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GRAS Notification
for
Pine Tree Phytosterols

Volume 1 of 1

Submitted by:

Arboris LLC
1101 W. Lathrop Avenue, Gate 16
Savannah, GA 31415

April 11, 2008

Cover Letter

pine tree extracts

April 11, 2008

Laura Tarantino, Ph.D., Director
Office of Food Additive Safety, (HFS 200)
Center for Food Safety and Applied Nutrition
5100 Paint Branch Parkway
College Park, MD 20740

VIA: Federal Express

Re: GRAS Notification for Pine Tree Phytosterols

Dear Dr. Tarantino:

Pursuant to the proposed 21 CFR § 170.36 (c) Arboris claims that the use of Arboris pine tree phytosterols are exempt from the premarket approval requirements of the Federal Food, Drug and Cosmetic Act because we have determined by scientific procedures that such use is Generally Recognized as Safe (GRAS) as a food grade phytosterol.

In accordance with proposed regulation, the following information is provided:

Proposed 21 CFR § 170.36 (c)(1)(i) The name and mailing address of the notifier:

Arboris LLC
P. O. Box 2008
Savannah, GA 31402

Proposed 21 CFR § 170.36 (c)(1)(ii) The common or usual name of the notified substance:

Pine Tree Phytosterol
Trade Name: Arboris® Sterol AS-2® and Arboris® Sterol AS-4™

Proposed 21 CFR § 170.36 (c)(iii) The applicable conditions of use of the notified substance:

For the existing uses of phytosterols in food.

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Proposed 21 CFR § 170.36 (c)(1)(iv) The basis for the GRAS determination:

This GRAS determination is based on scientific procedures.

Proposed 21 CFR § 170.36 (c)(1)(v) Availability of information:

A summary of the data and information supporting this GRAS notification is attached. If you have any questions or require additional information, please contact Dr. Clyde A. Takeguchi, Ph.D. at Phoenix Regulatory Associates, Ltd., 21525 Ridgetop Circle, Suite 240, Sterling, VA 20166 by telephone at (703)-406-0906 or by email at phoenix@phoenixrising.com.

Sincerely,

Sergio Maldonado
Technology Manager
Arboris LLC

Attachment: Original and two (2) copies

cc: Phoenix Regulatory Associates, Ltd. letter only

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GRAS
Notification

GRAS Notification for Pine Tree Phytosterols

1. Introduction

Arboris, LLC (Arboris) has evaluated the previously submitted Generally Recognized as Safe (GRAS) notices on phytosterols (GRN 00039, 00061, 00112, 00176, and 00181) and phytosterol esters (GRN 00048, 00053, 00177, and 00206) that are incorporated by reference. FDA is aware of the information submitted in the earlier GRAS notifications and did not have any additional questions on safety. Arboris has reviewed the uses proposed in the submissions, estimates of dietary exposure, methods of manufacture, specifications for the ingredient, and conducted a literature search on recently published studies on phytosterols and phytostanols. Arboris is not adding new uses, but is providing an additional source of phytosterols.

Arboris concludes that its pine tree phytosterols are substantially equivalent to other products currently in the marketplace and that there is general recognition by experts qualified in scientific experience that the use of phytosterols in food from plant and vegetable sources for the existing uses are safe based on scientific procedures. Therefore, the use of phytosterols manufactured by Arboris is safe for use in food.

2. Identity

The term "phytosterols" is used as a collective term for sterols and stanols. The source of these phytosterols can be from pine tree or vegetable oils and determines the relative amounts of individual phytosterols.

2.1. Pine Tree Phytosterols (Arboris® Sterol AS-2®)

Arboris® Sterol AS-2® is a mixture of naturally occurring phytosterols derived from GMO-free pine trees. Minimum purity is 99% total sterols consisting of beta Sitosterol, beta sitostanol, campesterol, stigmasterol, campestanol, and other sterols. (See Table 1.)

The Arboris® Sterol AS-2® meets specifications for polyaromatic hydrocarbon (PAH) compounds, dioxins, heavy metals, pesticides, and other impurities allowed in food-grade fats and oils. (See specification below.) Arboris® Sterol AS-2® was found to be substantially equivalent to the specifications listed in the Teriaka tall oil product and the EU tall oil products. (See Table 1.)

GRAS Notification for Pine Tree Phytosterols (continued)
2. Identity (continued)

2.2. Pine Tree Phytosterols (Arboris® Sterol AS-4™)

Arboris® Sterol AS-4™ is a mixture of naturally occurring phytosterols. Minimum purity is 95% total sterols consisting of beta Sitosterol, beta sitostanol, campesterol, stigmasterol, campestanol, and other sterols. (See Table 1.)

2.3. Food-Grade Specifications

Substance	AS-2 (%)*	AS-4 (%)**
Total Phytosterols	≥ 99	≥95
Cholesterol	≤ 1.0	≤1
Brassicasterol	≤ 2	
Campesterol	≤ 15	≤ 15
Campestanol	≤ 5	≤ 5
Stigmasterol	≤ 2	≤ 2
Sitosterol	70 - 80	≤ 85
Sitostanol	≤ 15	≤ 20
Other Sterols	≤ 3	≤ 5
Moisture	< 1.0	< 1.0
Appearance	Free-flowing prills	
Color	White to off-white	
Taste and Odor	Neutral, bland	

*Arboris® Sterol AS-2® Specifications, March 12, 2008a
**Arboris® Sterol AS-4™ Specifications, March 12, 2008b

Heavy Metals

Heavy metals are routinely assayed from representative batches in Arboris® sterols. The heavy metal content is very low. The level of arsenic and mercury is usually under 0.02 ppm, and under 0.01 ppm for cadmium and lead. (The NFL, 2007)

Pesticide Residues

The amount of pesticide residues is also monitored. The level of chlorinated pesticide residues (20 substances) are typically under the detection limit of 10 ppb. The level of organophosphosporus pesticide residues (18 substances) are also below the detection limit of 20 ppb. (SGS, 2007a)

GRAS Notification for Pine Tree Phytosterols (continued)
2. Identity (continued)

Polycyclic Aromatic Hydrocarbons

Benzo(a)pyrene levels are below 2 ppb, the limits set in the European Community for food-grade fats and oils. (SGS, 2007a; Intertek, 2008)

Dioxin and Dioxin-like PCBs

The values of dioxin from ten different batches are very low and close to the WHO-Toxicity Equivalence (TEQ) limit of reporting of 16-17 ng/kg (SGS 2007b).

Residual Solvents

Solvents like methanol, ethanol, acetone, propanol, are all under the detection limit of 0.1 mg/kg. (SGS, 2007a)

Microbiological Analysis

Microbiological contamination is very unlikely since sterols are a dry product that is not commonly metabolized by microorganisms, and the production process includes high temperatures, organic solvents and high vacuum conditions. The levels of microbiological contamination were clearly under the commonly accepted levels in food products for bacterial and fungal contamination. Both yeast and mold contamination was under 10 cfu/g. (Silliker, 2008)

GRAS Notification for Pine Tree Phytosterols (continued)
2. Identity (continued)

Table 1: Sterol Content

Sterols	Percentage(s)				
	EU Tall Oil[1]	GRN 39[2] Novartis	GRN 112[3] Teriaka	AS-2[4]	AS-4[5]
Cholesterol				≤ 1	≤ 1
Brassicasterol	-		0	≤ 2	≤ 2
Campesterol	8 - 11	9 - 18	6 - 9	≤ 15	≤ 15
Campestanol	1 - 4	2 - 14	0 - 2	≤ 5	≤ 5
Stigmasterol	-			≤ 2	≤ 2
ß-Sitosterol	70 - 80	38 - 60	75 - 80	70 - 80	≤ 85
ß-Sitostanol	10 - 15	14 - 34	10 - 14	≤ 15	≤ 20
Others	< 2		< 3	≤ 3	< 5
Total Free	> 95	> 86	> 95	≥ 99	≥ 95

[1] SCF 2003a
[2] GRN 39 Novartis
[3] GRN 112 Teriaka
[4] Arboris, 2008a
[5] Arboris, 2008b

3. Manufacture

3.1. Phytosterols

Phytosterols are derived from tall oil of pine trees (mainly *Pinus elliottii and Pinus taeda*) and are manufactured by a five step process. (See Figure 1 below.)

- Saponification. The tall oil pitch is saponified with caustic soda to obtain a mixture of free sterols and organic salts.
- Evaporation. The phytosterol concentrated stream is separated from residual organic salts by evaporation stages.
- Neutral distillation. The phytosterols are further concentrated by distillation to obtain a high sterol concentration fraction.

GRAS Notification for Pine Tree Phytosterols (continued)
3. Manufacturing (continued)

- Crystallization and filtration. The crude phytosterols are crystallized from a solvent mixture and the crystals recovered by filtration.

- Drying and prilling. The crystals are dried under vacuum to remove any remaining solvent and prilled to obtain the pure phytosterol product.

GRAS Notification for Pine Tree Phytosterols (continued)
3. Manufacturing (continued)

