of total stanols)	26.7	29.8	26.4
Sitostanol	Min 68 % (of total stanols)	73.3	70.3	73.7
Free fatty acids[c]	≤ 0.1 %	0.0	0.0	0.0
Peroxide value	max 0.5 meq/kg	0.0	0.0	0.0
Moisture	max 0.1 %	0.02	0.01	0.01
1-propanol[d]	≤50 ppm	<10	<10	<10

[a] Data presented in this table represents plant stanol ester esterified with canol oil fatty acids
[b] Derived from both free and esterified sitostanol and campestanol.
[c] Equivalent to acid value of max. 0.2 mg KOH/g.
[d] 1-propanol analyses conducted on lots 410085, 411099, 111003.

D. Specifications for Food Grade Plant Stanol Ester Formulations

As some food uses of Raisio's plant stanol ester are not conducive to the incorporation of plant stanol ester in liquid oil or solid fat formats. Raisio therefore may manufacture plant stanol ester as a powder formulation, which is a spray-dried powder mixture of plant stanol ester and a various food-grade carriers. Raw materials used for the production of plant stanol ester powder include maltodextrin, sodium caseinate, emulsifiers, anti-caking agents, and antioxidants. All ingredients and additives used in the production of Raisio's plant stanol ester powder are food grade ingredients that are GRAS or permitted for use in food in accordance with U.S. regulations. Raisio understands that addition of sodium caseinate to the ingredient will require allergenicity labeling for milk in accordance with Food Allergen Labeling and Consumer Protection Act of 2004 (FALCPA). Product specification for Raisio's plant stanol ester is displayed below in Table II-3.

Table II-3 Product Specifications for Raisio's Plant Stanol Ester Powder

Parameter	Specification	Method
Physical Properties		
Appearance	Creamy white free flowing powder	Visual
Flavor	Bland	Organoleptic
Chemical Composition wet weight		
Total Plant Stanols[a,b]	≥ 38% of product	Raisio BD 1121 or equal
Moisture	≤5% of product	AOCS Ca2e-84
Chemical Composition dry weight		
Plant stanol ester Of which: Plant stanols[a,b] Plant sterols[a] Fatty acids	Approx. 70% ≥ 38% ≤3 Approx. 29	
Carrier	Approx 30%	
Microbiological Quality		
Total aerobic plate count	Max. 5000 / 1 g	ISO 4833:1991 or equal
Yeast and moulds	Max. 300 / 1 g	ISO 7954:1987 o equal
Escherichia coli	Negative / 1 g	ISO 16649-1 or 2 or equal
Coliforms	Negative / 1 g	EN/ISO 9308-1:2001 or equal
Salmonella	Negative / 25 g	EN/ISO 6579:2002 or equal

AOCS = American Oil Chemists' Society; ISO = International Standards Organization; EN = European Standards
[a] is esterified and free forms
[b] sitostanol + campestanol

III. SELF-LIMITING LEVELS OF USE

The use of plant stanol ester in food and beverages is self-limiting by the need to balance the fat and oil contents of foods for maintenance of desired organoleptic and textural qualities to the finished products.

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IV. BASIS FOR GRAS DETERMINATION

The GRAS uses of plant stanol ester described herein was determined through scientific procedures, on the basis of information provided by published studies characterizing the metabolic fate, animal toxicity, estrogenic activity, mutagenicity/genotoxicity, and safety of plant stanol ester in human subjects. Plant stanol ester has a long-history of use in food, and history of safe use of plant stanols and plant stanol ester in food and beverage products globally was considered supportive of Raisio's GRAS determination. Data and information pivotal to the GRAS determination of the uses of plant stanol ester in spreads, dressings, and snack bars has been previously reviewed (U.S. FDA, 1999a); this information remains relevant to the amended GRAS uses of Raisio's plants stanol ester as described in Table I-1. To identify new data and information relevant to the safety of plant stanol ester, updated literature searches were conducted in January, February, and December 2011, using the electronic search tool Dialog. The literature searches were limited to articles published between 1999 and 2011 (inclusive). The 12 databases searched are listed in Table IV-1. Keywords used to identify studies conducted using plant stanols included plant()stanol?, plant()sterol?, phytostanol?, phytosterol?, sitostanol?, sitosterol?, beta()sitostanol?, and beta()sitosterol?. Words separated by parentheses [*i.e.*, ()] indicate that the 2 words had to occur together, in the order presented. The symbol "?" indicates a truncation so as to not place a limitation on the word ending (*e.g.*, "sitostanol?" would locate "sitostanol" and "sitostanols"). Keywords were searched for in all fields available within each database. No language limitations were placed on any of the literature searches. From the records identified, duplicate titles were removed.

Table IV-1 Electronic Databases Used to Retrieve Literature

Electronic Database	Date Range of Database
MEDLINE®	1999 to November 24, 2011
TOXFILE	1999 to second week of November 2011
AGRIS International	1999 to October 2011
AGRICOLA	1999 to November 2011
Allied and Complementary Medicine™	1999 to November 2011
BIOSIS Toxline®	1999 to first week of December 2011
Foodline®: SCIENCE	1999 to December 1, 2011
CAB ABSTRACTS	1999 to fourth week of November 2011
BIOSIS Previews®	1999 to fourth week of November 2011
Food Science and Technology Abstracts	1999 to fourth week of November 2011
NTIS	1999 to first week of December 2011
EMBASE®	1999 to December 1, 2011

Once the search strategy was implemented and the publication titles were retrieved, the relevance of the publications was determined at 3 stages using the titles, abstracts, and subsequently the full text of publications. Although Raisio notes that plant stanols and their unsaturated counterparts plant sterols have been previously viewed as biologically equivalent materials, it is now recognized that there are significant differences between the metabolism

and biological effects of plant stanols and plant sterols in humans, which suggests that it may not be appropriate to extrapolate findings from studies conducted using plant sterols to those expected from consumption of plant stanols and *vice-versa*. For example, the absorption rates of plant stanols are an order of magnitude lower than those of the corresponding plant sterols (*e.g.,* Ostlund *et al.*, 2002). Furthermore, although a substantial amount of ingested plant sterols are metabolized to "secondary metabolites" ("coprostanols" and "coprostanones") by micro-organisms in the colon (Weststrate *et al.,* 1999), Lütjohann *et al.* (1993) have verified, using deuterated sitostanol, that the metabolic transformation of sitostanol to its 3-oxo metabolite by intestinal microbes does not occur *in vivo*. **Based on the metabolic differences in humans between plant stanols and plant sterols, and given the existence of extensive safety data on plant stanols from *in vitro*, animal, and human studies, the GRAS determination of Raisio's plant stanol ester was based on a scientific review of predominantly plant stanol and plant stanol ester studies[2,3].** To be conservative, however, adverse findings reported for plant sterols (*e.g.*, association between plant sterols and cardiovascular disease) were reviewed for their relevance to the proposed GRAS uses of Raisio's plant stanol ester ingredient.

An overview of all relevant data and information identified, which includes both favorable and unfavorable information as available to Raisio is presented herein. This information, including pivotal data and information used to establish the GRAS use of plant stanol ester in spreads, dressings and snack bars (U.S. FDA, 1999a), was made available to the Expert Panel. A signed copy of the Expert Panel statement is provided in Appendix A.

A. History of Safe Use

Raisio's plant stanol ester has been evaluated by a large number of authoritative bodies world-wide and has a long history of food use. In Finland, for example, plant stanol ester has been safely consumed for approximately 16 years. Regulatory provisions permitting the sale of plant stanol ester have been granted in many jurisdictions, including (but not limited to) the European Union (EU), Australia/New Zealand, Canada, Switzerland, China, Russia, and Japan.

A summary of the regulatory status of plant stanol ester in various major regulatory jurisdictions is provided below.

1. United States

In the U.S., plant stanol ester manufactured by Raisio has been safely used in spreads, snack bars and dressings, at use levels of up to 1.7 g of plant stanol ester (equivalent to 1 g of plant stanols) per RACC, for approximately 12 years; these GRAS uses have been reviewed by the

[2] Because plant stanol ester is hydrolyzed to free plant stanols and free fatty acids in the intestinal tract, studies on both plant stanols and plant stanol ester were deemed to be relevant to the GRAS determination of plant stanol ester.
[3] If plant stanols and plant sterols were studied in the same trial, then results for both were discussed.

U.S. FDA without objection from the Agency (U.S. FDA, 1999a,b,c, 2000[4]). Since the introduction of Raisio's plant stanol ester to the U.S. marketplace for food use in 1999, GRAS self-affirmed uses of vegetable oil derived plant sterol ingredients containing small quantities of plant stanols (≤5% sitostanol + campestanol), and tall oil derived plant sterol ingredients containing higher quantities of plant stanols (≤20 sitostanol + campestanol) have been introduced to the U.S. marketplace. These plant sterol ingredients and fatty acid ester forms derived thereof, have been successfully notified to the FDA without objection from the Agency for use in food and beverage products across a variety of food categories. Based on these Notifications that were successfully submitted to the FDA, wherein results from conservative intake modeling were reported,[5] the consumption of at least 10 g of plant sterols per person per day may occur in 90th percentile consumers (GRN 061 – 2001).

2. Canada

Health Canada conducted a safety assessment for "phytosterols", which was used as a collective term for plant stanols and plant sterols, whether used in esterified or non-esterified forms (Health Canada, 2010a). Based on its review of the available evidence, the Food Directorate concluded that 3 g/day for adults and 1 g/day for children of plant stanols and/or plant sterols should be used as reference safe intakes (Health Canada, 2010a). According to their safety and efficacy reviews, Health Canada also concluded that plant stanols and/or plant sterols could be added to un-standardized spreads, mayonnaise, margarine, calorie-reduced margarine, salad dressings and un-standardized salad dressings, yoghurt and yoghurt drinks, and vegetable and fruit juices, so long as use-levels were between 0.65 to 1.0 g (plant stanols and/or plant sterols), per serving and per reference amount (Health Canada, 2010a,b).

3. European Union

Raisio's plant stanol ester is a food ingredient that was used for human consumption prior to the adoption of the Novel Food Regulation [Regulation (EC) No 258/97 (EC, 1997)]. On 8 November 1999, Mr. Liikanen stated, on behalf of the European Commission that "*In discussions with Member States and on the basis of information provided by them it has been established that none of the products currently marketed under the trade name Benecol contain a novel food ingredient since this was on the market to a significant degree before the entry into force of Regulation (EC) 258/97. Hence the Regulation does not apply*" (Mulder, 2000). Thus, Raisio's plant stanol ester was exempt from the Novel Food Regulation, and clearance for

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[4] "*... FDA is not aware of any evidence that provides a basis to reject the petitioner's position that the use of plant stanol esters in spreads, dressings for salad, snack bars, and other foods is safe and lawful*" (U.S. FDA, 2000).

[5] The food intake modeling is considered "conservative" because an inherent assumption in the modeling is that plant sterols are added to all permitted foods within each proposed food use category at the highest permitted use level.

market authorization was conducted by the local food safety authorities within each Member State.

4. Australia and New Zealand

In Australia and New Zealand, plant stanols/sterols and their ester forms are permitted for use in edible spreads, breakfast cereals, milk, and yogurt. These food uses are subject to limitations described within the Food Standards Code, as summarized in Table IV-2. The cumulative exposure to plant stanols in the diet under the permitted food uses by the Australian population has been estimated by the Food Standards Agency of Australia New Zealand (FSANZ). Based on intake modeling, FSANZ has estimated that under worst-case conditions, mean and 90th percentile intakes of 1.9 and 4.7 g per person per day could occur for frequent consumers (age 40 to 60) of foods containing plant stanols/plant sterols. Whole population estimates were similar at 1.8 and 4.6 g per person per day of plant stanols/plant sterols for mean and 90th percentile users.

Table IV-2 Food Standard Code Permitting Use of Plant Stanols in Food

Food Standard Code No.	Product	Uses[a]	Use Levels[b]
Standard 1.5.1 Standard 2.4.2	Plant stanols, plant sterols and their ester forms	Edible oil spreads	No more than 82 g/kg
Standard 1.5.1		Breakfast cereals	At least 15 g/kg and not to exceed 19 g/kg
Standard 1.5.1 Standard 2.5.1		Milk	No less than 3 g/L and not to exceed 4 g/L
Standard 1.5.1 Standard 2.5.3		Yogurt	No less than 0.81 g per package, and not more than 1.0 g per package

[a] Food uses also are subject to the conditions prescribed in standards 1.2.4 and 1.2.3 (FSANZ, 2011).
[b] As sterol equivalents (unless otherwise indicated) as plant sterols, plant stanols alone or in combination (derived from plant or vegetable sources) in their non-esterified form and consisting of ≥95% des-methylsterols.

B. Probable Consumption of Plant Stanol Ester

Background exposure to plant stanols such as sitostanol and campestanol is limited. Recent evaluations of the plant stanol content within a "typical" American diet have been reported by Phillips *et al.* (1999). The authors measured (*via* gas chromatography) the stanol content of 3 different diets considered representative of the typical American diet that were obtained from multi-center clinical feeding trials that were part of the Dietary Effects on Lipoproteins and Thrombogenic Activity (DELTA) and Dietary Approach to Stop Hypertension (DASH) programs. The campestanol concentrations of the diets ranged between 5 to 7 mg/2,000 kcal diet, and the sitostanol concentrations ranged between 7 to 9 mg/2,000 kcal diet. Based on the low background concentrations of stanols in the diet background intakes of plant stanols would not appreciably add to the exposure of stanols in the diet from the proposed food-uses of plant stanol ester described herein.

Estimates for the intake of plant stanols under the intended conditions of use of plant stanol ester in food, as described under Section I.D above, were based on the proposed food-uses and use-levels of plant stanol ester in conjunction with food consumption data included in the U.S. National Center for Health Statistics (NCHS) and National Health and Nutrition Examination Survey (NHANES) (CDC, 2006, 2009; USDA, 2009). The data from the 2003-2004 and 2005-2006 cycles of the NHANES survey were combined to provide a larger population from which to estimate plant stanols consumption. Calculations for the mean and 90th percentile all-person and all-user intakes were performed for each of the individual proposed food-uses of plant stanol ester and the percentage of consumers were determined. Similar calculations were used to estimate the total intake of plant stanols resulting from all proposed food-uses of plant stanol ester combined. In both cases, the per person and per kilogram body weight intakes were reported for the following population groups:

infants, ages 0 to 2;
children, ages 3 to 11;
female teenagers, ages 12 to 19;
male teenagers, ages 12 to 19;
female adults, ages 20 and up;
male adults, ages 20 and up; and
total population (all age and gender groups combined).

Estimates for the total daily intakes of plant stanols in g/day and mg/kilogram body weight/day from proposed food-uses of plant stanol ester are provided in Tables IV-3 and IV-4, respectively.

Within the total U.S. population, 97.2% of respondents were identified as potential consumers of plant stanol ester from proposed food-uses (16,221 actual users identified). Consumption of plant stanol ester from the proposed food-uses by the total U.S. population resulted in estimated mean all-person and all-user intakes of plant stanols of 4.98 and 5.02 g/person/day (91 and 92 mg/kg body weight/day, respectively), respectively. The 90th percentile all-person and all-user intakes of plant stanols from proposed food-uses of plant stanol ester by the total population were 8.81 and 8.82 g/person/day, respectively (185 and 186 mg/kg body weight/day, respectively).

The percentage of potential consumers of the proposed food–uses for plant stanol ester identified were very high producing very similar estimates for the all-person and all-user intakes. As a result, only the intake estimates for the all-user designation are discussed in detail. The greatest mean all-user intake of plant stanols on an absolute basis was determined to occur in male adults and teenagers, both with values of 5.54 g/person/day. Infants displayed the lowest mean all-user intake of plant stanols on an absolute basis with a value of 3.48 g/person/day. On a body weight basis, the mean all-user intake of plant stanols was highest in infants, with an intake of 283 mg/kg body weight/day. The lowest all-user mean intake on a per kilogram body weight basis was observed to occur in male adults with a value of 65 mg/kg body weight/day.

Table IV-3 Summary of the Estimated Daily Intake of Plant Stanols from Proposed Food-Uses of Plant Stanol Ester in the U.S. by Population Group (2003-2004, 2005-2006 NHANES Data)

Population Group	Age (Years)	Percent Users	Actual # of Users	All Person Consumption (g/person/day)		All User Consumption (g/person/day)	
				Mean	90th Percentile	Mean	90th Percentile
Infants	0 to 2	75.9	1,452	2.86	6.40	3.48	6.80
Children	3 to 11	100.0	2,733	4.95	8.12	4.95	8.12
Female Teenagers	12 to 19	99.9	1,985	4.65	8.06	4.66	8.06
Male Teenagers	12 to 19	99.9	1,939	5.54	9.67	5.54	9.67
Female Adults	20 and up	99.8	4,274	4.69	8.08	4.70	8.09
Male Adults	20 and up	99.9	3,838	5.54	9.57	5.54	9.57
Total Population	All Ages	97.2	16,221	4.98	8.81	5.02	8.82

When high consumers (*i.e.* intakes at the 90th percentile) were examined, the estimate for the all-user intake of plant stanols from proposed food-uses was determined to be greatest in male teenagers at 9.67 g/person/day. The lowest 90th percentile all-user intake estimate of plant stanols was observed to occur in infants, with a value of 6.80 g/person/day. On a body weight basis, infants were determined to have the greatest all-user 90th percentile intake of plant stanols, with a value of 521 mg/kg body weight/day. The lowest all-user 90th percentile intake of plant stanols on a body weight basis was observed to occur in male adults at 115 mg/kg body weight/day.

Table IV-4 Summary of the Estimated Daily Per Kilogram Body Weight Intake of Plant Stanols from Proposed Food-Uses of Plant Stanol Ester in the U.S. by Population Group (2003-2004, 2005-2006 NHANES Data)

Population Group	Age (Years)	Percent Users	Actual # of Users	All Person Consumption (mg/kg body weight/day)		All User Consumption (mg/kg body weight/day)	
				Mean	90th Percentile	Mean	90th Percentile
Infants	0 to 2	75.9	1,452	232	500	283	521
Children	3 to 11	100.0	2,733	195	339	195	339
Female Teenagers	12 to 19	99.9	1,985	80	140	80	140
Male Teenagers	12 to 19	99.9	1,939	87	157	87	157
Female Adults	20 and up	99.8	4,274	66	119	66	119
Male Adults	20 and up	99.9	3,838	65	115	65	115
Total Population	All Ages	97.2	16,221	91	185	92	186

1. Summary and Discussion

Raisio proposes to expand the current GRAS use of its plant stanol ester to include multiple food and beverage categories at use levels providing a maximum of 0.5, 1.0, or 2.0 g of plant stanols per RACC. The minimum use-level of 0.5 g of plant stanols (delivered as plant stanol

ester) per RACC was chosen based on the Agency's Proposed Rule for the authorized health claim related to "phytosterols"[6] and reduced risk of CHD, wherein it is explicitly stated that "*...the agency is proposing to amend § 101.83(c)(2)(iii)(A) to permit health claims on foods that contain at least 0.5 g per RACC of phytosterols, expressed as the weight of nonesterified phytosterols*" (U.S. FDA, 2012a).

Estimates for the intake of plant stanols were based on the proposed food-uses and use-levels in conjunction with food consumption data included in the U.S. NCHS NHANES. Background sources of plant stanols naturally occurring in foods, and currently permitted food uses of plant stanols also were considered. The campestanol and sitostanol concentrations of American diets range between 5 to 7 and 7 to 9 mg/2,000 Kcal diet, respectively (Phillips *et al.,* 1999), and therefore do not appreciably add to total dietary exposures. Similar to plant stanols, plant sterols are marketed for use by individuals seeking dietary management of cholesterol levels. Although market research by Raisio suggests that consumers are typically brand loyal, there is a possibility that consumers may purchase both plant stanol and plant sterol enriched food products. However, since Raisio's plant stanol ester is proposed for addition to the same types of food and beverage products that may be enriched with plant sterols, introduction of proposed expanded food uses of Raisio's plant stanol ester to the U.S. market will not result in additive exposure to both plant stanols and plant sterols, and therefore will not change total dietary intake of these ingredients in the U.S. food supply.

The entire U.S. population was identified as potential consumers of plant stanol ester from proposed food-uses (16,221 actual users identified). On an all-user basis, the mean intake of plant stanols (from consumption of plant stanol ester enriched foods) by the total U.S. population from proposed food uses was estimated to be 5.02 g/person/day (92 mg/kg body weight/day). The 90th percentile all-user intake of plant stanols by the total U.S. population from proposed food-uses was estimated to be 8.82 g/person/day (186 mg/kg body weight/day). Raisio notes that these intakes of plant stanols from proposed uses of plant stanol ester are less than total population 90th percentile exposures to plant sterols from current GRAS Notified uses of plant sterols in the U.S. food supply, which have been estimated to be 11 g per person per day among all-users (GRN 398 – 2012). Similar intake estimates for plant sterol consumption in the EU have been reported by Kuhlmann *et al.* (2005). Using consumption data from the German National Food Consumption Study (1985 through 1988 with 23,209 participants) and a selection of ten different foods identified within existing Novel Food applications, the authors determined that the addition of plant sterols to these products at use levels up to 2.0 g plant sterols to usual daily servings, would result in a maximum intake of 13 g of plant sterols per person per day in frequent consumers, defined as 95th percentile, all-user intakes.

[6] The term "phytosterols" was used by the Agency to refer collectively to plant stanols and plant sterols.

Consumption data and information pertaining to the individual proposed food-uses of Raisio's plant stanol ester were used to estimate the all-person and all-user intakes of plant stanols for specific demographic groups and for the total U.S. population. This type of intake methodology is generally considered to be "worst case" as a result of several conservative assumptions made in the consumption estimates. For example, it is often assumed that all food products within a food category contain the ingredient at the maximum specified level of use. 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 the typical 2-day dietary survey utilized in NHANES 2003-2004, 2005-2006, overestimate consumption over longer time periods (greater than 3 months) (Anderson, 1988).

Several post-market surveillance studies and theoretical modeling approaches have evaluated the consumption of plant stanols/plant sterols from the current regulated food uses of these ingredients across multiple food and beverage products in the EU (SCF, 2002; Wolfs *et al.,* 2006; de Jong *et al.,* 2008a; EFSA, 2008; Hearty *et al.*, 2008). These studies show that the addition of plant stanols/plant sterols to multiple food categories does not result in "over-consumption" of these ingredients. Moreover, as reported by the European Food Safety Authority (EFSA) (EFSA, 2008), a large number of consumers do not consume enough products enriched with plant stanols/plant sterols to gain a nutritional benefit from their use. In addition, EFSA also reported that consumption of plant stanol/plant sterol containing foods on more than 3 occasions per day, or consumption of 2 or more products at their respected recommended intakes is required to exceed a daily intake of 3 g of these ingredients. Thus, Raisio expects that expansion of the current food uses of plant stanols in spreads will be of nutritional benefit to target users by allowing increased food choices of products containing plant stanol ester. During their evaluation, EFSA also noted that relative to their non-supplemented counterparts, food products enriched with plant stanols/plant sterols are premium priced, a factor that appears to limit "overconsumption" of these ingredients and further acts as a deterrent to the regular purchase of foods enriched with plant sterols/plant stanols by consumers not part of the target group for the product and thus not benefiting directly by consuming them (EFSA, 2008).

C. Absorption, Distribution, Metabolism, and Elimination

Published and unpublished studies characterizing the absorption, distribution, metabolism and excretion of Raisio's plant stanol ester have been previously reviewed (U.S. FDA, 1999a). In brief, following consumption, Raisio's plant stanol ester is hydrolyzed into its free plant stanol and fatty acid form within the intestinal tract, possibly by pancreatic carboxylic ester lipase. The free fatty acid ester derived from plant stanol ester is absorbed and metabolized in a manner similar to other common dietary fatty acids. Studies in animals and humans suggest that only minimal quantities of dietary plant stanols are absorbed. For example, Ostlund *et al.* (2002) report that $0.044 \pm 0.004\%$ sitostanol and $0.16 \pm 0.017\%$ campestanol were absorbed from a single meal providing 600 mg plant stanols (Ostlund *et al.,* 2002). The small amount of

absorbed plant stanols is not further metabolized, and the administered dose can be accounted for quantitatively as unchanged sitostanol and campestanol excreted in the feces.

Since plant stanols are virtually unabsorbed, and plasma levels of plant stanols remain at low levels even with high daily intakes of plant stanol ester (Gylling *et al.,* 2010a), plant stanols are predicted to have minimal effects on normal physiological processes.

D. Toxicological Studies

Subchronic toxicity evaluations in rats demonstrate that plant stanol ester, manufactured by Raisio, is non-toxic when consumed by male and female Sprague-Dawley rats at dietary concentrations of up to 8.5% plant stanol ester (5% stanols in free form) (Turnbull *et al.*, 1999a). The only treatment-related effect at this dietary intake level was a reduction in plasma and liver concentrations of fat-soluble vitamins D, E, and K_1. Developmental and reproductive studies did not identify evidence suggestive that plant stanol ester causes adverse developmental, reproductive or estrogenic effects (Slesinski *et al.*, 1999; Turnbull *et al.*, 1999b; Whittaker *et al.*, 1999). Plant stanol ester also is not mutagenic in bacterial and mammalian cell assays (Turnbull *et al.*, 1999b), and has not demonstrated any effect that would indicate that the material represents a carcinogenic potential. These studies were reviewed previously during the GRAS evaluation of Raisio's plant stanol ester for use in spreads, salad dressings and snack bars (U.S. FDA, 1999a,b,c). The safety information provided by these studies remains pivotal to the GRAS assessment of the expanded food uses of Raisio's plant stanol ester.

Based on observations of reduced fat soluble vitamin absorption reported in the 90-day feeding studies using Wistar rats, a no-observed-adverse-effect level (NOAEL) of 1.7% plant stanol ester (1.0% plant stanols) in the diet was determined by the authors (Turnbull *et al.*, 1999a). The effects of plant stanol ester on fat soluble vitamin absorption have now been extensively evaluated using placebo controlled human intervention studies, which are further summarized in Section IV.G and IV.H; these studies demonstrate that consumption of plant stanol ester containing foods, even at intakes several fold above usual/expected daily intakes of plant stanol ester, do not affect dietary absorption of fat soluble vitamins in humans. Accordingly, it is now generally recognized that the impaired absorption of fat soluble vitamins observed in Wistar rats administered high dietary concentrations of plant stanol ester are not relevant to the situation occurring in humans consuming plant stanol ester in the diet from consumption of foods to which plant stanol esters will be added (JECFA, 2009). Raisio has similarly concluded that the previous NOAEL of 1.7% plant stanol ester in the diet is no longer appropriate, and a NOAEL of 8.5% plant stanol ester (5% stanols), the highest dietary concentration administered, can be determined for this study. This exposure corresponds to a daily intake of 4,600 mg of plant stanol ester (2,700 mg plant stanols) per kg body weight, or 322 g of Raisio's plant stanol ester (189 g plant stanols) for a 70 kg adult human.

As discussed, an updated comprehensive search of the literature was conducted by Raisio to identity new data and information published since previous GRAS self-affirmations of Raisio's plant stanol ester. A series of studies that reported the effects of plant stanol consumption in rat species with genotypes that manifest in predispositions to hypertension and increased plant stanol and sterol absorption were identified; these studies are discussed below in Section IV.E. New information relevant to the mutagenicity/genotoxicity of plant stanols also was identified and is discussed in Section IV.F.

E. Other Animal Studies

1. Spontaneously Hypertensive Stroke-prone Rats and WKY Inbred Rats

A number of studies have examined the effects of plant stanols and sterols in spontaneously hypertensive stroke-prone (SHRSP) rats, a model of human essential hypertension and hemorrhagic stroke. This strain of rats has a mutation in the gene encoding the adenosine triphospahte (ATP) transporter binding cassette (ABC) G5 transporter gene (*Abcg5*), resulting in increased absorption and impaired biliary elimination of dietary plant stanols and plant sterols (Scoggan *et al.*, 2003). Accordingly, plant stanols and plant sterols accumulate in the serum and are incorporated into tissues such as erythrocytes, livers and kidneys of SHRSP rats fed diets supplemented with these compounds (Ratnayake *et al.*, 2003).

Rats fed diets containing 10% canola oil, which is naturally rich in plant sterols, had shorter life spans and higher systolic blood pressures compared to rats fed diets containing soybean oil, which has a much lower plant sterol content (Ratnayake *et al.*, 2000a; Naito *et al.*, 2003). Furthermore, SHRSP rats fed diets enriched with plant sterols (14 g/kg diet) for at least 31 days had significantly shorter lifespans, lower platelet counts, and higher white blood cell counts compared to rats fed control diets ($p<0.05$) (Ratnayake *et al.*, 2003). In similar studies, Chen *et al.* (2009) found diets enriched with plant sterols (2.0 g/kg diet) for 5 weeks increased diastolic blood pressure, while Ogawa *et al.* (2003) found diets enriched with plant sterols (4.5 g/kg diet) for at least 24 days increased systolic blood pressure, promoted earlier onset of stroke, and shortened lifespan compared to control diets ($p<0.05$). In all of these studies, animals also were administered salted (NaCl) drinking water (1% NaCl) to accelerate the progress of hypertension.

Similar findings have been reported for SHRSP rats fed diets enriched with plant stanols. Animals fed diets enriched with plant stanols (11 g/kg diet) for at least 31 days had significantly shorter life spans compared to controls (79 days *vs.* 88 days, $p<0.01$) (Ratnayake *et al.*, 2003). Consumption of plant stanols did not alter hematological parameters or erythrocyte deformability, in contrast to the animals fed plant sterols in this study. In another report, animals fed diets enriched with plant stanols (2.0 g/kg diet) for 5 weeks had greater increases in diastolic blood pressure compared to the controls ($p<0.05$), although no significant differences in systolic blood pressure were observed (Chen *et al.*, 2009). Both of these studies were performed under salt-loading conditions where the drinking water contained 1% NaCl.

The effects of plant stanols and plant sterols have also been examined in Wistar-Kyoto (WKY) inbred rats, the parental strain from which SHRSP rats were derived. The WKY inbred rats also have a mutation in the *Abcg5* gene that increases tissue incorporation of plant sterols and plant stanols; however, these rats have normal blood pressure, unlike the hypertensive SHRSP rats. Consumption of plant stanols or plant sterols (2.0 g/kg diet) for 5 weeks, in the absence of salt-loading, significantly increased systolic and diastolic blood pressure compared to control WKY inbred rats ($p<0.01$) (Chen *et al.*, 2010). The increase in diastolic blood pressure was significantly higher for rats in the plant stanol group compared to the plant sterol group ($p<0.05$). In contrast, plant sterols and plant stanols administered at the same dose and duration had little to no effect on systolic and diastolic blood pressure in another study of WKY inbred rats under salt-loading conditions (Chen *et al.*, 2009). The authors postulated that the extra dietary intake of salt would exacerbate hypertensive states in these animals, thereby potentially masking any effects of the plant sterols and plant stanols on blood pressure.

The mechanism by which plant sterols and plant stanols shorten the lifespan of SHRSP rats remains unclear. Treatment with plant sterols and plant stanols may exacerbate the development of hemorrhagic stroke, and Ratnayake *et al.* (2003) speculated that the reduced platelet numbers and erythrocyte deformability may contribute to greater mortality in rats fed diets supplemented with plant sterols. As plant stanols were not associated with reduced platelet numbers and erythrocyte deformability, this mechanism does not explain the shorter lifespan observed with the administration of plant stanols to SHRSP rats. It is noteworthy that cholesterol appears to be protective against hemorrhagic stroke in this strain of rats, and plant sterols and plant stanols are cholesterol-lowering agents. Consumption of plant sterols and plant stanols may also elevate blood pressure in SHRSP rats. Ratnayake *et al.* (2003) speculated that incorporation of plant sterols and plant stanols into cellular membranes may potentially increase the activity of Na^+/K^+-ATPase, such as that present in the kidneys, where increased Na^+ reabsorption would lead to retention of body fluid and hypertension.

Although SHRSP rats fed plant stanols and plant sterols appear to have shortened lifespans, the validity of this animal model has been questioned. For example, dietary olive oil also was found to shorten the lifespan of SHRSP rats, an effect that is inconsistent with the low sterol content of the oil and its long history of safe human consumption and recognized nutritional benefits to cardiovascular health (Ratnayake *et al.*, 2000b). In addition, Tatematsu *et al.* (2004) did not find any significant correlations between plant sterol content in the diet, serum or liver with mean survival time. Rats may not be a suitable model for assessing the safety of vegetable oils as this species has limited capacity to metabolize large quantities of dietary fatty acids (Grice and Heggtveit, 1983). Rats can be sustained on diets containing 5% fat, and diets that contain 20% fat, resembling the levels found in the average North American diet, have been found to shorten lifespan (Grice and Heggtveit, 1983). Finally, it should be noted that SHRSP rats are an animal model predisposed to hypertension and stroke. SHRSP rats have less deformable erythrocytes that are more fragile compared to other rat strains as a result of lower

cholesterol levels in cell membranes (Yamori *et al.*, 1980). Weakened cell membranes, particularly within the small vasculature in the brain, may be more susceptible to damage and contribute to the increased propensity for cerebral hemorrhage and infarctions in these rats. Accordingly, increased dietary intake of cholesterol lengthens the lifespan of these rats, whereas an elevated cholesterol level is considered an important risk factor for the development of atherosclerosis, CHD, and stroke in humans. It is notable that the effects of plant sterols and plant stanols on diastolic and systolic blood pressure differed between rat strains and salt-loading conditions, and the relevance of these animal models to humans has not been established. Lastly, consumption of plant stanols has not been associated with changes in blood pressure or other hematological parameters, markers of inflammation or endothelial function in human clinical studies, as described in Section IV.G.

F. Mutagenicity and Genotoxicity Studies

Plant stanol ester was demonstrated to be non-mutagenic and non-genotoxic in *in vitro* studies [bacterial Ames assay, gene mutation assay in L5178Y mammalian cells, and chromosome aberration assay in Chinese hamster ovary (CHO) cells], at doses up to the limit of solubility, in the presence or absence of metabolic activation by rat liver S9 microsomal fractions (Turnbull *et al.*, 1999b). Additional studies characterizing the effects of plant stanol ester on cell proliferation in various *in vitro* and *in vivo* studies were identified (Jia *et al.,* 2006; Marttinen *et al.*, 2008; Daly *et al.*, 2009); although the data and information provided from these studies are not considered validated measures of mutagenicity/genotoxic potential in humans, a review of these studies and their relevance to the GRAS use of plant stanol ester in food is presented below.

1. Additional Studies

The effects of plant stanols on chemical induced mutagenesis using human epithelial colorectal adenocarcinoma (Caco-2) cell lines were examined Daly *et al.* (2009). The authors reported that pre-treatment of human Caco-2 cell lines with β-sitostanol at 25 μM for 48 hours did not protect against DNA damage (comet assay) elicited by the mutagens *N*-methyl-*N'*-nitro-*N'*-nitrosoguanidine and H_2O_2. The authors also reported that incubation of Caco-2 cells with β-sitostanol at a concentration of 25 μM for 48 hours did not alter cell membrane integrity, induce apoptosis or alter basal and interleukin- (IL-) 2 induced levels of cyclooxygenase-2.

The effects of plant stanols on colonic mucosal cell proliferation were evaluated by Jia *et al.* (2006). In this study, 70 male golden Syrian hamsters were randomly divided into 1 of 7 groups and fed the following experimental diets for 5 weeks:

Group 1 – the semipurified diet with no cholesterol
Group 2 – the control diet plus 0.25% cholesterol
Group 3 – Group 2 diet plus 1% plant sterols
Group 4 – Group 2 diet plus 1% plant stanols

Group 5 – Group 2 diet plus 1.76% plant sterol esters, esterified to fish oil
Group 6 – Group 2 diet plus 0.71% plant stanol esters, esterified to ascorbic acid
Group 7 – Group 2 diet plus 1.43% plant stanol esters, esterified to ascorbic acid.

Hamsters randomized to Group 6 had a significantly lower colonic mucosal cell proliferation relative to hamsters in the control group. All of the other groups displayed mucosal cell proliferation measures that were similar to the control group.

Preliminary findings by Marttinen *et al.* (2008) have suggested that plant stanols may influence the formation of intestinal adenomas and activate ERK1 signaling within intestinal mucosa cells in the Apc Min/+ mouse. The Apc Min/+ mouse carries a heterozygous mutation in the *Apc* tumor suppressor gene, a mutation that results in a dysfunction of multiple transcriptional pathways that regulate cell proliferation, resulting in the development of spontaneous adenomas in the these animals. Individuals with Familial Adenomatosis Polyposis (FAP) have are reported to display mutations in the human homologue to Apc, and the Apc Min/+ mouse is therefore used as a model to study hereditary colon cancer. General background information on the Apc Min/+ mouse model and discussion of the recognized uses and limitations of the model to cancer research has been summarized by Raisio and is presented in Appendix B; this information was available to the Expert Panel.

As described by Marttinen *et al.* (2008) a total of 28 Apc Min/+ male and female mice were fed either a control diet (n=14) or a diet containing 0.8% (w/w) plant stanols (n=14). Basal diets used for the study were semi-synthetic, high-fat formulations. The plant stanol ester diet was formulated using freeze-dried plant stanol ester enriched food products found on the market in Finland in 2003. The commercially available plant stanol ester enriched foods used in the study included:

- 27.5 g/1,000 g of diet of fresh cheese (a Philadelphia-type cheese spread based on vegetable oils and fats, no milk fat);
- 50 g/1,000 g of diet of pasta;
- 10 g/1,000 g of diet of ripened cheese; and
- 44 g/1,000 g of diet of sour milk.

The control diet also was semi-synthetic and was made with similar commercially available products, but that were not enriched with plant stanol ester. Raisio has noted that however, that the nutrient and additive compositions of the test diets were not balanced between groups, a limitation that significantly confounds interpretation of the data generated by the study.

Marttinen and colleagues reported that compared to the feeding of the control diet, the feeding of the plant stanol ester diet (containing 0.8% plant stanols, w/w) resulted in a statistically significant 60% increase in the number of small intestinal adenomas (P=0.002), although no difference in the size of intestinal adenomas was seen. The authors also reported that the plant

stanol diet increased the level of activated extracellular-signal-regulated kinase 1 (ERK1, the ratio of phosphorylated p44 to total p44) and nuclear cyclin D1 in the intestinal mucosa. Raisio notes however, that in the absence of information characterizing the methodology used for analyses of these signal transduction and cell cycle mediators, interpretation of the mechanistic data is not possible. For example, it is unclear what specific tissues were examined, what locations were assessed, or whether the mucosa or full thickness intestine was evaluated. Information on the inclusion/exclusion of inflammatory cells and Peyer's patches within the tissue isolates subject to the analyses, and information describing the analytical methods (gross *vs.* microscopic) were not provided.

Although the level of intestinal mucosal cholesterol was not affected by plant stanol diet feeding, the levels of sitosterol and campesterol were significantly decreased, and those of sitostanol and campestanol significantly increased in the intestinal mucosa of plant stanol fed Apc Min/+ mice. This observation is expected given that plant stanols have been demonstrated to reduce the absorption of plant sterols (Hallikainen *et al.,* 2000b; Miettinen *et al.,* 2000) and that an increased daily intake of plant stanols results in reduced serum levels of plant sterols (Hallikainen *et al.,* 2000b). An increased daily intake of plant stanols is expected to reduce plant sterol concentrations in small intestinal mucosal cells. The authors reported a negative correlation between the number of intestinal adenomas and the level of mucosal plant sterols (r=0.517; P=0.007); however, the biological relevance of this finding is unclear as it is widely recognized that it is inappropriate to infer causational relationships from correlation analyses (Greenhalgh, 1997). As discussed in Appendix B, the Apc Min/+ mouse model is subject to significant biological variability, and in the absence of a dose-response relationship, and inclusion of a reproducible positive control group and sterol comparator control group into the study design, it is not possible to demonstrate that the reported effects are attributed to plant stanol ester consumption, or are simply a function of the recognized variability that has been previously acknowledged for this study model. Moreover, based on generally available information on the use and relevance of the Apc Min/+ mouse to human colon carcinogenesis, Raisio has further concluded that the model is inappropriate for use in evaluating the effects of dietary interventions on the development of colonic tumorigenesis in humans. Additional discussion on the findings by Marttinen *et al.* (2008) and use of the Apc Min/+ mouse has been deliberated by the Expert Panel, who concluded that the findings by the authors were not relevant to the safety of plant stanol ester (see Appendix B).

Information provided by validated *in vitro* mutagenesis studies in *Salmonella* and *Escherichia coli* (*E. coli*) tester strains, and *in vitro* studies in CHO cells studies of mutagenicity/genotoxicity show that plant stanols are not mutagenic or genotoxic (Turnbull *et al.*, 1999b). Toxicity studies in animals show that plant stanols are innocuous compounds, which is consistent with their long-history of safe consumption by humans world-wide. Unlike plant sterols, which are metabolized to "secondary metabolites" (coprostanols and coprostanones) by micro-organisms in the colon (Weststrate *et al.,* 1999), plant stanols are not subjected to metabolic transformation

by intestinal microbes to putative carcinogens (Lütjohann *et al.,* 1993). Based on the above points and considerations, and unanimous consensus by the Expert Panel, the findings by Marttinen *et al.,* do not change the conclusion that plant stanols and plant stanol esters are not mutagenic/genotoxic substances, nor is there any reason to conclude that dietary plant stanols may adversely affect colonic epithelial cell proliferation in humans.

G. Studies in Humans

A search of the literature for human studies evaluating the safety of plant stanols/plant stanol ester consumption published between 1999 and 2011 identified several studies, which are summarized in Table IV-5. These studies have primarily focused on individuals with mild to moderate hypercholesterolemia, and included both non-medicated individuals and those concurrently taking cholesterol-lowering medications, such as statins. Many of the identified clinical studies have included measures such as low-grade chronic inflammation and endothelial function as predictors of atherosclerosis and future CHD risk. Recent studies also have examined the effects of consuming plant stanol ester for prolonged periods (85 weeks) or at higher doses (up to 9 g plant stanols per day) in healthy subjects. The available dataset of human trials was reviewed on a case by case basis to identify studies providing information relevant to the GRAS use of plant stanol ester. Clinical supplementation studies at high doses, and for prolonged periods of time, which also included adverse event reporting within the publication, and/or other measures relevant to safety such as clinical chemistry and hematology monitoring, and measurements of fat soluble vitamin and carotenoids absorption, were considered particularly important in the current GRAS determination. Studies evaluating the effects of plant stanol ester on endothelial function or neurcognition, or wherein study populations included pregnant women or children, also were carefully reviewed and are presented in detail within their respective sections below. **As already stated, clinical studies on plant sterols were not considered relevant to the GRAS determination of Raisio's plant stanol ester, given that plant stanols and plant sterols differ with regards to their molecular structures and their metabolic fates. Nevertheless, to remain conservative, adverse findings from studies conducted with plant sterols (*e.g.*, associations between plant sterol consumption and cardiovascular disease risk) were reviewed for their relevance to the safety assessment of Raisio's plant stanol ester.**[7,8]

1. Healthy Individuals with Normal Cholesterol Levels

Several clinical studies of plant stanol ester published between 1999 and 2011 have been conducted in healthy subjects with normal cholesterol levels[9]. These studies are summarized in Table IV-5. In most of these studies, the objective of the trial was to determine the efficacy of

[7] Because plant stanol ester is hydrolyzed to free plant stanols and free fatty acids in the intestinal tract, studies on both plant stanols and plant stanol ester were deemed to be relevant to the GRAS determination of plant stanol ester.
[8] If plant stanols and plant sterols were studied in the same trial, then results for both were discussed.
[9] Subjects were considered to be "healthy" and "normal" with regards to cholesterol levels if they were described as such by the study authors.

plant stanol ester in reducing blood lipid levels, particularly low-density lipoprotein (LDL)-cholesterol, and although no "formal" assessments of safety were conducted in these studies, no adverse events were reported [Plat *et al.,* 2000; Ishiwata *et al.,* 2002; Cater *et al.,* 2005 (Study 1); Hyun *et al.,* 2005; McPherson *et al.,* 2005; Alhassan *et al.,* 2006]. Because these studies were predominantly efficacy, as opposed to safety trials, they are summarized in Table IV-5, but are not discussed any further in this section. In several of these studies, effects on serum fat-soluble vitamin and carotenoid levels also were assessed; results are discussed in Section IV.H.

In 2 clinical studies, extended safety monitoring was conducted. Therefore, the results of these 2 studies are summarized in detail below.

Mensink *et al.* (2002) examined the effects of 3 g/day plant stanols, delivered as plant stanol ester, on fasting concentrations of plasma lipids, lipid-soluble antioxidants, and on serum chemistry and hematology in 60 non-hypercholesterolemic subjects. The delivery matrix was a low-fat yoghurt, which was consumed 3 times daily (1 g plant stanols/serving) for 4 weeks, following a 3-week run-in period with a placebo yoghurt. Results related to fasting serum concentrations of lipid-soluble antioxidants are discussed in Section 4.6.2. Although data were not shown, values for alanine transaminase (ALAT), aspartate aminotransferase (ASAT), *gamma*-glutamyl transpeptidase (γ-GT), creatinine and C-RP (C-reactive protein), and for hematological parameters were reported to not have changed from the start until the end of the study. Moreover, the study authors noted that there was no suggestion that consumption of the yoghurt with plant stanol ester caused any physical complaints.

In a randomized, placebo-controlled study conducted to compare the effects of vegetable oil- and wood-based stanol ester mixtures on cholesterol absorption, 112 non-hypercholesterolemic men and women (41 men, 71 women; mean age of 33 years) were given low erucic acid rapeseed oil-based margarine and shortening for 4 weeks (Plat and Mensink, 2000). At the end of the 4-week run-in period, subjects were randomized to either the group that continued to consume the control products or test products containing vegetable oil-based stanol esters (providing 3.8 g/day of stanols), or wood-based stanol esters providing (4.0 g/day of stanols) for 8 weeks. Coagulation and fibrinolytic parameters were evaluated at the end of the run-in period and the treatment period. One subject withdrew during the treatment period due to lack of motivation. Changes in body weight were reported to be marginal in all treatment groups. There were no significant differences in changes in plasma fibrinogen or factor VII amidolytic (FVIIam), factor VII coagulant (FVIIc), AT-111 (anti-thrombin III), plasminogen activator inhibitor (PAI-1), and tissue plasminogen activator t-PA activities reported among groups. Based on their findings, the investigators concluded that neither plant stanol ester mixture caused any short-term side effects.

2. Individuals with Hypercholesterolemia[10], but not Taking Cholesterol Lowering Medication

2.1 Short-Term Studies with Safety Monitoring

The majority of clinical studies on plant stanol ester published between 1999 and 2011 were short-term (up to 10 weeks in duration, but typically 4 to 6 weeks in duration). The primary objective in the majority of these short-term studies was to assess the efficacy of plant stanol ester in reducing LDL-cholesterol levels; as such, safety measures were not formally assessed in most studies. These studies are summarized in Table IV-5. Below, detailed summaries of short-term plant stanol ester studies that included safety outcome measures are provided (Hallikainen *et al.,* 2006; Raitakari *et al.,* 2008).

Hallikainen *et al.* (2006) examined the effects of plant stanol ester consumption on endothelial function. Eighty hypercholesterolemic subjects (total cholesterol <8 mmol/L) were randomized into an intervention or control group for a period of 20 weeks. The intervention group was a cross-over design where individuals consumed spreads containing either plant stanol ester or plant sterol ester in random order, each for a period of 10 weeks, at an estimated daily stanol or plant sterol intake of 2.0 g. High compliance was found for both the control spread and the spreads fortified with plant stanol ester or plant sterol ester. BMI increased slightly in both the control and intervention groups by the end of treatment compared to baseline. Significant reductions in total and LDL-cholesterol levels, ranging from 4 to 12% compared to baseline, were observed following consumption of plant stanols or plant sterols ($p<0.05$). Plant stanol and plant sterol consumption reduced systolic blood pressure by approximately 3% from baseline levels; however, similar reductions were also seen in the control group. Serum ALAT increased by 16% following plant sterol consumption ($p<0.05$) and a trend towards higher levels was seen following plant stanol consumption (12%, $p=0.09$); however, no such changes were observed in the control group. Biomarkers of inflammation [hs-CRP, IL-6, tumor necrosis *alpha* (TNF-α)], plasma glucose, and insulin levels were not altered by consumption of plant stanols or plant sterols. Indicators of endothelial function [brachial artery diameter, flow-mediated dilatation (FMD) and nitrate-mediated vasodilatation] did not change over the course of the study for individuals in both the plant stanol and control groups; however, consumption of plant sterols reduced brachial artery diameter by 3% compared to baseline ($p=0.06$), although FMD and vasodilation in response to nitroglycerin were not altered. Reduction in vascular diameter is generally considered an unfavorable response since it is related to an increase in vascular resistance and limitations in blood flow. However, systolic blood pressure was also reduced following plant sterol consumption, and the authors stated that "the clinical relevance of diminished brachial artery diameter during STEEST (sterol ester) remains unclear".

Raitakari *et al.* (2008) investigated the effects of plant stanol ester on arterial function in a randomized, double-blind, placebo-controlled study of 200 subjects, of whom 190 completed the

[10] Subjects were considered to have hypercholesterolemia if they were described as such by the study authors.

study. Subjects had mild hypercholesterolemia (total cholesterol >5 mmol/L) but were not taking any cholesterol-lowering medications and reported no regular use of products containing plant stanols or plant sterols. Subjects were assigned to either control vegetable oil spread (50 subjects), or treatment groups where plant stanol ester was added to spreads made with camelina oil (50 subjects), rapeseed oil (50 subjects), or sunflower oil (50 subjects). Treatment lasted for 3 months and an estimated daily dose of 2.0 g of plant stanols was consumed. Used containers were returned at the beginning of each month to assess compliance and intake. Fasting blood samples were collected at baseline and at the end of treatment. Body weight measurements and tests of arterial function also were performed at these time points. High compliance was found in both the control spread group and the group administered a spread enriched with plant stanol ester. Body weight was not altered across the treatment groups. Consumption of plant stanol ester decreased LDL-cholesterol levels by approximately 6% from baseline ($p<0.001$), regardless of the type of oil spread it was added to. In contrast, LDL-cholesterol levels for subjects in the control group increased by 3.6% between baseline and end of treatment. High-density lipoprotein (HDL)-cholesterol levels and triacylglycerides did not differ among the treatment groups. Using ultrasonic imaging, carotid arterial compliance (CAC) and endothelium dependent brachial artery FMD did not significantly differ among the treatment groups. However, consumption of plant stanol ester improved CAC and FMD scores in those who had below average baseline scores, suggesting plant stanol ester may have beneficial effects in individuals with already poor arterial function.

2.2 Long-Term Consumption

In the majority of plant stanol ester clinical studies in subjects with hypercholesterolemia but not taking cholesterol-lowering medication published between 1999 and 2011, the duration of treatment ranged from 3 to 10 weeks; however, in 1 study, the administration of plant stanol ester was longer in duration, specifically 52 weeks (Gylling *et al.,* 2009). This study is summarized in Table IV-5 and also is discussed in detail below.

Gylling *et al.* (2009) investigated the effect of long-term plant stanol ester and plant sterol ester consumption in a randomized, double-blind, placebo-controlled study of 282 subjects with mild to moderate hypercholesterolemia. Subjects were assigned the control spread (96 subjects), or spread containing plant stanol ester (93 subjects) or plant sterol ester (93 subjects) for 12 months. Plant stanols or plant sterols were consumed at an estimated daily intake of approximately 2 g. Fasting blood samples were collected at baseline and 12 months, while blood pressure was measured at baseline, 3, 6, 9, and 12 months. Subjects were also genotyped for 5 common genetic polymorphisms in *ABCG5* and *ABCG8*. Body weight, waist circumference, blood pressure and a marker of inflammation, hs-CRP, did not significantly differ among the treatment groups or change over course of the study. Blood glucose levels were significantly increased by 1 to 3% from baseline levels in all 3 groups; but, no differences were found among treatment groups. Plant stanols and plant sterols consumption (as their ester forms) reduced serum total cholesterol levels by approximately 5% compared to baseline

(p=0.001 for plant stanol ester and p<0.01 for plant sterol ester). Parameters of endothelial function (brachial artery diameter, FMD, carotid artery intima media thickness, and CAC) were significantly altered over the course of the study; however, the effects were observed in both the intervention and control groups, and no significant group x time interaction was detected. Genetic polymorphisms in *ABCG5* and *ABCG8* were not associated with reductions in serum cholesterol or changes in markers of cholesterol synthesis and absorption or vascular functions over the course of the study. It is notable that some of these polymorphisms were associated with baseline levels of markers of cholesterol synthesis and absorption, as well as FMD and brachial artery diameter, suggesting these genes may contribute to inherent differences in cholesterol metabolism and vascular function and structure.

2.3 High-Dose Consumption

In the majority of plant stanol ester studies identified, the dose of plant stanols administered ranged from 0 to 3 g/day (see Table IV-5); however, in 2 plant stanol ester studies, the dose of plant stanols administered was exceptionally high; specifically, in the study by Gylling *et al.* (2010a,b) approximately 9 g of plant stanol, as plant stanol ester, were administered daily for 10 weeks, and in the study by Mensink *et al.* (2010), approximately 3, 6, or 9 g of plant stanols, as plant stanol ester, were administered daily for 4 weeks. The results of these studies are summarized in Table IV-5 and discussed below in greater detail.

Gylling *et al.* (2010a,b) investigated the safety and efficacy of high doses of plant stanol ester in a randomized, double-blind, placebo-controlled study of 49 subjects over a period of 10 weeks. As inclusion criteria, subjects had to have mild/moderate hypercholesterolemia, with serum total cholesterol levels between 4.5 to 7.5 mmol/L. The control group of subjects (n=24) consumed vegetable oil-based spreads and an oat-based drink for a period of 10 weeks, while the treatment group (n=25) consumed the same products to which plant stanol ester had been added. The estimated daily intake of plant stanols was 9 g, and used containers were returned to validate compliance. Fasting blood samples were collected and body weights were assessed at weeks 0, 9, 10, and 14, while blood pressure was measured at weeks 0 and 9. Body weight, body mass index (BMI), and blood pressure did not change over the course of the study or differ between groups. Hematological parameters and indices of liver function also did not differ between treatment groups. Serum total and LDL-cholesterol levels in the treatment group were significantly reduced by 12.0 and 17.1% from the control group, respectively, demonstrating efficacy of high-dose plant stanols, as plant stanol ester, in reducing cholesterol levels (p<0.001). Similar reductions were seen when comparing serum total and LDL-cholesterol levels at end-of-intervention from baseline levels for subjects in the treatment group, and only 2 of the 25 subjects did not see reductions in LDL-cholesterol levels following plant stanol ester consumption. The high dose of plant stanols consumed in this study did not alter serum levels of HDL-cholesterol, triacylglycerides, or levels of vitamins A and D and α- and β-tocopherols, after adjustment for total cholesterol. Serum levels of α-carotene and β-carotene were reduced from baseline by approximately 40% in subjects consuming plant stanol ester (p<0.001), with

differences remaining even after controlling for total cholesterol levels. However, the functional impact of reduced carotene absorption is thought to be minimal, as discussed further in Section 4.6.2.

Mensink *et al.* (2010) performed a randomized, double-blind, placebo-controlled dose-response study of plant stanol ester in 93 healthy individuals with baseline serum total cholesterol between 5 and 8 mmol/L and no history of cardiovascular diseases or other severe medical conditions. During a 3-week run-in period, subjects consumed a placebo margarine and soy-based yogurt. After this period, subjects were randomized to 1 of 4 groups and administered, for 4 weeks, either:

(i) Placebo (0 g plant stanols/day) – continued to consume the placebo margarine and soy-based yoghurt;
(ii) ~3 g plant stanols/day – consumed margarine supplemented with plant stanol ester plus placebo yogurt;
(iii) ~6 g plant stanols/day – consumed placebo margarine plus yogurt supplemented with plant stanol ester; or
(iv) ~9 g plant stanols/day – consumed margarine plus yogurt supplemented with plant stanol ester.

Fasting blood samples were collected at baseline and at weeks 2 and 3 of the run-in period, and weeks 6 and 7 of the treatment period; lipid results were based on average data from samples collected at weeks 2 and 3 and at weeks 6 and 7. Clinical biochemistry indicators of kidney and liver function were not altered by consumption of plant stanol ester, and no differences in the marker of inflammation (hs-CRP) were reported. The placebo-adjusted LDL-cholesterol reductions were 7.5, 12, and 17.4% for individuals in the 3, 6, and 9 g/day plant stanol groups, respectively. Thus, LDL-cholesterol seems to be dose-dependently reduced by plant stanols (as plant stanol ester), although it is notable that the LDL-cholesterol reduction in the 6 g/day plant stanols group was not statistically significant relative to the 3 g/day group. Changes in total cholesterol among the treatment groups were similar to those observed for LDL-cholesterol. Plant stanol consumption had no effects on HDL-cholesterol or triacylglyceride levels, and no substantial changes in nutrient status of antioxidants (β-carotene, lutein) and fat-soluble vitamins (α-tocopherol) were reported, as further discussed in Section IV.H.2.

3. Individuals with Hypercholesterolemia Taking Statin Medications

Several clinical studies published in 1999 to 2011 have evaluated the safety of plant stanol ester when co-consumed with a statin [Blair *et al.*, 2000; O'Neill *et al.*, 2004; Cater *et al.*, 2005 (study 2 and study 3); Cabezas *et al.*, 2006; de Jong *et al.*, 2006, 2008b,c; Goldberg *et al.*, 2006; Berendschot *et al.*, 2009; Plat *et al.*, 2009; Kelly *et al.*, 2011]; results for these studies are summarized in Table IV-5. In several of these studies, the primary objective was to assess the efficacy of plant stanol ester, when added to statin treatment, in reducing blood lipid levels; as

such, formal measures of safety were not conducted [Blair *et al.,* 2000; O'Neill *et al.,* 2004; Cater *et al.,* 2005 (study 2 and study 3); Cabezas *et al.,* 2006; Goldberg *et al.,* 2006]. Nonetheless, it is noteworthy that no adverse events were reported in these studies. The remaining studies, which also are summarized in Table IV-5, included formal measures of safety and are particularly useful in assessing the safety of plant stanol ester when co-consumed with statins (de Jong *et al.,* 2006, 2008b; Plat *et al.,* 2009). These studies are described in detail below.

de Jong *et al.* (2008b) examined the safety of plant stanol ester and plant sterol ester in a randomized, double-blind, placebo-controlled study of 41 hypercholesterolemic subjects (20 men, 21 women) who had been taking statin medication (simvastatin, atorvastatin, or pravastatin) for at least the past 6 months. Following a 4-week run-in period, during which subjects consumed a light margarine (40% fat) with no added plant stanol ester or plant sterol ester, subjects were divided into 3 different groups. One group continued to consume the control margarine (11 subjects) while others consumed margarine enriched with either plant stanol ester (15 subjects) or plant sterol ester (15 subjects) for a period of 16 weeks. Subjects were instructed to consume 30 g of margarine daily over at least 2 meals, providing an estimated daily intake of 2.5 g of plant stanols or plant sterols. Blood samples were collected following an overnight fast at the end of the run-in phase (baseline) and at end of treatment. Body weight and margarine usage among the treatment groups did not differ over the course of the study. Clinical biochemistry indicators of liver and kidney function, as well as hematological parameters, also were not altered by consumption of plant stanol ester or plant sterol ester. Plant stanol ester and plant sterol ester consumption for 16 weeks significantly lowered serum total and LDL-cholesterol levels by approximately 7 to 10% ($p \leq 0.05$). No differences in HDL-cholesterol, triacylglycerol, aproprotein A1 and aproprotein B100 levels were observed among the treatment groups. No significant differences in the levels of hydrocarbon carotenoid antioxidants (α- and β-carotene, lycopene), other fat soluble antioxidants (oxygenated carotenoids, tocopherols), or activities of antioxidant enzymes (red blood cell catalase, superoxide dismutase, or glutathione peroxidase) were reported by treatment groups in this study. Consumption of plant stanol ester or plant sterol ester also did not alter biomarkers of lipid peroxidation (oxidized LDL-cholesterol, malondialdehyde, 15-keto-dihydro-prostaglandin F2α), DNA damage (7-dihydro-8-oxo-2-deoxyguanosine), endothelial dysfunction [intracellular adhesion molecules (ICAM) and vascular cell adhesion molecules (VCAM)] and low-grade inflammation (E-selectin, monocyte chemotactic protein-1, CRP).

The effect of plant stanol ester on erythrocyte fragility was published in another report of the same clinical trial as consumption of plant stanol ester may potentially alter the viscoelasticity of cell membranes (de Jong *et al.*, 2006). Erythrocyte deformability, or the ability of red blood cells to change their shape while passing through small arterioles and capillaries, is important for proper microcirculation and delivery of oxygen to tissues. Fragile erythrocytes are also less resistant to hemolysis upon changes in osmotic pressures. Erythrocyte fragility was measured

by the concentration of saline solution (NaCl) that results in 50% hemolysis of blood samples collected at baseline and at the end of treatment. Cholesterol-standardized serum levels of plant stanols (sitostanol, campestanol) did not differ from baseline at the end of treatment in all treatment groups. In contrast, cholesterol-standardized levels of campesterol and sitosterol in serum were significantly elevated at the end of treatment for subjects in the plant sterol group only ($p<0.001$). Protein-standardized levels of campesterol and cholesterol in erythrocyte membranes did not differ significantly among treatment groups. Sitosterol, sitostanol, and campestanol were not detectable in erythrocytes. Osmotic fragility was similar for all treatment groups at both baseline and at end of treatment, and was not associated with serum levels of plant sterols or plant stanols, or with erythrocyte levels of campesterol and cholesterol. As such, consumption of plant stanol ester and plant sterol ester for 16 weeks did not significantly alter serum or erythrocyte levels of sterols, or affect erythrocyte fragility in hypercholesterolemic subjects taking statin medications.

Plat *et al.* (2009) tested the effects of plant stanol ester on the serum lipid profile and markers of low-grade inflammation and endothelial dysfunction in 36 subjects with metabolic syndrome, defined as having a family history of CHD in a first-degree relative, or elevated BMI of >25 kg/m^2 or abdominal obesity with waist circumference of >102 cm for men and >88 cm for women. The total cholesterol level in serum was approximately 6 to 7 mmol/L for these participants, and they had to meet the criteria to use low-dose (10 mg) statin medications that are available over-the-counter in the United Kingdom (UK). After a 3-week run-in period, during which subjects consumed placebo yogurt drinks and capsules, they were randomized into the control group (9 subjects) who continued to consume these products, or groups receiving 10 mg of simvastatin plus placebo yogurt (10 subjects), placebo tablet plus yogurt containing stanol ester (9 subjects), or simvastatin plus yogurt containing stanol ester (8 subjects) for a period of 8 weeks. Plant stanol ester was consumed at an estimated daily intake of 2.0 g of plant stanols. Fasting blood samples were collected twice during the run-in period (week 2 and 3) and at weeks 10 and 11 (end of treatment), and lipid data were presented as averages. High compliance to treatment was observed and there were no changes in body weight over the course of the study. Clinical biochemistry parameters indicative of liver and kidney function, as well as hematological parameters, were not altered by either plant stanol ester or simvastatin treatment. Plant stanol ester, simvastatin and the combination treatment significantly lowered levels of total and non-HDL-cholesterol, triacylglyceride, and the ratio of total cholesterol to HDL-cholesterol when compared to placebo. Plant stanol ester consumption did not alter levels of apoB, apoA-I, apoCIII, cholesterol ester transfer protein, or free fatty acids. An increase in absolute levels of apoCII was observed from plant stanol ester and combination treatment with simvastatin; however, the ratio of apoCII:CIII was unchanged suggesting the changes in triacylglyceride levels cannot be accounted for by changes in the activity of lipoprotein lipase. No differences in the markers of low-grade inflammation and endothelial dysfunction [sE-selectin, sICAM, sVCAM-1, monocyte chemoattractant protein-1, IL-6, metallopeptidase-9 (MMP-9), CD40, hs-CRP] were observed among the treatment groups.