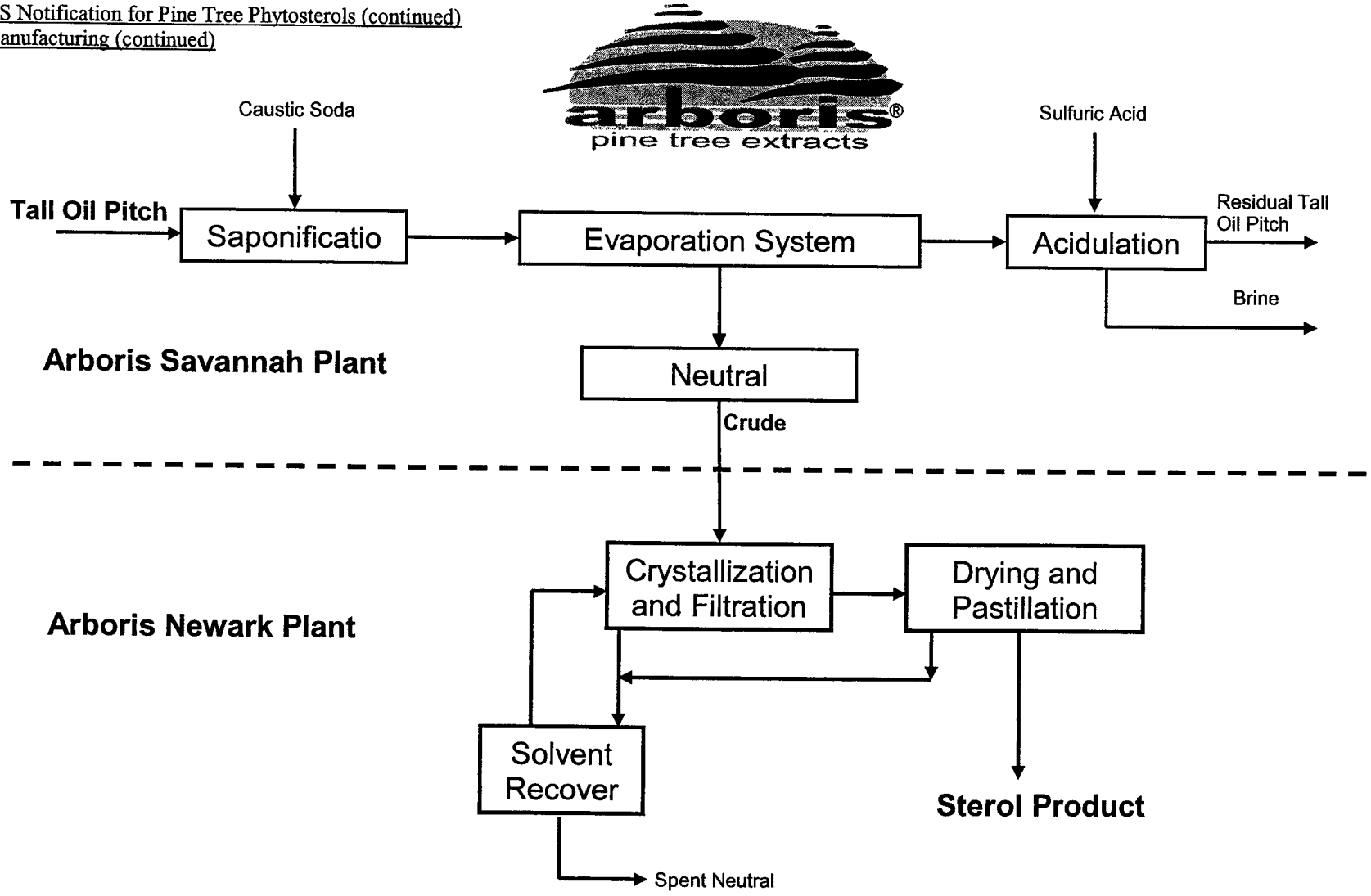

Figure 1: Basic Process Flow Diagram

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4. Conditions of Use

The various GRNs reviewed by FDA included the following uses: Margarine and vegetable-based spreads; yogurt and yogurt-like products; milk-based juice beverages; ice cream and non-standardized ice cream products; cream cheese and cream cheese-like products; snack bars; salad dressing; standardized and non-standardized bread products; baked foods; beverages; dairy analogs; cheese and cream; breakfast cereal; mayonnaise; pasta and noodles; sauces; salty snacks; processed soups; puddings; confections; vegetarian meat analogs; fruit/vegetable juice; vegetable oils; egg products, including egg whites and substitute egg products. (See Tables 2 and 3.)

The estimates of dietary exposure for all proposed uses from the GRN submissions were 5.5 to 7.3 g/p/d at the mean and 10.6 to 12.9 g/p/d at the 90th percentile. (See Tables 2 and 3.)

Arboris believes that its phytosterol products will be used as an alternative source of phytosterols currently used as ingredients in commercial food products.

GRAS Notification for Pine Tree Phytosterols (continued)
4. Conditions of Use (continued)

Table 2: GRAS Notification for Sterols

GRN No.	Novartis 39	ADM 61	ADM 176	Teriaka 112	Michael Fd 181
ID	Tall oil phytosterols	Plant sterols and plant sterol esters	Plant sterols and plant sterol esters	phytosterols	Plant phytosterols
Mfg	Tall oil soap; by product of paper pulping process; organic solvent extraction; crystallized from alcohol	Phytosterols from vegetable oils are esterified with vegetable oil fatty acids.	Phytosterols from vegetable oils and tall oil	Phytosterols, vegetable fat mixture, vegetable oil mixture, or anhydrous milk fat mixture Phytosterols from veg oils or tall oil	Phytosterols from vegetable oils and tall oil (< 5%)
Use	In vegetable oil spread	In vegetable oil spreads; salad dressing; health drinks; health bars; yogurt-type products	In margarines and vegetable oil spreads; salad dressing; beverages; snack bars; dairy analogs; cheese and cream; baked foods; breakfast cereal; mayonnaise; pasta and noodles; sauces; salty snacks; processed soups; puddings; yogurt; confections; vegetarian meat analogs; fruit/vegetable juice; vegetable oils	In margarine and vegetable-based spreads; yogurt and yogurt-like products; milk-based juice beverages; ice cream and non-standardized ice cream products; cream cheese and cream cheese-like products; snack bars; salad dressing; standardized and non-standardized bread products	Egg products, including egg whites and egg substitutes
Level	Up to 12% free phytosterols	EDI 5.5 g/p/day @ mean, 10.6 g @ 90th percentile	EDI 3.9 g/p/day @ 90th percentile	EDI 1.6g/p/day @ mean 3.6 g/p/day 90th percentile	Up to 20 mg plant sterol/g egg product; 1100 mg phytosterol/serving
GRAS Panel	yes	yes	yes	no	yes

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GRAS Notification for Pine Tree Phytosterols (continued)
4. Conditions of Use (continued)

Table 3: GRAS Notification for Sterol Esters

GRN No.	Cargill 48	P&G 53	HeartBlend 177	Enzymotec 206
ID	vegetable oil phytosterol esters	Phytosterol esters	Plant sterol esters	Phytosterol esters and diglycerides
Mfg	Soy sterols are esterified with vegetable oil fatty acids from soy canola, sunflower, or other veg oils. After esterification, adsorbants are used to remove trace components, vacuum stripped, and filtered.	Cargill	ADM	Enzymatic and chemical transesterification of vegetable fats/oils with vegetable phytosterols
Use	In vegetable oil spreads; salad dressing; bars; yogurt	In vegetable oil at a level up to 13.3% for home use, and salad dressings	In ground roasted coffee	In baked goods and baking mixes; fats and oils; frozen dairy desserts and mixes; gelatins, puddings, and fillings; grain products and pastas; gravies and sauces; hard candy; milk; milk products; soft candy; soups and soup mixes; and snack foods at a level of 0.65 g/ esters/RACC
Level	3 g plant sterol or stanols, or 5 g plant sterol or stanol esters/person/ day	Additional consumption of 0.75 g/ person/day to 2.1 g/person/day @ 90th percentile	1 g plant sterol esters/8 fl oz serving 1.4 g/person/day mean, 2.5 g/person/day 90th percentile	7.3 g/p/d @ mean, 12.9 g/p/d @ 90th percentile
GRAS Panel	yes	yes	yes	yes

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5. Safety and Effectiveness

5.1 Regulatory Status

FDA

FDA has not made its own determination on the GRAS status of phytosterols, but has listed its responses to several GRAS notices on phytosterols (GRN 00039, 00061, 00112, 00176, and 00181) and phytosterol esters (GRN 00048, 00053, 00177, and 00206) obtained from vegetables, plants, and pine trees. (See Tables 2 and 3.)

The GRAS panels for earlier notices have concluded that phytosterols meeting food-grade specifications are GRAS by scientific procedures for their intended use.

On September 8, 2000, FDA issued an interim final rule (FDA 2000) allowing the use of health claims on the association between plant sterol/stanol esters and the reduced risk of coronary heart disease. To be eligible for using the health claim, a food product must contain at least 0.65 g of plant sterol esters per reference amount customarily consumed (RACC) in spreads and salad dressing, or at least 1.7 g of plant stanol esters per RACC in spreads, salad dressings, snack bars, and dietary supplements in softgel form.

On February 14, 2003, FDA issued a letter regarding enforcement discretion with respect to expanded use of the health claims to all the forms and sources of phytosterols (FDA 2003). This letter expands the health claims to free forms of phytosterols from several sources and allows a wider range of uses.

The European Union

The Scientific Committee on Food previously determined that the use of phytosterols is safe provided that the sterol-containing foodstuffs are not consumed in amounts resulting in total phytosterol intakes exceeding 3 g/day (SCF, 2002; 2003a, b, and c). In principle, this can be applied also to the phytosterol and phytosterol ester mixtures.

However, the mixtures can only be accepted if the composition complies with the phytosterol/phytostanol profile accepted by the Committee (SCF, 2003b). The Committee reemphasized that appropriate risk management measures should be developed to minimize the likelihood of a daily intake exceeding 3 g phytosterols/phytostanols, in particular from the cumulative intakes of different types of products (SCF, 2002; 2003a, b, and c).

The Committee also stated that the recommendations:

- *that the small number of people with inborn error of phytosterol metabolism (phytosterolaemia) should be made aware of the presence of higher levels of phytosterols in the product,*
- *that patients on cholesterol-lowering medication should only consume the products under medical supervision, and*
- *that the potential β-carotene lowering effect should be communicated to the consumer, together with appropriate dietary advice regarding the regular consumption of fruits and vegetables.*

5.2 Safety and Toxicity of Phytosterols

It is clear from current literature review on phytosterols that no difference exists between vegetable or plant oil derived sterols and tall oil or pine tree oil derived sterols concerning toxicity or safety aspects (Ostlund, 2007; Rozner and Garti, 2006).

Rozner and Garti (2006) summarized the mechanism by which phytosterols interact with cholesterol based on three absorption stages: 1) Physico-chemical effects; 2) Effects on the absorption site; and 3) Effects on intra-cellular trafficking of cholesterol. The first stage deals with the competitive solubilization between cholesterol and phytosterols in dietary mixed micelles. The second deals with intestinal absorption, and the third stage deals with the transport from intestinal lumen to the lymph.

Table 4 shows a comparison of the physiological aspects of cholesterol, phytosterols and phytostanols based on data from several sources cited in Clifton (2002).

GRAS Notification for Pine Tree Phytosterols (continued)
5. Safety and Effectiveness (continued)

Table 4: Comparison of the Physiological Aspects of Cholesterol, Phytosterols and Phytostanols

	Cholesterol	Phytosterols	Phytostanols
Dietary intake	300 – 500 mg/d	200 – 400 mg/d; vegetarians up to 1000 mg/d	< 10 mg/d
Dietary sources	Eggs; butter; dairy products; meat	Vegetable oils; nuts; seeds; grains	Coconut oil; tall oil extracts; some vegetable oils
Endogenous Synthesis	Biliary cholesterol: 800 – 1200 mg/d	Not synthesized	Not synthesized
Rate of absorption	40% – 60%	< 5%	0.1% – 2%
Plasma Concentration	140-320 mg/dl	0.3-1.7 mg/dl	0.3 – 0.6 mg/dl
Rate of excretion	40% – 60%	> 95%	98%

From Clifton, 2002

Recent studies confirm that the difference between the use of free or esterified phytosterols is not significant. There was an overall decrease in LDL cholesterol of 5.1% to 12.8% when delivering sterol and stanol esters in yoghurt (Platt, et al., 2006; Doornbos, et al., 2006) and an overall decrease of 9.4% to 21% when delivering free sterols in an oil mixture or in a juice beverage (Rudkowska, et al., 2006; Deveraj, et al., 2006). (See Table 5.)