Although safety measures were not formally conducted in the study by de Jong *et al.* (2008c), the study is important in the demonstration of the safety of plant stanol ester when co-consumed with statins, given that this study was 85 weeks in duration. For this reason, this study, as well as the subsequent follow-up studies (Berendschot *et al.*, 2009; Kelly *et al.,* 2011) of the same population group as was studied by de Jong *et al.* (2008c) are summarized below.

The long-term effects of consuming plant stanol ester or plant sterol ester were assessed in a randomized, double-blind, placebo-controlled study of 54 subjects receiving stable statin treatment (de Jong *et al.*, 2008c). Following a 5-week run-in period, during which subjects consumed a light margarine (40% fat) with no added plant stanol ester or plant sterol ester, subjects were randomly allocated to 1 of 3 groups. One group continued to consume the control margarine (17 subjects) while others consumed margarine enriched with either plant sterol ester (18 subjects) or plant stanol ester (19 subjects) for a period of 85 weeks. Subjects were instructed to consume 30 g of margarine daily to achieve an estimated daily intake of 2.5 g of plant sterols or plant stanols. Results were based on fasting blood samples that were collected at week 5 (end of run-in), week 45 (half-way of treatment) and week 90 (end of treatment). Results from 6 subjects were excluded from analyses because they changed their dose or type of statin medication, and 1 subject was considered an outlier and excluded from analyses because their serum total cholesterol decreased by more than 5 standard deviations from the group mean. Body weight and margarine usage did not significantly differ among the treatment groups over the course of the study. Serum sitostanol and campestanol levels were significantly increased in the plant stanol ester treatment group, while serum sitosterol and campesterol levels were significantly increased in the plant sterol ester treatment group. Long-term consumption of plant sterol ester or plant stanol ester was efficacious in lowering cholesterol levels; in the plant stanol ester group, total and LDL-cholesterol were reduced by 6.9 to 9.4% and 8.7 to 13.1%, respectively, with similar results observed at weeks 45 and 90. Apolipoprotein B levels were also reduced by 5.8% at week 45 ($p=0.06$) and by 10.0% at week 90 ($p<0.05$) for those consuming plant stanol ester compared to controls. No significant differences in HDL cholesterol, triacylglycerides, or apolipoprotein (apo) A1 levels were observed among treatment groups. Serum levels of biomarkers of bile acid synthesis (oxysterols and 7α-hydroxy-4-cholesten-3-one) did not significantly differ among treatment groups. Berendschot *et al.* (2009) also published, in a subsequent report, that plant stanol ester had no effect on lutein levels or macular pigment optical density (MPOD) in this study sample, as described in Section 4.6.2. Together, these results suggest plant stanol ester and plant sterol ester can safely and effectively reduce LDL-cholesterol levels in hypercholesterolemic subjects who are already taking statin medications.

In a subsequent study, the effect of long-term plant sterol ester and plant stanol ester consumption on the microcirculation of blood vessels in the retina, which can serve as a predictor of atherosclerosis and CHD risk, was assessed (Kelly *et al.*, 2011). Retinal images were taken at baseline (end of run-in period) and at the end of treatment. Data from 30 of the

subjects who participated in the original study by de Jong *et al.* (2008c) were used for this analysis. Data could not be collected for all subjects because subjects could not travel the long distance to reach the testing facilities for retinal photography, did not complete the 85-week treatment, changed type or dose of statin medication during the study, failed to attend the second retinal photograph session, or had extreme changes in serum total cholesterol levels. Cholesterol levels for subjects in this report did not differ from those excluded from analyses at either baseline or end of treatment. Plant stanol ester consumption did not significantly change the diameter of the retinal arteriole and venule over the course of the study. A trend towards greater increase in venular diameter between end of treatment and baseline was observed for individuals consuming plant sterol ester, independently of changes in LDL-cholesterol levels. The authors noted that plant sterol ester may have adverse effects on vascular functions, although the mechanism of this observation is unclear and the functional significance of increased retinal venular diameter on cardiovascular health needs to be examined further.

Table IV-5 Summary of Human Studies Published Between 1999 and 2011

Study Objective	Study Design and Treatment Duration	Study Population (Sex, Age, BMI or Body Weight, Health Status)	Dose of Plant Stanols and Delivery Matrix[a]	Safety-Related Results[b,c]	Reference
Studies Conducted in Normocholesterolemic Subjects					
To examine in humans the effects on serum lipids, lipoproteins and fat-soluble antioxidants of a daily consumption of 2.5 g plant stanols, consumed either once per day at lunch or divided over 3 meals	Randomized, double-blind, placebo-controlled, cross-over 4 weeks (no washout period)	39 subjects (11 M, 28 F; mean age of 31 years; mean BMI of 22.7±2.6 kg/m^2; healthy)	0 (control) or 2.5 g/day (once per day) or 2.5 g/day (3 times per day) Once per day period: Margarine (20 g/day at lunch) 3 times per day period: Margarine (10 g/day at breakfast, 20 g/day at lunch) and shortening in cakes and cookie (10 g/day after dinner)	• One subject dropped out during the 1st week because she could not combine the study protocol with her lifestyle • NSD in body weight • NSD in retinol concentrations • No adverse events were reported in the study • Refer to Table 4.6-1 for results for carotenoids and fat-soluble vitamins	Plat *et al.*, 2000
To investigate the effect of apolipoprotein E phenotype on changes in plasma levels of lipids and apoproteins by PSE ingestion in Japanese subjects whose diet is low in fat and cholesterol	Randomized, placebo-controlled, parallel Blinding NR 4-weeks, followed by a 4-week observation period	96 subjects (34 M, 62 F; mean age of 46 years; mean BMI of 23.5 kg/m^2; healthy)	0 (control), 2 (low-dose), or 3 (high-dose) g/day Spread (amount NR)	• No adverse events were reported in the study	Ishiwata *et al.*, 2002

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Table IV-5 Summary of Human Studies Published Between 1999 and 2011

Study Objective	Study Design and Treatment Duration	Study Population (Sex, Age, BMI or Body Weight, Health Status)	Dose of Plant Stanols and Delivery Matrix[a]	Safety-Related Results[b,c]	Reference
To examine the cholesterol-lowering capacity of PSE emulsified into a low-fat yogurt	Randomized, double-blind, placebo-controlled, parallel 4 weeks	60 subjects (16 M, 44 F; mean age of 36.0 years; mean BMI of 23.3±2.7 kg/m^2; healthy)	0 (control) or 2.98 g/day Yogurt (450 mL/day)	• NSD in body weight • NSD in serum ALT, AST, GGT, creatinine, and hs-CRP • NSD in hematology parameters (details not provided) • Authors stated that "*there were no suggestions that consumption of the yogurt with PSE caused any physical complaints*" • Refer to Table 4.6-1 for results for carotenoids and fat-soluble vitamins	Mensink *et al.*, 2002
To determine the effects of plant stanol ester on serum lipid profile and LDL oxidation in individuals with normal to mild hypercholesterolemia who may have the potential to develop more severe hypercholesterolemia	Randomized, double-blind, placebo-controlled, parallel 4 weeks (2 week run-in)	51 subjects (26M, 25F; mean age 29; mean BMI 23; healthy)	0 (control) or 2.0 g/day Yogurt	• No adverse events were reported in the study • Refer to Table 4.6-1 for results for carotenoids and fat-soluble vitamins	Hyun *et al.*, 2005
To investigate whether PSE given in higher doses than the 2 g/day dose recommended by the ATP III of the National Cholesterol Education Program will provide additional LDL-cholesterol lowering efficacy	Randomized, double-blind, placebo-controlled, parallel 6 weeks	8 subjects (6 M, 2 F; mean age of 58 years; mean BMI of 28±4; healthy)	0 (control), 2 (low-dose), 3 (mid-dose), or 4 (high-dose) g/day Margarine (24 g/day)	• No adverse events were reported in the study	Cater *et al.*, 2005 (Study 1)

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Table IV-5 Summary of Human Studies Published Between 1999 and 2011

Study Objective	Study Design and Treatment Duration	Study Population (Sex, Age, BMI or Body Weight, Health Status)	Dose of Plant Stanols and Delivery Matrix[a]	Safety-Related Results[b,c]	Reference
To investigate the feasibility of using solid dosage forms containing stanol lecithin to lower human LDL-cholesterol	Randomized, double-blind, placebo-controlled, parallel 6 weeks	25 subjects (11 M, 14 F; mean age of 46.5±8.1 years; mean BMI of 26 kg/m^2; healthy)	0 (control) or 1.26 g/day (provided as free plant stanols) Tablets (6/day)	• No adverse events were reported in the study	McPherson *et al.*, 2005
		27 subjects (9 M, 18 F; mean age 50.7±12.5 years; mean BMI of 27.8 kg/m^2; healthy)	0 (control) or 1.01 g/day (provided as free plant stanols) Capsules (8/day)		
To examine if a vegetable oil-based stanol ester mixture with 68% sitostanol and 32% campestanol is less effective than the wood-based stanol ester mixture in reducing cholesterol absorption	Randomized, double-blind, placebo-controlled, parallel 8 weeks	112 subjects (41 M, 71 F; mean age of 33.0 years; mean BMI of 22.9 kg/m^2; healthy)	0 (control), 3.8 (vegetable derived stanol provided as stanol ester), 4.0 (wood derived stanol provided as stanol ester) g/day Margarine (20 g/day) and shortening (10 g/day)	• 1 subject (group not specified) withdrew due to lack of motivation • NSD in body weight • changes in fibrinogen, FVIIc, FVIIam, AT-111, PAI-1 and t-PA were not significantly different between the groups • Refer to Table 4.6-1 for results for carotenoids and fat-soluble vitamins	Plat and Mensink, 2000, 2001a,b; Plat *et al.*, 2009 (Study 2)
To examine the independent and combined effects of PSE supplementation and aerobic exercise on blood lipids in health middle-aged men and postmenopausal women with varying baseline blood cholesterol concentrations	Randomized, double-blind, placebo-controlled, parallel 9 weeks	26 subjects (14 M, 12 F; mean age of 52.7 years; mean BMI of 27±1 kg/m^2; healthy)	0 (control) or 3.0 g/day Margarine (42 g/day)	• 1 subject in control group dropped out of the study due to medical problems • 9 subjects (3 from PSE group, 6 from control group) were removed from the statistical analyses because changes in body weight exceeded the *a priori* cutoff of ± 2 kg	Alhassan *et al.*, 2006

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Table IV-5 Summary of Human Studies Published Between 1999 and 2011					
Study Objective	**Study Design and Treatment Duration**	**Study Population (Sex, Age, BMI or Body Weight, Health Status)**	**Dose of Plant Stanols and Delivery Matrix[a]**	**Safety-Related Results[b,c]**	**Reference**
				• NSD in body weight, BMI, waist girth, and body fat • No adverse events were reported in the study	
Studies Conducted in Non-Medicated Hypercholesterolemic Subjects					
To examine the effects of feeding esterified plant sterols and stanols in margarine mixtures on sterol metabolism in hyperlipidemic males consuming fixed foods diets	Randomized, double-blind, placebo-controlled, crossover 3 weeks with a 5-week washout period	15 M subjects (age and BMI NR; hyperlipidemic)	0 (control) or 1.76 g/day Margarine (23 g/day)	• 2 subjects dropped out of the study during the 1st phase due to personal reasons • 1 subject was withdrawn from the study due to poor compliance • NSD in body weight • NSD in clinical chemistry, complete blood counts, and urinalysis (parameters not specified) • No clinical irregularities observed during regular physical exams • Subjects tolerated diet without any reports of adverse effects, and did not report any abnormal or atypical taste, smell, color, or mouth-feel effects • Refer to Table 4.6-1 for results for carotenoids and fat-soluble vitamins	Jones *et al.*, 2000; Raeini-Sarjaz *et al.*, 2002

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Table IV-5 Summary of Human Studies Published Between 1999 and 2011

Study Objective	Study Design and Treatment Duration	Study Population (Sex, Age, BMI or Body Weight, Health Status)	Dose of Plant Stanols and Delivery Matrix[a]	Safety-Related Results[b,c]	Reference
To assess whether advice to consume specific daily amounts of foods high in carotenoids prevents a reduction in plasma carotenoid concentrations in subjects who consume plant sterol or stanol ester	Randomized, double-blind, placebo-controlled, cross-over 3 weeks (no washout period)	46 subjects (20 M, 26 F; mean age of 56.5 years; mean BMI of 26.2 kg/m^2; hypercholesterolemia)	0 (control) or 2.5 g/day Spread (25 g/day)	• 2 subjects (group not specified) dropped out during the study ○ 1 because of an unrelated hospitalization ○ 1 because of travel commitments • No adverse events were reported in the study • Refer to Table 4.6-1 for results for carotenoids and fat-soluble vitamins	Noakes *et al.*, 2002
To examine the effect of supplementation with unesterified plant sterols and stanols on plasma lipid and plant sterol concentrations and cholesterol absorption, synthesis, and turnover	Randomized, double-blind, placebo-controlled, crossover 3 weeks with a 4-week washout period	15 subjects (9 M, 6 F; mean age of 47.8 years; mean BMI of 30.8±1.3 kg/m^2; primary familial hyperlipidemia)	0 (control) or 1.8 g/day (provided as free plant stanols) Butter (amount NR)	• 1 subject dropped out at the end of the first period because of difficulties with daily transportation to the unit • NSD in body weight • NSD in clinical chemistry, hematology, and urinalyses (parameters not specified) • All individuals tolerated the diet without any reported adverse events • Results of regular physical exams showed no suggestion of any clinical irregularities	Vanstone *et al.*, 2002; Charest *et al.*, 2004

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Table IV-5 Summary of Human Studies Published Between 1999 and 2011

Study Objective	Study Design and Treatment Duration	Study Population (Sex, Age, BMI or Body Weight, Health Status)	Dose of Plant Stanols and Delivery Matrix[a]	Safety-Related Results[b,c]	Reference
To assess the cholesterol-lowering effect of plant sterol ester and PSE enriched low-fat yogurt in modestly hypocholesterolemic subjects	Randomized, double-blind, placebo-controlled, cross-over 3 weeks (no washout period)	40 subjects (17 M, 23 F; mean age of 60.4 years; mean BMI of 26.5±3.2 kg/m^2; hypercholesterolemia)	0 (control) or 1.7 g/day Low-fat yogurt (300 g/day)	• No adverse events were reported in the study	Noakes *et al.*, 2005
To determine the cholesterol-lowering efficacy of plant stanol ester capsules taken as part of a normal diet	Randomized, double-blind, placebo-controlled, parallel 3 weeks	42 subjects (20M, 22F; mean age 40; mean BMI 25; mild to moderate hypercholesterolemia)	0 (control0 or 2 g/day Capsule	• NSD in hematology and biochemistry values (blood count, GGT, ALT) • Subjects did not report any side effects	Lagström *et al.*, 2006
To study the effects of PSE-containing spread on plasma levels of TC, LDL-cholesterol, and apolipoprotein B in a randomized, placebo-controlled trial in Japanese subjects whose diet is low in fat and cholesterol. The effects of plant stanol ester on plasma levels of arteriosclerosis-promoting factors, namely remnants of TAG-rich lipoproteins, cholesteryl ester transfer protein, and oxidized LDL, were also studied. The assessment of safety was also made.	Randomized, double-blinded, placebo-controlled, parallel 4 weeks	105 subjects (38 M, 67 F; mean age of 47.4 years; mean BMI of 23.7 kg/m^2; healthy with elevated serum TC concentrations)	0 (control), 2 (low-dose), or 3 (high-dose) g/day Low-fat spread (12.6 or 18.9 g/day)	• NSD in body weight • NSD in ALT, AST, ALP, GGT, total protein, albumin, uric acid, urea nitrogen, creatinine, glucose, and electrolytes • NSD in complete blood count and urinalysis (parameters not specified) • No subjective findings with the exception of mild diarrhea in 1 subject in low-dose group and 1 subject in high-dose group • Refer to Table 4.6-1 for results for carotenoids and fat-soluble vitamins	Homma *et al.*, 2003

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Table IV-5 Summary of Human Studies Published Between 1999 and 2011

Study Objective	Study Design and Treatment Duration	Study Population (Sex, Age, BMI or Body Weight, Health Status)	Dose of Plant Stanols and Delivery Matrix[a]	Safety-Related Results[b,c]	Reference
To investigate cholesterol-lowering effects of stanol ester and sterol ester-enriched margarines as part of a low-fat diet.	Randomized, double-blind, cross-over 4 weeks (each period)	34 subjects (M and F; number per sex NR; mean age of 48.8±8.1 years; mean BMI of 24.9±2.4 kg/m^2; hypercholesterolemia)	0 (control) or 1.92 g/day Margarine (20 g/day)	• NSD in BMI • ↓ mean fasting plasma glucose and serum GGT • ↑ mean serum TSH concentration • 1 subject had marginally elevated serum TSH concentrations at the beginning and end of the study; however, excluding this subject from statistical analyses did not affect the results • Authors reported that the changes in routine laboratory measurements cannot be connected with the treatments • ↓ mean blood Hb concentration in M subjects; however, the authors attributed this finding to the fact that 7 M subjects also participated in a fat-loading test with many blood samples at the end of all test margarine periods • NSD in blood thrombocyte counts • NSD in serum ALT and creatinine • No adverse events were reported in the study • Refer to Table 4.6-1 for results for carotenoids and fat-soluble vitamins	Hallikainen *et al.*, 2000a

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Table IV-5 Summary of Human Studies Published Between 1999 and 2011

Study Objective	Study Design and Treatment Duration	Study Population (Sex, Age, BMI or Body Weight, Health Status)	Dose of Plant Stanols and Delivery Matrix[a]	Safety-Related Results[b,c]	Reference
To determine the efficacy on plasma cholesterol-lowering of plant sterol ester or non-esterified stanols eaten within low-fat foods as well as margarine	Randomized, single-blind, placebo-controlled, cross-over 4 weeks (no washout period)	22 subjects (18 M, 4 F; mean age of 60 years; mean BMI of 24±1.9 kg/m^2; hypercholesterolemia)	0 (control) or 2.4 g/day (provided as free plant stanols) Cereal (40 g/day), sliced bread (70 g/day), and margarine (20 g/day)	• No adverse events were reported in the study • Refer to Table 4.6-1 for results for carotenoids and fat-soluble vitamins	Nestel *et al.*, 2001
To show that fat-free, lecithin-formulated soy stanols lower cholesterol absorption and serum LDL cholesterol	Randomized, double-blind, placebo-controlled, parallel 4 weeks with a 6-week run-in period	24 subjects (9 M, 15 F; mean age of 50.6 years; mean BMI of 26.1±4.5 kg/m^2; healthy with elevated serum TC levels)	0 (control) or 1.9 g/day (provided as free plant stanols) 180 mL/day lemonade-flavored beverage	• 2 subjects in the plant stanol group were excluded from analysis as they failed to provide a blood sample at Week 10 • No adverse events were reported in the study	Spilburg *et al.*, 2003
To examine the effects of sitostanol as sitostanol ester in softgel form on serum lipid concentrations in hypercholesterolemic individuals	Randomized, double-blind, placebo-controlled, parallel 4 weeks	30 subjects (21 M, 9 F; mean age of 53.7 years; mean BMI of 27.5±3.4 kg/m^2; mild to moderate hypercholesterolemia)	0 (control) or 1.6 g/day Capsules	• 1 subject (group not specified) dropped out for reasons unrelated to the study (details not provided) • NSD in body weight, blood pressure, and heart rate compared to baseline • Only adverse event reported was rash in the placebo group (number of subjects NR)	Woodgate *et al.*, 2006

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Table IV-5 Summary of Human Studies Published Between 1999 and 2011

Study Objective	Study Design and Treatment Duration	Study Population (Sex, Age, BMI or Body Weight, Health Status)	Dose of Plant Stanols and Delivery Matrix[a]	Safety-Related Results[b,c]	Reference
To study the relation between plant stanols provided as PSE on changes in serum concentrations of LDL cholesterol and fat-soluble antioxidants	Randomized, double-blind, placebo-controlled, parallel 4 weeks	93 subjects (49 M, 44 F; mean age of 56.1 years; mean BMI of 25.1 kg/m^2; healthy with elevated serum TC concentrations)	0 (control), 3 (low-dose), 6 (mid-dose), or 9 (high-dose) g/day Margarine (20 g/day) and soy-based yogurt (300 mL/day)	• 3 subjects (group not specified) dropped out during the course of the study for reasons not related to the study protocol • 1 subject (group not specified) was excluded because of poor compliance • NSD in body weight • NSD in serum creatinine, ALP, ALT, AST, GGT, bilirubin, and hs-CRP • 6 subjects (group not specified) had on 1 or more occasions hs-CRP >7 mg/L; however, excluding these subjects from statistical analysis did not affect the results • No adverse events were reported in the study • Refer to Table 4.6-1 for results for carotenoids and fat-soluble vitamins	Mensink *et al.*, 2010
To investigate the cholesterol-lowering effects of a low-fat cheese enriched with PSE in mildly hypercholesterolemic subjects, as part of their normal diet	Randomized, double-blind, controlled, parallel 5 weeks	67 subjects (24 M, 43 F; mean age 43.3 years; mean body weight of 75.2 kg; mild hypercholesterolemia)	0 (control) or 2.0 g/day Low-fat cheese (50 g/day)	• NSD in body weight • 14 subjects in PSE group and 21 subjects in control group reported adverse effects, such as headaches, tiredness, flatulence, diarrhea, and constipation, most of which were mild and occurred only occasionally	Jauhiainen *et al.*, 2006

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Table IV-5 Summary of Human Studies Published Between 1999 and 2011					
Study Objective	**Study Design and Treatment Duration**	**Study Population (Sex, Age, BMI or Body Weight, Health Status)**	**Dose of Plant Stanols and Delivery Matrix[a]**	**Safety-Related Results[b,c]**	**Reference**
				• 1 subject in control group reported that constipation and abdominal discomfort were very disturbing during the first 3 weeks of the study • NSD in changes in routine safety parameters (GGT, creatinine, glucose, uric acid)	
To investigate the effects of low-fat milk products enriched with plant stanol ester on serum lipids	Randomized, double-blind, placebo-controlled, parallel 5 weeks	202 subjects (73M, 129 F; mean age 46 years; mean BMI 25; mild or moderate hypercholesterolemia)	0 (control) or 2 g/day Yogurt, yogurt (single-shot drink), milk	• NSD in GGT, creatinine, glucose or uric acid	Seppo *et al.*, 2007
To evaluate the effects of 2 commonly available strategies (PSE drink and 10 mg simvastatin) on CHD risk variables in participants with metabolic syndrome	Randomized, double-blind, placebo-controlled, parallel 8 weeks	18 subjects (12 M, 6 F; mean age of 60.0 years; mean BMI of 29.2 kg/m^2; metabolic syndrome)	0 (control) or 2.0 g/day 60 mL/day of low-fat yogurt drink	• NSD in body weight • NSD in serum total bilirubin, ALT, AST, ALP, GGT, and creatinine • NSD in WBC, lymphocytes, mononuclear cells, granulocytes, RBC, Hb, hematocrit, MCV, and platelet count and volume • NSD in markers of low-grade inflammation (hs-CRP, SAA, and IL-6) or endothelial function (sICAM, sVCAM, sE selectin, MCP-1, MMP-9, and CD40L)	Plat *et al.*, 2009 (Study 1)

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Table IV-5 Summary of Human Studies Published Between 1999 and 2011

Study Objective	Study Design and Treatment Duration	Study Population (Sex, Age, BMI or Body Weight, Health Status)	Dose of Plant Stanols and Delivery Matrix[a]	Safety-Related Results[b,c]	Reference
				• No adverse events were reported in the study	
1) To study the effects of 8.8 g/d of plant stanols as ester on serum lipids and safety variables in subjects with mild to moderate hypercholesterolemia; 2) To evaluated the effects of very high daily intake of plant stanols (8.8 g/day) as ester on cholesterol metabolism, and serum levels of plant sterols and stanols	Randomized, double-blind, placebo-controlled, parallel 10 weeks	49 subjects (17 M, 32 F; mean age of 61.8 years; mean BMI of 25.4 kg/m^2; mild to moderate hypercholesterolemia)[d]	0 (control) or 8.8 g/day Spread (20 g/day) and an oat-based drink (2.5 dL/day)	• NSD in body weight, BMI, and blood pressure • NSD in serum ALT, ALP, GGT, or TSH • NSD in Hb, hematocrit, number of erythrocytes, MCH, MCV, or MCHC • NSD in haptoglobin and bilirubin concentrations • NSD in counts of blood leucocytes or thrombocytes • PSE treatment was reported to be well-tolerated with no side effects other than ↓ serum β-carotene concentrations • Refer to Table 4.6-1 for results for carotenoids and fat-soluble vitamins	Gylling *et al.*, 2010a,b
To investigate the effects of stanol and sterol ester on endothelial function in hypercholesterolemic subjects	Randomized, double-blind, cross-over 10 weeks (no washout period) An age-matched parallel control group was included in the study	80 subjects (48 M, 32 F; mean age of 50.1 years; mean BMI of 25.7 kg/m^2; hypercholesterolemia)	0 (control) or 2.07 g/day Spread (25 g/day)	• ↓ systolic blood pressure • NSD in inflammatory markers, plasma glucose, serum insulin, or serum ALT • NSD in endothelial function markers • No adverse events were reported in the study	Hallikainen *et al.*, 2006

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Table IV-5 Summary of Human Studies Published Between 1999 and 2011

Study Objective	Study Design and Treatment Duration	Study Population (Sex, Age, BMI or Body Weight, Health Status)	Dose of Plant Stanols and Delivery Matrix[a]	Safety-Related Results[b,c]	Reference
	Control group received spread containing no added PSE for 20 weeks				
To evaluate whether PSEs mixed with different vegetable oil spreads improved arterial health	Randomized, double-blind, placebo-controlled, parallel 12 weeks	190 subjects (100 M, 90 F; mean age of 42.4 years; mean BMI of 25.8 kg/m^2; healthy)	0 (control) or 2.0 g/day Spread (25 g/day)	• 7 subjects in PSE group and 3 subjects in control group dropped out from the study for reasons unrelated to the study (details not provided in study publication) • NSD in body weight • No adverse events were reported in the study	Raitakari *et al.*, 2008
To investigate whether serum levels of vitamin D, retinol, α-tocopherol, and α- and β-carotene are altered by 1-year sitostanol ester margarine diet	Randomized, double-blind, controlled 1 year	153 subjects (mean age of 50 years; mean BMI of 25 kg/m^2; moderate hypercholesterolemia)[e]	0 (control), 2.0, or 3.0[d] g Margarine (24 g/day)	• 2 subjects in control group discontinued study (reasons not provided) • No adverse events were reported in the study • Refer to Table 4.6-1 for results for carotenoids and fat-soluble vitamins	Gylling *et al.*, 1999a
To investigate whether any of the common polymorphic sites of the ABCG5 and ABCG8 genes regulate vascular function and structure and cholesterol metabolism in hypercholesterolemic subjects at baseline and during 1-year inhibition of cholesterol absorption with PSE or sterol esters	Randomized, double-blind, placebo-controlled, parallel 52 weeks	189 subjects (87 M, 102 F; mean age of 53.7 years; mean BMI of 25.8±0.2 kg/m^2; mild to moderate hypercholesterolemia)[d]	0 (control) or 2.13 g/day Spread (25 g/day)	• Most frequent reasons for drop-out were reported to be unwillingness to use the test spread in the indicated amount or personal reasons not related to the study (number of drop-outs NR) • NSD in body weight, waist circumference, and blood pressure	Gylling *et al.*, 2009

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Table IV-5 Summary of Human Studies Published Between 1999 and 2011

Study Objective	Study Design and Treatment Duration	Study Population (Sex, Age, BMI or Body Weight, Health Status)	Dose of Plant Stanols and Delivery Matrix[a]	Safety-Related Results[b,c]	Reference
				• No adverse events were reported in the study	
Studies Conducted in Medicated Hypercholesterolemic Subjects					
To investigate whether substantial reduction in LDL cholesterol can be obtained in postmenopausal women with hypercholesterolemia by addition of PSE to the diet	Randomized, double-blind, placebo-controlled, crossover 6 weeks (no washout period)	13 F subjects (mean age of 57±6 years; mean BMI of 27.5±3.6 kg/m^2; postmenopausal, hypercholesterolemia)	0 (control) or 3.0 g/day, or 3.0 g/day in combination with 10 mg/day of simvastatin Margarine (24 g/day)	• NSD in hs-CRP • No adverse events were reported in the study	Cater *et al.*, 2005 (Study 2)
To investigate the effect of plant stanols in margarines, combined with a lipid-lowering dietary intervention, in patients already on lipid-lowering medications at maximal doses not reaching their target lipid levels	Randomized, single-blind, placebo-controlled, parallel 6 weeks	20 subjects (8 M, 12 F; mean age of 48.7 years; mean BMI of 26.3 kg/m^2; primary hyperlipidemia)	0 (control) or 3.0 g/day Margarine (30 to 35 g/day)	• NSD in body weight or BMI • Treatment reported to be well-tolerated without significant side effects • No significant effects on quality of life	Cabezas *et al.*, 2006
To determine whether plant-stanol containing tablets could be used as an adjunctive therapy for patients who were already on a stable statin dose	Randomized, double-blind, placebo-controlled, parallel 6 weeks	26 subjects (9 M, 17 F; mean age of 59.5 years; mean BMI of 27.2 kg/m^2; on statin treatment for ≥90 days)	0 (control) or 1.80 g/day (provided as free plant stanols) Tablets	• No adverse events were reported in the study	Goldberg *et al.*, 2006
To compare the cholesterol-lowering efficacy and other metabolic effects of plant sterol and stanol ester	Randomized, double-blind, placebo-controlled, parallel 8 weeks with 4-week washout period	88 subjects (38 M, 50 F; mean age of 47.5 years; BMI and weight NR; 42 were normocholesterolemic and 46 had FH)[d]	1.6 (low-dose) or 2.6 (high-dose) g/day Margarine (20 g/day) and cereal bar (25 g/day)	• 3 subjects were withdrawn from study because of protocol violations • NSD in body weight • No adverse events were reported in the study	O'Neill *et al.*, 2004

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Table IV-5 Summary of Human Studies Published Between 1999 and 2011

Study Objective	Study Design and Treatment Duration	Study Population (Sex, Age, BMI or Body Weight, Health Status)	Dose of Plant Stanols and Delivery Matrix[a]	Safety-Related Results[b,c]	Reference
To examine the effect of plant stanol ester on cholesterol in patients taking statin medications but still have elevated cholesterol levels	Randomized, double-blind, placebo-controlled, parallel 8 weeks (6 week follow-up)	167 subjects (100M, 67F; mean age 56±10 years; mean BMI 185 ±37 kg/m^2; stable statin treatment)	0 or 3.0 g/day Margarine (24 g/day)	• No treatment-related adverse events were reported	Blair *et al.*, 2000
To investigate whether ATP III goals can be obtained by adding PSE to an LDL-lowering regimen in high-risk patients who retain LDL cholesterol levels in the above-optimal range, despite ongoing statin therapy	Randomized, double-blind, placebo-controlled, crossover 8 weeks (no washout period)	10 M subjects (mean age of 66 years; BMI and weight NR; CHD and receiving statin therapy)	0 (control) or 3.0 g/day Margarine (24 g/day)	• ↓ in serum hs-CRP • No adverse events were reported in the study	Cater *et al.*, 2005 (Study 3)
To evaluate the effects of 2 commonly available strategies (PSE drink and 10 mg simvastatin) on CHD risk variables in participants with metabolic syndrome	Randomized, double-blind, placebo-controlled, parallel 8 weeks	18 subjects (11 M, 7 F; mean age of 60.6 years; mean BMI of 29.2 kg/m^2; metabolic syndrome)	0 (control) or 2.0 g/day (provided as free plant stanols), in combination with 10 mg of simvastatin Low-fat yogurt drink (60 mL/day)	• NSD in body weight • NSD in serum total bilirubin, ALT, AST, ALP, GGT, and creatinine • NSD in WBC, lymphocytes, mononuclear cells, granulocytes, RBC, Hb, hematocrit, MCV, and platelet count and volume • NSD in markers of low-grade inflammation (hs-CRP, SAA, and IL-6) or endothelial function (sICAM, sVCAM, sE selectin, MCP-1, MMP-9, and CD40L) • No adverse events were reported in the study	Plat *et al.*, 2009 (Study 1)