5.3 The Effects of Sterols on Intestinal Cholesterol Absorption

Phytosterols are inactive as supplied in pure form and must be solubilized or emulsified to achieve biological activity. Ostlund (2007) reviewed the emerging role of dietary phytosterols in the reduction of LDL cholesterol levels. Phytosterols reduce cholesterol absorption but are poorly absorbed and excreted. He cited data to demonstrate that phytosterols must be solubilized or formulated to become bioavailable, and compared the solubility of sitostanol dried in the presence and absence of lecithin in artificial bile. He concluded that the principal mechanism of action appears to be competition with cholesterol in the intestine, resulting in reduced cholesterol absorption.

Rozner and Garti (2006) reviewed recent research on cholesterol health benefits and risks, absorption pathway of cholesterol, cholesterol-lowering drugs and phytosterols. Large quantities of powdered phytosterols were initially used because of its insoluble nature. Esterified phytosterols were oil soluble and increased bioavailability 10 times higher than that of pure phytosterols. The current practice for increased bioavailability is to use methods to suspend, precipitate and make micro-emulsions or micro-crystals of the free phytosterols in the food matrix.

A new mechanism for the anticholesterolemic action of sterols has been proposed by Kozlowska-Wojciechowska (2006), according to which sterols reduce absorption and formation of oxysterols (and reduce inflammation which is inherently associated with atherosclerosis), thus inhibiting the absorption of cholesterol in the small intestine through stimulation of the Liver X Receptor (LXR). However, more studies are needed on this proposed sterol action.

5.4 Effects of Sterols on Cholesterol and Lipid Metabolism

Combination therapy with two different hypocholesterolemic compounds, each affecting separate pathways of cholesterol metabolism, is expected to be more effective than single drug therapy. A recent study by Plat and his colleagues (2006) reported that plant stanols and statins have additive, not synergistic, effects.

Marinangeli, Varady, and Jones (2006) reviewed and evaluated individual effects of plant sterols and exercise training on lipid levels while attempting to elucidate the possible independent and synergistic mechanism of action responsible for these modulations. They found adopting a healthy lifestyle that included the consumption of phytosterols and physical activity, may significantly reduce one's risk of CHD by favorably altering each of four key lipid parameters: a decrease of both total and LDL cholesterol levels by the use of phytosterols, and a decrease of triglyceride level and an increase of HDL cholesterol level from the physical activity.

5.5 Recent Clinical Studies

Recently, several studies have shown that consumption of sterols enriched low-fat or nonfat foods like yoghurt or orange juice significantly lowered total and LDL cholesterol, demonstrating their cholesterol-lowering efficacy (Devaraj, et al., 2006, Doornbos, et al., 2006). The study by Doornbos and coworkers suggest that the optimal cholesterol-lowering effect may depend on the physical form of the sterol formulation, the solubility in the food matrix, and possibly on the fat content of the particular food or its consumption with regular meals as part of the daily diet (Doornbos, et al., 2006).

GRAS Notification for Pine Tree Phytosterols (continued)
5. Safety and Effectiveness (continued)

Devaraj and associates (2006) added plant sterols (1 g/240 ml) to a reduced calorie orange juice beverage (50 cal/240 ml) to study the effect of plant sterols on cholesterol, C-reactive protein and lipid levels. The reduced calorie beverage (with and without sterols) was fed to 72 subjects two times per day with meals for eight weeks. Supplementation with sterols decreased total and LDL cholesterol (5% and 9%) compared to baseline and also lowered C-reactive protein levels (12%). There were no significant changes in triglycerides, plasma vitamin E, and carotenoids.

Doornbos, et al. (2006) determined the effect of a phytosterol-enriched yoghurt drink (3 g/day) with or without a meal, and with different fat levels. The study was for four-week treatments using 184 moderate hypocholesterolaemic subjects. The results indicated that the single-dose drink effectively reduced LDL cholesterol irrespective of the fat content of the product, and there was a substantially larger decrease in serum cholesterol concentration when the yoghurt drink was consumed with a meal.

Rudkowska and colleagues (2006) studied the effect of feeding phytosterols in a fat medium with functional properties. The objective was to identify the existence of combining the biological actions of a functional oil containing high-oleic canola (45% – 47%) with medium-chain triglycerides (45% – 47%) and sterol esters (6% – 10%) compared to a control (extra-virgin olive oil). Twenty-three hyperlipidemic men consumed olive oil or the functional oil as part of a controlled diet in a randomized, crossover trial for six weeks each. The results indicate that the men fed the functional oil diet lowered plasma LDL cholesterol level without significantly changing the HDL cholesterol or the triacylglycerol levels. The decrease in total cholesterol was similar for both oil groups.

Plat and colleagues (2006) conducted a study with metabolic syndrome (MS) and non-MS patients (36 MS patients and 94 non-MS patients) fed a yogurt drink alone, with 2 g stanol, with 10 mg simvastatin, or with the stanol and statin combined for nine weeks. They found that the supplementation with sterol, statin, or the combination lowered non-HDL cholesterol by 12.8%, 30.7%, and 35%, respectively, compared to placebo. Triglycerides (TG) were also lowered and HDL cholesterol increased, resulting in a decreased TG/TC-HDL ratio of 16%, 40%, and 45%, respectively. Non-MS patients on the stanol drink had a 3.8% decrease in non-HDL cholesterol but the TG/TC-HDL ratio was not affected.

GRAS Notification for Pine Tree Phytosterols (continued)
5. Safety and Effectiveness (continued)

In terms of carriers, there is abundant evidence suggesting LDL cholesterol-lowering efficacy of phytosterols either as plant sterols or stanols in food forms, including water emulsions, water as lecithin micelles, yogurt, low fat milk, chocolate, cereal, snack bars, breads, and beverages. However, there are very few studies that investigated if these compounds provided as pharmaceutical forms, such as tablets and capsules, offer the same benefits.

Recent studies demonstrate the efficacy of free stanols and stanol esters delivered in tablets and sterol esters delivered in capsules has been proved, achieving LDL cholesterol-lowering effects ranging between 7% and 14% (Goldberg, et al., 2006; Nissinen, et al., 2006; Acuff, et al., 2007). Considering plant sterol ester is a more dispersible form in oil than free phytosterols, phytosterol esters may be more suitable choice for soft gel capsules than free plant sterols/stanols (Acuff, et al., 2007).

Table 5 summarizes the recent human clinical trials assessing the effects of phytosterols and phytosterol esters on hypercholesterolemic subjects when delivered in different vehicles.

GRAS Notification for Pine Tree Phytosterols (continued)
5. Safety and Effectiveness (continued)

Table 5: Effects of phytosterols or phytosterol esters on hypercholesterolemic subjects when delivered in different food vehicles

Product	Daily dose (g/d)[1]	Duration of intervention (weeks)	Observations	Reduction in plasma cholesterol	Reference
Stanol ester in yoghurt	2.0	9	Sterol Statin Combination	12.8 LDL[2] 30.7 LDL[2] 35.4 LDL[2]	Plat, *et al.*, 2006
Sterol ester in yoghurt[3,4]	3.0	4	w/ meal, low-fat w/ meal, regular w/o meal, low-fat w/o meal, regular	9.5 LDL 9.5 LDL 5.1 LDL 6.9 LDL	Doornbos, *et al.*, 2006
Sterols in oil mixture[4]	3.9	6	Hyperlipidemic men	17.0 total, 21.0 LDL	Rudkowska, *et al.*, 2006
Sterols in orange juice beverage	2	8	12% decrease in C-reactive protein	5.0 total, 9.4 LDL	Deveraj, *et al.*, 2006
Stanols in tablets	1.8[5]	6	Lecithin solubilized emulsion	9.1 LDL[6]	Goldberg, *et al.*, 2006
Stanol esters in tablets (pastilles)	2.8	1	Colectomized patients	9 total, 14 LDL	Nissinen, *et al.*, 2006
Sterol esters in capsules	1.3	3 4	Lowest FDA dose recommendation	7 LDL 4 LDL	Acuff, *et al.*, 2007

[1] g free sterol/stanol equivalent
[2] Reduction compared to placebo in metabolic syndrome patients
[3] Emulsion
[4] Low fat: 0.1% dairy fat, 2.2% total fat; regular: 1.5% dairy fat, 3.3% total fat
[5] Rapidly disintegrating
[6] In addition to statin regime

000026

5.6 Other Secondary Effects

Jansen, et al. (2006) reported that mice deficient for ATP-binding cassette transporter G5 (Abcg5) or Abcg8 (genes encoding for transporters of plant sterols from endoplasmic reticulum back to the luminal membrane to be re-secreted into the lumen of the intestine and present in the liver as mediators of the efflux of cholesterol and plant sterols into bile), with strongly elevated serum plant sterol levels, display dramatically increased (7- to 16-fold) plant sterol levels in the brain, but not in brain of ApoE-deficient mice. Mutations in the genes also lead to sitosterolemia, an inborn error of metabolism characterized by high levels of sitosterol, campesterol and stigmasterol. They suggest an important role for HDL and/or ApoE (both lipoproteins involved in cholesterol metabolism) in the transfer of plant sterols into the brain. They conclude that dietary plant sterols pass the blood–brain barrier and accumulate in the brain, and suggest that an accumulation of plant sterols in the brain may exert brain cell type-specific effects and as a consequence may affect brain functioning. The implications of these findings in humans remain to be established. Plant sterols are predominantly transported by LDL particles (Jansen, et al., 2006). Also, as noted in Table 4, more than 95% of the phytosterols are excreted and the plasma levels are insignificant compared to cholesterol plasma levels.

5.7 Conclusions on Safety

Arboris believes that there is general recognition that the use of Arboris sterols as another source of phytosterol is safe based on the following:

- GRAS Panels convened for eight GRAS Notifications submitted to FDA have concluded that the use of phytosterols and the phytosterol esters are safe for the proposed uses.

- FDA has issued an interim final rule allowing the use of health claims on the association between plant sterol/stanol esters and the reduced risk of coronary heart disease. FDA concluded that the petitioner of the health claim has satisfied the requirement to demonstrate that the use of these sterol/stanol esters at the levels necessary to justify a claim is safe. FDA has also issued a letter stating that it would consider enforcement discretion for foods with appropriate health claims that contain free and/or esterified phytosterols from tall oil and vegetable oil sources for the uses listed in the GRAS Notifications.