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Table IV-5 Summary of Human Studies Published Between 1999 and 2011

Study Objective	Study Design and Treatment Duration	Study Population (Sex, Age, BMI or Body Weight, Health Status)	Dose of Plant Stanols and Delivery Matrix[a]	Safety-Related Results[b,c]	Reference
1) To assess the effects of plant sterol or stanol ester consumption on their incorporation into erythrocytes and their effects on osmotic fragility of RBC in subjects on stable statin therapy; 2) To examine the effects of plant sterol and stanol consumption on lipid metabolism and markers of antioxidant status, oxidative stress, endothelial dysfunction and low-grade inflammation in subjects on stable statin treatment	Randomized, double-blind, placebo-controlled, parallel 16 weeks (4-week run-in)	41 subjects (20 M, 21 F; mean age 58.5±8.3 years, mean BMI 26.8±2.6 kg/m^2; on stable statin treatment)[c]	0 (control) or 2.5 g/day Margarine (30 g/day)	• No adverse events were reported in the study	de Jong *et al.*, 2006, 2008b

1) To investigate the long-term effects of combining statin treatment with plant sterol- or stanol ester-enriched functional foods on markers of endothelial dysfunction and arterial stiffness; 2) to evaluate the possible effects of plant stanol and sterol ester on serum lutein/zeaxanthin concentration in relation to the MPOD; and 3) to examine the effects of long-term plant sterol or stanol consumption on neurocognitive functioning and mood	Randomized, double-blind, placebo-controlled, parallel
	
	85 weeks
		36 subjects (22 M, 14 F; mean age of 59.4 years; mean BMI of 26 kg/m^2; on stable statin treatment)[d]	0 (control) or 2.5 g/day
			
			Margarine (30 g/day)
				• 2 subjects in PSE group dropped out
				  ○ 1 in Week 48, because of discontinuation of statin treatment due to side effects
				  ○ 1 in Week 70, when starting in a weight loss program
				• NSD in body weight
				• No adverse events were reported in the study
					de Jong *et al.*, 2008c; Berendschot *et al.*, 2009; Schiepers *et al.*, 2009

To examine the association between changes in serum plant sterol concentrations and changes in retinal vessel diameter during an 85-week follow-up study in which plant sterol or stanol ester were consumed	Randomized, double-blind, placebo-controlled, parallel 85-weeks with 5-week run-in period	19 subjects (11 M, 8 F; mean age of 61.3 years; mean BMI of 25.9 kg/m^2; current statin treatment)[d]	0 (control) or 2.5 g/day (provided as free plant stanols) Margarine (30 g/day)	• 3 subjects (group not specified) dropped out of study (reasons not reported in study publication) • No adverse events were reported in the study	Kelly *et al.*, 2011

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Table IV-5 Summary of Human Studies Published Between 1999 and 2011

Study Objective	Study Design and Treatment Duration	Study Population (Sex, Age, BMI or Body Weight, Health Status)	Dose of Plant Stanols and Delivery Matrix[a]	Safety-Related Results[b,c]	Reference
Studies Conducted in Special Populations					
To evaluate the effects of PSE supplementation on lipid metabolism in a population with elevated fasting TAG concentrations	Randomized, double-blind, placebo-controlled, parallel 3 weeks	28 subjects (16 M, 12 F; mean age of 54.0 years; mean BMI of 28±3 kg/m^2; familial combined hyperlipidemia)	0 (control) or 2.5 g/day Margarine (20 g/day)	• NSD in body weight • Liver function parameters (ALT, AST, GGT, ALP, total bilirubin) remained within normal range for all subjects and no treatment effects were observed • NSD in serum hs-CRP • NSD in plasma glucose and insulin • NSD in HOMA index • No adverse events were reported in the study	Theuwissen *et al.*, 2009
To assess the lipid-lowering properties and gastrointestinal effects of a spread containing plant stanol esters in healthy 2- to 5-year old children	Non-blinded, uncontrolled, crossover 4 weeks with a 2-week washout period	19 children (8 M, 11 F; mean age of 3.58±1.07 years; BMI and body weight NR: healthy)	3.0 g/day or wheat bran fiber (Raisin Bran) Spread (24 g/day) or cereal (1.4 oz/day during the first 2 weeks, and 2.8 oz per week during the last 2 weeks)	• NSD in body weight gain, BMI, blood pressure, or heart rate • 23 GI events reported during spread phase (including stomachache, more gas than usual, constipation, poor appetite, vomiting, and loose/watery stools) ○ 4 children vomited during the 3rd week of spread use, 2 of which were ill with viral-type ailments (no further details were provided) • 46 GI events reported during the bran fiber phase (including stomachache, more gas than usual, diarrhea,	Williams *et al.*, 1999

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Table IV-5 Summary of Human Studies Published Between 1999 and 2011					
Study Objective	**Study Design and Treatment Duration**	**Study Population (Sex, Age, BMI or Body Weight, Health Status)**	**Dose of Plant Stanols and Delivery Matrix[a]**	**Safety-Related Results[b,c]**	**Reference**
				poor appetite, vomiting, and loose/watery stools) ○ Most of these events (91%) occurred when the serving size was doubled • 17 children reported at least 1 adverse event during the study • 13 children reported 1 or more GI event during the study • 10 children reported at least 1 GI event during the run-in and washout periods • 5 children reported 5 or more GI events during the study; in 4 of these children, more events occurred during the bran fiber phase compared to the spread phase • Authors stated that "*consumption of stanol esters was not associated with any short-term adverse health effects*"	

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Table IV-5 Summary of Human Studies Published Between 1999 and 2011

Study Objective	Study Design and Treatment Duration	Study Population (Sex, Age, BMI or Body Weight, Health Status)	Dose of Plant Stanols and Delivery Matrix[a]	Safety-Related Results[b,c]	Reference
To examine the effect of plant stanols on lipids and endothelial function in pre-pubertal children with FH	Randomized, double-blind, placebo-controlled, crossover 4 weeks with a 6-week washout period	42 children (22 M, 19 F; mean age of 9.8±1.5 years; mean BMI of 17.7±2.8 kg/m^2; FH but no clinical signs of hypercholesterolemia)	0 (control) or 2.0 g/day (provided as free plant stanols) Low-fat yogurt (500 mL)	• One child dropped out of the study after 1 week because he was not able to drink the required daily amount of yogurt • No serious adverse events were reported • 6 children complained of stomachaches during the plant stanol period compared to 8 children during the placebo period • NSD in blood pressure, body length, weight, or BMI	Jakulj *et al.*, 2006
To show whether the ratios of squalene and cholesterol precursor sterols to cholesterol and cholestanol and plant sterols to cholesterol change differently in plasma and especially in the red cells of hypercholesterolemic children during consumption of plant stanol and sterol ester spreads	Randomized, double-blind, crossover 5 weeks with a 5-week washout period	23 children (9 M, 13 F; mean age of 6.8 ± 0.5 years; mean weight of 23.1±1.5 kg; hypercholesterolemia, 17 had FH or family history of FH)	2.0 g/day Spread (25 g/day)	• No adverse effects were reported by the children	Ketomaki *et al.*, 2003
To investigate the cholesterol-lowering efficacy and safety of PSE margarine in healthy 6 year old children already consuming a low saturated fat, low-cholesterol diet	Randomized, double-blind, placebo-controlled, crossover 3 months with a 6-week washout period	72 children (40 M, 32 F; age of 6 years; mean body weight of 22.6±2.7 kg; healthy)	0 (control) or 1.6 g/day Margarine (20 g/day)	• 9 subjects withdrew from the study (4 during the PSE period, 3 during the control period, and 2 during the washout period) mainly because the child refused to participate in the frequent blood draws or to eat margarine • No adverse clinical signs of symptoms were reported	Tammi *et al.*, 2000

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Table IV-5 Summary of Human Studies Published Between 1999 and 2011

Study Objective	Study Design and Treatment Duration	Study Population (Sex, Age, BMI or Body Weight, Health Status)	Dose of Plant Stanols and Delivery Matrix[a]	Safety-Related Results[b,c]	Reference
				• Refer to Table 4.6-1 for results for carotenoids and fat-soluble vitamins	
To examine whether stanols can exert their cholesterol lowering effect in renal transplant recipients who develop hypercholesterolemia	Randomized, double-blind, controlled, parallel 3 months	84 subjects (48 M, 36 F; mean age of 49 years; mean BMI of 26 kg/m^2; renal transplant recipient)	0 (control) or 2.0 g/day Milk, yoghurt, margarine and muesli bars Control group only received routine clinical support but no additional advice	• Plant stanols reported to be well-tolerated by subjects	Sutton *et al.*, 2009
To investigate the effect of PSE serum total and LDL cholesterol concentrations and endothelial function in subjects with type 1 diabetes	Randomized, double-blind, placebo-controlled, parallel 12 weeks	22 subjects (8 M, 14 F; mean age of 40 years; mean BMI of 25 kg/m^2; type 1 diabetes)	0 (control) or 2.0 g/day Spread (20 g/day)	• 3 subjects dropped out during the beginning of the study due to personal reasons • NSD in BMI, waist circumference, blood pressure, or HbA1c • No adverse events were reported in the study	Hallikainen *et al.*, 2008
To evaluate the clinical safety of PSE spread consumption during pregnancy and breast-feeding, and the impact of intervention on serum and breast-milk lipid and sterol concentrations	Randomized, open-label, controlled, parallel 3 trimesters of pregnancy and 52 months post-partum	21 F subjects and their infants (mean age of 30.7 years; BMI and weight NR; healthy)	0 (control) or 1.3 g/day Margarine and soft cheese (amounts of foods consumed NR)	• NSD in maternal body weight gain during pregnancy • All pregnancies were uncomplicated and full-term • NSD in infant height and body weight at birth, 1, 6, or 12 months • NSD in infant cognitive development assessed on Griffiths scale at 12 months	Laitinen *et al.*, 2009

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Table IV-5 Summary of Human Studies Published Between 1999 and 2011

Study Objective	Study Design and Treatment Duration	Study Population (Sex, Age, BMI or Body Weight, Health Status)	Dose of Plant Stanols and Delivery Matrix[a]	Safety-Related Results[b,c]	Reference
				• No adverse effects on the mother or infant were reported	

↑ = increase; ↓ = decrease; ALP= alkaline phosphatase; ALT = alanine aminotransferase; apoE = apolipoprotein E; AST = aspartate aminotransferase; ATP = Adult Treatment Panel; BMI = body mass index; CD40L = CD40 ligand; CHD = coronary heart disease; F = female; FH = familial hypercholesterolemia; FVIIc = factor VII coagulant activity; FVIIam = factor VII amidolytic activity; GGT = gamma-glutamyltransferase; GI = gastrointestinal; Hb = hemoglobin; HbA1c = glycated hemoglobin; HOMA = homeostatic model assessment; hs-CRP = high-sensitivity C-reactive protein; IL-6 = interleukin-6; LDL = low-density lipoprotein cholesterol; M = male; MCP = monocyte chemoattractant protein; MCV = mean corpuscular volume; MMP = matrix metallopeptidase; MPOD = macular pigment optical density; NR = not reported; NSD = no significant differences; PAI-1 = plasminogen activator inhibitor type 1 activity; PSE = plant stanol ester; RBC = red blood cells; SAA = serum myloid A; SD = standard deviation; sICAM = soluble intercellular adhesion molecule; sVCAM = soluble vascular adhesion molecule; TAG = triacylglycerol; TC = total cholesterol; t-PA = tissue plasminogen activator; TSH = thyroid stimulating hormone; WBC = white blood cells

[a] Unless specified otherwise, all plant stanols were delivered as plant stanol esters. All doses are expressed as free equivalents.

[b] Unless stated otherwise, all reported effects are statistically significant for the plant stanol ester group relative to control group(s). Results related to plant sterols, if evaluated in the study, are not presented. Details on the plant sterol treatment are provided in the text.

[d] A third parallel arm that received plant sterols was included in this study; however, only data for subjects that received plant stanols or the control groups are presented. Details on the plant sterol group are provided in the text.

[e] Subjects in the treatment group received 3 g/day of plant stanols during the first 6 months of the study, after which they were randomized to continue the intake of 3 g/day or to reduce the intake to 2 g/day for 6 months.

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4. Special Populations

4.1 Pregnant and Lactating Women and Their Infants

The safety of plant stanol ester during pregnancy and lactation has been examined in 21 women (Laitinen *et al.,* 2009). Women were enrolled into the study if they were at less than 17 weeks gestation and did not consume products supplemented with plant stanol ester at baseline. Subjects were randomized into either the control group, where no dietary counseling or food products were provided (n=10), or the intervention group where they were provided with food products enriched with plant stanol ester (Benecol® margarine and soft cheese). Instructions were given to consume a recommended quantity of spread to achieve an intake of 1.8 g/day of plant stanols. Subjects were followed at each trimester of pregnancy and when infants reached 1, 6, and 12 months of age. Dietary intakes recorded in food diaries showed similar intakes of energy, energy-yielding nutrients, cholesterol, dietary fiber and fat-soluble vitamins between the control and intervention groups. Based on the food diaries, an average of 1.1 g of plant stanols was consumed per day during the third trimester and 1.4 g of plant stanols was consumed per day at one month post-partum for those assigned the intervention diet. Fasting blood samples were collected from the women at the first and third trimesters, and at one month post-partum.

Higher serum sitostanol levels were observed in the intervention group, indicative of treatment compliance. Levels of total, LDL- and HDL-cholesterol and triacylglycerides did not significantly differ between the plant stanol and control groups at the time points examined, and levels of biomarkers indicative of cholesterol synthesis (squalene, cholestenol, and desmosterol) were not different between the intervention and control groups at all time points examined. No complications from the pregnancy were reported in all 21 subjects. All infants were born full term and had similar gestation weights in both the control and intervention groups. Infants in both treatment groups were breast-fed for similar durations (11 to 16 weeks) and there were no differences in infant growth (in weight and height) from birth to 12 months of age or in cognitive development assessed at 12 months of age by the Griffiths scale. Breast milk collected at one month post-partum contained similar levels of total cholesterol, sterols reflecting cholesterol synthesis, sterols reflecting cholesterol absorption or sitostanols for the intervention and control groups. Accordingly, the serum levels of total cholesterol and sterols in infants at either 1 or 6 months of age did not differ by treatment group. Absolute levels of β-carotene in the infants also did not significantly differ by treatment group. However, β-carotene levels adjusted by total cholesterol levels were significantly lower in infants in the plant stanol intervention group at 1 month of age (29.1 *vs.* 59.3 nmol/mmol cholesterol, $p<0.01$). No such differences were found in the infants at 6 months of age, and the authors concluded that plant stanol ester consumption appeared safe, although additional studies are needed to assess the clinical significance of transiently reduced levels of β-carotene among infants.

4.2 Other Populations

A number of additional studies have examined the effects of plant stanol ester in individuals with familial hypercholesterolemia (FH), an inherited autosomal dominant disorder of lipoprotein metabolism resulting from a number of mutations identified in the gene encoding the LDL-cholesterol receptor. Individuals with this disorder have elevated cholesterol levels from birth and develop premature artherosclerosis. As such, cholesterol reduction in children with FH is essential for preventing artherosclerotic development early on. Four additional randomized, double-blind, cross-over studies comparing plant stanols with placebo or other forms of treatment (plant sterols, bran diet) have provided further support for the efficacy and safety of plant stanol ester in children or adults with FH.

Theuwissen *et al.* (2009) recruited 28 individuals with familial combined hyperlipidemia (serum total cholesterol >6.5 mmol/L, elevated serum triacylglycerols >1.7 mmol/L) to examine the effects of plant stanol ester supplementation in a randomized, double-blind, placebo-controlled study. Participants stopped taking any cholesterol-lowering medications prescribed for their conditions 2 weeks prior to the start of the study, and were given the control margarine for consumption as part of a 3-week run-in period. Further participation was allowed if total cholesterol levels were <8.0 mmol/L and triacylglycerols <4.0 mmol/L at week 2 of the run-in period. Participants were randomized into either the group that continued to consume the control margarine (14 subjects), or the group that consumed margarine containing plant stanol ester, for an estimated daily intake of 2.5 g plant stanols (14 subjects) over a period of 3 weeks. Used containers were returned to assess compliance and intake, and 5 fasting blood samples were collected over the study at days 14 and 21 (of the run-in period) and on days 32, 37 and 42 (of the treatment period). High compliance was found for both the control spread and the spread enriched with plant stanol ester, and changes in body weight did not differ across the treatment groups. Consumption of plant stanol ester significantly reduced levels of total and LDL-cholesterol (by 6.7 and 9.5%, respectively) compared to control treatment ($p<0.05$). No differences in HDL-cholesterol and apoA-1 levels were observed, although plant stanol ester reduced triacylglyceride levels in those who had levels that were high at baseline (>2.3 mmol/L) ($p<0.01$). There were no differences in clinical biochemistry indicators of liver function, or changes in the levels of high-sensitivity C-reactive protein (hs-CRP, a marker of inflammation), plasma glucose and insulin, or the Homeostatic Model Assessment (HOMA) index, a measure of insulin sensitivity, between individuals in the stanol ester and control groups.

Consumption of plant stanol ester reduced total and LDL- cholesterol levels by 8 to 16% without any serious adverse effects or gastrointestinal discomfort reported (Williams *et al.*, 1999; Ketomaki *et al.*, 2003; Jakulj *et al.*, 2006). Two additional randomized, double-blind, placebo-controlled trials tested the effects of plant stanol ester (both at estimated intakes of 2 g/day for 12 weeks) in diabetics and renal transplant patients, with no serious adverse events reported (Hallikainen *et al.*, 2008; Sutton *et al.*, 2009). Although these studies may be limited in terms of

their relevance to the general population, consumption of plant stanol ester in this population also appears to be safe and well-tolerated for this group of individuals.

5. Summary and Discussion

Earlier clinical trials published before 1999 have extensively evaluated the safety of plant stanol ester, including reports of adverse events, HDL-cholesterol levels, HDL/LDL ratio, triglycerides, vitamins, blood biochemistry and hematological parameters, changes in body weight, and changes in serum or plasma levels of plant stanols and plant sterols. Together, these studies represent approximately 2,000 subjects who have consumed products containing plant stanols or plant stanol ester for periods ranging from several weeks up to one year. Participants in these earlier studies included healthy adults, adults with hypercholesterolemia (medicated and non-medicated), CHD, non-insulin dependent diabetes mellitus, as well as children with normal and elevated cholesterol. Critical evaluation of these earlier findings supported the conclusion that consumption of plant stanols appears to be safe and well tolerated at levels of up to 3 g/day.

No adverse events attributable to consumption of plant stanol ester were reported in studies identified in the literature that were published after 1999. A number of studies have included measurements of markers of inflammation (such as hs-CRP, E-selectin, monocyte chemotactic protein-1, IL-6, TNF-α) and ultrasonic imaging of the brachial artery as part of investigations to determine whether reductions in cholesterol levels were associated with improved arterial functions, and to examine whether plant stanols themselves have artherogenic effects. Consumption of plant stanols at relevant dosage (approximately 2 g/day) generally had no significant effects on these parameters among individuals with mild to moderate hypercholesterolemia. Blood pressure, erythrocyte fragility and retinal microcirculation were also not altered by plant stanol consumption in these individuals. Similar to earlier reports, consumption of plant stanols also did not result in additional adverse effects in mild to moderate hypercholesterolemic individuals taking statin medications. Long-term consumption of plant stanols is safe, even among individuals taking statin medications, and no evidence of tolerance was observed, with plant stanol ester being efficacious in lowering cholesterol levels even after 85 weeks of treatment. High doses of plant stanol consumption (up to 9 g/day) are well tolerated, with no alterations in hematological parameters or measures of liver and kidney function observed. Serum levels of α- and β-carotene were significantly reduced in some studies as a result of plant stanol ester consumption; however, as discussed in Section IV.H, the functional significance of this is likely minor. Together, these studies provide additional support for the safety of consuming plant stanol ester.

H. Nutritional Considerations

1. Cholesterol-lowering Properties of Plant Stanol Ester

Cholesterol is a critical component in the construction and maintenance of cell membranes, and in the regulation of membrane fluidity and permeability. It can be produced endogenously or acquired through dietary sources. In excess, cholesterol can result in the formation of artherogenic plaques, fatty lesions that can build up on the luminal walls of arteries and occlude blood flow. Elevated plasma levels of cholesterol, particularly the LDL form, is a well established risk factor for cardiovascular diseases. Although the precise mechanism is unclear, there are several hypotheses proposed to explain the inhibition of intestinal cholesterol absorption by plant stanols. Free plant stanols might displace cholesterol from mixed micelles, inhibit the enzymes involved in cholesterol absorption (such as lipases and esterases), affect intra-cellular trafficking and/or alter the influx and efflux transport of cholesterol at the brush border of enterocytes (Rozner and Garti, 2006). The ABCG5 and ABCG8 transporters are believed to be responsible for preventing the absorption of free plant stanols and enhancing their efflux from enterocytes (Berge *et al.*, 2000). A reduction in hepatic cholesterol levels leads to an increase in cholesterol synthesis in the liver and upregulation of LDL-cholesterol receptors, although a net overall reduction in plasma cholesterol remains despite these compensatory changes. Alterations in levels of HDL-cholesterol, very low density lipoprotein cholesterol, and triacylglycerol are generally not observed with the consumption of plant stanol ester (Katan *et al.*, 2003).

The ability of plant stanol ester to lower blood cholesterol levels has been extensively investigated in healthy and hypercholesterolemic individuals. Dietary intake of plant stanols and plant sterols at approximately 2 g/day reduces LDL-cholesterol levels by 7 to 11% (Katan *et al.*, 2003). A more recent meta-analysis of 59 randomized clinical trials published between 1992 and 2006 found consumption of plant stanols or plant sterols significantly decreased LDL-cholesterol levels by 0.31 mmol/L (95% CI: -0.35 to -0.27, $p<0.0001$) compared to placebo control (AbuMweis *et al.,* 2008). The reductions in LDL-cholesterol were greater in studies testing higher doses of plant stanols and plant sterols (>2.5 g/day) compared to lower doses (<1.5 g/day). LDL-cholesterol reductions by plant stanols and plant sterols were greater among those who had higher baseline levels of LDL-cholesterol, although age was not a contributing factor to the efficacy of plant stanols and plant sterols. That LDL-cholesterol levels are dose-dependently reduced by plant stanols, delivered as plant stanol ester, is supported by the study by Mensink *et al.* (2010), and also by the recent meta-analysis by Musa-Veloso *et al.* (2011), wherein a continuous dose-response was observed for plant stanol ester and LDL-cholesterol, as described by a first-order elimination function.

Statin medications are a class of drugs that inhibit 3-hydroxy-3-methylglutaryl-CoA reductase, the main enzyme responsible for cholesterol synthesis in the liver. They are currently widely used for the treatment of hypercholesterolemia and prevention of cardiovascular diseases.

Although statins are one of the most effective pharmacotherapy used in the management of cholesterol levels, combination therapy is needed to achieve the recommended target lipoprotein levels in certain individuals. Combining statin medication with dietary intake of plant sterols or plant sterols has been shown to lower cholesterol levels to a greater extent than either given alone (Katan *et al.*, 2003). Dietary consumption of plant stanol and sterol concurrently as statin medications reduce LDL-cholesterol levels by an additional 10 to 20%, whereas doubling of statin dosages typically only produce additional reductions of 5 to 7% (Katan *et al.*, 2003).

No studies have provided direct evidence for whether plant stanols or plant sterols are associated with lower risk of cardiovascular diseases. Large-scale randomized clinical trials to ascertain this are likely to be cost-prohibitive due to the large sample size and long study duration required. However, interventions that reduce cholesterol levels are generally associated with reductions in risk of CHD. A reduction in LDL-cholesterol levels by approximately 10%, similar to effects observed from plant stanol ester consumption, is estimated to reduce the risk of ischemic heart disease by 12 to 20% over 5 years (cited in Katan *et al.*, 2003). As such, this provides further support for the consumption of plant stanol ester, particularly among individuals with elevated cholesterol levels who are at an increased risk of cardiovascular diseases.

2. Effects of Plant Stanols on Circulating Levels of Dietary Fat Soluble Vitamins and Carotenoids

In addition to their effects in decreasing cholesterol absorption, plant sterols and stanols may potentially decrease the absorption of dietary fat soluble vitamins [such as vitamins A (retinol), D, E, K)] and carotenoids (such as α- and β-carotene, lycopene, lutein, zeaxanthin, β-cryptoxanthin, canthaxanthin). These nutrients require intestinal solubilization into mixed micelles for absorption through enterocytes. Consumption of plant stanols or their ester lowered plasma levels of fat-soluble vitamins D, E, and K in 1 study of Sprague-Dawley rats fed diets containing 5% stanols as plant stanol ester (equivalent to daily intake of 189 g plant stanols for a 70 kg human), although no functional consequences were observed (Turnbull *et al.*, 1999a). In a 2-generation reproduction study in Wistar rats, no functional or developmental deficits attributable to altered nutritional status were observed at diets containing 5% plant stanols as plant stanol ester, the highest dose tested (Whittaker *et al.*, 1999). Similarly, earlier studies reviewed previously did not find evidence of clinically relevant changes in the levels of fat-soluble vitamins D, E, and K or carotenoids at daily intakes of up to 6.8 g plant stanol ester and for periods of up to 1 year (U.S. FDA, 1999a).

Since the original GRAS application, a number of published reports have provided additional data on how consumption of plant stanols or their ester forms alters nutritional status. The results from these additional studies are summarized below and in Table IV-6 and are generally similar to the findings from earlier reports. The levels of fat-soluble nutrients are highly dependent on the levels of their lipoprotein carriers; as such, accurate assessment of the effect

of plant stanols on the levels of these compounds must also take into account concurrent changes in total cholesterol levels mediated by plant stanol ester. Many of the studies tested doses of plant stanols similar to the recommended daily dosage for their cholesterol-lowering effects (approximately 1.5 to 3.0 g); however, even in studies that tested dosages as high as 9 g/day of plant stanols, no detrimental effects were observed.

2.1 Vitamin D

Vitamin D is not commonly found in natural food sources and is produced primarily in the skin of vertebrates from exposure to ultraviolet B radiation. Vitamin D is important in the absorption and maintenance of calcium and phosphorus homeostasis in the body; deficiency can lead to osteoporosis in adults or rickets in children. Consumption of plant stanols in 3 clinical trials did not result in significantly altered levels of 25-hydroxyvitamin D_3, the activated form of the vitamin that is commonly used as an indicator of deficiency (Hallikainen *et al.*, 2000a; Raeini-Sarjaz *et al.*, 2002; Gylling *et al.*, 2010b). A slight increase in 25-hydroxyvitamin D_3 levels (10%) was observed in one study (Hallikainen *et al.*, 2000b); however, this occurred only for individuals consuming the lowest test dose of plant stanols (0.8 g/day) and was not observed at higher doses tested (1.6, 2.4, 3.2 g/day). Earlier randomized, placebo-controlled, double-blinded clinical trials also did not find any clinically significant changes in 25-hydroxyvitamin D_3 levels following consumption of plant stanol ester.

2.2 Vitamin K

Green vegetables, dark berries, and certain vegetable oils are foods naturally rich in vitamin K; also, bacteria in the lower gastrointestinal tract can also produce small amounts. Vitamin K is important in the production of proteins involved in the blood coagulation cascade, and blood coagulation time is often used as an indicator of vitamin K levels. One additional clinical trial did not report any differences in serum levels of vitamin K among hypercholesterolemic males consuming 1.8 g/day of plant stanols compared to controls (Raeini-Sarjaz *et al.*, 2002). No significant changes in blood clotting proteins, indicative of normal vitamin K status, were reported in an earlier clinical trial of plant stanols.

2.3 Tocopherols

Dietary vitamin E is comprised of mainly α- and γ-tocopherols; these are found naturally in many food sources such as vegetable oils, grains, nuts and green leafy vegetables. Slight reductions in absolute α-tocopherol levels (6 to 10%) were observed for individuals consuming plant stanols (as plant stanol ester) compared to controls; however, these differences did not remain after adjustment for total cholesterol levels in serum (Hallikainen *et al.*, 2000a,b; Homma *et al.*, 2003; Gylling *et al.*, 2010b; Mensink *et al.*, 2010). Earlier randomized, placebo-controlled, double-blinded clinical trials also did not find any changes in tocopherol levels following consumption of plant stanols.

2.4 Retinol (Vitamin A) and Carotenoids

Vitamin A refers to a large family of structurally related compounds that are obtained through ingestion of the 2 main dietary forms, either directly as retinol (found in fish and dairy products), or indirectly through the retinol precursors known as provitamin A carotenoids (includes α and β-carotene and β-cryptoxanthin). Other carotenoids, like lycopene, lutein, and zeaxanthin, do not have vitamin A activity, but are currently being studied for their potential benefits in the promotion of health and prevention of disease. Similar to earlier reports, consumption of plant stanols did not significantly alter levels of retinol, lycopene, zeaxanthin or cryptoxanthin. The change in cholesterol-standardized lutein levels following plant stanol treatment from baseline was significantly different from the change observed in the control group in 1 study (Berendschot *et al.*, 2009); however, there were three groups in this study (placebo, plant stanol ester, and plant sterol ester), and a *post-hoc* test was not conducted to determine which of the groups differed from each other. The changes from baseline in cholesterol-standardized lutein levels were -0.004, -0.013, and 0.005 μmol/mmol in the placebo, plant sterol ester, and plant stanol ester groups, respectively. The plant stanol ester group was actually the only group to experience an increase from baseline in cholesterol-standardized serum lutein levels. Despite the nominal changes from baseline in cholesterol-standardized serum lutein levels, changes from baseline in macular pigment optical density did not differ between the 3 groups.

Some studies have found a significant effect of plant stanols on α- and β-carotene levels, even after accounting for changes in total cholesterol levels; however, a number of other studies did not report such effects (Table 4.6-1). In earlier reports, the β-carotene/total cholesterol ratio was reduced significantly by 20 to 30% in individuals consuming plant stanols at a dose of 3 g/day (Gylling *et al.*, 1999a,b). The β-carotene/total cholesterol ratio was also reduced to a similar extent (approximately 30%) in individuals consuming plant stanols in a study where 8.8 g/day was given (Gylling *et al.*, 2010b). In infants whose mothers consumed a diet containing plant stanols at a daily intake of approximately 1.1 to 1.4 g/day during pregnancy and lactation, β-carotene levels, after accounting for total cholesterol, were reduced by 51% at 3 months, but not at 6 months (Laitinen *et al.,* 2009). No differences in the absolute levels of β-carotene were observed. Even though no differences in the indicators of cholesterol synthesis were observed, the authors speculated the lower β-carotene to cholesterol ratio may be an artifact of elevated cholesterol synthesis in the infants rather than reduced absorption of β-carotene. The lowered β-carotene levels were observed only at 1 month of age and no significant differences were found at 6 months of age.

The functional significance of lowered β-carotene levels following plant stanol consumption is unknown. β-Carotene has anti-oxidant properties *in vitro*, and higher blood concentrations have been associated with lower risk of several chronic diseases (IOM, 2006). However, these associations may be mediated by other compounds present in foods that are rich in β-carotenes, or other behavioral correlates of increased fruit and vegetable consumption. Since β-carotene is a vitamin A precursor, it may be important in preventing deficiency of this nutrient.

There are currently no daily recommended intakes for pro-vitamin carotenoids (α- and β-carotenes, β-cryptoxanthin), although increased consumption of fruits and vegetables rich in carotenoids is promoted. As such, reductions in β-carotene levels by plant stanols are predicted to be of minimal functional consequences. Daily consumption of 5 or more servings (125 mL) of fruits and vegetables that includes at least 1 serving of carrots, tomatoes, spinach or broccoli can prevent the reduction of plasma carotenoid levels resulting from consumption of plant stanol ester (Noakes *et al.*, 2002).

2.5 Meta-analysis of Plant Stanol and Sterol Consumption on Nutritional Status

A meta-analysis was performed summarizing the effects of either plant stanol or plant sterol consumption on levels of fat-soluble nutrients from 18 clinical trials testing doses of at least 1.5 g/day or more (Katan *et al.*, 2003). The average change across these studies was significant for α-tocopherol (-5.9%, $p<0.001$), α-carotene (-8.7%, $p<0.001$), β-carotene (-19.9%, $p<0.001$), and lycopene (-7.3%, $p<0.01$). However, after adjustment for total serum cholesterol, only β-carotene remained significantly reduced by plant stanol or plant sterol consumption (-12.1%, $p<0.001$). Plant stanol or plant sterol consumption did not significantly alter the levels of retinol or vitamin D. Although this meta-analysis combined the effects of plant stanols with those of plant sterols, the results from this study are in agreement with those summarized above and in the previous GRAS notifications, providing further support that plant stanols do not substantially alter nutritional status.

2.6 Authoritative Opinions of Plant Stanol and Sterol Consumption on Nutritional Status

A number of regulatory authorities have evaluated whether cautionary statements were required for products containing plant stanols or plant sterols due to their potential effects on the absorption of fat-soluble vitamins and carotenoids. The EC Scientific Committee on Food (SCF) recommended that consumers should be made aware that foods enriched with plant stanols and plant sterols may lower beta-carotene, and appropriate dietary advice regarding consumption of fruits and vegetables should be provided (SCF, 2000, 2003). As such, EC regulation requires the following statement on the labeling of products containing added plant stanols or plant sterols: "(1) Phytosterol-enriched foods may not be nutritionally appropriate for pregnant or breastfeeding women and children under the age of 5 years; and (2) phytosterol-enriched foods should be used as part of a balanced and varied diet, including regular consumption of fruits and vegetables to help maintain carotenoid levels" (EC, 2000, 2004).

Similarly, FSANZ also requires that food containing plant stanols or plant sterols to have a label statement advising that the product be consumed in moderation as part of a diet low in saturated fats and high in fruits and vegetables, and that the product is not recommended for infants, children, pregnant or lactating women unless under medical supervision (FSANZ, 2011). Although the FDA recognizes these labeling requirements in other jurisdictions, it was stated in the most recent Proposed Rule (75 Fed. Reg. 76525) that there was "no basis for concluding

that any reduction in the intestinal absorption of these nutrients caused by consuming phytosterols amounts to an adverse health consequence" (U.S. FDA, 2010). Even though some studies showed a reduction in cholesterol-adjusted levels of beta-carotene at doses of plant stanols and sterols ranging from 3 to 9 g/day, these were not considered by the FDA to be of safety concern as no corresponding effects on serum retinol levels were observed. As such, it was concluded that a cautionary statement regarding a potential effect on fat soluble vitamins or carotenoids was not warranted. Moreover, Health Canada has conducted an assessment of the safety of foods containing plant stanols and plant sterols (Health Canada, 2010a). Although there is evidence to suggest that plant stanols and plant sterols may significantly decrease serum β-carotene levels, it was noted that the circulating levels of this nutrient can be highly variable, with serum concentrations reported to range from 2.2 to 122.7 g/dL. Various lifestyle and physiological factors may contribute to the observed variability.

Table IV-6 Human Clinical Studies Published Between 1999 and 2011 Reporting the Effects of Plant Stanol Ester Consumption on the Absorption of Fat-Soluble Nutrients[a]

Reference & Study Population	Study Details (Plant Stanol Dose[b], Duration, Matrix, Study Design)	Statistical Analyses	Tocopherols	Retinol (Vitamin A)	Carotenes	Lycopene	Other
Studies Conducted in Normocholesterolemic Subjects							
Mensink *et al.*, 2010 93 subjects (males, females) Age = 18 to 70	0, 3, 6, or 9 g 4 weeks Margarine and yogurt RPC, double-blind	Differences in the relative change from baseline among treatment groups	α-tocopherol: NS	NR	β-carotene: NS	NR	Lutein: NS Canthaxanthin: NS Zeaxanthin: NS
Homma *et al.*, 2003 105 subjects (males, females) Age = ≥20	0, 2 or 3 g/day 4 weeks Spread RPC	Levels at end of treatment compared to baseline	α-tocopherol: NS (not standardized to TC)	NS (not standardized to TC)	α-carotene: NR β-carotene: NS (not standardized to TC)	NR	---
Plat and Mensink, 2001a 112 subjects (males, females) Age (mean) = 33	0, 3.8 g/day (vegetable oil- derived), or 4.0 g/day (wood-derived) 8 weeks Margarine RPC, double-blind	Differences in the relative change from baseline among treatment groups	Total tocopherols: NS (standardized to LDL-C)	+12 to 16% from baseline (standardized to LDL-C)	Total carotenoid: NS (standardized to LDL-C)	NS	Lutein/zeaxanthin: NS (standardized to LDL-C) β-Cryptoxanthin: NS (standardized to LDL-C)

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Table IV-6 Human Clinical Studies Published Between 1999 and 2011 Reporting the Effects of Plant Stanol Ester Consumption on the Absorption of Fat-Soluble Nutrients[a]

Reference & Study Population	Study Details (Plant Stanol Dose[b], Duration, Matrix, Study Design)	Statistical Analyses	Tocopherols	Retinol (Vitamin A)	Carotenes	Lycopene	Other
Laitinen *et al.*, 2009 21 infants	Mother consumed plant stanols (0, 1.1 g/day) from 1st trimester of pregnancy and lactation until infant reached 1 year of age	Levels in infants exposed to plant stanols compared to controls	NR	NR	α-carotene: NR β-carotene: Levels were 51% lower in infants exposed to plant stanols compared to unexposed infants at 1 month of age, but not at 6 months	NR	---
Mensink *et al.*, 2002 60 subjects (males, females) Age = 18 to 65	0 or 3 g/day 4 weeks Yogurt RPC, double-blind	Differences in the relative change from baseline among treatment groups	δ-tocopherol: +31.4% from control (standardized to LDL-C) β+γ-tocopherol: +27.9% from control (standardized to LDL-C) α-tocopherol: +12.7% from control (standardized to LDL-C)	NR	α-carotene: NS β-carotene: -12.9% from baseline (standardized to LDL-C)	NS	Lutein/zeaxanthin: NS (standardized to LDL-C) β-Cryptoxanthin: NS (standardized to LDL-C)
Hyun *et al.*, 2005 51 subjects (males, females) Age : 20 to 39	0 (control) or 2 g/day 4 weeks Yogurt RPC, double-blind	Differences in the relative change from baseline among treatment groups	α-tocopherols: +5% compared to baseline	NS	β-carotene: NS	NR	---

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Table IV-6 Human Clinical Studies Published Between 1999 and 2011 Reporting the Effects of Plant Stanol Ester Consumption on the Absorption of Fat-Soluble Nutrients[a]

Reference & Study Population	Study Details (Plant Stanol Dose[b], Duration, Matrix, Study Design)	Statistical Analyses	Tocopherols	Retinol (Vitamin A)	Carotenes	Lycopene	Other
Studies Conducted in Hypercholesterolemic Subjects							
Gylling *et al.*, 1999a 151 subjects (males, females) Age = 25 – 64 On stable medication for hypertension, diabetes, or coronary heart disease	0 (control), 2, or 3[c] g Margarine 1 year RPC, double-blind	Levels at end of treatment compared among treatment groups and controls	α-tocopherol: NS	NS (not standardized to TC)	α-carotene: NS β-carotene: -33.3% from control	NR	Vit. D: -17.3% from control (not standardized to TC)
Hallikainen *et al.*, 2000a 34 subjects (males, females) Age = 30 – 65 Not taking lipid-lowering medications	0, 0.1, or 2.0 g/day 4 weeks Margarine, also enriched with vit. A and D RPCX, double-blind, no washout period	Levels at end of treatment compared among treatment groups and controls	α-tocopherol: NS γ–tocopherol: NS	NS	α-carotene: NS β-carotene: NS	NS	Vit. D: NS

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Table IV-6 Human Clinical Studies Published Between 1999 and 2011 Reporting the Effects of Plant Stanol Ester Consumption on the Absorption of Fat-Soluble Nutrients[a]

Reference & Study Population	Study Details (Plant Stanol Dose[b], Duration, Matrix, Study Design)	Statistical Analyses	Tocopherols	Retinol (Vitamin A)	Carotenes	Lycopene	Other
Hallikainen *et al.*, 2000b 22 subjects (males, females) Age (mean) = 50 Not taking lipid-lowering medications	0, 0.8, 1.6, 2.4, or 3.2 g/day 4 weeks Margarine also enriched with vit. A and D RPCX, single-blind, no washout period; all subjects consumed doses in this randomly determined order (2.4, 3.2, 1.6, 0, 0.8 g)	Levels at end of treatment compared among treatment groups and controls	α-tocopherol: NS γ-tocopherol: NS	NS	α-carotene: NS β-carotene: NS	NS	Vit. D: NS
Raeini-Sarjaz *et al.*, 2002 15 subjects (males only) Age = 37 to 61 Not taking lipid-lowering medications	0 or 1.8 g/day 3 weeks Margarine RPCX, double-blind, with 5- week washout	Differences in nutrient levels at baseline, end of treatment, and their percentage changes relative to baseline among treatment groups	α-tocopherol: NS γ-tocopherol: NS	NS (not standardized to TC)	α-carotene: NS β-carotene: NS	NS	Vit. D: NS (not standardized to TC) Vit. K: NS (not standardized to TC) Lutein: NS (not standardized to TC) Cryptoxanthin: NS (not standardized to TC)

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Table IV-6 Human Clinical Studies Published Between 1999 and 2011 Reporting the Effects of Plant Stanol Ester Consumption on the Absorption of Fat-Soluble Nutrients[a]

Reference & Study Population	Study Details (Plant Stanol Dose[b], Duration, Matrix, Study Design)	Statistical Analyses	Tocopherols	Retinol (Vitamin A)	Carotenes	Lycopene	Other
Gylling *et al.*, 2010b 49 subjects (males, females) Age = 18 to 75 Not taking lipid-lowering medications	0 or 8.8 g/day 10 weeks Spreads and drinks RPC, double-blind	Levels compared between treatment and control groups AND Levels following intervention compared to baseline	α-tocopherol: NS γ–tocopherol: NS	NS (not standardized to TC)	α-carotene: -31% compared to baseline β-carotene: -34% compared to control α + β carotene: -34% at end of treatment compared to control	NR	Vit. D: NS (not standardized to TC)
Berendschot *et al.*, 2009 47 subjects (males, females) Age = 18 to 65 Medicated with statins	0 or 2.5 g/day 85 weeks Margarine RPC, double-blind	Differences in nutrient levels at baseline, end of treatment, and the relative change from baseline among treatment groups	NR	NR	NR	NR	Lutein: +8% from baseline
de Jong *et al.*, 2008b 41 subjects (males, females) Age = 18 to 65 Medicated with statins	0 or 2.5 g/day 16 weeks Margarine RPC, double-blind	Differences in the relative change from baseline among treatment groups	Total tocopherols: NS (standardized to LDL-C)	NR	α-carotene: NS (standardized to LDL-C) β-carotene: NS (standardized to LDL-C)	NS (standardized to LDL-C)	Cryptoxanthin: NS (standardized to LDL-C)

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Table IV-6 Human Clinical Studies Published Between 1999 and 2011 Reporting the Effects of Plant Stanol Ester Consumption on the Absorption of Fat-Soluble Nutrients[a]

Reference & Study Population	Study Details (Plant Stanol Dose[b], Duration, Matrix, Study Design)	Statistical Analyses	Tocopherols	Retinol (Vitamin A)	Carotenes	Lycopene	Other
Studies Conducted in Special Populations							
Tammi *et al.*, 2000 72 children (males, females) Age of 6 years	0 (control) or 1.6 g/day 3 months Margarine RPCx, double-blind	Differences in the relative change from baseline among treatment groups	α-tocopherol: NS (standardized to LDL-C)	NS (not standardized to TC or LDL-C)	β-carotene: -18.8% from baseline (standardized to LDL-C)	NR	Vit. D: NS (standardized to LDL-C)
Meta-Analysis of Plant Sterol and Stanol Consumption[d]							
Katan *et al.*, 2003	Meta-analysis of 18 RPC clinical trials where doses of at least 1.5 g/day of plant stanols or plant sterols were tested	Mean change of nutrient levels across different clinical trials	α-tocopherol: NS	NS (not standardized to TC)	α-carotene: NS β-carotene: -12.1% (95% CI: -17.4 to -6.8; p <0.001)	NS	Vit. D: NS (not standardized to TC)

CI = confidence interval; LDL-C = low-density lipoprotein cholesterol; NR = not reported; NS = not significant; RPC = randomized placebo-controlled; RPCX = randomized placebo-controlled cross-over; TC = total cholesterol; Vit. = vitamin

[a] Unless otherwise noted, data are presented for changes in nutrient levels following plant stanol treatment compared to baseline, standardized by total cholesterol levels.

[b] Unless otherwise noted, doses are expressed as free plant stanols (*i.e.*, un-esterified plant stanols) administered in the form of plant stanol esters.

[c] Subjects in the treatment group received 3 g/day of plant stanols during the first 6 months of the study, after which they were randomized to continue intake of 3 g/day or to reduce the intake to 2 g/day for 6 months.

[d] Effects were not differentiated between individuals in the plant sterol or plant stanol treatment group.

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2.7 Summary and Discussion

Overall, findings from clinical reviewed above that obtained measures of fat-soluble vitamin and carotenoid absorption are consistent with the general consensus that the effects of dietary plant stanol and sterol consumption, on absorption of cholesterol adjusted concentrations of fat soluble vitamins and carotenoids, are limited to reductions in the absorption efficiency of carotenoids. Data from some human clinical trials suggest a 25 to 30% reduction in β-carotene levels from the consumption of plant stanol ester; similar observations are observed with consumption of plant sterols. Plasma β-carotene levels display a broad interindividual variation across the population, and are highly influenced by diet and lifestyle. The ranges for β-carotene reduction reported in the literature for individuals consuming plant stanols is within the natural variation in plasma β-carotene that is expected in the general North American population (Health Canada, 2010a). The nutritional function of β-carotene is currently limited to its role as a source of provitamin A, and in the absence of clinical evidence supporting reduced levels of vitamin A in plant stanol users, the functional impact of the observed reductions in β-carotene is likely to be minimal. Finally, the effect of the consumption of plant stanols and plant sterols on the absorption of fat soluble vitamins and β-carotene has been comprehensively reviewed by the FDA, who concluded that:

"Collectively, these studies provided phystosterols[11] ranging from 0.8 to 9 g per day. After adjusting for plasma total or LDL-cholesterol levels, only one study showed that vitamin E levels were significantly reduced with phytosterols intake (3 g per day) (Ref. 88[12]). Vitamin E levels were not altered at higher phytosterol intake levels (3.2 to 9 g per day) (Refs. 51, 55, 88, and 89[13]). There was no effect of phytosterol intake on adjusted levels of other fat soluble vitamins (*i.e.,* vitamin A, vitamin D, vitamin K)" (U.S. FDA, 2010).

and

"although there is some evidence that consuming phytosterols reduces plasma levels of carotenoids such as beta-carotene, lutein, and lycopene, those carotenoids have no established health benefits at particular levels" (U.S. FDA, 2010).

Raisio has therefore concluded that regular consumption of plant stanol ester under the proposed food uses described herein would not have detrimental effects on nutrient absorption in healthy individuals consuming a regular balanced diet.

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[11] FDA defines term 'phytosterols "as a collective term for plant sterols and their hydrogenated stanol forms, whether used in the free form or esterified with fatty acids".
[12] Mensink *et al.,* 2002.
[13] Hendricks *et al.*, 1999; Davidson *et al.*, 2001; Plat and Mensink, 2001a; Mensink *et al.*, 2002

I. Other Considerations

1. Association Studies of Plant Stanols and Sterols with Cardiovascular Disease Risk

Sitosterolemia (also known as phytosterolemia) is a rare inherited autosomal recessive disorder characterized by mutations in either the ATP-binding cassette G5 or ATP-binding cassette G8 half-transporter genes (*ABCG5, ABCG8*). These ABC transporters are involved in the transport of cholesterol, plant sterols, and plant stanols in enterocytes and hepatocytes. Accordingly, individuals with sitosterolemia have enhanced absorption and impaired elimination of plant sterols, with plasma levels being 20 to 100 times higher compared to healthy individuals (Salen *et al.*, 2002). Case reports of individuals with sitosterolemia suggest they have unique clinical presentations including accelerated atherosclerosis and premature coronary artery disease, particularly affecting males at a young age (Bhattacharyya and Connor, 1974; Salen *et al.*, 1985; Hidaka *et al.*, 1990). It is notable that these effects have only been found in a few case reports and do not necessarily support a causal relationship between increased plant sterol levels with cardiovascular ailments. It is possible that the higher incidence of atherosclerosis and CHD may be due to other genetic abnormalities that are also present in patients with sitosterolemia.

A number of epidemiological studies have found elevated serum levels of plant sterols with increased risk of cardiovascular diseases (Glueck *et al.,* 1991; Rajaratnam *et al.*, 2000; Sudhop *et al.*, 2002; Assmann *et al.*, 2006). However, other studies have failed to replicate these findings (Wilund *et al.*, 2004; Kesaniemi *et al.*, 2009). These epidemiological studies often do not control for possible confounders such as individual differences in lifestyle (diets, smoking, physical activity), diabetes and other metabolic syndromes, and use of statin medications. As such, there is a lack of conclusive evidence supporting a causal relationship between plant sterols and increased risk of adverse cardiovascular events.

Sitosterolemia is extremely rare, and 1 in 5 million people are estimated to be homozygous for this disorder (Katan *et al.*, 2003). Heterozygosity is more common with an estimated frequency of 1 in 1,000 individuals; however, enhanced absorption of plant sterols is observed only in homozygotes while heterozygotes appear clinically and biochemically normal (Salen *et al.*, 2002). The plasma levels of campestanol, β-sitostanol, campesterol and β-sitosterol following consumption of plant stanols was 2- to 3-fold higher in heterozygous individuals for sitosterolemia compared to individuals without this disorder (Kratz *et al.*, 2007). The extent of which plant stanol absorption is altered in individuals with sitosterolemia is unknown. However, given the low frequency of this condition, and that dietary plant stanols are minimally absorbed in healthy individuals (Ostlund *et al.,* 2002), the impact of increased uptake of dietary plant stanols is predicted to be relatively minor for the general population. In addition, consumption of plant stanol has actually been shown to reduce serum levels of the potentially artherogenic plant sterols (Thompson and Grundy, 2005). In animal models of artherosclerosis, New Zealand

White (NZW) rabbits fed plant stanols (8 g/kg of diet) showed less atherosclerotic plaque development in the aorta ($p<0.01$) and coronary arteries ($p<0.001$) compared to controls when animals were fed an artherogenic diet (Ntanios *et al.*, 1998). Furthermore, in heterozygous Watanabe heritable hyperlipidemic rabbits fed an artherogenic diet, consumption of plant stanols (10 or 20 g/kg of diet) led to fewer artherosclerotic lesions in the cranial, thoracic and abdominal aorta (Schroder *et al.*, 2009). Together, these results suggest it is unlikely that consumption of plant stanols will increase the risk of adverse cardiovascular effects.

2. Effect of Plant Stanol Ester on Neurocognition

Plant sterols and plant stanols were not originally thought to cross the blood-brain barrier, given their close structural resemblance to cholesterol, which is formed *in situ* in the brain rather than entering from peripheral circulation. However, the identification of plant sterols in the brain tissue of mice and humans has been reported (Fricke *et al.*, 2007). In addition, studies by Fricke *et al.* (2007) also have demonstrated, under controlled conditions, that dietary administration of plant sterol ester and plant stanol ester to Watanabe rabbits results in retention of plant stanols and plant sterols in the brain tissue of the animals. In this study the authors administered plant stanol ester or plant sterol ester at concentrations of 34 g/kg in diet to 6 week old male and female Watanabe rabbits (n=9/group) for 120 days. Plasma concentrations of plant stanols increased from 480 µg/dl at baseline to 9787 µg/dl in the homozymgous animals consuming the plant stanol ester diet. Corresponding plant stanols concentration in the brain increased from 1.66 ng/mg in control animals to 10.7 ng/mg ($P<0.001$) in animals consuming the plant stanol ester diet. Higher transport of plant sterols were shown in the brains of homozygous animals in the plant sterol ester group, where plant sterol concentrations increased from 51 ng/mg in control animals to 223 ng/mg in animals fed the plant sterol ester supplemented diets. No effect on cholesterol metabolism could be identified in the animals. It remains unknown if similar increases in plant stanols would be observed in humans consuming plant stanol ester or plant sterol ester supplemented foods.

Despite findings in Watanabe rabbits showing that plant stanols can be transported to the brain in humans, plant stanols have a widespread and long-history of safe use in food and there is currently no epidemiological or case-findings suggestive that consumption of plant stanols may influence brain function. Moreover, the high plasma concentrations observed in this animal model is not consistent with the highly limited absorption of plant stanols reported in human clinical studies (Ostlund *et al.*, 2002), which suggest that findings from the Watanabe rabbit model are of limited relevance to humans.

As part of a larger intervention study where hypercholesterolemic subjects receiving stable statin treatment consumed margarine fortified with plant sterol ester or plant stanol ester for 85 weeks (de Jong *et al.*, 2008c) (see Section IV.G.3), analyses have been performed to obtain qualitative/quantitative measures of neurocognition and mood in subjects consuming plant sterols and plant stanols (Schiepers *et al.*, 2009). A battery of tests to measure memory, ability

to process simple and complex information, and mood were administered to the subjects at baseline (end of run-in period) and end of treatment. Results from the Mini-Mental State Examination to assess dementia, Visual Verbal Word Learning Task to assess learning capacity and long-term memory, Stroop Color-Word Interference Test to assess selective attention, Concept Shifting Test to assess behavioral planning, and Letter-Digit Substitution Test to assess working memory were not significantly different among individuals consuming margarine fortified with either plant sterol ester or plant stanol ester compared to controls. Thus, supplementation of plant sterol ester or plant stanol ester did not significantly alter neurocognitive function as measured by these tests. Differences in subjective cognitive function (measured with Cognitive Failure Questionnaire) or mood (measured with depression subscale of the Symptom Checklist 90) were also not reported.

J. GRAS Conclusion

Based on the above data and information presented herein, Raisio Nutrition has concluded that the intended food uses of the company's plant stanol ester, as described in Table II-1, are GRAS based on scientific procedures. General recognition of Raisio's GRAS determination is supported by the unanimous consensus rendered by an independent Panel of Experts, qualified by experience and scientific training, to evaluate the use of plant stanol ester in food, who similarly concluded that the intended uses of plant stanol ester described herein are GRAS.

A summary of the data and information described herein as reviewed by the Expert Panel is provided in Appendix A.

V. REFERENCES

AbuMweis SS, Barake R, Jones PJ (2008). Plant sterols/stanols as cholesterol lowering agents: a meta-analysis of randomized controlled trials. Food Nutr Res 52:doi: 10.3402/fnr.v52i0.1811.

Alhassan S, Reese KA, Mahurin J, Plaisance EP, Hilson BD, Garner JC *et al.* (2006). Blood lipid responses to plant stanol ester supplementation and aerobic exercise training. Metabolism 55(4):541-549.

Anderson SA, editor (1988). Estimation of Exposure to Substances in the Food Supply (Contract no FDA 223-84-2059). Bethesda (MD): Federation of American Societies for Experimental Biology (FASEB), Life Science Research Office (LSRO).

Assmann G, Cullen P, Erbey J, Ramey DR, Kannenberg F, Schulte H (2006). Plasma sitosterol elevations are associated with an increased incidence of coronary events in men: results of a nested case-control analysis of the Prospective Cardiovascular Mü nster (PROCAM) study. Nutr Metab Cardiovasc Dis 16(1):13-21.

Berendschot TT, Plat J, de Jong A, Mensink RP (2009). Long-term plant stanol and sterol ester-enriched functional food consumption, serum lutein/zeaxanthin concentration and macular pigment optical density. Br J Nutr 101(11):1607-1610.

Berge KE, Tian H, Graf GA, Yu L, Grishin NV, Schultz J *et al.* (2000). Accumulation of dietary cholesterol in sitosterolemia caused by mutations in adjacent ABC transporters. Science 290(5497):1771-1775.

Bhattacharyya AK, Connor WE (1974). β-sitosterolemia and xanthomatosis. A newly described lipid storage disease in two sisters. J Clin Invest 53(4):1033-1043.

Blair SN, Capuzzi DM, Gottlieb SO, Nguyen T, Morgan JM, Cater NB (2000). Incremental reduction of serum total cholesterol and low-density lipoprotein cholesterol with the addition of plant stanol ester-containing spread to statin therapy. Am J Cardiol 86(1):46-52.

Cabezas MC, De Vries JH, Van Oostrom AJ, Iestra J, Van Staveren WA (2006). Effects of a stanol-enriched diet on plasma cholesterol and triglycerides in patients treated with statins. J Am Diet Assoc 106(10):1564-1569.

Cater NB, Garcia-Garcia AB, Vega GL, Grundy SM (2005). Responsiveness of plasma lipids and lipoproteins to plant stanol esters. Am J Cardiol 96(1A):23D-28D.

CDC (2006). *Analytical and Reporting Guidelines: The National Health and Nutrition Examination Survey (NHANES)*. Hyattsville (MD): Centers for Disease Control and Prevention (CDC), National Center for Health Statistics (NCHS). Available at: http://www.cdc.gov/nchs/data/nhanes/nhanes_03_04/nhanes_analytic_guidelines_dec_2005.pdf.

CDC (2009). *National Health and Nutrition Examination Survey (NHANES): 2005-2006.* Hyattsville (MD): Centers for Disease Control and Prevention (CDC), National Center for Health Statistics (NCHS). Available at: http://www.cdc.gov/nchs/nhanes/nhanes2005-2006/nhanes05_06.htm.

Charest A, Desroches S, Vanstone CA, Jones PJ, Lamarche B (2004). Unesterified plant sterols and stanols do not affect LDL electrophoretic characteristics in hypercholesterolemic subjects. J Nutr 134(3):592-595.

Chen Q, Gruber H, Swist E, Pakenham C, Ratnayake WM, Scoggan KA (2009). Influence of dietary phytosterols and phytostanols on diastolic blood pressure and the expression of blood pressure regulatory genes in SHRSP and WKY inbred rats. Br J Nutr 102(1):93-101.

Chen Q, Gruber H, Swist E, Coville K, Pakenham C, Ratnayake WMN *et al.* (2010). Dietary phytosterols and phytostanols decrease cholesterol levels but increase blood pressure in WKY inbred rats in the absence of salt-loading. Nutr Metab 7:11. doi:10.1186/1743-7075-7-11.

Daly TJ, Aherne SA, O'Connor TP, O'Brien NM (2009). Lack of genoprotective effect of phytosterols and conjugated linoleic acids on Caco-2 cells. Food Chem Toxicol 47(8):1791-1796.

Davidson MH, Maki KC, Umporowicz DM, Ingram KA, Dicklin MR, Schaefer E *et al.* (2001). Safety and tolerability of esterified phytosterols administered in reduced-fat spread and salad dressing to healthy adult men and women. J Am Coll Nutr 20(4):307-319.

de Jong A, Plat J, Mensink RP (2006). Plant sterol or stanol consumption does not affect erythrocyte osmotic fragility in patients on statin treatment. Eur J Clin Nutr 60(8):985-990.

de Jong N, Ros MM, Ocke MC, Verhagen H (2008a). A general postlaunch monitoring framework for functional foods tested with the phytosterol/-stanol case. Trends Food Sci Technol 19(10):535-545.

de Jong A, Plat J, Bast A, Godschalk RW, Basu S, Mensink RP (2008b). Effects of plant sterol and stanol ester consumption on lipid metabolism, antioxidant status and markers of oxidative stress, endothelial function and low-grade inflammation in patients on current statin treatment. Eur J Clin Nutr 62(2):263-273.

de Jong A, Plat J, Lütjohann D, Mensink RP (2008c). Effects of long-term plant sterol or stanol ester consumption on lipid and lipoprotein metabolism in subjects on statin treatment. Br J Nutr 100(5):937-941.

EC (1997). Regulation (EC) No 258/97 of the European Parliament and of the Council of 27 January 1997 concerning novel foods and novel food ingredients. Off J Eur Communities 40(L43):1-6. Available at: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CONSLEG:1997R0258:20090120:EN:PDF [Amended to 2009].

EC (2000). Commission Decision of 24 July 2000 on authorising the placing on the market of 'yellowfat 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) (Only the English text is authentic) (2000/500/EC). Off J Eur Communities 43(L200):59-60. Available at: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2000:200:0059:0060:EN:PDF.

EC (2004). Commission Regulation (EC) No 608/2004 of 31 March 2004 concerning the labelling of foods and food ingredients with added phytosterols, phytosterol esters, phytstanols and/or phytostanol esters (Text with EEA relevance). Off J Eur Union 47(L97):44-45. Available at: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2004:097:0044:0045:EN:PDF.

EFSA (2008). A Report from the Data Collection and Exposure Unit in Response to a Request from the European Commission. Consumption of Food and Beverages with Added Plant Sterols in the European Union. (EFSA/DATEX 03). EFSA J 133:1-21. Available at: http://www.efsa.europa.eu/en/scdocs/doc/datex_report_ej133_phytosterols_en.pdf.

Fricke,CB, Schroder M, Poulsen M, von Bergmann K, Wester I *et al.* (2007). Increased plant sterol and stanol levels in brain of Watanabe rabbits fed rapeseed oil derived plant sterol or stanol esters. Br J Nutr 98(5):890-899.

FSANZ (2011). Chapter 1—General food standards. Part 1.5—Foods requiring pre-market clearance. Standard 1.5.1—Novel foods; Chapter 2—Food product standards, Part 2.4—Edible oils. Standard 2.4.2—Edible oils spreads; Part 2.5—Dairy products. Standard 2.5.1—Milk; 2.5.3—Fermented milk products. In: *Australia New Zealand Food Standards Code*. Canberra, Australia / Wellington, NZ: Foods Standards Australia New Zealand (FSANZ). Available at: http://www.foodstandards.gov.au/foodstandards/foodstandardscode.cfm.

Glueck CJ, Speirs J, Tracy T, Streicher P, Illig E, Vandegrift J (1991). Relationships of serum plant sterols (phytosterols) and cholesterol in 595 hypercholesterolemic subjects, and familial aggregation of phytosterols, cholesterol and premature coronary heart disease in hyperphytosterolemic probands and their first-degree relatives. Metabolism 40(8):842-848.

Goldberg AC, Ostlund RE Jr, Bateman JH, Schimmoeller L, McPherson TB, Spilburg CA (2006). Effect of plant stanol tablets on low-density lipoprotein cholesterol lowering in patients on statin drugs. Am J Cardiol 97(3):376-379.

Greenhalgh T (1997). How to read a paper. Statistics for the non-statistician. II: "Significant" relations and their pitfalls. BMJ 315(7105):422-425.

Grice HC, Heggtveit HA (1983). The relevance to humans of myocardial lesions induced in rats by marine and rapeseed oils. In: Kramer JKG, Sauer FD, Pigden WJ, editors. *High and Low Erucic Acid, Rapeseed Oils: Production, Usage, Chemistry, and Toxicological Evaluation*. New York (NY): Academic Press, pp. 551-562.

GRN 061 (2001). *GRN No. 061 [Archer Daniels Midland Company, re:* Plant sterols/Plant sterol esters*]*. (GRAS Notice Inventory). U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety and Applied Nutrition (CFSAN), Office of Premarket Approval. Available at: http://www.fda.gov/Food/FoodIngredientsPackaging/GenerallyRecognizedasSafeGRAS/GRASListings/ucm153969.htm [Date of filing: 2001-04-18].

GRN 398 (2012). *GRN No. 398 [Cargill, re: Vegetable oil and tall oil derived phytosterol and phytosterol ester formulations]*. (GRAS Notice Inventory). U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety and Applied Nutrition (CFSAN), Office of Premarket Approval. Available at: http://www.fda.gov/Food/FoodIngredientsPackaging/GenerallyRecognizedasSafeGRAS/GRASListings/ucm295936.htm [Date of filing: August 18, 2012; Date of approval/closure: February 13, 2012].

Gylling H, Puska P, Vartiainen E, Miettinen TA (1999a). Retinol, vitamin D, carotenes and α-tocopherol in serum of a moderately hypercholesterolaemic population consuming sitostanol ester margarine. Atherosclerosis 145(2):279-285.