GRAS Notification for Pine Tree Phytosterols (continued)
5. Safety and Effectiveness (continued)

- The European Union Committee, the Scientific Committee on Food, has determined that the use of phytosterols is safe for the proposed uses. However, there was still a concern of: 1) total phytosterol intakes exceeding 3 g/day, 2) consumption of phytosterol-containing foods by people with inborn error of phytosterol metabolism (phytosterolaemia), and 3) consumption of phytosterol-containing foods by patients on cholesterol-lowering medication because of the potential β-carotene-lowering effect.

  Therefore, the SCF recommended that appropriate risk management measures should be developed to minimize the likelihood of exceeding the 3 g/d daily intake of phytosterols, recommended that the small number of people with phytosterolaemia should be made aware of foods containing high levels of phytosterols, and that consumers using cholesterol-lowering medication be notified about the potential β-carotene-lowering effect by phytosterols in the diet.

- The recent literature obtained since the last GRAS notification supports the safety of the continued use of phytosterols in food and confirms that when phytosterols are formulated to become bioavailable, they have the same activity as the esterified phytosterols.

- These phytosterols obtained by Arboris from pine trees are substantially equivalent to other pine-tree derived phytosterols. Arboris believes that its products will provide an additional source of free phytosterols for the existing uses listed in earlier GRAS submissions and will not change the cumulative dietary intake of phytosterols.

  Arboris concludes that there is general recognition by experts qualified in scientific experience that the use of phytosterols in food from plant and vegetable sources at the existing and proposed uses are safe based on scientific procedures. Therefore, the use of Arboris' phytosterols is safe.

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COA Issue Date:	9/18/2007		
Product Name:	Arboris® Sterols AS-2™		

Manufacture Date			6/1/2007
Lot No.		Specifications	F7M0101
Quantity – Bags			26
Total Sterols	%	>99.0%	99.1
Cholesterol	%	<=1.0%	<0.25
Brassicasterol*	%	<=2.0%	<0.5
Campesterol*	%	<=15.0%	7.1
Campestanol*	%	<=5.0%	1.2
Stigmasterol*	%	<=2.0%	0.8
Sitosterol*	%	60.0-85.0%	76.5
Sitostanol*	%	<=15.0%	10.9
D5-Avenasterol	%	<=2%	0.4
Other Sterols	%	<=5%	2.2
Steradienes	%	<=0.3%	<0.05
Loss on Drying	%	<1.0%	0.17
Iron	ppm	<=20	<10
Copper	ppm	<=2	<2
Sulfur	ppm	<=10	4
Heptane	ppm	<=15	<5
Hexane	ppm	<=15	<5
Ethanol	ppm	<=1000	<10
Methanol	ppm	<=40	<5
Color		White/Off White	Pass
Appearance		Free-Flowing pastilles	Pass
Taste and Odor		Neutral, Bland	Pass
Arsenic	Ppm	<=0.1	Certified every 5 lots
Cadmium	Ppm	<=0.1	Certified every 5 lots
Lead	Ppm	<=0.1	Certified every 5 lots
Mercury	Ppm	<=0.1	Certified every 5 lots
BaP	Ppb	<=2	Certified every 6 Months
Pesticides		Absent	Pass

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Manufacture Date			6/14/2007
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Campesterol*	%	<=15.0%	7.2
Campestanol*	%	<=5.0%	1.2
Stigmasterol*	%	<=2.0%	0.7
Sitosterol*	%	60.0-85.0%	77.7
Sitostanol*	%	<=15.0%	10.9
D5-Avenasterol	%	<=2%	0.4
Other Sterols	%	<=5%	2.4
Steradienes	%	<=0.3%	<0.05
Loss on Drying	%	<1.0%	0.17
Iron	ppm	<=20	<10
Copper	ppm	<=2	<2
Sulfur	ppm	<=10	5
Heptane	ppm	<=15	<5
Hexane	ppm	<=15	<5
Ethanol	ppm	<=1000	<10
Methanol	ppm	<=40	<5
Color		White/Off White	Pass
Appearance		Free-Flowing pastilles	Pass
Taste and Odor		Neutral, Bland	Pass
Arsenic	Ppm	<=0.1	Certified every 5 lots
Cadmium	Ppm	<=0.1	Certified every 5 lots
Lead	Ppm	<=0.1	Certified every 5 lots
Mercury	Ppm	<=0.1	Certified every 5 lots
BaP	Ppb	<=2	Certified every 6 Months
Pesticides		Absent	Pass

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Campesterol*	%	<=15.0%	7.2
Campestanol*	%	<=5.0%	1.2
Stigmasterol*	%	<=2.0%	0.8
Sitosterol*	%	60.0-85.0%	76.8
Sitostanol*	%	<=15.0%	10.8
D5-Avenasterol	%	<=2%	0.3
Other Sterols	%	<=5%	2.3
Steradienes	%	<=0.3%	<0.05
Loss on Drying	%	<1.0%	0.11
Iron	ppm	<=20	<10
Copper	ppm	<=2	<2
Sulfur	ppm	<=10	5
Heptane	ppm	<=15	<5
Hexane	ppm	<=15	<5
Ethanol	ppm	<=1000	<10
Methanol	ppm	<=40	<5
Color		White/Off White	Pass
Appearance		Free-Flowing pastilles	Pass
Taste and Odor		Neutral, Bland	Pass
Arsenic	Ppm	<=0.1	Certified every 5 lots
Cadmium	Ppm	<=0.1	Certified every 5 lots
Lead	Ppm	<=0.1	Certified every 5 lots
Mercury	Ppm	<=0.1	Certified every 5 lots
BaP	Ppb	<=2	Certified every 6 Months
Pesticides		Absent	Pass

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Manufacture Date			6/19/2007
Lot No.		Specifications	F7M0119
Quantity – Bags			29
Total Sterols	%	>99.0%	99.4
Cholesterol	%	<=1.0%	<0.25
Brassicasterol*	%	<=2.0%	<0.5
Campesterol*	%	<=15.0%	7.2
Campestanol*	%	<=5.0%	1.2
Stigmasterol*	%	<=2.0%	0.8
Sitosterol*	%	60.0-85.0%	76.7
Sitostanol*	%	<=15.0%	10.6
D5-Avenasterol	%	<=2%	0.3
Other Sterols	%	<=5%	2.3
Steradienes	%	<=0.3%	<0.05
Loss on Drying	%	<1.0%	0.14
Iron	ppm	<=20	<10
Copper	ppm	<=2	<2
Sulfur	ppm	<=10	5
Heptane	ppm	<=15	<5
Hexane	ppm	<=15	<5
Ethanol	ppm	<=1000	<10
Methanol	ppm	<=40	<5
Color		White/Off White	Pass
Appearance		Free-Flowing pastilles	Pass
Taste and Odor		Neutral, Bland	Pass
Arsenic	Ppm	<=0.1	Certified every 5 lots
Cadmium	Ppm	<=0.1	Certified every 5 lots
Lead	Ppm	<=0.1	Certified every 5 lots
Mercury	Ppm	<=0.1	Certified every 5 lots
BaP	Ppb	<=2	Certified every 6 Months
Pesticides		Absent	Pass

APPROVED BY:
Jesse A. Boyer
Quality Manager

arboris®
pine tree extracts

CALL CUSTOMER SERVICE
At 1-912-238-6685
1201 West Lathrop Avenue, Gate 16
Savannah, GA 31415
Manufacturing Loc: Savannah

Certificate of Analysis

Arboris® Order #:			
Customer/Product:		Tall Oil Sterols	
Customer Ref #:			
Date Shipped:			
COA Issue Date:	10/3/2007		
Product Name:	Arboris® Sterols AS-2™		

Manufacture Date			6/25/2007
Lot No.		Specifications	F7M0125
Quantity – Bags			30
Total Sterols	%	>99.0%	99.4
Cholesterol	%	<=1.0%	<0.25
Brassicasterol*	%	<=2.0%	<0.5
Campesterol*	%	<=15.0%	7.0
Campestanol*	%	<=5.0%	1.2
Stigmasterol*	%	<=2.0%	0.9
Sitosterol*	%	60.0-85.0%	76.8
Sitostanol*	%	<=15.0%	10.8
D5-Avenasterol	%	<=2%	0.3
Other Sterols	%	<=5%	2.4
Steradienes	%	<=0.3%	<0.05
Loss on Drying	%	<1.0%	0.09
Iron	ppm	<=20	<10
Copper	ppm	<=2	<2
Sulfur	ppm	<=10	6
Heptane	ppm	<=15	<5
Hexane	ppm	<=15	<5
Ethanol	ppm	<=1000	<10
Methanol	ppm	<=40	<5
Color		White/Off White	Pass
Appearance		Free-Flowing pastilles	Pass
Taste and Odor		Neutral, Bland	Pass
Arsenic	Ppm	<=0.1	Certified every 5 lots
Cadmium	Ppm	<=0.1	Certified every 5 lots
Lead	Ppm	<=0.1	Certified every 5 lots
Mercury	Ppm	<=0.1	Certified every 5 lots
BaP	Ppb	<=2	Certified every 6 Months
Pesticides		Absent	Pass

APPROVED BY:
Jesse A. Boyer
Quality Manager

3

Arboris® Sterol AS-2®

High Purity-Grade Pine Tree Phytosterols

Description: Arboris® Sterol AS-2® is a mixture of naturally occurring phytosterols derived from GMO-free pine trees.

Properties	Specification
Total Sterols, including others, %	99% min
Cholesterol, %	1.0% max
Beta-Sitosterol, %	70% min ;80% max
Beta-Sitostanol, %	15% max
Campesterol, %	15% max
Stigmasterol, %	2% max
Campestanol, %	5% max
Brassicasterol, %	2% max
Other Sterols	3 % max
Appearance at 25°C	Free-flowing prills
Color	White to Off-White
Taste and Odor	Neutral, Bland
Moisture, %	1% max

March 12, 2008

Arboris LLC.
Phone: 912-238-6685 ❁ Fax: 912-238-7424 ❁
Mailing Address: P.O. Box 2008, Savannah, GA 31402, USA

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Arboris® Sterol AS-4™

High Purity-Grade Pine Tree Phytosterols

Description: Arboris® Sterol AS-4™ is a mixture of naturally occurring phytosterols.

Properties	Specification
Total Sterols, including others, %	95% min
Cholesterol, %	1.0% max
Beta-Sitosterol, %	85% max
Beta-Sitostanol, %	20% max
Campesterol, %	15% max
Stigmasterol, %	2% max
Campestanol, %	5% max
Other Sterols	5 % max
Appearance at 25°C	Free-flowing prills
Color	White to Off-White
Taste and Odor	Neutral, Bland
Moisture, %	1% max

March 12, 2008

Arboris LLC.
Phone: 912-238-6685 ❁ Fax: 912-238-7424 ❁
Mailing Address: P.O. Box 2008, Savannah, GA 31402, USA

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8

Federal Register

Friday,
September 8, 2000

Part III

Department of Health and Human Services

Food and Drug Administration

21 CFR Part 101

Food Labeling: Health Claims; Plant Sterol/Stanol Esters and Coronary Heart Disease; Interim Final Rule

DEPARTMENT OF HEALTH AND HUMAN SERVICES

Food and Drug Administration

21 CFR Part 101

[Docket Nos. 00P–1275 and 00P–1276]

Food Labeling: Health Claims; Plant Sterol/Stanol Esters and Coronary Heart Disease

AGENCY: Food and Drug Administration, HHS.

ACTION: Interim final rule.

SUMMARY: The Food and Drug Administration (FDA) is authorizing the use, on food labels and in food labeling, of health claims on the association between plant sterol/stanol esters and reduced risk of coronary heart disease (CHD). FDA is taking this action in response to a petition filed by Lipton (plant sterol esters petitioner) and a petition filed by McNeil Consumer Healthcare (plant stanol esters petitioner). Based on the totality of publicly available evidence, the agency has concluded that plant sterol/stanol esters may reduce the risk of CHD.

DATES: This rule is effective September 8, 2000. Submit written comments by November 22, 2000. The Director of the Office of the Federal Register approves the incorporation by reference in accordance with 5 U.S.C. 552(a) and 1 CFR part 51 of certain publications in 21 CFR 101.83(c)(2)(ii)(A)(*2*) and (c)(2)(ii)(B)(*2*), as of September 8, 2000.

ADDRESSES: Submit written comments to the Dockets Management Branch (HFA 305), Food and Drug Administration, 5630 Fishers Lane, rm. 1061, Rockville, MD 20852.

FOR FURTHER INFORMATION CONTACT: Sharon A. Ross, Center for Food Safety and Applied Nutrition (HFS 832), Food and Drug Administration, 200 C St. SW., Washington, DC 20204, 202 205 5343.

SUPPLEMENTARY INFORMATION:

I. Background

The President signed into law, on November 8, 1990, the Nutrition Labeling and Education Act of 1990 (the 1990 amendments) (Public Law 101 535). This new law amended the Federal Food, Drug, and Cosmetic Act (the act) in number of important ways. One of the most notable aspects of the 1990 amendments was that they provided procedures whereby FDA is to regulate health claims on food labels and in food labeling.

In the **Federal Register** of January 6, 1993 (58 FR 2478), FDA issued a final rule that implemented the health claim provisions of the act for conventional foods (hereinafter referred to as the 1993 health claims final rule). In that final rule, FDA adopted §101.14 (21 CFR 101.14), which sets out the rules for the authorization of health claims by regulation and prescribes general requirements for the use of health claims. Additionally, §101.70 (21 CFR 101.70) establishes a process for petitioning the agency to authorize health claims about a substance-disease relationship (§101.70(a)) and sets out the types of information that any such petition must include (§101.70(d)). On January 4, 1994 (59 FR 395), FDA issued a final rule applying the requirements of §§101.14 and 101.70 to health claims for dietary supplements.

FDA also conducted an extensive review of the evidence on 10 substance-disease relationships listed in the 1990 amendments. As a result of its review, FDA authorized claims for 8 of these 10 relationships, one of which focused on the relationship between dietary saturated fat and cholesterol and reduced risk of CHD. CHD is the most common, most frequently reported, and most serious form of cardiovascular disease (CVD) (58 FR 2739, January 6, 1993). Further, while the agency denied the use on food labeling of health claims relating dietary fiber to reduced risk of CVD (58 FR 2552, January 6, 1993), it authorized a health claim relating fiber-containing fruits, vegetables, and grain products to a reduced risk of CHD.

In the proposed rule entitled "Health Claims and Label Statements; Lipids and Cardiovascular Disease" (56 FR 60727 at 60727, 60728, and 60732, November 27, 1991), FDA set out the criteria for evaluating evidence on diet and CVD relationships, including the relationship between diet and CHD. FDA noted that, because of the public health importance of CHD, identification of "modifiable" risk factors for CHD had been the subject of considerable research and public policy attention. The agency also noted that there is general agreement that elevated blood cholesterol levels are one of the major modifiable risk factors in the development of CHD. FDA cited Federal Government and other reviews that concluded that there is substantial epidemiologic and clinical evidence that high blood levels of total and low density lipoprotein (LDL) cholesterol are a cause of atherosclerosis (inadequate blood circulation due to narrowing of the arteries) and represent major contributors to CHD. Further, factors that decrease total blood cholesterol and LDL cholesterol will also decrease the risk of CHD. FDA concluded that it is generally accepted that blood total and LDL cholesterol levels are major risk factors for CHD, and that dietary factors affecting blood cholesterol levels affect the risk of CHD. High intakes of dietary saturated fat and, to a lesser degree, of dietary cholesterol are consistently associated with elevated blood cholesterol levels. FDA concluded that the publicly available data supported an association between diets low in saturated fat and cholesterol and reduced risk of CHD (58 FR 2739 at 2751).

The agency has authorized other health claims for reducing the risk of CHD using the aforementioned criteria. In the final rule entitled "Health Claims; Dietary Fiber and Cardiovascular Disease" (58 FR 2552), FDA concluded that the publicly available scientific information supported an association between fruits, vegetables, and grain products (i.e., foods that are low in saturated fat and cholesterol and that are good sources of dietary fiber) and reduced risk of CHD through the intermediate link of blood cholesterol (58 FR 2552 at 2572) (codified at §101.77)). In response to two petitions documenting that dietary consumption of soluble fiber from beta-glucan from oat products and psyllium seed husk significantly reduced blood cholesterol levels, FDA authorized health claims for soluble fiber from certain foods and reduced risk of CHD in §101.81 (21 CFR 101.81) (62 FR 3584 at 3600, January 23, 1997, and amended at 62 FR 15343 at 15344, March 31, 1997, pertaining to beta-glucan from oat products, and 63 FR 8103 at 8119, February 18, 1998 pertaining to psyllium seed husk). More recently, FDA authorized a health claim for soy protein and reduced risk of CHD in §101.82 (21 CFR 101.82) (64 FR 57700, October 26, 1999). In the final rule authorizing the claim, the agency concluded, based on the totality of publicly available scientific evidence, that there is significant scientific agreement that soy protein, included at a level of 25 grams (g) per day (d) in a diet low in saturated fat and cholesterol, can help reduce total and LDL cholesterol levels, and that such reductions may reduce the risk of CHD (64 FR 57700 at 57713). The dietary fiber and CVD (56 FR 60582 at 60583 and 60587, November 27, 1991), soluble fiber from beta-glucan from oat products and CHD (61 FR 296 at 298, January 4, 1996), soluble fiber from psyllium seed husk and CHD (62 FR 28234 at 28236 and 28237, May 22, 1997), and soy protein and CHD (63 FR 62977 at 62979 and 62980, November 10, 1998) health claim reviews in the proposed rules were conducted in accordance with the

1991 criteria for evaluating the evidence between diet and CHD (56 FR 60727 at 60727, 60728, and 60732.

The present rulemaking is in response to two health claim petitions. One health claim petition concerns the relationship between plant sterol esters and the risk of CHD, and the other concerns the relationship between plant stanol esters and the risk of CHD. Although the plant sterol esters petition characterizes the petitioned substance as vegetable oil sterol esters, FDA believes it is more accurately characterized as plant sterol esters. The petition states that vegetable oil sterol esters consist of esterified plant sterols (Ref. 1, page 3). The petition also mentions that canola oil is one of the oils used as a source for the sterol component of vegetable oil sterol esters (Ref. 1, page 82). Canola oil is derived from a seed (rapeseed). Although seeds are clearly part of the plant kingdom, they are not ordinarily thought of as vegetables. Therefore, FDA is concerned that the term "vegetable oil sterol esters" may not be understood to cover esterified sterols from sources like canola oil. Accordingly, the agency is using the term "plant sterol esters" throughout this document. For purposes of this rule, plant sterol esters and plant stanol esters will be referred to collectively as "plant sterol/stanol esters."

II. Petitions for Plant Sterol/Stanol Esters and Reduced Risk of CHD

A. Background

Lipton submitted a health claim petition to FDA on February 1, 2000, requesting that the agency authorize a health claim on the relationship between consumption of certain plant sterol ester-containing foods and the risk of CHD (Refs.1 through 4). Specifically, Lipton requested that spreads and dressings for salad[1] containing at least 1.6 grams of plant sterol esters per reference amount customarily consumed be authorized to bear a health claim about reduced risk of CHD. On May 11, 2000, the agency sent this petitioner a letter stating that FDA had decided to file the petition for further review (Ref. 5). On June 26, 2000, Lipton submitted a request asking FDA to exercise its authority under section 403(r)(7) of the act (21 U.S.C. 343(r)(7)) to make any proposed regulation for its petitioned health claim effective upon publication, pending consideration of public comment and publication of a final rule (Ref. 6). If the agency does not act, by either denying the petition or issuing a proposed regulation to authorize the health claim, within 90 days of the date of filing, the petition is deemed to be denied unless an extension is mutually agreed upon by the agency and the petitioner (section 403(r)(4)(a)(i) of the act and 21 CFR 101.70(j)(3)(iii)). On August 2, 2000, FDA and the plant sterol ester petitioner agreed to an extension of 30 days, until September 6, 2000 (Ref. 7).

On February 15, 2000, McNeil Consumer Healthcare submitted a health claim petition to FDA requesting that the agency authorize a health claim on the relationship between consumption of plant stanol ester-containing foods and dietary supplements and the risk of CHD (Refs. 8 through 14). On May 25, 2000, the agency sent this petitioner a letter stating that FDA had decided to file the petition for further review (Ref. 15). On June 14, 2000, McNeil Consumer Healthcare submitted a request asking FDA to exercise its authority under section 403(r)(7) of the act to make any proposed regulation for its petitioned health claim effective upon publication, pending consideration of public comment and publication of a final rule (Ref. 16). On July 17, 2000, FDA and the plant stanol ester petitioner agreed to an extension of the deadline to publish a proposed regulation until September 6, 2000 (Ref. 17).

In this interim final rule, the agency concludes that a health claim about plant sterol/stanol esters and reduced risk of CHD should be authorized under the standard in section 403(r)(3)(B)(i) of the act and §101.14(c) of FDA's regulations and should be made effective upon publication under section 403(r)(7) of the act, pending consideration of public comment and publication of a final regulation. The agency is requesting comments on this interim final rule. Firms should be aware that a final rule on this health claim may differ from this interim final rule and that they would be required to revise their labels to conform to any changes adopted in the final rule.