Gylling H, Puska P, Vartiainen E, Miettinen TA (1999b). Serum sterols during stanol ester feeding in a mildly hypercholesterolemic population. J Lipid Res 40(4):593-600.

Gylling H, Hallikainen M, Raitakari OT, Laakso M, Vartiainen E, Salo P *et al.* (2009). Long-term consumption of plant stanol and sterol esters, vascular function and genetic regulation. Br J Nutr 101(11):1688-1695.

Gylling H, Hallikainen M, Nissinen MJ, Simonen P, Miettinen TA (2010a). Very high plant stanol intake and serum plant stanols and non-cholesterol sterols. Eur J Nutr 49(2):111-117.

Gylling H, Hallikainen M, Nissinen MJ, Miettinen TA (2010b). The effect of a very high daily plant stanol ester intake on serum lipids, carotenoids, and fat-soluble vitamins. Clin Nutr 29(1):112-118.

Hallikainen MA, Sarkkinen ES, Gylling H, Erkkila AT, Uusitupa MI (2000a). Comparison of the effects of plant sterol ester and plant stanol ester-enriched margarines in lowering serum cholesterol concentrations in hypercholesterolaemic subjects on a low-fat diet. Eur J Clin Nutr 54(9):715-725.

Hallikainen MA, Sarkkinen ES, Uusitupa MI (2000b). Plant stanol esters affect serum concentrations of hypercholesterolemic men and women in a dose-dependent manner. J Nutr 130(4):767-776.

Hallikainen M, Lyyra-Laitinen T, Laitinen T, Agren JJ, Pihlajamä ki J, Rauramaa R *et al.* (2006). Endothelial function in hypercholesterolemic subjects: effects of plant stanol and sterol esters. Atherosclerosis 188(2):425-432.

Hallikainen M, Lyyra-Laitinen T, Laitinen T, Moilanen L, Miettinen TA, Gylling H (2008). Effects of plant stanol esters on serum cholesterol concentrations, relative markers of cholesterol metabolism and endothelial function in type 1 diabetes. Atherosclerosis 199(2):432-439.

Health Canada (2010a). *Notice of [Safety] Assessment of Certain Categories of Foods Containing Added Phytosterols.* Ottawa (ON): Health Canada, Food Directorate, Health Products and Food Branch, Bureau of Nutritional Sciences. Available at: http://www.hc-sc.gc.ca/fn-an/gmf-agm/appro/phytosterols-eng.php.

Health Canada (2010b). *Plant Sterols and Blood Cholesterol Lowering Summary of Health Canada's Assessment of a Health Claim about Plant Sterols in Foods and Blood Cholesterol Lowering.* Ottawa (ON): Health Canada, Food Directorate, Health Products and Food Branch, Bureau of Nutritional Sciences. Available at: http://www.hc-sc.gc.ca/fn-an/alt_formats/pdf/label-etiquet/claims-reclam/assess-evalu/phytosterols-claim-allegation-eng.pdf.

Hearty A, Duffy E, Joyce J, O'Conner C, Gibney MJ (2008). Phytosterol-enriched products on the Irish market:examination of intake and consumption patterns. Public Health Nutr 12(1):51-58.

Hendriks HF, Weststrate JA, van Vliet T, Meijer GW (1999). Spreads enriched with three different levels of vegetable oil sterols and the degree of cholesterol lowering in normocholesterolaemic and mildly hypercholesterolaemic subjects. Eur J Clin Nutr 53(4):319-327.

Hidaka H, Nakamura T, Aoki T, Kojima H, Nakajima Y, Kosugi K *et al.* (1990). Increased plasma plant sterol levels in heterozygotes with sitosterolemia and xanthomatosis. J Lipid Res 31(5):881-888.

Homma Y, Ikeda I, Ishikawa T, Tateno M, Sugano M, Nakamura H (2003). Decrease in plasma low-density lipoprotein cholesterol, apolipoprotein B, cholesteryl ester transfer protein, and oxidized low-density lipoprotein by plant stanol ester-containing spread: a randomized, placebo-controlled trial. Nutrition 19(4):369-374.

Hyun YJ, Kim OY, Kang JB, Lee JH, Jang Y, Liponkoski L *et al.* (2005). Plant stanol esters in low-fat yogurt reduces total and low-density lipoprotein cholesterol and low-density lipoprotein oxidation in normocholesterolemic and mildly hypercholesterolemic subjects. Nutr Res 25:743-753.

IOM (2006). Carotenoids. In: *Dietary Reference Intakes: The Essential Guide to Nutrient Requirements.* Institute of Medicine (IOM) of the National Academies. Washington (DC): National Academy Press (NAP), pp. 211-217, 8510-862 (references).

Ishiwata K, Homma Y, Ishikawa T, Nakamura H, Handa S (2002). Influence of apolipoprotein E phenotype on metabolism of lipids and apolipoproteins after plant stanol ester ingestion in Japanese subjects. Nutrition 18(7&8):561-565.

Jakulj L, Vissers MN, Rodenburg J, Wiegman A, Trip MD, Kastelein JJ (2006). Plant stanols do not restore endothelial function in pre-pubertal children with familial hypercholesterolemia despite reduction of low-density lipoprotein cholesterol levels. J Pediatr 148(4):495-500.

Jauhiainen T, Salo P, Niittynen L, Poussa T, Korpela R (2006). Effects of low-fat hard cheese enriched with plant stanol esters on serum lipids and apolipoprotein B in mildly hypercholesterolaemic subjects. Eur J Clin Nutr 60(11):1253-1257.

JECFA (2008). Phytosterols, phytostanols and their esters. In: *Compendium of Food Additive Specifications*. Joint FAO/WHO Expert Committee on Food Additives (JECFA), 69th Meeting, June 17-26, 2008, Rome, Italy. (FAO JECFA Monographs 5). Rome, Italy: Food and Agriculture Organization of the United Nations, World Health Organization (WHO). Available at: http://www.fao.org/ag/agn/jecfa-additives/specs/monograph5/additive-509-m5.pdf.

JECFA (2009). Phytosterols, phytostanols and their esters. In: *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). Geneva, Switz.: World Health Organization (WHO), pp. 117-163, 618. Available at: http://whqlibdoc.who.int/publications/2009/9789241660600_eng.pdf.

Jia X, Ebine N, Wang Y, Awad AB, Jones PJ (2006). Effects of different phytosterol analogs on colonic mucosal cell proliferation in hamsters. J Nutr Biochem 17(6):396-401.

Jones RJ, Raeini-Sarjaz M, Ntanios FY, Vanstone CA, Feng JY, Parsons WE (2000). Modulation of plasma lipid levels and cholesterol kinetics by phytosterol versus phytostanol esters. J Lipid Res 41(5):697-705.

Katan MB, Grundy SM, Jones P, Law M, Miettinen T, Paoletti R (2003). Efficacy and safety of plant stanols and sterols in the management of blood cholesterol levels. Mayo Clin Proc 78(8):965-978.

Kelly ER, Plat J, Mensink RP, Berendschot TT (2011). Effects of long term plant sterol and-stanol consumption on the retinal vasculature: a randomized controlled trial in statin users. Atherosclerosis 214(1):225-230.

Kesaniemi AY, Luetjohann D, Rossebo AB, Holme I, Pedersen TR, SEAS Investigators (2009). Effect of baseline phytosterol levels on aortic valve and ischemic cardiovascular events and the incidence and mortality of cancer in the patients with aortic valve stenosis: The SEAS Trial. J Am Coll Cardiol 53(10, Suppl. 1):A209 [abstract 1021-89].

Ketomaki AM, Gylling H, Antikainen M, Siimes MA, Miettinen TA (2003). Red cell and plasma plant sterols are related during consumption of plant stanol and sterol ester spreads in children with hypercholesterolemia. J Pediatr 142(5):524-531.

Kratz M, Kannenberg F, Gramenz E, Berning B, Trautwein E, Assmann G *et al.* (2007). Similar serum plant sterol responses of human subjects heterozygous for a mutation causing sitosterolemia and controls to diets enriched in plant sterols or stanols [including supplementary table]. Eur J Clin Nutr 61(7):896-905.

Kuhlmann K, Lindtner O, Bauch A, Ritter G, Woerner B, Niemann B (2005). Simulation of prospective phytosterol intake in Germany by novel functional foods. Br J Nutr 93(3):377-385.

Lagström H, Helenius H, Salo P (2006). Serum cholesterol-lowering efficacy of stanol ester incorporated in gelatin capsules. Scand J Food Nutr 50(3):124-130.

Laitinen K, Isolauri E, Kaipiainen L, Gylling H, Miettinen TA (2009). Plant stanol ester spreads as components of a balanced diet for pregnant and breast-feeding women: evaluation of clinical safety. Br J Nutr 101(12):1797-1804.

Lütjohann D, Meese CO, Crouse JR III, von Bergmann K (1993). Evaluation of deuterated cholesterol and deuterated sitostanol for measurement of cholesterol absorption in humans. J Lipid Res 34(6):1039-1046.

Marttinen M, Päivärinta E, Piironen V, Pajari A-M, Mutanen M [2008]. Plant stanols induce the formation of intestinal adenomas and activate ERK1 signaling in the *Apc*Min/+ mouse. [Re-printed In: *6th Science Day at Hanasaari,* August 25, 2011]. Helsinki, Finland: University of Helsinki, Department of Food and Environmental Sciences [poster].

McPherson TB, Ostlund RE, Goldberg AC, Bateman JH, Schimmoeller L, Spilburg CA (2005). Phytostanol tablets reduce human LDL-cholesterol. J Pharm Pharmacol 57(7):889-896.

Mensink RP, Ebbing S, Lindhout M, Plat J, Van Heugten MMA (2002). Effects of plant stanol esters supplied in low-fat yoghurt on serum lipids and lipoproteins, non-cholesterol sterols and fat soluble antioxidant concentrations. Atherosclerosis 160(1):205-213.

Mensink RP, de Jong A, Lütjohann D, Haenen GRMM, Plat J (2010). Plant stanols dose-dependently decrease LDL-cholesterol concentrations, but not cholesterol-standardized fat-soluble antioxidant concentrations, at intakes up to 9 g/d. Am J Clin Nutr 92(1):24-33.

Miettinen TA, Vuoristo M, Nissinen M, Jarvinen HJ, Gylling H (2000). Serum, biliary, and fecal cholesterol and plant sterols in colectomized patients before and during consumption of stanol ester margarine. Am J Clin Nutr 71(5):1095-1102.

Mulder J (2000). (2000/C 203 E/004) Written question P-1506/99 by Jan Mulder (ELDR) to the Commission (1 September 1999). Subject: Application of Regulation 258/97 (novel foods and novel ingredients). Off J Eur Communities 43:4-5. Available at: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:C:2000:203E:0004:0005:EN:PDF.

Musa-Veloso K, Poon TH, Elliot JA, Chung C (2011). 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 Leukot Essent Fatty Acids 85(1):9-28.

Naito Y, Nagata T, Takano Y, Nagatsu T, Ohara N (2003). Rapeseed oil ingestion and exacerbation of hypertension-related conditions in stroke prone spontaneously hypertensive rats. Toxicology 187(2&3):205-216.

Nestel P, Cehun M, Pomeroy S, Abbey M, Weldon G (2001). Cholesterol-lowering effects of plant sterol esters and non-esterified stanols in margarine, butter and low-fat foods. Eur J Clin Nutr 55(12):1084-1090.

Noakes M, Clifton P, Ntanios F, Shrapnel W, Record I, McInerney J (2002). An increase in dietary carotenoids when consuming plant sterols or stanols is effective in maintaining plasma carotenoid concentrations 1-3. Am J Clin Nutr 75(1):79-86.

Noakes M, Clifton PM, Doornbos AM, Trautwein EA (2005). Plant sterol ester-enriched milk and yoghurt effectively reduce serum cholesterol in modestly hypercholesterolemic subjects. Eur J Nutr 44(4):214-222.

Ntanios FY, Jones PJ, Frohlich JJ (1998). Dietary sitostanol reduces plaque formation but not lechithin: Chloesterol acyltransferase activity in rabbits. Atherosclerosis 138(1):101-110.

O'Neill FH, Brynes A, Mandeno R, Rendell N, Taylor G, Seed M *et al.* (2004). Comparison of the effects of dietary plant sterol and stanol esters on lipid metabolism. Nutr Metab Cardiovasc Dis 14(3):133-142.

Ogawa H, Yamamoto K, Kamisako T, Meguro T (2003). Phytosterol additives increase blood pressure and promote stroke onset in salt-loaded stroke-prone spontaneously hypertensive rats. Clin Exp Pharmacol Physiol 30(12):919-924.

Ostlund RE Jr, McGill JB, Zeng C-M, Covey DF, Stearns J, Stenson WF *et al.* (2002). Gastrointestinal absorption and plasma kinetics of soy Δ(5)-phytosterols and phytostanols in humans. Am J Physiol Endocrinol Metab 282(4):E911-E916.

Phillips KM, Tarragó-Trani MT, Stewart KK (1999). Phytosterol content of experimental diets differing in fatty acid composition. Food Chem 64(3):415-422.

Plat J, Mensink RP (2000). Vegetable oil based versus wood based stanol ester mixtures: Effects on serum lipids and hemostatic factors in non-hypercholesterolemic subjects. Atherosclerosis 148(1):101-112.

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

Plat J, Mensink RP (2001b). Effects of plant sterols and stanols on lipid metabolism and cardiovascular risk. Nutr Metab Cardiovasc Dis 11(1):31-40.

Plat J, van Onselen EN, van Heugten MM, Mensink RP (2000). Effects on serum lipids, lipoproteins and fat soluble antioxidant concentrations of consumption frequency of margarines and shortenings enriched with plant stanol esters. Eur J Clin Nutr 54(9):671-677.

Plat J, Brufau G, Dallinga-Thie GM, Dasselaar M, Mensink RP (2009). A plant stanol yogurt drink alone or combined with a low-dose statin lowers serum triacylglycerol and non-HDL cholesterol in metabolic syndrome patients [includes supplemental data]. J Nutr 139(6):1143-1149.

Raeini-Sarjaz M, Ntanios FY, Vanstone CA, Jones PJH (2002). No changes in serum fat-soluble vitamin and carotenoid concentrations with the intake of plant sterol/stanol esters in the context of a controlled diet. Metabolism 51(5):652-656.

Raitakari OT, Salo P, Gylling H, Miettinen TA (2008). Plant stanol ester consumption and arterial elasticity and endothelial function. Br J Nutr 100(3):603-608.

Rajaratnam RA, Gylling H, Miettinen TA (2000). Independent association of serum squalene and noncholesterol sterols with coronary artery disease in postmenopausal women. J Am Coll Cardiol 35(5):1185-1191.

Ratnayake WM, Plouffe L, Hollywood R, L'Abbé MR, Hidiroglou N, Sarwar G *et al.* (2000a). Influence of sources of dietary oils on the life span of stroke-prone spontaneously hypertensive rats. Lipids 35(4):409-420.

Ratnayake WMN, Abbe MRL, Mueller R, Hayward S, Plouffe L, Hollywood R *et al.* (2000b). Vegetable oils high in phytosterols make erythrocytes less deformable and shorten the life span of stroke-prone spontaneously hypertensive rats. J Nutr 130(5):1166-1178.

Ratnayake WMN, Plouffe L, L'Abbé MR, Trick K, Mueller R, Hayward S (2003). Comparative health effects of margarines fortified with plant sterols and stanols on a rat model for hemorrhagic stroke. Lipids 38(12):1237-1247.

Rozner S, Garti N (2006). The activity and absorption relationship of cholesterol and phytosterols. Colloids Surf A 282/283:435-456.

Salen G, Kwiterovich PO Jr, Shefer S, Tint GS, Horak I, Shore V *et al.* (1985). Increased plasma cholestanol and 5α-saturated plant sterol derivatives in subjects with sitosterolemia and xanthomatosis. J Lipid Res 26(2):203-209.

Salen G, Patel S, Batta AK (2002). Sitosterolemia. Cardiovasc Drug Rev 20(4):255-270.

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

SCF (2002). *Opinion of the Scientific Committee on Food on a Report on Post Launch Monitoring of 'Yellow Fat Spreads With Added Phytosterol Esters'(Expressed on 26 September 2002)*. (SCF/CS/NF/DOS/21 ADD 2 Final). Brussels, Belgium: European Commission, Health & Consumer Protection Directorate-General, Scientific Committee for Food (SCF). Available at: http://www.ec.europa.eu/food/fs/sc/scf/out144_en.pdf.

SCF (2003). *Opinion of the Scientific Committee on Food on Applications for Approval of a Variety of Plant Sterol-Enriched Foods (expressed on 5 March 2003)*. (SCF/CS/NF/DOS/15 ADD 2 Final). Brussels, Belgium: European Commission, Health & Consumer Protection Directorate-General, Scientific Committee for Food (SCF). Available at: http://ec.europa.eu/food/fs/sc/scf/out174_en.pdf.

Schiepers OJ, de Groot RH, van Boxtel MP, Jolles J, de Jong A, Lütjohann D *et al.* (2009). Consuming functional foods enriched with plant sterol or stanol esters for 85 weeks does not affect neurocognitive functioning or mood in statin-treated hypercholesterolemic individuals [including supplementary data]. J Nutr 139(7):1368-1373.

Schroder M, Fricke C, Pilegaard K, Poulsen M, Wester I, Lutjohann D *et al.* (2009). Effect of rapeseed oil-derived plant sterol and stanol esters on atherosclerosis parameters in cholesterol-challenged heterozygous watanabe heritable hyperlipidaemic rabbits. Br J Nutr 102(12):1740-1751.

Scoggan KA, Gruber H, Lariviere K (2003). A missense mutation in the *Abcg5* gene causes phytosterolemia in SHR, stroke-prone SHR, and WKY rats. J Lipid Res 44(5):911-916.

Seppo L, Jauhiainen T, Nevala R, Poussa T, Korpela R (2007). Plant stanol esters in low-fat milk products lower serum total and LDL cholesterol. Eur J Nutr 46(2):111-117.

Slesinski RS, Turnbull D, Frankos VH, Wolterbeek AP, Waalkens-Berendsen DH (1999). Developmental toxicity study of vegetable oil-derived stanol fatty acid esters. Regul Toxicol Pharmacol 29(2, Part 1):227-233.

Spilburg CA, Goldberg AC, McGill JB, Stenson WF, Racette SB, Bateman J *et al.* (2003). Fat-free foods supplemented with soy stanol-lecithin powder reduce cholesterol absorption and LDL cholesterol. J Am Diet Assoc 103(5):577-581.

Sudhop T, Gottwald BM, von Bergmann K (2002). Serum plant sterols as a potential risk factor for coronary heart disease (a preliminary report). Metabolism 51:1519-1521.

Sutton D, Davey T, Venkatraman G, Hart K (2009). Can a functional food exert a cholesterol lowering effect in renal transplant patients? J Ren Care 35(1):42-47.

Tammi A, Rönnemaa T, Gylling H, Rask-Nissilä L, Viikari J, Tuominen J *et al.* (2000). Plant stanol ester margarine lowers serum total and low-density lipoprotein cholesterol concentrations of healthy children: the STRIP project. J Pediatr 136(4):503-510.

Tatematsu K, Fuma SY, Nagase T, Ichikawa Y, Fujii Y, Okuyama H (2004). Factors other than phytosterols in some vegetable oils affect the survival of SHRSP rats. Food Chem Toxicol 42(9):1443-1451.

Theuwissen E, Plat J, Van Der Kallen CJ, Van Greevenbroek M M, Mensink RP (2009). Plant stanol supplementation decreases serum triacylglycerols in subjects with overt hypertriglyceridemia. Lipids 44(12):1131-1140.

Thompson GR, Grundy SM (2005). History and development of plant sterol and stanol esters for cholesterol-lowering purposes. Am J Cardiol 96(1A):3D-9D.

Turnbull D, Whittaker MH, Frankos VH, Jonker D (1999a). 13-week oral toxicity study with stanol esters in rats. Regul Toxicol Pharmacol 29(2, Part 1):216-226.

Turnbull D, Frankos VH, van Delft JH, DeVogel H (1999b). Genotoxicity evaluation of wood-derived and vegetable oil-derived stanol esters. Regul Toxicol Pharmacol 29(2, Part 1):205-210.

U.S. FDA (1999a). *Letter from Alan M. Rulis, Director, Office of Premarket Approval, CFSAN to Vivian A. Chester and Edward B. Nelson of McNeil Consumer Healthcare dated May 17,1999 [Re: Food Master File 000626, Plant Stanol Esters GRAS Submission].* Washington (DC): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety and Applied Nutrition (CFSAN). Available at: http://www.fda.gov/ohrms/dockets/DOCKETS/95s0316/rpt0054_01.pdf (See p. 14-17 of PDF).

U.S. FDA (1999b). *Letter from John C. Young, McNeil Consumer Healthcare, to Alan Rulis, FDA, July 21, 1999.* Cited In: U.S. FDA, 2000.

U.S. FDA (1999c). *Letter from John C. Young, McNeil Consumer Healthcare, to Alan Rulis, FDA, October 13, 1999.* Cited In: U.S. FDA, 2000.

U.S. FDA (2000). Food labeling: health claims; plant sterol/stanol esters and coronary heart disease: interim final rule [21 CFR Part 101, Docket Nos. 00P-1275 and 00P-1276]. Fed Regist (US) 65(175):54685-54739.

U.S. FDA (1997). Substances generally recognized as safe; Proposed rule (21 CFR Parts 170, 184, 186, and 570) [Docket No. 97N-0103]. Fed Regist (US) 62(74):18937-18964.

U.S. FDA (2010). Food labeling; health claim; phytosterols and risk of coronary heart disease; proposed rule [21 CFR Part 101, Docket Nos. FDA-2000-P-0102, FDA-2000-P-0133, and FDA-2006-P-0033; Formerly Docket Nos. 2000P-1275, 2000P-1276, and 2006P-0316, Respectively]. Fed Regist (US) 75(235):76525-76571.

U.S. FDA (2012a). Part 101—Food labeling. Section §101.83—Health claims: plant sterol/stanol esters and risk of coronary heart disease (CHD). In: *U.S. Code of Federal Regulations (CFR). Title 21: Food and Drugs (U.S. Food and Drug Administration)*. Washington (DC): U.S. Food and Drug Administration (U.S. FDA), U.S. Government Printing Office (GPO). Available at: http://www.gpo.gov/fdsys/browse/collectionCfr.action?collectionCode=CFR.

U.S. FDA (2012b). Part 101—Food labeling. Section §101.12—Reference amounts customarily consumed per eating occasion. In: *U.S. Code of Federal Regulations (CFR). Title 21: Food and Drugs (U.S. Food and Drug Administration)*. Washington (DC): U.S. Food and Drug Administration (U.S. FDA), U.S. Government Printing Office (GPO). Available at: http://www.gpo.gov/fdsys/browse/collectionCfr.action?collectionCode=CFR.

U.S. FDA (2012c). Part 170—Food additives. Section §170.30— Eligibility for classification as generally recognized as safe (GRAS). In: *U.S. Code of Federal Regulations (CFR). Title 21: Food and Drugs (U.S. Food and Drug Administration)*. Washington (DC): U.S. Food and Drug Administration (U.S. FDA), U.S. Government Printing Office (GPO). Available at: http://www.gpo.gov/fdsys/browse/collectionCfr.action?collectionCode=CFR.

USDA (2009). *What We Eat in America: National Health and Nutrition Examination Survey (NHANES): 2003-2004, 2005-2006.* Riverdale (MD): U.S. Department of Agriculture (USDA). Available at: http://www.ars.usda.gov/Services/docs.htm?docid=13793#release.

Vanstone CA, Raeini-Sarjaz M, Parsons WE, Jones PJ (2002). Unesterified plant sterols and stanols lower LDL-cholesterol concentrations equivalently in hypercholesterolemic persons. Am J Clin Nutr 76(6):1272-1278.

Weststrate JA, Ayesh R, Bauer-Plank C, Drewitt PN (1999). 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 Chem Toxicol 37(11):1063-1071.

Whittaker MH, Frankos VH, Wolterbeek AP, Waalkens-Berendsen DH (1999). Two-generation reproductive toxicity study of plant stanol esters in rats. Regul Toxicol Pharmacol 29(2, Part 1):196-204.

Williams CL, Bollella M, Strobino BA, Boccia L, Campanaro L (1999). Lipid-lowering effects of a plant stanol ester spread in young children. Eur Heart J Suppl 1(Suppl. S):S96-S103.

Wilund KR, Yu L, Xu F, Vega GL, Grundy SM, Cohen JC *et al.* (2004). No association between plasma levels of plant sterols and atherosclerosis in mice and men. Arterioscler Thromb Vasc Biol 24(12):2326-2332.

Wolfs M, de Jong N, Ocké MC, Verhagen H, Monique Verschuren WM (2006). Effectiveness of customary use of phytosterol/-stanol enriched margarines on blood cholesterol lowering. Food Chem Toxicol 44(10):1682-1688.

Woodgate D, Chan CH, Conquer JA (2006). Cholesterol-lowering ability of a phytostanol softgel supplement in adults with mild to moderate hypercholesterolemia. Lipids 41(2):127-132.

Yamori Y, Nara Y, Horie R, Ooshima A (1980). Abnormal membrane characteristics of erythrocytes in rat models and men with predisposition to stroke. Clin Exp Hypertens 2(6):1009-1021.

APPENDIX A

APPENDIX A

Expert Panel Report on the Generally Recognized as Safe (GRAS) Use of Plant Stanol Ester as a Direct Food Ingredient in Multiple Food and Beverage Products

Expert Panel Report on the Generally Recognized as Safe (GRAS) Use of Plant Stanol Ester as a Direct Food Ingredient in Multiple Food and Beverage Products

June 27, 2012

ABBREVIATIONS

Apc Min/+	adenomatous polyposis coli, multiple intestinal neoplasia
BMI	body mass index
CaCO-2	colorectal adenocarcinoma cells
cGMP	current Good Manufacturing Practice
EFSA	European Food Safety Authority
ERK1	extracellular-signal-regulated kinase 1
FAP	familial adenomatous polyposis
FDA	Food and Drug Administration
FSANZ	Food Standards Australia New Zealand
GRAS	Generally Recognized as Safe
HDL	high density lipoprotein
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LDL	low density lipoprotein
NHANES	National Health and Nutrition Examination Surveys
NOAEL	no-observed-adverse-effect level
RACC	reference amount customarily consumed
SCF	Scientific Committee on Food (European Commission)
U.S.	United States

Expert Panel Report On the Generally Recognized as Safe (GRAS) Use of Plant Stanol Ester as a Direct Food Ingredient in Multiple Food and Beverage Products

June 18, 2012

Plant stanol ester manufactured by Raisio Nutrition Ltd. (Raisio) is Generally Recognized as Safe (GRAS) for addition to spreads, dressings, and snack bars, at a use level of 1.0 g of plant stanols (or 1.7 g of plant stanol ester) per reference amount customarily consumed (RACC) (U.S. FDA, 1999a,b,c). General Recognition of Raisio's GRAS self affirmation, determined using scientific procedures, was established through the deliberations of a Panel of Experts[1], qualified by their training and scientific experience to evaluate the safety of plant stanol ester as a food ingredient. The Expert Panel evaluated a comprehensive dossier of information pertaining to the method of manufacture, product specifications, supporting analytical data, intended uses and estimated intakes, and published and unpublished literature supporting the safety of plant stanols, delivered as plant stanol ester, under the proposed conditions of use in spread. On February 18th, 1999, documentation supporting Raisio's GRAS self-affirmation of the use of plant stanol ester in spreads was submitted to the offices of the United States (U.S.) Food and Drug Administration (FDA) by its partner McNeil Consumer Healthcare (McNeil), and was designated as Food Master File 000626 by the FDA on February 19th, 1999. Following a review of the material provided to the Agency, the FDA stated that "based on its evaluation, the agency has no questions at this time regarding McNeil's conclusion that plant stanol ester is GRAS under the intended conditions of use" (U.S. FDA, 1999a). The FDA also understands that uses of plant stanol ester in dressings and spreads has been determined to be GRAS by McNeil (U.S. FDA, 1999b,c); the Agency has not objected to such uses and has stated that "FDA is not aware of any evidence that provides a basis to reject the petitioner's position that the use of plant stanol esters in spreads, dressings for salad, snack bars, and other foods is safe and lawful." (U.S. FDA, 2012a). **Raisio proposes to expand the GRAS uses of its plant stanol ester to include the following food categories: baked goods and baking mixes; beverages and beverage bases; breakfast cereals; cheeses; coffee and tea; dairy product analogs; fats and oils; grain products and pastas; gravies and sauces; milk; milk products; processed fruits and fruit juices; processed vegetables and vegetable juices; soft candy; and soups and soup mixes. Use levels of plant stanols in these products will range between 0.5 to 2.0 g of plant stanols per serving, provided as a plant stanol ester (see Table A-1 in Attachment A).**

[1] The Expert Panel consisted of the following qualified Experts: Robert M. Lees, M.D. (Harvard-MIT Division of Health Sciences and Technology); W. Virgil Brown, M.D. (Atlanta VA Medical Center); Thomas Q. Garvey III, M.D. (Garvey Associates, Inc.); David Kritchvesky, Ph.D. (The Wistar Institute); Joyce L. Beare-Rogers, C.M., Ph.D., D.Sc., F.R.S.C. (Consultant); Mark A. Levine, M.D. (National Institutes of Health); Joseph V. Rodricks, Ph.D. (Life Sciences Trust).

Raisio convened an Expert Panel (the "Expert Panel") of independent scientists, qualified by their relevant national and international experience and scientific training to evaluate the safety of food ingredients, to conduct a critical and comprehensive evaluation of the available pertinent data and information, and determine whether the expanded food and beverage uses of Raisio's plant stanol ester would be GRAS based on scientific procedures. The Expert Panel consisted of the following individuals: G. Harvey Anderson Ph.D. (University of Toronto); Samuel M. Cohen, M.D., Ph.D. (University of Nebraska Medical Center); Stephen L. Taylor Ph.D. (University of Nebraska); and Gilbert R. Thompson M.D., FRCP (Imperial College, London).

The Expert Panel, independently and collectively, critically evaluated a dossier which included a summary of the scientific data and information relevant to the safety of the proposed uses of Raisio's plant stanol ester. In addition to documentation presented by Raisio for support of the company's GRAS self-affirmed uses of plant stanol ester in spreads, dressings, and snack bars, the Expert Panel was provided with additional information prepared from a comprehensive search of the scientific literature to retrieve studies on the safety of plant stanols and plant stanol ester, published since the first Expert Panel evaluation conducted in 1999. Updated literature searches were conducted in January, February, and December 2011, using the electronic search tool Dialog. Literature searches were limited to articles published between 1999 and 2011 (inclusive). The Expert Panel also reviewed details pertaining to the method of manufacture and product specifications, supporting analytical data, intended conditions of use of plant stanol ester in food, estimated exposure under the proposed uses, and a comprehensive assessment of the available scientific literature pertaining to the safety of the ingredient. In addition, the Expert Panel evaluated other information deemed appropriate or necessary.

Following its independent critical evaluation of such data and information, the Expert Panel convened on Thursday, March 8, 2012 and unanimously concluded that the proposed uses for Raisio's plant stanol ester, meeting appropriate food-grade specifications as described in the supporting dossier **[Documentation Supporting the Generally Recognized as Safe (GRAS) Determination of a Plant Stanol Ester for Use in Multiple Food Categories]** and manufactured consistent with current Good Manufacturing Practice (cGMP), are GRAS based on scientific procedures. A summary of the basis for the Expert Panel's conclusion is provided below.

Raisio's plant stanol ester has a long-history of safe consumption as an ingredient in food and supplement products marketed in various jurisdictions throughout the world, including the U.S., the European Union, Australia/New Zealand, China, Russia and Japan. Worldwide, Raisio's plant stanol ester has been consumed for approximately 12 years, and consumption of plant stanol ester enriched products in Finland spans approximately 16 years. Post-market reports suggesting that food uses of plant stanols have been associated with adverse or undesirable effects were not identified within the updated literature search. The Expert Panel therefore

concluded that plant stanol ester, manufactured by Raisio, has an established long-history of safe consumption.

Plant stanol ester manufactured by Raisio, is GRAS for addition to spreads, dressings and snack bars at a use level of 1.7 g plant stanol ester per RACC (U.S. FDA, 1999a,b,c). Raisio proposes to expand the food uses of its plant stanol ester to include uses in multiple food and beverage products at use levels of between 500 and 2,000 mg plant stanols per RACC, provided as plant stanol ester. Current food uses of plant stanol ester will be targeted to consumers seeking foods for cholesterol management. The dietary exposure to plant stanol ester from all proposed food uses without regard to product labeling instructions was modeled using consumption data from the 2003-2004 and 2005-2006 National Health and Nutrition Examination Surveys (NHANES). The entire U.S. population was identified as potential consumers of plant stanols from proposed food-uses of plant stanol ester (16,221 actual users identified) (CDC, 2006, 2009; USDA, 2009). On an all-user basis, the mean intake of plant stanols by the total U.S. population from proposed food-uses of plant stanol ester was estimated to be 5.02 g/person/day (92 mg/kg body weight/day). The 90th percentile all-user intake of plant stanols by the total U.S. population from proposed food-uses of plant stanol ester was estimated to be 8.82 g/person/day (186 mg/kg body weight/day). On a body weight basis, exposure to plant stanol ester was highest in children aged 0 to 2 years with mean and 90th percentile exposures of 283 and 521 mg/kg body weight. However, foods containing plant stanol ester will be exclusively marketed to consumers seeking foods for the dietary management of cholesterol; these individuals are typically >45 years of age (EFSA, 2008), and therefore the presence of children under the age of 2 residing in these households is expected to be uncommon. The premium pricing of plant stanol ester containing foods above the costs of comparator foods not containing plant stanol ester also will serve as a deterrent to significant consumption by non-target consumers such as infants. Finally, the results of post-market surveillance studies have shown that consumption of plant sterol containing foods among children was low, with reported occasional consumption less than 8% and regular use less than 1% for children below the age of 5 (EFSA, 2008). The Expert Panel therefore concluded that the intake modeling of plant stanol ester exposure in children aged 0 to 2 would not be representative of actual intakes of plant stanol ester containing foods by these individuals.

Raisio noted that the estimated 90th percentile intakes of plant stanols from the proposed food uses of the company's plant stanol ester are less than the estimated 90th percentile exposures to plant sterols from current GRAS Notified uses of plant sterols in the U.S. food supply, which has been estimated to be 10 g per person per day amongst all-users (GRN 061 – 2001). Since foods to which Raisio's plant stanol ester are added encompass the existing permitted food uses of plant sterols, the proposed food uses for plant stanol ester will serve as alternatives to existing plant sterol containing foods currently available on the U.S. marketplace, and therefore will not add to total dietary intakes of plant stanols and plant sterols.

The Expert Panel recognized that the current intake modeling practices would result in the overestimation of actual exposures in heavy (90th percentile) users of plant stanol containing products by several fold. This conclusion was supported by 2 observations: (i) The intake modeling was highly conservative, given that all foods were assumed to contain plant stanol ester at the maximum use-level; and (ii) Actual intakes of plant stanols and plant sterols, as reported in published post-market surveillance and market-based intake modeling studies of supplemented food products introduced in the European Union, are lower than predicted intake levels (SCF, 2002; Wolfs *et al.*, 2006; de Jong *et al.*, 2008a; EFSA, 2008; Hearty *et al.*, 2008). These studies confirm that the incorporation of plant stanols/plant sterols in multiple food and beverage products does not result in "over-consumption" of these ingredients.

Finally, the Expert Panel also recognized that the 90th percentile estimates are not representative of a pattern of habitual intake of foods that may contain plant stanols. The 90th percentile estimates are a reflection of potential acute exposures to plant stanols by a group of heterogeneous individuals on a given day during the survey; habitual consumption patterns by these individuals will typically revert to the mean over time.

Raisio's plant stanol ester is esterified with fatty acids and used in ester form to improve solubility in fat, and to allow them to be properly distributed in the fat phase in the gut prior to digestion (Gylling and Miettinen, 2005). Once in the small intestine, plant stanol ester is hydrolyzed to free plant stanols and fatty acids, and the fatty acid moiety is absorbed and metabolized as is any dietary fatty acid. Studies in animals and humans show that only minimal quantities of dietary plant stanols are absorbed (Ostlund *et al.,* 2002; Gylling and Miettinen, 2005). The small amount of absorbed plant stanols is not further metabolized, and the administered dose can be accounted for quantitatively as unchanged sitostanol and campestanol excreted in the feces. The Expert Panel therefore concluded that systemic exposure to plant stanols in the diet from consumption of plant stanol ester containing foods, even at high dietary levels, would not result in significant systemic exposure to the material.

Data from published studies evaluating the subchronic toxicity, and reproductive and developmental toxicity using male and female Wistar rats have demonstrated that plant stanol ester and plant stanols exerted no toxic effects at the doses administered (Robinson *et al.*, 1998; Slesinski *et al.*, 1999; Turnbull *et al.*, 1999a; Whittaker *et al.*, 1999). Published *in vitro* and *in vivo* observations have further reported that plant stanol ester is not estrogenic (Mellanen *et al.*, 1996; Turnbull *et al.*, 1999a,b). Plant stanol ester also has been demonstrated to be non-mutagenic and non-genotoxic (Turnbull *et al.*, 1999c). These studies were reviewed by Raisio during the company's GRAS determination of the uses of plant stanol ester in spreads, dressings and snack bars, and the Expert Panel also considers these studies to be relevant to the GRAS determination of the expanded food uses of its plant stanol ester. Raisio has noted that the only treatment related effect that has been reported in rodents administered plant stanol ester in the diet is a significant reduction in the plasma and liver concentrations of the fat-soluble

vitamins D, E, and K (vitamin K was not measured in the liver) and carotenoids (β-carotene), and a slight decrease in F_2 Wistar rat pup weights due to nutritional imbalances attributed to caloric dilution at high dietary plant stanol ester intakes. Based on the reduction in fat-soluble vitamins reported in the subchronic toxicity study by Turnbull and colleagues, a no-observed-adverse-effect level (NOAEL) of 1.7% plant stanol ester (1% plant stanols) in the diet was determined by the authors (Turnbull *et al.*, 1999a). However, since the original GRAS determination in 1999, a large body of clinical data, which includes information on the effect of plant stanol ester on vitamin availability, has been published in the peer-reviewed literature. These studies demonstrate that consumption of plant stanol ester by human volunteers at doses that are comparable to, or exceed those estimated under the expanded food uses described herein, do not affect fat-soluble vitamin absorption. The effects of plant stanol ester on fat soluble vitamin absorption reported by Turnbull *et al.* (1999a) have been reviewed by multiple regulatory Agencies, and it is now generally recognized that the adverse changes in fat-soluble vitamin absorption reported by the authors are not relevant to humans (JECFA, 2009; U.S. FDA, 2012a). Raisio therefore considers that an appropriate NOAEL for plant stanol ester, as evaluated by Turnbull *et al.* (1999a), would correspond to 8.5% plant stanol ester (5% stanols) in the diet. This exposure corresponds to a daily intake of 4,600 mg of plant stanol ester (2,700 mg plant stanols) per kg body weight, or 322 g of plant stanol ester (189 g plant stanols) for a 70 kg adult human. The Expert Panel considered this "revised" NOAEL to be appropriate.

Since the original GRAS self-affirmation in 1999, a limited number of rodent studies evaluating endpoints relevant to the safety of the proposed food uses of plant stanol ester were identified within the publicly available literature. Raisio presented the results from a series of rodent studies investigating the effects of vegetable oil, plant stanol and plant sterol consumption using Stroke-Prone Spontaneously Hypertensive rats (SHRSP), and Wistar-Koyoto inbred rats (Ratnayake *et al.*, 2000a, 2003; Naito *et al.*, 2003; Chen *et al.*, 2009, 2010). These rat strains contain multiple genetic mutations that result in a genetic predisposition to developing cerebral stroke; the SHRSP rat also is severely hypertensive, whereas WSK rats are normo-tensive. These rodents have been shown to contain multiple genetic defects including a mutation in the adenosine triphosphate (ATP) transporter binding cassette (ABC) G5 transporter (*Abcg5*), resulting in an increased capacity for absorption of sterols. Although a reduction in the lifespan of animals consuming plant stanols and plant sterols has been reported by multiple investigators, the Expert Panel considers these animal models to be of questionable relevance to humans. For example, the spontaneously hypertensive rat strains used in these studies also display reduced life-spans following dietary administration of olive oil, a "heart healthy" vegetable oil that has a long history of safe consumption by humans (Ratnayake *et al.*, 2000b). Raisio noted that the absorption and kinetics of plant stanols and plant sterols in these rodents are not representative of the situation in humans. For example, these rodent strains display abnormal elevations in intestinal plant sterol/plant stanol absorption rate and decreased biliary excretion, resulting in the excessive accumulation of dietary plant stanols/plant sterols in the

body. Based on these significant species differences in the absorption and kinetics of plant stanols/plant sterols information provided from studies evaluation the dietary administration of plant stanols to SHRSP or Wistar-Koyoto inbred rats is not relevant to humans. The Expert Panel also noted that the test articles administered in these studies were provided at dietary concentrations in excess of 10%, which far exceed the internationally accepted 5% limit that is recommended for toxicity testing of chemicals; these dietary formulations would therefore be expected to produce nutritional imbalances in the animals. Finally, these studies have been reviewed by multiple regulatory bodies, including Health Canada, FDA, and Food Standards Australia New Zealand (FSANZ), without cause for concern. The Expert Panel therefore considered the authors' findings to be of questionable relevance to the safety assessment.

Preliminary observations by Marttinen *et al.* (2008) who evaluated the effects of plant stanol ester in the adenomatous polyposis coli, multiple intestinal neoplasia (APC Min/+) mouse, a putative model of human familial adenomatous polyposis (FAP), were reviewed by Raisio. A total of 28 Apc Min/+ male and female mice were fed either a control diet (n=14) or a diet containing 0.8% (w/w) plant stanols (n=14). Both diets were semi-synthetic, high-fat diets, and the plant stanol ester diet was formulated using freeze-dried plant stanol ester enriched food products (cheese spread, pasta, ripened cheese, and sour milk) found on the market in Finland in 2003. The control diet was formulated with similar commercially available products. The authors reported that compared to the feeding of the control diet, the feeding of the plant stanol ester diet (containing 0.8% plant stanols, w/w) resulted in a statistically significant 60% increase in the number of small intestinal adenomas (P=0.002). No difference in the size of intestinal adenomas was observed. Several deficiencies in the study methodology were identified by Raisio, a primary limitation being confounding compositional differences between the plant stanol ester and control diets.

The APC Min/+ mouse is intended for use in the investigation of the molecular biology of intestinal tumorigenesis, and the Expert Panel did not consider the APC Min/+ mouse model to represent an appropriate tool for use in predictive modeling of the effects of dietary substances or environmental influences on intestinal or colonic tumorigenesis in humans; these limitations were highlighted in the 2004 National Cancer Institute (NCI) Mouse Models of Human Cancers Consortium (MMHCC) Pre-cancers Workshop, where the panel[2] concluded that there were "hazards" in using the Apc Min/+ mouse model in drawing associations with cancer, given the biological discordance of the model with human colon cancer (Cardiff *et al.*, 2006). For example, in patients with FAP, aberrant crypt foci (ACF) and adenomatous polyps appear before there is any evidence of carcinoma; however, in Apc Min/+ mice, ACF are rare. Thus, the established biological course of ACF to carcinoma progression is not established in this model.

[2] Dr. Samuel M. Cohen, M.D., Ph.D. was a panel member of the 2004 National Cancer Institute (NCI) Mouse Models of Human Cancers Consortium (MMHCC) Precancers Workshop

The Expert Panel also reviewed published studies evaluating the effects of dietary substances on intestinal adenoma formation in Apc Min/+ mice, which further illustrate that this mouse strain is not appropriate for use in predictive modeling. For example, it is widely accepted that the consumption of diets rich in fruits and vegetables may have protective effects against cancer; however, the dietary administration of chow diets supplemented with freeze-dried fruit and vegetable preparations has been reported to increase the average number of intestinal adenomas in the APC Min/+ mouse model (van Kranen *et al.*, 1998). Finally, the Panel also has noted that all agents producing an effect on colon cancer will show an early effect on proliferative lesions in the colon before there is development of tumors, and that plant stanols have not displayed proliferative effects in other investigations. For example, the dietary administration of plant stanols (1% w/w) to Syrian Golden hamsters (n=10/group) for 5 weeks did not affect colonic musosal cell proliferation as measured by immunohistochemistry using monoclonal antibodies against the cell proliferation marker Ki-67 (Jia *et al.,* 2006), and proliferative effects have not been observed following incubation of human epithelial colorectal adenocarcinoma cells (CaCO-2) with β-sitostanol at concentrations up to 400 μM (Daly *et al.*, 2009).To date, the findings by Marttinen *et al.* (2008) have not been peer reviewed or published. The authors findings have not been repeated to date, and are inconsistent with earlier unpublished studies conducted by the same group, where the dietary administration of plant stanols had no effect on incidence of intestinal adenoma formation in this same mouse model; these findings further corroborate the conclusion that the increase in intestinal adenoma in rodents consuming plant stanol fortified diets reported by Marttinen *et al.* (2008) are a spurious finding that is consistent with the well established variability of the model. The Expert Panel therefore concluded that the data reported by Marttinen *et al.* (2008) were not relevant to the safety assessment of Raisio's plant stanol ester.

An extensive body of studies evaluating the consumption of plant stanol ester by human subjects has been reported in the literature. These studies were reviewed by Raisio, and were described in detail in the supporting GRAS dossier. Raisio considers the results from these studies to be consistent with findings in rodents, and the long-history of safe consumption of plant stanol ester, that the daily consumption of plant stanol ester is not associated with adverse effects. In human studies representing approximately 3,800 subjects who have consumed plant stanol or plant stanol ester containing products for periods of several weeks to 85 weeks (approximately 1.5 years), no serious adverse events related to plant stanol/plant stanol ester consumption were reported; these observations are consistent with available post-market surveillance studies obtained following the introduction of plant stanol ester containing spreads to Finland, and the current history of safe consumption of plant stanol ester containing foods in the U.S., and world-wide. In these studies, plant stanol ester was administered to healthy individuals, pregnant and lactating women, and children as well as individuals with mild to moderate hypercholesterolemia, including non-medicated individuals and those taking lipid-lowering medications, such as statins. The daily consumption of plant stanol ester containing foods providing up to 9 g of plant stanols per day for 10 weeks or 2 to 3 g of plant stanols per

day for as long as 85 weeks (approximately 1.5 years) did not result in adverse events or adverse changes in serum chemistry, hematology, and urinalysis measurements. Data from these and previous studies in healthy humans, and target market populations (hypercholesterolemic subjects) support the safety of the current and proposed expanded uses of plant stanol ester in multiple food and beverage categories, as described in Table A-1 in Attachment A. Specifically, Raisio considered the data presented by Gylling *et al.* (2010a,b) and Mensink *et al.* (2010) to be relevant to the safety assessment of the expanded food uses of the company's plant stanol ester. These studies were conducted using daily intakes of plant stanols that are several-fold in excess of the expected daily intake of plant stanols among regular consumers of foods products to which plant stanol ester may be added under the food uses described in Table A-1 in Attachment A.

Gylling *et al.* (2010a,b) investigated the safety and cholesterol lowering efficacy of high doses of plant stanol ester in a randomized, double-blind, placebo-controlled study of 49 subjects over a period of 10 weeks. The estimated daily intake of plant stanols in the treatment group was 9 g, administered as plant stanol ester. The authors reported that body weight, body mass index (BMI), and blood pressure did not change over the course of the study or differ between groups. Hematological parameters and indices of liver function also did not differ between treatment groups. Serum total and low-density lipoprotein- (LDL-) cholesterol levels in the treatment group were significantly reduced by 12.0 and 17.1% from the control group ($p<0.001$), respectively, demonstrating compliance within the treatment group. Plant stanol ester consumption did not alter serum levels of high-density lipoprotein- (HDL-) cholesterol, triacylglycerides, or vitamins A and D and α- and β-tocopherols, after adjustment for total cholesterol. Serum levels of α-carotene and β-carotene were reduced from baseline by approximately 40% in subjects consuming plant stanol ester ($p<0.001$), with differences remaining significant even after controlling for total cholesterol levels.

Mensink *et al.* (2010) performed a randomized, double-blind, placebo-controlled dose-response study of plant stanol ester in 93 healthy individuals with baseline serum total cholesterol between 5 and 8 mmol/L and no history of cardiovascular diseases or other severe medical conditions. Subjects participating in the trial consumed 0, 3, 6, or 9 g of plant stanols/day, delivered as plant stanol ester, for a duration of 4 weeks. Clinical biochemistry indicators of kidney and liver function were not altered by consumption of plant stanol ester, and no changes in C-reactive protein, a marker of inflammation, were reported. LDL-cholesterol was dose-dependently reduced by plant stanols. Plant stanol ester consumption had no effects on HDL-cholesterol or triacylglyceride levels, and no substantial changes in nutrient status of antioxidants (β-carotene, lutein) and fat-soluble vitamins (α-tocopherol) were reported.

The use of statins for control of hypercholesterolemia is common in the United States, with 24 million individuals reporting the use of these drugs in 2003 through 2004 (Mann *et al.*, 2008). The Expert Panel recognized that use of statins by regular consumers of plant stanol ester

containing foods is therefore expected to be common. A number of placebo controlled randomized studies evaluating the use of plant stanol containing foods by statin users were reviewed by The Expert Panel [Blair *et al.*, 2000; O'Neill *et al.*, 2004; Cater *et al.*, 2005 (study 2 and study 3); Cabezas *et al.*, 2006; Goldberg *et al.*, 2006; de Jong *et al.*, 2006, 2008b,c; Berendschot *et al.*, 2009; Plat *et al.*, 2009; Kelly *et al.*, 2011]. These studies included an investigation of the long-term (85 weeks) consumption of plant stanol ester containing foods by statin users, and several studies also obtained safety related measures (clinical chemistry and hematology evaluations) during the investigations (de Jong *et al.*, 2006, 2008b; Plat *et al.*, 2009). Overall, the consumption of plant stanol containing food by statin users is associated with additive beneficial effects on cholesterol status among statin users. The Expert Panel did not identify effects, showing or suggesting, that consumption of plant stanol ester by individuals under prophylactic treatment with statins was of concern.

Raisio has noted that FSANZ requires foods containing plant stanols and plant sterols to include the following warning statement "this product may not be suitable for children under the age of five years and pregnant or lactating women" (FSANZ, 2011a). Similar labeling restrictions have been imposed by the European Commission whereby foods to which plant stanols, plant sterols and their ester have been added must contain the following statements pertaining to use by pregnant and lactating women and children: "*there shall be an easily visible and legible statement that the product may not be nutritionally appropriate for pregnant and breastfeeding women and children under the age of five years*" (EC, 2011).

The safety of plant stanol ester during pregnancy and lactation was evaluated in randomized controlled study in 21 women less than 17 weeks gestation (Laitinen *et al.*, 2009). Subjects were randomized into either the control group (n=10), or the intervention group (n=11) where participants were provided with cheese and margarine products enriched with plant stanol ester providing an average of 1.1 g of plant stanols per day during the third trimester and 1.4 g of plant stanols was consumed per day at one month post-partum. All infants were born full term and had similar gestation weights in both the control and intervention groups. Infants in both treatment groups were breast-fed for similar durations (11 to 16 weeks) and there were no differences in infant growth (in weight and height) from birth to 12 months of age or in cognitive development assessed at 12 months of age by the Griffiths scale. Breast milk collected at one month post-partum contained similar levels of total cholesterol, sterols reflecting cholesterol synthesis, sterols reflecting cholesterol absorption or sitostanols for the intervention and control groups. Accordingly, the serum levels of total cholesterol and sterols in infants at either 1 or 6 months of age did not differ by treatment group. Absolute levels of serum β-carotene in the infants also did not significantly differ by treatment group. However, cholesterol adjusted serum β-carotene concentrations were significantly lower in the infants in the plant stanol intervention group at 1 month of age (29.1 *vs.* 59.3 nmol/mmol cholesterol, $p<0.01$). No such differences were found in the infants at 6 months of age, and the authors concluded that plant stanol ester consumption appeared safe, although additional studies are needed to assess the clinical

significance of transiently reduced levels of serum β-carotene among infants. As discussed previously, post-market evaluations show that frequent consumption of plant stanol ester products by healthy pregnant women and/or children will be uncommon.

The Expert Panel also notes that the effects of daily consumption of plant stanol ester containing foods by children between ages of 2 and 12 have been reported (Williams *et al.*, 1999; Ketomaki *et al.*, 2003; Jakulj *et al.*, 2006). These studies evaluated the daily administration plant stanol ester containing foods, providing between 2 to 3 g plant stanols, to healthy male and female children, and children with hypercholesterolemia, for durations of between 4 to 5 weeks. No adverse events or clinical observations were reported to suggest that the consumption of plant stanol ester by this population group should be avoided. Beneficial effects of plant stanol ester on serum cholesterol status were observed in both normo, and hypercholesterolemic children. There are currently 12.5 million (~17%) children categorized as obese in United States, and the prevalence of children with elevated total serum cholesterol levels >200 mg/dL has been reported to be as high as 18.7% among black American children (Hickman *et al.*, 1998; Ogden *et al.*, 2010). Recent policy statements on cholesterol in childhood issued by the American Academy of Pediatrics (AAP) Committee on Nutrition, have reported that overweight children represent a special risk category, and that these individuals are in need of cholesterol screening regardless of family history or their risk factors; the use of plant stanol ester containing foods as a non-pharmacologic approach for the dietary management of childhood hypercholesterolemia was acknowledged by the AAP Committee (Daniels and Greer, 2008).

The Expert Panel therefore concluded that cautionary statements restricting use of plant stanol ester containing foods by pregnant and lactating women, or children, are unjustified. The FDA similarly concluded that cautionary statements restricting the use of plant stanol ester by pregnant and lactating women and children was not supported by the current scientific data (U.S. FDA, 2010).

Finally, as discussed previously, the daily consumption of plant stanol ester by Wistar rats produced significant reductions in the absorption of fat soluble vitamins and carotenoids. A large number of randomized placebo controlled studies have evaluated the effects of plant stanols on the absorption of fat soluble vitamins and carotenoids, and these studies have been extensively evaluated by various authoritative bodies including the FDA, Health Canada, FSANZ, European Food Safety Agency (EFSA) and the Joint FAO/WHO Expert Committee on Food Additives (JECFA). In some instances, the outcomes of these assessments have resulted in the requirements for cautionary statements to be included on the labeling of foods containing plant stanols/plant sterols. For example, the European Commission, Scientific Committee on Food (SCF) recommended that:

"... *the beta-carotene lowering effect of phytosterol enriched foods be communicated to the consumer, together with appropriate dietary advice regarding the regular consumption of fruits and vegetables.*" (SCF, 2000, 2003)

In Australia and New Zealand, FSANZ requires:

"*that phytosterol-enriched foods have a label statement advising that the product should be consumed in moderation as part of a diet low in saturated fat and high in fruits and vegetables...*" (FSANZ, 2011b).

The most current comprehensive assessment of the effects of plant sterols and plant stanols on fat soluble and vitamin and carotenoid absorption was conducted by the FDA, and was discussed within the Agency's interim proposed rule on food labeling pertaining to health claims on phytosterols[3] and risk of coronary heart disease (U.S. FDA, 2010). Within the proposed rule, the Agency acknowledged that the effects of plant stanols/plant sterols on fat soluble vitamin status has been a concern to regulatory bodies in some countries; however, following the Agency's review of 19 intervention studies evaluating the effect of plant stanol/plant sterol intake on fat soluble and carotenoid status including studies administering quantities of plant sterols/plant stanols ranging from 0.8 to 9 g per day, "*the FDA has determined that available scientific evidence does not demonstrate that consuming phytosterols has an effect on intestinal absorption of fat soluble vitamins.*" Although the FDA did recognize that plant stanols/plant sterols may reduce beta-carotene absorption and potentially other carotenoids such as lutein and lycopene, "*these food components likewise do not have an established health benefit at a particular level. Thus, FDA has no basis for concluding that any reduction in the intestinal absorption of these nutrients caused by consuming phytosterols amounts to an adverse health consequence.*"

Since the previous GRAS determination of Raisio's plant stanol ester 15 clinical studies evaluating the effects of plant stanols on fat soluble vitamin and carotenoid absorption have been published. These studies, in conjunction with previous evaluations by various authoritative bodies were considered by the Expert Panel. Based on the available evidence the Expert Panel considered the aforementioned conclusions by the FDA to be appropriate, and similarly concluded that regular consumption of plant stanol ester under the proposed food uses of plant stanol ester by frequent and/or heavy consumers would not place these individuals at risk for nutritional insufficiencies of fat soluble vitamins. The Panel did, however, point out that the body of clinical data characterizing the effects of plant stanols or plant sterols on vitamin K absorption was sparse and available studies employed methodological designs that limit their use for the evaluation of vitamin K absorption. One study was identified in which a group of (112) healthy male and female non-hypercholesterolemic subjects consumed either a placebo margarine or margarine supplemented with 6.8 g of plant stanol ester (4 g plant stanol) daily for 8 weeks (Plat

[3] FDA defines the term 'phytosterols' as a "collective term for plant sterols and their hydrogenated stanol forms, whether used in the free form or esterified with fatty acids."

and Mensink, 2000). The effects of the dietary interventions on vitamin K dependent hemostatic factors were evaluated at the run-in period and upon completion of treatment. The authors reported that neither coagulation nor fibrinolytic parameters were affected by plant stanol ester consumption. Users of vitamin K antagonists (*e.g.*, warfarin) represent target consumers of plant stanol ester containing products, and slight reductions in vitamin K status in these individuals, insignificant in healthy subjects, may be clinically relevant to this patient population. However, Nguyen and Dale (1999) reported that the daily consumption of plant stanol ester (4.5 g/day) by a subgroup of 9 warfarin users for an average duration of 7.7 weeks did not result in statistically significant changes in medication dosing or anticoagulation status. With respect to the effects of plant stanols on carotenoids, the Expert Panel recognized that the nutritional value of dietary antioxidants, and the association between carotenoids and ocular function represent areas of active and evolving scientific research; however, the Expert Panel agreed with the FDA, that at the current time, outside of the role of the pro-vitamin A carotenoids as a source of vitamin A, there is no established nutritional benefit to consuming carotenoids, and no requirement that any part of the dietary reference intakes for vitamin A come from the pro-vitamin A carotenoids.

Conclusion

We, the members of the Expert Panel, have independently and collectively critically evaluated the information summarized above and conclude that Raisio's plant stanol ester, meeting appropriate food-grade specifications and manufactured in accordance with current Good Manufacturing Practice, is Generally Recognized as Safe, based on scientific procedures, for use within the proposed food categories described within Table A-1 in Attachment A.

It is our opinion that other qualified experts would concur with these conclusions.

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REFERENCES

Berendschot TT, Plat J, de Jong A, Mensink RP (2009). Long-term plant stanol and sterol ester-enriched functional food consumption, serum lutein/zeaxanthin concentration and macular pigment optical density. Br J Nutr 101(11):1607-1610.

Blair SN, Capuzzi DM, Gottlieb SO, Nguyen T, Morgan JM, Cater NB (2000). Incremental reduction of serum total cholesterol and low-density lipoprotein cholesterol with the addition of plant stanol ester-containing spread to statin therapy. Am J Cardiol 86(1):46-52.

Cabezas MC, De Vries JH, Van Oostrom AJ, Iestra J, Van Staveren WA (2006). Effects of a stanol-enriched diet on plasma cholesterol and triglycerides in patients treated with statins. J Am Diet Assoc 106(10):1564-1569.

Cardiff RD, Anver MR, Boivin GP, Bosenberg MW, Maronpot RR, Molinolo AA et al. (2006). Precancer in mice: animal models used to understand, prevent, and treat human precancers. Toxicol Pathol 34(6):699-707.

Cater NB, Garcia-Garcia AB, Vega GL, Grundy SM (2005). Responsiveness of plasma lipids and lipoproteins to plant stanol esters. Am J Cardiol 96(1A):23D-28D.

CDC (2006). *Analytical and Reporting Guidelines: The National Health and Nutrition Examination Survey (NHANES)*. Hyattsville (MD): Centers for Disease Control and Prevention (CDC), National Center for Health Statistics (NCHS). Available at: http://www.cdc.gov/nchs/data/nhanes/nhanes_03_04/nhanes_analytic_guidelines_dec_2005.pdf.

CDC (2009). *National Health and Nutrition Examination Survey (NHANES): 2005-2006*. Hyattsville (MD): Centers for Disease Control and Prevention (CDC), National Center for Health Statistics (NCHS). Available at: http://www.cdc.gov/nchs/nhanes/nhanes2005-2006/nhanes05_06.htm.

Chen Q, Gruber H, Swist E, Pakenham C, Ratnayake WM, Scoggan KA (2009). Influence of dietary phytosterols and phytostanols on diastolic blood pressure and the expression of blood pressure regulatory genes in SHRSP and WKY inbred rats. Br J Nutr 102(1):93-101.

Chen Q, Gruber H, Swist E, Coville K, Pakenham C, Ratnayake WMN et al. (2010). Dietary phytosterols and phytostanols decrease cholesterol levels but increase blood pressure in WKY inbred rats in the absence of salt-loading. Nutr Metab 7:11. doi:10.1186/1743-7075-7-11.

Daly TJ, Aherne SA, O'Connor TP, O'Brien NM (2009). Lack of genoprotective effect of phytosterols and conjugated linoleic acids on Caco-2 cells. Food Chem Toxicol 47(8):1791-1796.

Daniels SR, Greer FR (2008). Lipid screening and cardiovascular health in childhood (Committee on Nutrition). Pediatrics 122(1):198-208.

de Jong A, Plat J, Mensink RP (2006). Plant sterol or stanol consumption does not affect erythrocyte osmotic fragility in patients on statin treatment. Eur J Clin Nutr 60(8):985-990.

de Jong N, Ros MM, Ocke MC, Verhagen H (2008a). A general postlaunch monitoring framework for functional foods tested with the phytosterol/-stanol case. Trends Food Sci Technol 19(10):535-545.

de Jong A, Plat J, Bast A, Godschalk RW, Basu S, Mensink RP (2008b). Effects of plant sterol and stanol ester consumption on lipid metabolism, antioxidant status and markers of oxidative stress, endothelial function and low-grade inflammation in patients on current statin treatment. Eur J Clin Nutr 62(2):263-273.

de Jong A, Plat J, Lütjohann D, Mensink RP (2008c). Effects of long-term plant sterol or stanol ester consumption on lipid and lipoprotein metabolism in subjects on statin treatment. Br J Nutr 100(5):937-941.

EC (2011). Regulation (EU) No 1169/2011 of the European Parliament and of the Council of 25 October 2011 on the provision of food information to consumers, amending Regulations (EC) No 1924/2006 and (EC) No 1925/2006 of the European Parliament and of the Council, and repealing Commission Directive 87/250/EEC, Council Directive 90/496/EEC, Commission Directive 1999/10/EC, Directive 2000/13/EC of the European Parliament and of the Council, Commission Directives 2002/67/EC and 2008/5/EC and Commission Regulation (EC) No 608/2004. Off J Eur Union 47(L304):18-63. Available at: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2011:304:0018:0063:EN:PDF.

EFSA (2008). A Report from the Data Collection and Exposure Unit in Response to a Request from the European Commission. Consumption of Food and Beverages with Added Plant Sterols in the European Union. (EFSA/DATEX 03). EFSA J 133:1-21. Available at: http://www.efsa.europa.eu/en/scdocs/doc/datex_report_ej133_phytosterols_en.pdf.

FSANZ (2011a). Standard 1.2.3: Mandatory Warning and Advisory Statements and Declarations (F2011C00610). In: *Australia New Zealand Food Standards Code.* Canberra, Australia / Wellington, NZ: Foods Standards Australia New Zealand (FSANZ). Available at: http://www.comlaw.gov.au/Details/F2011C00610.

FSANZ (2011b). Chapter 1—General food standards. Part 1.5—Foods requiring pre-market clearance. Standard 1.5.1—Novel foods. In: *Australia New Zealand Food Standards Code*. Canberra, Australia / Wellington, NZ: Foods Standards Australia New Zealand (FSANZ). Available at: http://www.foodstandards.gov.au/foodstandards/foodstandardscode.cfm.