B. Review of Preliminary Requirements for a Health Claim

1. The Substances Are Associated With a Disease for Which the U.S. Population Is at Risk

Several previous rules establish that CHD is a disease for which the U.S. population is at risk. These include rules authorizing claims for dietary saturated fat and cholesterol and risk of CHD §101.75 (21 CFR 101.75)); fiber-containing fruits, vegetables, and grain products and risk of CHD (§101.77); soluble fiber from certain foods and risk of CHD (§101.81); and soy protein and risk of CHD (§101.82). FDA stated in these rules that CHD remains a major public health problem and the number one cause of death in the United States. Despite the decline in deaths from CHD over the past 30 years, this disease is still exacting a tremendous toll in morbidity (illness and disability) and mortality (premature deaths) (Refs. 18 through 20). There are more than 500,000 deaths each year for which CHD is the primary cause, and another 250,000 deaths for which CHD is a contributing cause. About 20 percent of adults (male and female; black and white) ages 20 to 74 years have blood total cholesterol (or serum cholesterol) levels in the "high risk" category (total cholesterol greater than (>) 240 milligrams (mg) / deciliter (dL) and LDL cholesterol > 160mg/dL) (Ref. 21). Another 31 percent have "borderline high" cholesterol levels (total cholesterol between 200 and 239 mg/dL and LDL cholesterol between 130 and 159 mg/dL) in combination with two or more other risk factors for CHD.

CHD has a significant effect on health care costs. In 1999, total direct costs related to CHD were estimated at $53.1 billion, and indirect costs from loss of productivity due to illness, disability, and premature deaths from this disease were an estimated $46.7 billion (Ref. 22). Based on these facts, FDA concludes that, as required in §101.14(b)(1), CHD is a disease for which the U.S. population is at risk.

2. The Substances Are Food

The substances that are the subject of this interim final rule are plant sterol esters and plant stanol esters (Refs. 1 through 4 and 8 through 14).

a. *Plant sterol esters.* The substance that is the subject of the plant sterol ester petition is a mixture of plant sterols esterified to food-grade fatty acids. The sterols are primarily (beta-sitosterol, campesterol, and stigmasterol and are extracted from plant sources (Ref. 1, page 6). Plant sterols occur widely throughout the plant kingdom

[1] The agency is using the term "dressings for salad" throughout this document in lieu of the term "salad dressing" used by the petitioners because the standard of identity for "salad dressing" in §169.150 (21 CFR 164.150) refers to a limited class of dressings for salad, i.e., those that contain egg yolk and meet certain other specifications. "Salad dressing" as defined in §169.150 does not include a number of common types of dressings for salad, such as Italian dressing.

and are present in many edible fruits, vegetables, nuts, seeds, cereals, and legumes (Refs. 23 and 24). The plant sterols in foods may occur as either the free sterol or esterified with a fatty acid.

Several studies have estimated dietary plant sterol intake. From a population in the Los Angeles area, Nair et al. (Ref. 25) found that plant sterol (beta-sitosterol and stigmasterol) intake ranged from 77.9 mg/d in the general population to 343.6 mg/d in lacto-ovo vegetarians. The 1991 British diet was estimated to contain about 158 mg/d of sterols (beta-sitosterol, stigmasterol, and campesterol) (Ref. 26). Scandinavian vegetarians consume, on average, 513 mg/d and nonvegetarians 398 mg/d (Ref. 27). Plant sterol intake in the Japanese diet has been estimated at 373 mg/d (Ref. 28). In an analysis of diets of participants in the Seven Countries Study, deVries et al. (Ref. 29) found plant sterol intake (sitosterol, stigmasterol and campesterol) to range from 170 mg/d among U.S. railroad workers to 358 mg/d in Corfu, Greece. In a review, Ling and Jones (Ref. 30) estimated average U.S. intake at 250 mg/d; it was speculated that this level was doubled among vegetarians. Thus, plant sterols are a constituent of the diet for Americans and other population groups.

According to the plant sterol ester petitioner, the solubility of free sterols in oil is only 2 percent, but the solubility of sterol esters in oil exceeds 20 percent (Ref. 1, pages 14 and 99). Therefore, the free plant sterols are esterified with fatty acids from sunflower to improve solubility. The petitioner also notes that improved solubility of plant sterols creates a palatable product and is associated with more uniform distribution in the product and in the gastrointestinal tract (Ref. 1, page 14). In vegetable oils, typically between 25 and 80 percent of the sterol is in the ester form (Refs. 31 through 34). One gram of plant sterols is equivalent to about 1.6 g of plant sterol esters (Refs. 35 and 36).

Under §101.14(b)(3)(i), the substance that is the subject of a health claim must contribute taste, aroma, or nutritive value, or any other technical effect listed in §170.3(o) (21 CFR 170.3(o)), to the food and must retain that attribute when consumed at the levels that are necessary to justify a claim. Plant sterol esters do not contribute taste, aroma, or any other technical effect listed in §170.3(o), and thus the plant sterol esters must contribute nutritive value to meet the requirement in §101.14(b)(3)(i).

The term 'nutritive value' is defined in §101.14(a)(3) as "value in sustaining human existence by such processes as promoting growth, replacing loss of essential nutrients, or providing energy." In the proposed rule entitled "Labeling; General Requirements for Health Claims for Food" (56 FR 60537, November 27, 1991), FDA proposed this definition and explained its interpretation of nutritive value in the context of whether a substance is a food and thus appropriately the subject of a health claim (56 FR 60537 at 60542). The agency indicated that the definition was formulated based on the common meaning of the words that make up the term "nutritive value." The agency also added that use of the phrase "such processes as" in the definition of nutritive value was intended to provide a measure of flexibility that the agency believed would be necessary in evaluating future petitions. In the final rule adopting the proposed definition, the agency noted that the evaluation of the nutritive value of substances would be done on a case-by-case basis to best ensure that the definition retains its intended flexibility (58 FR 2478 at 2488). In a subsequent final rule on health claims for dietary supplements (59 FR 395 at 407), FDA further explained that nutritive value "includes assisting in the efficient functioning of classical nutritional processes and of other metabolic processes necessary for the normal maintenance of human existence."

The scientific evidence suggests that the cholesterol-lowering effect of plant sterol esters is achieved through an effect on the digestive process (Ref. 1, pages 62 through 64). The digestive process is one of the metabolic processes necessary for the normal maintenance of human existence. Therefore, the agency concludes that the preliminary requirement of §101.14(b)(3)(i) is satisfied.

b. *Plant stanol esters.* The substance that is the subject of the plant stanol ester petition is a mixture of plant stanols esterified to food-grade fatty acids. The stanols are primarily sitostanol and campestanol and may be derived from hydrogenated plant sterol mixtures or extracted from plant sources (Ref. 8, page 18). Sitostanol and campestanol occur naturally in small quantities in the lipid fractions of cereal grains such as wheat, rye, and corn (Refs. 37 through 39) and in vegetable oils such as corn and olive oil (Refs. 40 and 41). The average western diet provides 20 to 50 mg of plant stanols daily (Ref. 42).

According to the plant stanol ester petitioner, esterification of free stanols with fatty acids renders plant stanols readily soluble in foods and makes an effective vehicle for delivery of plant stanols to the small intestine (Ref. 8, page 9). One gram of wood-derived plant stanols is equivalent to about 1.7 g of plant stanol esters (Ref. 43), and 1 g of vegetable oil plant stanols is equivalent to about 1.8 g of plant stanol esters (Ref. 43).

As discussed in section II.B.2.a of this document, the substance that is the subject of a health claim must contribute taste, aroma, or nutritive value, or any other technical effect listed in §170.3(o), to the food and must retain that attribute when consumed at levels that are necessary to justify a claim (§101.14(b)(3)(i)). Plant stanol esters do not contribute taste, aroma or any other technical effect listed in §170.3(o) and thus must contribute nutritive value to meet the requirement in §101.14(b)(3)(i). The term "nutritive value" is defined in §101.14(a)(3) as "value in sustaining human existence by such processes as promoting growth, replacing loss of essential nutrients, or providing energy."

The scientific evidence suggests that the cholesterol-lowering effect of plant stanol esters is achieved through an effect on the digestive process (Ref. 8, pages 11 through 12). As discussed in section II.B.2.a of this document and in the final rule on health claims for dietary supplements (59 FR 395 at 407), nutritive value includes assisting in the efficient functioning of classical nutritional processes and of other metabolic processes necessary for the normal maintenance of human existence, such as digestive processes. Therefore, the agency concludes that the preliminary requirement of §101.14(b)(3)(i) is satisfied.

3. The Substances Are Safe and Lawful

a. *Plant sterol esters.* The plant sterol ester petitioner asserts that plant sterol esters are generally recognized as safe (GRAS) for certain uses. In a submission dated January 11, 1999, the petitioner informed FDA of its conclusion that plant sterol esters are GRAS for use in vegetable oil spreads at levels up to 20 percent (corresponding to 1.6 g of plant sterol esters per serving) to supplement the nutritive value of the spread, and to help structure the fat phase and reduce the fat and water content of the spread. The January 11, 1999, submission included the supporting data on which this conclusion was based. FDA responded to this submission in a letter dated April 30, 1999 (Ref. 44). In its response, the agency stated, "Based on its evaluation, the agency has no questions at this time regarding Lipton's conclusion that vegetable oil sterol esters are GRAS under the intended conditions of use. Furthermore, FDA is not aware of any scientific evidence that

vegetable oil sterol esters would be harmful. The agency has not, however, made its own determination regarding the GRAS status of the subject use of vegetable oil sterol esters" (Ref. 44). In a letter dated September 24, 1999, the petitioner informed FDA of an additional use of plant sterol esters in dressings for salad (Ref. 45). The letter contained additional safety information to support the new use.

The agency notes that authorization of a health claim for a substance should not be interpreted as affirmation that the substance is GRAS. A review of Lipton's January 11, 1999, submission and of its September 24, 1999, letter to the agency, however, reveals significant evidence supporting the safety of the use of plant sterol esters at the levels necessary to justify a health claim. Moreover, FDA is not aware of any evidence that provides a basis to reject the petitioner's position that the use of plant sterol esters in spreads and dressings for salad up to 1.6 g/serving is safe and lawful. As discussed in section V.B of this document, the level of plant sterol esters necessary to justify a claim is 1.3 g per day. Therefore, FDA concludes that the petitioner has satisfied the requirement of §101.14(b)(3)(ii) to demonstrate that the use of plant sterol esters in spreads and dressings for salad at the levels necessary to justify a claim is safe and lawful.

b. *Plant stanol esters.* Under the health claim petition process, FDA evaluates whether the substance is "safe and lawful" under the applicable food safety provisions of the act (§101.14(b)(3)(ii)). For conventional foods, this evaluation involves considering whether the ingredient that is the source of the substance is GRAS, listed as a food additive, or authorized by a prior sanction issued by FDA (see §101.70(f)). Dietary ingredients in dietary supplements, however, are not subject to the food additive provisions of the act (see section 201(s)(6) of the act (21 U.S.C. 321(s)(6)). Rather, they are subject to the new dietary ingredient provisions in section 413 of the act (21 U.S.C. 350b) and the adulteration provisions in section 402 of the act (21 U.S.C. 342). The term "dietary ingredient" is defined in section 201(ff)(1) of the act and includes vitamins; minerals; herbs and other botanicals; dietary substances for use by man to supplement the diet by increasing the total daily intake; and concentrates, metabolites, constituents, extracts, and combinations of the preceding ingredients.