Goldberg AC, Ostlund RE Jr, Bateman JH, Schimmoeller L, McPherson TB, Spilburg CA (2006). Effect of plant stanol tablets on low-density lipoprotein cholesterol lowering in patients on statin drugs. Am J Cardiol 97(3):376-379.

GRN 061 (2001). *GRN No. 061 [Archer Daniels Midland Company, re:* Plant sterols/Plant sterol esters*]*. (GRAS Notice Inventory). U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety and Applied Nutrition (CFSAN), Office of Premarket Approval. Available at: http://www.fda.gov/Food/FoodIngredientsPackaging/GenerallyRecognizedasSafeGRAS/GRASListings/ucm153969.htm [Date of filing: 2001-04-18].

Gylling H, Miettinen TA (2005). The effect of plant stanol- and sterol-enriched foods on lipid metabolism, serum lipids and coronary heart disease. Ann Clin Biochem 42(4):254-263.

Gylling H, Hallikainen M, Nissinen MJ, Simonen P, Miettinen TA (2010a). Very high plant stanol intake and serum plant stanols and non-cholesterol sterols. Eur J Nutr 49(2):111-117.

Gylling H, Hallikainen M, Nissinen MJ, Miettinen TA (2010b). The effect of a very high daily plant stanol ester intake on serum lipids, carotenoids, and fat-soluble vitamins. Clin Nutr 29(1):112-118.

Hearty A, Duffy E, Joyce J, O'Conner C, Gibney MJ (2008). Phytosterol-enriched products on the Irish market:examination of intake and consumption patterns. Public Health Nutr 12(1):51-58.

Hickman TB, Briefel RR, Carroll MD, Rifkind BM, Cleeman JI, Maurer KR et al (1998). Distributions and trends of serum lipid levels among United States children and adolescents ages 4-19 years: data from the Third National Health and Nutrition Examination Survey. Prev Med 27(6):879-890.

Jakulj L, Vissers MN, Rodenburg J, Wiegman A, Trip MD, Kastelein JJ (2006). Plant stanols do not restore endothelial function in pre-pubertal children with familial hypercholesterolemia despite reduction of low-density lipoprotein cholesterol levels. J Pediatr 148(4):495-500.

JECFA (2009). Phytosterols, phytostanols and their esters. In: *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). Geneva, Switz.: World Health Organization (WHO), pp. 117-163, 618. Available at: http://whqlibdoc.who.int/publications/2009/9789241660600_eng.pdf.

Jia X, Ebine N, Wang Y, Awad AB, Jones PJH (2006). Effects of different phytosterol analogs on colonic mucosal cell proliferations in hamsters. J Nutr Biochem 17(6):396-401.

Kelly ER, Plat J, Mensink RP, Berendschot TT (2011). Effects of long term plant sterol and-stanol consumption on the retinal vasculature: a randomized controlled trial in statin users. Atherosclerosis 214(1):225-230.

Ketomaki AM, Gylling H, Antikainen M, Siimes MA, Miettinen TA (2003). Red cell and plasma plant sterols are related during consumption of plant stanol and sterol ester spreads in children with hypercholesterolemia. J Pediatr 142(5):524-531.

Laitinen K, Isolauri E, Kaipiainen L, Gylling H, Miettinen TA (2009). Plant stanol ester spreads as components of a balanced diet for pregnant and breast-feeding women: evaluation of clinical safety. Br J Nutr 101(12):1797-1804.

Mann D, Reynolds K, Smith D, Muntner P (2008). Trends in statin use and low-density lipoprotein cholesterol levels among US adults: impact of the 2001 National Cholesterol Education Program guidelines. Ann Pharmacother 42(9):1208-1215.

Marttinen M, Päivärinta E, Piironen V, Pajari A-M, Mutanen M [2008]. Plant stanols induce the formation of intestinal adenomas and activate ERK1 signaling in the *Apc*Min/+ mouse. [Re-printed In: *6th Science Day at Hanasaari,* August 25, 2011]. Helsinki, Finland: University of Helsinki, Department of Food and Environmental Sciences [poster].

Mellanen P, Petänen T, Lehtimäki J, Mäkelä S, Bylund G, Holmbom B et al. (1996). Wood-derived estrogens: Studies *in vitro* with breast cancer cell lines and *in vivo* in trout. Toxicol Appl Pharmacol 136(2):381-388.

Mensink RP, de Jong A, Lütjohann D, Haenen GRMM, Plat J (2010). Plant stanols dose-dependently decrease LDL-cholesterol concentrations, but not cholesterol-standardized fat-soluble antioxidant concentrations, at intakes up to 9 g/d. Am J Clin Nutr 92(1):24-33.

Naito Y, Nagata T, Takano Y, Nagatsu T, Ohara N (2003). Rapeseed oil ingestion and exacerbation of hypertension-related conditions in stroke prone spontaneously hypertensive rats. Toxicology 187(2&3):205-216.

Nguyen TT, Dale LC (1999). Plant stanol esters and vitamin K. Mayo Clin Proc 74(6):642-643.

O'Neill FH, Brynes A, Mandeno R, Rendell N, Taylor G, Seed M et al. (2004). Comparison of the effects of dietary plant sterol and stanol esters on lipid metabolism. Nutr Metab Cardiovasc Dis 14(3):133-142.

Ogden CL, Carroll MD, Curtin LR, Lamb MM, Flegal KM (2010). Prevalence of high body mass index in US children and adolescents, 2007–2008. JAMA 303(3):242–249.

Ostlund RE Jr, McGill JB, Zeng C-M, Covey DF, Stearns J, Stenson WF et al. (2002). Gastrointestinal absorption and plasma kinetics of soy Δ(5)-phytosterols and phytostanols in humans. Am J Physiol Endocrinol Metab 282(4):E911-E916.

Plat J, Mensink RP (2000). Vegetable oil based versus wood based stanol ester mixtures: Effects on serum lipids and hemostatic factors in non-hypercholesterolemic subjects. Atherosclerosis 148(1):101-112.

Plat J, Brufau G, Dallinga-Thie GM, Dasselaar M, Mensink RP (2009). A plant stanol yogurt drink alone or combined with a low-dose statin lowers serum triacylglycerol and non-HDL cholesterol in metabolic syndrome patients [includes supplemental data]. J Nutr 139(6):1143-1149.

Ratnayake WM, Plouffe L, Hollywood R, L'Abbé MR, Hidiroglou N, Sarwar G et al. (2000a). Influence of sources of dietary oils on the life span of stroke-prone spontaneously hypertensive rats. Lipids 35(4):409-420.

Ratnayake WMN, Abbe MRL, Mueller R, Hayward S, Plouffe L, Hollywood R et al. (2000b). Vegetable oils high in phytosterols make erythrocytes less deformable and shorten the life span of stroke-prone spontaneously hypertensive rats. J Nutr 130(5):1166-1178.

Ratnayake WMN, Plouffe L, L'Abbé MR, Trick K, Mueller R, Hayward S (2003). Comparative health effects of margarines fortified with plant sterols and stanols on a rat model for hemorrhagic stroke. Lipids 38(12):1237-1247.

Robinson M, Wnorowski G, Dreher M (1998). Dietary stanols as anti-hypercholesterterolemic agents: a 90-day sub-chronic feeding trial as a safety assessment in the rat. FASEB J 12:A206 [abstract 1202].

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

SCF (2002). *Opinion of the Scientific Committee on Food on a Report on Post Launch Monitoring of 'Yellow Fat Spreads With Added Phytosterol Esters' (Expressed on 26 September 2002).* (SCF/CS/NF/DOS/21 ADD 2 Final). Brussels, Belgium: Scientific Committee on Food (SCF), European Commission, Health & Consumer Protection Directorate-General, Final. Available at: http://www.ec.europa.eu/food/fs/sc/scf/out144_en.pdf.

SCF (2003). *Opinion of the Scientific Committee on Food on Applications for Approval of a Variety of Plant Sterol-Enriched Foods* (expressed on 5 March 2003). (SCF/CS/NF/DOS/15 ADD 2 Final). Brussels, Belgium: Scientific Committee on Food (SCF), European Commission, Health & Consumer Protection Directorate-General, Final. Available at: http://ec.europa.eu/food/fs/sc/scf/out174_en.pdf.

Slesinski RS, Turnbull D, Frankos VH, Wolterbeek AP, Waalkens-Berendsen DH (1999). Developmental toxicity study of vegetable oil-derived stanol fatty acid esters. Regul Toxicol Pharmacol 29(2, Part 1):227-233.Turnbull D, Frankos VH, van Delft JH, DeVogel H (1999a). Genotoxicity evaluation of wood-derived and vegetable oil-derived stanol esters. Regul Toxicol Pharmacol 29(2, Part 1):205-210.

Turnbull D, Whittaker MH, Frankos VH, Jonker D (1999a). 13-week oral toxicity study with stanol esters in rats. Regul Toxicol Pharmacol 29(2, Part 1):216-226.

Turnbull D, Frankos VH, Leeman WR, Jonker D (1999b). Short-term tests of estrogenic potential of plant stanols and plant stanol esters. Regul Toxicol Pharmacol 29(2, Part 1):211-215.

Turnbull D, Frankos VH, van Delft JH, DeVogel H (1999c). Genotoxicity evaluation of wood-derived and vegetable oil-derived stanol esters. Regul Toxicol Pharmacol 29(2, Part 1):205-210.

U.S. FDA (1999a). *Letter from Alan M. Rulis, Director, Office of Premarket Approval, CFSAN to Vivian A. Chester and Edward B. Nelson of McNeil Consumer Healthcare dated May 17,1999 [Re: Food Master File 000626, Plant Stanol Esters GRAS Submission].* Washington (DC): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety and Applied Nutrition (CFSAN). Available at: http://www.fda.gov/ohrms/dockets/DOCKETS/95s0316/rpt0054_01.pdf (See p. 14-17 of PDF).

U.S. FDA (1999b). *Letter from John C. Young, McNeil Consumer Healthcare, to Alan Rulis, FDA, July 21, 1999.* Cited In: U.S. FDA (2011a). Part 101—Food labeling. Section §101.83—Health claims: plant sterol/stanol esters and risk of coronary heart disease (CHD). In: *U.S. Code of Federal Regulations (CFR). Title 21: Food and Drugs (U.S. Food and Drug Administration)*. Washington (DC): U.S. Food and Drug Administration (U.S. FDA), U.S. Government Printing Office (GPO). Available at: http://www.gpo.gov/fdsys/browse/collectionCfr.action?collectionCode=CFR.

U.S. FDA (1999c). *Letter from John C. Young, McNeil Consumer Healthcare, to Alan Rulis, FDA, October 13, 1999.* Cited In: U.S. FDA (2011a). Part 101—Food labeling. Section §101.83—Health claims: plant sterol/stanol esters and risk of coronary heart disease (CHD). In: *U.S. Code of Federal Regulations (CFR). Title 21: Food and Drugs (U.S. Food and Drug Administration)*. Washington (DC): U.S. Food and Drug Administration (U.S. FDA), U.S. Government Printing Office (GPO). Available at: http://www.gpo.gov/fdsys/browse/collectionCfr.action?collectionCode=CFR.

U.S. FDA (2010). Food labeling; health claim; phytosterols and risk of coronary heart disease; proposed rule [21 CFR Part 101, Docket Nos. FDA-2000-P-0102, FDA-2000-P-0133, and FDA-2006-P-0033; Formerly Docket Nos. 2000P-1275, 2000P-1276, and 2006P-0316, Respectively]. Fed Regist (US) 75(235):76526-76571.

U.S. FDA (2012a). Part 101—Food labeling. Section §101.83—Health claims: plant sterol/stanol esters and risk of coronary heart disease (CHD). In: *U.S. Code of Federal Regulations (CFR). Title 21: Food and Drugs (U.S. Food and Drug Administration)*. Washington (DC): U.S. Food and Drug Administration (U.S. FDA), U.S. Government Printing Office (GPO). Available at: http://www.gpo.gov/fdsys/browse/collectionCfr.action?collectionCode=CFR.

U.S. FDA (2012b). Part 101—Food labeling. Section §101.12—Reference amounts customarily consumed per eating occasion. In: *U.S. Code of Federal Regulations (CFR). Title 21: Food and Drugs (U.S. Food and Drug Administration)*. Washington (DC): U.S. Food and Drug Administration (U.S. FDA), U.S. Government Printing Office (GPO). Available at: http://www.gpo.gov/fdsys/browse/collectionCfr.action?collectionCode=CFR.

USDA (2009). *What We Eat in America: National Health and Nutrition Examination Survey (NHANES): 2003-2004, 2005-2006.* Riverdale (MD): U.S. Department of Agriculture (USDA). Available at: http://www.ars.usda.gov/Services/docs.htm?docid=13793#release.

van Kranen HJ, van Iersel PW, Rijnkels JM, Beems DB, Alink GM, van Kreijl CF (1998). Effects of dietary fat and a vegetable-fruit mixture on the development of intestinal neoplasia in the *Apc Min/+* mouse. Carcinogenesis 19(9):1597-601.

Whittaker MH, Frankos VH, Wolterbeek AP, Waalkens-Berendsen DH (1999). Two-generation reproductive toxicity study of plant stanol esters in rats. Regul Toxicol Pharmacol 29(2, Part 1):196-204.

Williams CL, Bollella M, Strobino BA, Boccia L, Campanaro L (1999). Lipid-lowering effects of a plant stanol ester spread in young children. Eur Heart J Suppl 1(Suppl. S):S96-S103.

Wolfs M, de Jong N, Ocké MC, Verhagen H, Monique Verschuren WM (2006). Effectiveness of customary use of phytosterol/-stanol enriched margarines on blood cholesterol lowering. Food Chem Toxicol 44(10):1682-1688.

ATTACHMENT A

Individual Proposed Food-Uses and Use-Levels for Plant Stanol Ester in the U.S.

Table A-1 Summary of the Individual Proposed Food-Uses and Use-Levels for Plant Stanols (Provided as Plant Stanol Ester) in the United States (2003-2004, 2005-2006 NHANES Data)

Food Category	Proposed Food-Uses	Serving Size (g or mL)[a]	Plant Stanols Use Level[b]	
			mg/Serving[c]	%
Baked Goods and Baking Mixes	Bagels	55 g	500	0.91
	Biscuits	55 g	500	0.91
	Breads and Rolls	50 g	500	1.0
	Low-Fat or Calorie Reduced Cookies and Brownies	30 g (cookies) 40 g (brownies)	1,000 500	3.34 1.25
	Cornbread, Corn Muffins, and Tortillas	55 g	500	0.91
	Crackers	30 g	500	1.67
	English Muffins	55 g	500	0.91
	Muffins	55 g	500	0.91
	Quick Breads	55 g	500	0.91
Beverages and Beverage Bases	Sports, Energy, and Isotonic Drinks (including enhanced waters)	240 mL	500	0.21
	Meal Replacement Beverages (Slimming-Type)	240 mL	1,000	0.42
Breakfast Cereals	Instant and Regular Hot Breakfast Cereals	240 mL	2,000	0.80
	Ready-to-Eat Breakfast Cereals (Adult-oriented)	15 g 30 g 55 g	1,000	6.67 3.33 1.82
Cheeses	Imitation Cheese	30 g	1,000	3.33
	Low-Fat or Calorie Reduced Natural Cheese and Cream Cheese	5 to 110 g	500	0.45 to 10.0
	Processed Cheese and Spreads	30 g	1,000	3.33
Coffee and Tea	Instant Coffee	240 mL	1,000	0.42
	Instant Tea	240 mL	1,000	0.42
Dairy Product Analogs	Coffee Whiteners	2 g (powder) or 15 mL (liquid)	500	25.0 or 3.33
	Non-Dairy Milk and Imitation Cream	240 mL	2,000	0.83
Fats and Oils	Fat-Based Sauces	55 g	500	0.91
	Margarine, and Margarine-Like Spreads	15 mL	1,000	6.67
	Mayonnaise and Mayonnaise-Type Dressings	15 g	1,000	6.67
	Salad Dressings	30 g	1,000	3.34
	Vegetable Oil	15 mL	500	3.33
Grain Products and Pastas	Cereal and Grain-Based Bars	40 g	2,000	5.00
	Meal Replacement Bars	40 g	1,000	2.5
	Pasta and Noodle Products	140 g (prepared)	1,000	0.71

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Table A-1 Summary of the Individual Proposed Food-Uses and Use-Levels for Plant Stanols (Provided as Plant Stanol Ester) in the United States (2003-2004, 2005-2006 NHANES Data)

Food Category	Proposed Food-Uses	Serving Size (g or mL)[a]	Plant Stanols Use Level[b]	
			mg/Serving[c]	%
Gravies and Sauces	Tomato-Based Sauces	125 g	500	0.40
	White Sauces and Milk Gravies	60 mL	500	0.83
Milk	Reduced Fat Milks	240 mL	1,000	0.42
Milk Products	Cream	15 mL	500	3.33
	Flavored Milk and Milk-Type Drinks	240 mL	1,000	0.42
	Sour Cream	30 g	500	1.67
	Yogurt	225 g	2,000	0.89
	Yogurt Drinks[d]	240 g	2,000	0.83
	Yogurt Shot[e]	65 mL - 150 mL	2,000	1.33
Processed Fruits and Fruit Juices	Fruit-Flavored Drinks and Ades	240 mL	1,000	0.42
	Fruit Juices	240 mL	1,000	0.42
	Fruit Smoothies	240 mL	2,000	0.83
	Juice Shot[e]	65 mL - 150 mL	2,000	1.33
	Nectars	240 mL	2,000	0.83
Processed Vegetables and Vegetable Juices	Vegetable Juices	240 mL	2,000	0.83
Soft Candy	Low-Fat or Calorie Reduced Chocolate Bars	40 g	500	1.25
	Soft Candy	5[f] g	500	10.0
Soups and Soup Mixes	Prepared Soups	245 g	1,000	0.41

[a] Serving sizes based on the US FDA Reference amounts customarily consumed per eating occasion (RACCs), April 1, 2012, 21 CFR §101.12 (U.S. FDA, 2012b). When a range of values is reported for a proposed food-use, particular foods within that food-use may differ with respect to their RACC.
[b] Use levels represent maximum/up to amounts.
[c] Plant stanols per serving, resulting from the addition of plant stanol ester to the food.
[d] No food codes were identified for yogurt drinks in the NHANES 2003-2004, 2005-2006 databases; therefore, food codes of dairy-based fruit smoothie drinks were selected as surrogates for yogurt drinks.
[e] No food codes for juice shots and yogurt shots were available in the NHANES 2003-2004, 2005-2006 databases.
[f] No RACC exists for these products; thus, the serving size was based on commercially available chews.

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APPENDIX B

APPENDIX B

THE Apc GENE, FAMILIAL ADENOMATOUS POLYPOSIS, AND THE Apc Min/+ MOUSE MODEL

THE Apc GENE, FAMILIAL ADENOMATOUS POLYPOSIS, AND THE Apc Min/+ MOUSE MODEL

ABBREVIATIONS

ACF	aberrant crypt foci
Apc Min/+	adenomatous polyposis coli, multiple intestinal neoplasia
EGFR	epidermal growth factor receptor
ERK1	extracellular-signal-regulated kinase 1
FAP	Familial adenomatous polyposis
GSK3β	glycogen synthase kinase 3 *beta*
IL	interleukin
K-ras	Kirsten rat sarcoma viral oncogene homolog
LEF	lymphoid enhancer-binding factor
MTT	3,-4,5 dimethylthiazol-2,5 diphenyltetrazolium bromide
p53	protein 53
PEG	Polyethylene glycol
q	long arm
TCF	transcription factor

1.0 Introduction

In humans, the Apc gene encodes for the protein *adenomatous polyposis coli*. The Apc gene is classified as a tumor suppressor gene. The human Apc gene is located on the long arm (q) of chromosome 5; the full-length protein contains 2,843 amino acids with a predicted molecular mass of 311,646 Da. The Apc protein is important in suppressing the cellular overgrowth that leads to the formation of polyps, which can become cancerous. The Apc protein forms a complex with axin and helps glycogen synthase kinase 3 *beta* (GSK3β) to phosphorylate N-terminal serine/threonine residues of β-catenin, accelerating its rapid degradation through ubiquitination (Polakis, 1999).

Familial adenomatous polyposis (FAP) is a hereditary disease that is inherited in an autosomal dominant pattern. FAP is associated with the formation of numerous polyps, mainly in the epithelium of the large intestine (colon). Although these polyps are initially benign, some of the polyps will eventually develop into malignant adenocarcinomas, if left untreated.

FAP is caused by mutations in the Apc gene; over 800 mutations in the Apc gene have been identified in families with FAP. Most of these mutations cause the production of an Apc protein that is abnormally short and nonfunctional, resulting in the accumulation of β-catenin in the cytoplasm and in the nucleas, the subsequent activation of transcription factor / lymphoid enhancer-binding factor (TCF/LEF) transcription factors, and the induction of a set of new genes: Wnt target genes (Korinek *et al.,* 1997). The activation of the Wnt pathway in the colonic epithelium appears to be one of the key events in the polyp initiation process (Oshima *et al.,* 1997).

2.0 The Apc Min/+ Mouse Model

In mice, the Apc gene is located on chromosome 18. Moser *et al.* (1990) was the first to describe a mouse model for intestinal tumorigenesis. Apc Min/+ (multiple intestinal neoplasia) is an ethylnitrosouria-induced mutation in the mouse Apc gene. Apc Min/+ encodes a stop codon at codon 850, resulting in premature truncation of the *adenomatous polyposis coli* polypeptide. Apc Min/+ mice carrying a heterozygous[1] mutation develop approximately 30 small intestinal polyps (both predunculated polyps and flat adenomas) that typically do not progress to invasive adenocarcinomas (Moser *et al.*, 1995; McCart *et al.,* 2008).

2.1 Limitations of the Apc Min/+ Mouse Model

There are several major differences between rodents and humans, including lifespan, body weight, intestinal morphology (*e.g.,* cecum), gut microflora, pattern of eating (*e.g.,* meals, chewing, coprophagia), and gene regulation (Corpet and Pierre, 2005). These differences may

[1] Homozygosity for mutant Apc results in embryonic death (McCart *et al.,* 2008).

impact the outcomes of various dietary interventions in rodents *versus* humans. With regards to the relevance of the Apc Min/+ mouse model to humans, there are several major limitations, including:

- The Apc Min/+ mouse putatively models tumorigenesis in patients with FAP, which represents less than 5% of all the patients with colorectal cancer; thus, the relevance of the Apc Min/+ mouse model to sporadic human colon cancer, even sporadic human colon cancer involving a mutation in the Apc gene, is unknown (Heyer *et al.,* 2010).

- The Apc Min/+ mouse manifests predominantly small intestinal adenomas; in contrast, the vast majority of gastrointestinal tumors in FAP patients are found in the colon and rectum (Hinoi *et al.,* 2007; McCart *et al.,* 2008)[2]. There remains no adequate explanation for this discrepancy (McCart *et al.,* 2008). Small intestinal adenomas and adenocarcinomas are uncommon in humans with FAP, and also are uncommon in the general population.

- In patients with FAP, there is a long period of years during which the colon appears normal; in contrast, in Apc Min/+ mice, small intestinal adenomas arise very soon after birth (Bruce, 2003).

- In patients with FAP, aberrant crypt foci (ACF) and adenomatous polyps appear before there is any evidence of carcinoma; however, in Apc Min/+ mice, ACF are rare (Boivin *et al.,* 2003; Bruce, 2003). Thus, the ACF to carcinoma progression is not established in this model (Pool-Zobel, 2005).

- The polyps that develop in Apc Min/+ mice are predominantly predunculated polyps and flat adenomas in the small intestine; the polyps only occasionally progress to adenocarcinomas[3] (Haigis and Dove, 2003; Sansom *et al.*, 2007). In contrast, human FAP patients have predominantly colonic adenomas, which routinely progress to invasive adenocarcinoma (Moser *et al.*, 1990; Halberg *et al.*, 2000; Caldwell *et al.*, 2007).

- The intestinal polyps that develop in Apc Min/+ mice do not have Kirsten rat sarcoma viral oncogene homolog (K-ras) mutations that are observed in many human tumors (Shoemaker *et al.,* 1997), and the protein 53 (p53) inactivation that is so frequently observed in human cancers was reported not to raise adenoma number in Apc Min/+ mice (Fazeli *et al.,* 1997).

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[2] In humans, the prevalence of small intestine carcinomas is about one-thirteenth that of colon carcinomas (Jemal *et al.,* 2007).

[3] Apc Min/+ mice usually die before 140 days of age due to anemia or intestinal obstruction; thus, few adenomas actually progress to carcinoma in Apc^{Min} mice (Hinoi *et al.,* 2007).

- Humans with identical mutant Apc alleles display diverse phenotypes, including but not limited to colonic polyp burden (Kinzler and Vogelstein, 1996; McCart *et al.,* 2008)[4]. Patients with germline mutations are predisposed to cancer, but will not necessarily be afflicted with the disease (Kinzler and Vogelstein, 1996). In contrast all Apc Min/+ mice will develop small intestinal adenomas.

- Additional mutations are required for the development of colorectal cancer in humans, and environmental factors can affect the rate of genetic mutation (Kinzler and Vogelstein, 1996). Environmental factors, such as diet, may also affect other cellular processes, such as apoptosis, which could limit tumor initiation or progression (Bellamy *et al.,* 1995). Thus, the relevance of the Apc Min/+ mouse model, which is considered to be environmentally and genetically controlled, to free-living, genetically diverse humans with mutations in the Apc gene, is unclear (McCart *et al.,* 2008).

- In the Apc Min/+ mouse model, several genetic modifiers[5] have been identified (called *Mom, or modifiers of* Min), including *Mom1, Mom2, Mom3, and Mom7* (Kinzler and Vogelstein, 1996; McCart *et al.,* 2008). It is unclear how these genetic modifiers relate to those in humans. Furthermore, the reliability and isogenicity of well-characterized strains cannot be assumed and may, indeed, contribute to inconsistent findings in studies involving the Apc Min/+ mouse model (Watkins-Chow and Pavan, 2008).

- There is considerable variability in tumor number and size between Apc Min/+ mice, making it difficult if not impossible to interpret differences between groups in a given study.

As indicated by McCart *et al.* (2008), "*Despite the wealth of information that mouse models of Apc deficiency have provided about the in vivo biology of this protein, there are several critical drawbacks in relating these models to human intestinal neoplasia".*

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[4] For example, some patients with identical truncating mutations develop features of Gardner's syndrome (mandibular osteomas and desmoid tumors) while others do not (Nishisho *et al.,* 1991).
[5] Genetic modifiers are genes that alter disease severity by interacting, directly or indirectly, with the primary genetic mutation causing the disease.

4.0 REFERENCES

Bellamy CO, Malcomson RD, Harrison DJ, Wyllie AH (1995). Cell death in health and disease: the biology and regulation of apoptosis. Semin Cancer Biol 6(1):3-16. Cited In: Kinzler and Vogelstein, 1996.

Boivin GP, Washington K, Yang K, Ward JM, Pretlow TP, Russell R et al. (2003). Pathology of mouse models of intestinal cancer: consensus report and recommendations. Gastroenterology 124(3):762-777. Cited In: McCart *et al.,* 2008 [Ref. #9].

Bruce WR (2003). Counterpoint: From animal models to prevention of colon cancer. Criteria for proceeding from preclinical studies and choice of models for prevention studies. Cancer Epidemiol Biomarkers Prev 12(5):401-404.

Caldwell CM, Green RA, Kaplan KB (2007). APC mutations lead to cytokinetic failures in vitro and tetraploid genotypes in Min mice. J Cell Biol 178(7):1109-1120.Cited In: McCart *et al.,* 2008 [Ref. #10].

Corpet DE, Pierre F (2005). How good are rodent models of carcinogenesis in predicting efficacy in humans? a systematic review and meta-analysis of colon chemoprevention in rats, mice and men. Eur J Cancer 41(13):1911-1922.

Fazeli A, Steen RG, Dickinson SL, Bautista D, Dietrich WF, Bronson RT et al. (1997). Effects of p53 mutations on apoptosis in mouse intestinal and human colonic adenomas. Proc Natl Acad Sci U S A 94(19):10199-10204. Cited In: Pool-Zobel, 2005.

Haigis KM, Dove WF (2003). A Robertsonian translocation suppresses a somatic recombination pathway to loss of heterozygosity. Nat Genet 33(1):33-39. Cited In: McCart *et al.,* 2008 [Ref. #24].

Halberg RB, Katzung DS, Hoff PD, Moser AR, Cole CE, Lubet RA et al. (2000). Tumorigenesis in the multiple intestinal neoplasia mouse: redundancy of negative regulators and specificity of modifiers. Proc Natl Acad Sci U S A 97(7):3461-3466. Cited In: McCart *et al.,* 2008 [Ref. #26].

Heyer J, Kwong LN, Lowe SW, Chin L (2010). Non-germline genetically engineered mouse models for translational cancer research. Nat Rev Cancer 10(7):470-480.

Hinoi T, Akyol A, Theisen BK, Ferguson DO, Greenson JK, Williams BO et al. (2007). Mouse model of colonic adenoma-carcinoma progression based on somatic *Apc* inactivation. Cancer Res 67(20):9721-9730.

Jemal A, Siegel R, Ward E, Murray T, Xu J, Thun MJ. (2007) Cancer statistics, 2007. CA Cancer J Clin 57(1):43-66. Cited In: Hinoi et al., 2007 [Ref. #7].

Kinzler KW, Vogelstein B (1996). Lessons from hereditary colorectal cancer. Cell 87(2):159-170.

Korinek V, Barker N, Morin PJ, van Wichen D, de Weger R, Kinzler KW et al. (1997). Constitutive transcriptional activation by a beta-catenin-Tcf complex in APC-/- colon carcinoma. Science 275(5307):1784-1787. Cited In: Taketo, 2006 [Ref. # 4].

McCart AE, Vickaryous NK, Silver A (2008). *Apc* mice: models, modifiers and mutants. Pathol Res Pract 204(7):479-490.

Moser AR, Pitot HC, Dove WF (1990). A dominant mutation that predisposes to multiple intestinal neoplasia in the mouse. Science 247(4940):322-324. Cited In: Moser et al., 1995 [Ref. #1]; McCart *et al.,* 2008 [Ref. #42].

Moser AR, Luongo C, Gould KA, McNeley MK, Shoemaker AR, Dove WF (1995). Apc^{Min}: a mouse model for intestinal and mammary tumorigenesis. Eur J Cancer 31A(7&8):1061-1064.

Nishisho I, Nakamura Y, Miyoshi Y, Miki Y, Ando H, Horii A et al. (1991). Mutations of chromosome 5q21 genes in FAP and colorectal cancer patients. Science 253(5020):665-669. Cited In: Kinzler and Vogelstein, 1996.

Oshima H, Oshima M, Kobayashi M, Tsutsumi M, Taketo MM (1997). Morphological and molecular processes of polyp formation in Apc(delta716) knockout mice. Cancer Res 57(9):1644-1649. Cited In: Taketo, 2006 [Ref. # 5].

Polakis P (1999). The oncogenic activation of beta-catenin. Curr Opin Genet Dev 9(1):15-21. Cited In: Taketo, 2006 [Ref. # 3].

Pool-Zobel BL (2005). Inulin-type fructans and reduction in colon cancer risk: Review of experimental and human data. Br J Nutr 93(Suppl. 1):S73-S90.

Sansom OJ, Meniel VS, Muncan V, Phesse TJ, Wilkins JA, Reed KR et al. (2007). Myc deletion rescues Apc deficiency in the small intestine. Nature 446(7136):676-669. Cited In: McCart *et al.,* 2008 [Ref. #62].

Shoemaker AR, Luongo C, Moser AR, Marton LJ, Dove WF (1997). Somatic mutational mechanisms involved in intestinal tumor formation in Min mice. Cancer Res 57(10):1999-2006. Cited In: Pool-Zobel, 2005.

Taketo MM (2006). Mouse models of gastrointestinal tumors. Cancer Sci 97(5):355-361.

Watkins-Chow DE, Pavan WJ (2008). Genomic copy number and expression variation within the C57BL/6J inbred mouse strain. Genome Res 18(1):60-66. Cited In: McCart *et al.,* 2008 [Ref. #82, as: Watkins-Chow and Pavan, 2007 – e-pub].

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