A "new dietary ingredient" is a dietary ingredient that was not marketed in the United States before October 15, 1994 (section 413(c) of the act). If a dietary supplement contains a new dietary ingredient that has not been present in the food supply as an article used for food in a form in which the food has not been chemically altered, section 413(a)(2) of the act requires the manufacturer or distributor of the supplement to submit to FDA, at least 75 days before the dietary ingredient is introduced or delivered for introduction into interstate commerce, information that is the basis on which the manufacturer or distributor has concluded that a dietary supplement containing such new dietary ingredient will reasonably be expected to be safe. FDA reviews this information to determine whether it provides an adequate basis for such a conclusion. Under section 413(a)(2) of the act, there must be a history of use or other evidence of safety establishing that the dietary ingredient, when used under the conditions recommended or suggested in the labeling of the dietary supplement, will reasonably be expected to be safe. If FDA believes that this requirement has not been met, the agency responds to the notification within 75 days from the date of its receipt. Otherwise, no response is sent. If a new dietary ingredient notification has been submitted and a history of use or other evidence of safety exists that establishes a reasonable expectation of safety, the new dietary ingredient may be lawfully marketed in dietary supplements 75 days after the notification is submitted.

As previously noted, the plant stanol ester petitioner requested authorization to make a health claim about plant stanol esters and the risk of CHD in the labeling of both conventional foods and dietary supplements. Because the standards under which the safety and legality of conventional foods and dietary supplements are evaluated differ, the agency is discussing these two proposed uses separately.

i. *Conventional foods.* The plant stanol ester petitioner asserts that plant stanol esters are GRAS. In a submission dated February 18, 1999, the petitioner informed FDA of its conclusion that plant stanol esters are GRAS for use as a nutrient in spreads at a level of 1.7g of plant stanol esters per serving of spread. The February 18, 1999, submission included the supporting data on which this conclusion was based. FDA responded to this submission in a letter dated May 17, 1999 (Ref. 46). In its response, the agency stated, "Based on its evaluation, the agency has no questions at this time regarding McNeil's conclusion that plant stanol esters are GRAS under the intended conditions of use. Furthermore, FDA is not aware of any scientific evidence that plant stanol esters would be harmful. The agency has not, however, made its own determination regarding the GRAS status of the subject use of plant stanol esters" (Ref. 46). The petitioner's GRAS determination applies to plant stanol esters whose stanol components are prepared by the hydrogenation of commercially available plant sterol blends, which are obtained as distillates from vegetable oils or as byproducts of the kraft paper pulping process (Ref. 46). In letters dated July 21, 1999, and October 13, 1999, the petitioner informed FDA of additional uses of plant stanol esters in dressings for salad and snack bars (Refs. 47 and 48).

The agency notes that authorization of a health claim for a substance should not be interpreted as affirmation that the substance is GRAS. A review of McNeil's February 18, 1999, submission, however, reveals significant evidence supporting the safety of the use of plant stanol esters at the levels necessary to justify a health claim. Moreover, 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. FDA therefore concludes that the petitioner has satisfied the requirement of §101.14(b)(3)(ii) to demonstrate that the use of plant stanol esters in conventional foods at the levels necessary to justify a claim is safe and lawful.

ii. *Dietary supplements.* The petitioner submitted a new dietary ingredient notification for plant stanol esters on August 19, 1999.[2] The new dietary ingredient notification contained several papers that reported the results of studies conducted in humans to test hypocholesterolemic effects of plant stanol esters as well as a reference to the plant stanol ester petitioner's GRAS submission of February 18, 1999, and the agency's response to this submission in a letter dated May 17, 1999 (Ref. 46). In FDA's judgment, the studies submitted in the plant stanol esters new dietary ingredient notification and GRAS submission appeared to provide an adequate basis that a dietary

[2] The notification states that McNeil does not believeplant stanol esters to be a new dietary ingredient requiring submission of a premarket notification, but that McNeil is voluntarily submitting the information that would be required as part of such a notification "for the purpose of providing the Food and Drug Administration with advance notice concerning its dietary ingredient" (Ref. 49).

supplement containing plant stanol esters would reasonably be expected to be safe. Therefore, the agency did not respond to the new dietary ingredient notification. Because the safety standard in section 413(a)(2) of the act has been met and the new dietary ingredient notification was submitted more than 75 days ago, plant stanol esters may now be lawfully marketed as dietary ingredients in dietary supplements. Therefore, FDA concludes that the petitioner has satisfied the requirement of §101.14(b)(3)(ii) to demonstrate that the use of plant stanol esters in dietary supplements at the levels necessary to justify a claim is safe and lawful.

III. Review of Scientific Evidence of the Substance-Disease Relationship

A. Basis for Evaluating the Relationship Between Plant Sterol/Stanol Esters and CHD

FDA's review examined the relationship between plant sterol/stanol esters and CHD by focusing on the effects of dietary intake of this substance on blood cholesterol levels and on the risk of developing CHD. In the 1991 lipids-CVD and dietary fiber-CVD health claim proposals, the agency set forth the scientific basis for the relationship between dietary substances and CVD (56 FR 60727 at 60728 and 56 FR 60582 at 60583). In those documents, the agency stated that there are many risk factors that contribute to the development of CVD, and specifically CHD, one of the most serious forms of CVD and among the leading causes of death and disability. The agency also stated that there is general agreement that elevated blood cholesterol levels are one of the major modifiable risk factors in the development of CVD and, more specifically, CHD.

Several Federal agencies and scientific bodies that have reviewed the matter have concluded that there is substantial epidemiologic evidence that high blood levels of total cholesterol and LDL cholesterol are a cause of atherosclerosis and represent major contributors to CHD (56 FR 60727 at 60728, 56 FR 60582 at 60583, Refs. 18 through 20). Factors that decrease total cholesterol and LDL cholesterol will also tend to decrease the risk of CHD. High-intakes of saturated fat and, to a lesser degree, of dietary cholesterol are associated with elevated blood total and LDL cholesterol levels (56 FR 60727 at 60728). Thus, it is generally accepted that blood total cholesterol and LDL cholesterol levels can influence the risk of developing CHD, and, therefore, that dietary factors affecting these blood cholesterol levels affect the risk of CHD (Refs. 18 through 20).

When considering the effect that the diet or components of the diet have on blood (or serum) lipids, it is important to consider the effect that these factors may have on blood levels of high density lipoprotein (HDL) cholesterol. HDL cholesterol appears to have a protective effect against CHD because it is involved in the regulation of cholesterol transport out of cells and to the liver, from which it is ultimately excreted (Refs. 18 and 50).

For these reasons, the agency based its evaluation of the relationship between consumption of plant sterol/stanol esters and the risk of CHD primarily on changes in blood total and LDL cholesterol resulting from dietary intervention with plant sterol/stanol ester-containing products. A secondary consideration was that beneficial changes in total and LDL cholesterol should not be accompanied by potentially adverse changes in HDL cholesterol. This focus is consistent with that used by the agency in deciding on the dietary saturated fat and cholesterol and CHD health claim, §101.75 (56 FR 60727 and 58 FR 2739); the fiber-containing fruits, vegetables, and grain products and CHD claim, §101.77 (56 FR 60582 and 58 FR 2552); the soluble fiber from certain foods and CHD claim, §101.81 (61 FR 296, 62 FR 3584, 62 FR 28234, and 63 FR 8119) and the soy protein and CHD claim, §101.82 (63 FR 62977 and 64 FR 57700).

B. Review of Scientific Evidence

1. Evidence Considered in Reaching the Decision

a. *Plant sterol esters and CHD.* The plant sterol esters petitioner submitted 15 scientific studies (Refs. 51 through 60, 61 and 62 (1 study), 63 and 64 (1 study), and 65 through 67) evaluating the relationship between plant sterol esters or plant sterols and blood cholesterol levels in humans. The studies submitted were conducted between 1953 and 2000. The petition included tables that summarized the outcome of each of the studies and a summary of the evidence.

The plant sterol ester petitioner states that since plant sterol esters are hydrolyzed to free sterols and fatty acids in the gastrointestinal tract (see Refs. 68 through 70), and free sterols are the active moiety of plant sterol esters (see Refs. 69 and 71), the literature on free plant sterols has a direct bearing on this petition (Ref. 1, page 14). The agency agrees that the active moiety of the plant sterol ester is the plant sterol and has concluded that studies of the effectiveness of free plant sterols in blood cholesterol reduction are relevant to the evaluation of the evidence in the plant sterol esters petition. Accordingly, FDA included such studies in its evaluation of the relationship between plant sterol esters and reduced risk of CHD if they met the study selection criteria specified in section III.B.2 of this document.

In several previous diet and CHD health claim rulemakings, the agency began its review of scientific evidence in support of the health claim by considering those studies that were published since 1988, the date of publication of the "Surgeon General's Report on Nutrition and Health" (Ref. 18), which is the most recent and comprehensive Federal review of the scientific evidence on dietary factors and CHD. That approach was not possible in this instance, however, as the "Surgeon General's Report on Nutrition and Health" does not discuss the effects of dietary plant sterols or plant sterol esters on blood cholesterol or CHD. A discussion of the role of dietary sterols in CHD does appear in another roughly contemporaneous source, the National Academy Press publication "Diet and Health: Implications for Reducing Chronic Disease Risk" (Ref. 19), which was issued in 1989. That publication states:

Long ago, plant sterols (beta-sitosterol and related compounds) were found to prevent absorption of dietary cholesterol (Best et al., 1955; Farquhar and Sokolow, 1958; Farquhar et al., 1956; Lees et al., 1977; Peterson et al., 1959), apparently by blocking absorption of cholesterol in the intestine (Davis, 1955; Grundy and Mok, 1977; Jandacek et al., 1977; Mattson et al., 1977). More recent reports indicate that these compounds may be more effective in small doses than previously believed (Mattson et al., 1982).

This discussion highlights the previous and current emphasis of research on the topic. Investigations in the 1950's reported the effects of plant sterols on cholesterol absorption using animal models and in a few human studies; work in the 1970's examined beta-sitosterol in the form of a drug product to lower cholesterol in humans. In fact, beta-sitosterol is approved for use as a drug to lower cholesterol (Refs. 72 and 73). More recent research has focused on smaller amounts of plant sterols that are solubilized as fatty acid esters of plant sterols in food products. The agency considers the older research to be of little relevance to the petitioned health claim because it concerned forms and amounts of the substance different from those that are the subject of the

petition. Therefore, FDA included in its review only those studies published from 1982 (the date the National Academy Press publication refers to for the more recent research reports (Ref. 19)) to the present among those submitted by the petitioner (Refs. 51, 52, 57, 58, 61 and 62 (1 study), 63 and 64 (1 study), 65, and 67). In addition to eight studies submitted by the petitioner, FDA also considered two other studies (Refs. 74 and 75) concerning the effects of plant sterol esters on blood cholesterol. These two studies were identified by a literature search (Ref. 76) performed to verify that the totality of publicly available scientific evidence had been submitted to the agency.

In addition to the human studies previously discussed, the plant sterol esters petition also presented some findings from studies that employed animal models. Human studies are weighted most heavily in the evaluation of evidence on a diet and disease relationship; animal model studies can be considered as supporting evidence but cannot serve as the sole basis for establishing that a diet and disease relationship exists. Because there were enough well-controlled studies in humans to evaluate the relationship between plant sterol esters and CHD, FDA did not closely review the studies in animals.

b. *Plant stanol esters and CHD.* The plant stanol ester petitioner submitted 21 scientific studies (Refs. 63 and 64 (1 study), and 67, 77 through 80, 81 and 82 (1 study), and 83 through 96) evaluating the relationship between plant stanol esters or plant stanols and blood cholesterol levels in humans. The studies submitted were conducted between 1993 and 2000. The petition included tables that summarized the outcome of each of the studies and a summary of the evidence.

Stanol esters are hydrolyzed in the gastrointestinal tract to fatty acids and free stanols, and investigators believe there is physiological equivalence of free stanols and stanol esters in affecting blood cholesterol concentrations. Accordingly, the agency concludes that studies of the effectiveness of free plant stanols in blood cholesterol reduction are relevant to the evaluation of the relationship between plant stanol esters and reduced risk of CHD when such studies meet the study selection criteria specified in section III.B.2 of this document.

In several previous diet and CHD health claim rulemakings, the agency began its review of scientific evidence in support of the health claim by considering those studies that were published since 1988, the date of publication of the "Surgeon General's Report on Nutrition and Health" (Ref. 18), which is the most recent and comprehensive Federal review of the scientific evidence on dietary factors and CHD. The "Surgeon General's Report on Nutrition and Health," however, did not discuss the effects of dietary plant stanol esters on blood cholesterol or CHD. Although a discussion of the role of dietary sterols in CHD appears in the 1989 National Academy Press publication "Diet and Health: Implications for Reducing Chronic Disease Risk," there is no mention of plant stanol esters in this publication (Ref. 19). In fact, research on the cholesterol-lowering capacity of plant stanol esters has been a recent development. The agency used 1992 as a starting point for its scientific evaluation, because this is the year that the earliest study evaluating the effects of plant stanol esters on blood cholesterol was published. The agency included in its review 24 studies published from 1992 to present that were submitted by the petitioner or otherwise identified (Refs. 58, 63 and 64 (1 study), 67, 74, 77 through 80, 81 and 82 (1 study), and 83 through 97). Of these, 21 studies (Refs. 63 and 64 (1 study), 67, 77 through 80, 81 and 82 (1 study), and 83 through 96) were submitted by the petitioner. Two studies (Refs. 74 and 97) were identified by a literature search (Ref. 76) performed to verify that the totality of publicly available scientific evidence had been submitted to the agency. In addition, one recently published study that was submitted in the plant sterol esters petition included administration of plant stanol esters (Ref. 58). This study was included in the plant stanol ester review.

In addition to the published studies previously discussed, the plant stanol ester petitioner submitted a summary of 10 unpublished studies (Ref. 8, pages 59 through 69). The unpublished studies did not weigh heavily in the agency's review because health claims are authorized based on the totality of publicly available scientific evidence (see section 403(r)(3)(B)(i) of the act and §101.14(c)) and because the summaries of these studies lacked sufficient detail on study design and methodologies.

2. Criteria for Selection of Human Studies on Plant Sterol/Stanol Esters and CHD

The criteria that the agency used to select the most pertinent studies in both health claim petitions were consistent with those that the agency used in evaluating the relationship between other substances and CHD. These criteria were that the studies: (1) Present data and adequate descriptions of the study design and methods; (2) be available in English; (3) include estimates of, or enough information to estimate, intakes of plant sterols or stanols and their esters; (4) include direct measurement of blood total cholesterol and other blood lipids related to CHD; and (5) be conducted in persons who represent the general U.S. population. In the case of criterion (5), these persons can be considered to be adults with blood total cholesterol levels less than 300 mg/dL, as explained below.

In a previous rulemaking (62 FR 28234 at 28238 and 63 FR 8103 at 8107), the agency concluded that hypercholesterolemic study populations were relevant to the general population because, based on data from the National Health and Nutrition Examination Surveys (NHANES) III, the prevalence of individuals with elevated blood cholesterol (i.e., 200 mg/dL or greater) is high, i.e., approximately 51 percent of adults (Ref. 21). The proportion of adults having moderately elevated blood cholesterol levels (i.e., between 200 and 239 mg/dL) was estimated to be approximately 31 percent, and the proportion of adults with high blood cholesterol levels (240 mg/dL or greater) was estimated to be approximately 20 percent (Ref. 21). It is also estimated that 52 million Americans 20 years of age and older would be candidates for dietary intervention to lower blood cholesterol (Ref. 21). As the leading cause of death in this country, CHD is a disease for which the general U.S. population is at risk. Since more than half of American adults have mildly to moderately elevated blood cholesterol levels, FDA considers studies in these populations to be representative of a large segment of the general population. Accordingly, in this rule, the agency has reviewed and considered the evidence of effects of plant sterol/stanol esters on blood cholesterol in mildly and moderately hypercholesterolemic subjects as well as subjects with cholesterol levels in the normal range.

In selecting human studies for review, the agency excluded studies that were published in abstract form because they lacked sufficient detail on study design and methodologies, and because they lacked necessary primary data. Studies using special population groups, such as adults with very high serum cholesterol (mean greater than 300 mg/dL), children with hypercholesterolemia, and persons who had already experienced a myocardial infarction (heart attack) or

who had a diagnosis of noninsulin dependent diabetes mellitus, were also excluded because of questions about their relevance to the general U.S. population.

3. Criteria for Evaluating the Relationship Between Plant Sterol/Stanol Esters and CHD

The evaluation of study design, protocol, measurement, and statistical issues for individual studies serves as the starting point from which FDA determines the overall strengths and weaknesses of the data and assesses the weight of the evidence. FDA's "Guidance for Industry: Significant Scientific Agreement in the Review of Health Claims for Conventional Foods and Dietary Supplements" articulates the agency's approach to evaluating studies supporting diet/disease relationships (Ref. 98). The criteria that the agency used in evaluating the studies for this rulemaking include: (1) Adequacy and clarity of the design (e.g., was the methodology used in the study clearly described and appropriate for answering the questions posed by the study?); (2) population studied (e.g., was the sample size large enough to provide sufficient statistical power to detect a significant effect?); (3) assessment of intervention or exposure and outcomes (e.g., was the dietary intervention or exposure well defined and appropriately measured?); and (4) statistical methods (e.g., were appropriate statistical analyses applied to the data?).

The general study design characteristics for which the agency looked included selection criteria for subjects, appropriateness of controls, randomization of subjects, blinding, statistical power of the studies, presence of recall bias and interviewer bias, attrition rates (including reasons for attrition), potential for misclassification of individuals with regard to dietary intakes, recognition and control of confounding factors (for example, monitoring body weight and control of weight loss), and appropriateness of statistical tests and comparisons. The agency considered whether the intervention studies that it evaluated had been of long enough duration, greater than or equal to 3 weeks duration, to ensure reasonable stabilization of blood lipids.

As discussed above, dietary saturated fat and cholesterol affect blood cholesterol levels (Refs. 19 and 20). Previous reviews by FDA and other scientific bodies have generally concluded that, in persons with relatively higher baseline levels of blood cholesterol, responses to dietary intervention tend to be of a larger magnitude than is seen in persons with more normal blood cholesterol levels (56 FR 60582 at 60587 and Refs. 19 and 20). To take into account these factors, FDA separately evaluated studies on mildly to moderately hypercholesterolemic individuals (persons with elevated blood total cholesterol levels of 200 to 300 mg/dL) and studies on normocholesterolemic individuals (persons with blood total cholesterol levels in the normal range (< 200 mg/dL)). FDA also separately evaluated studies in which the effects of plant sterol/stanol esters were evaluated as part of a "typical" American diet (approximately 37 percent of calories from fat, 13 percent of calories from saturated fat, and more than 300 mg of cholesterol daily) and studies in which the test protocols incorporated a dietary regimen that limits fat intake such as the National Heart, Lung, and Blood Institute's National Cholesterol Education Program Step I Diet (intake of 8 to 10 percent of total calories from saturated fat, 30 percent or less of calories from total fat, and cholesterol less than 300 mg/d) (Ref. 99).

C. Review of Human Studies

1. Studies Evaluating the Effects of Plant Sterol Esters on Blood Cholesterol

As discussed in section III. B.1.a of this document, FDA reviewed 10 human clinical studies on plant sterol esters or other plant sterols (Refs. 51, 52, 57, 58, 61 and 62 (1 study), 63 and 64 (1 study), 65, 67, and 74 and 75). Of these, nine met the selection criteria listed in section III.B.2 of this document (Refs. 51, 57, 58, 61 and 62 (1 study), 63 and 64 (1 study), 65, 67 and 74 and 75). These studies are summarized in table 1 at the end of this document and discussed below. The remaining study (Ref. 52) failed to meet the inclusion criteria because the population studied (children with familial hypercholesterolemia) was not representative of the general U.S. population. As supporting evidence, the results of one research synthesis study (Ref. 100) that included a number of the plant sterol ester studies submitted in the petition are discussed in section III.C.1.d of this document.

Studies typically report the amount of free plant sterol consumed rather than the amount of plant sterol ester administered. Where possible, we report both the amount of plant sterol ester and the equivalent free sterol.

(a) *Hypercholesterolemics (serum cholesterol < 300 mg/dL): low saturated fat and cholesterol diets.* One study was submitted as a draft in the plant sterol esters petition because it has been submitted for publication, but has not yet been published other than in abstract form (Ref. 62). FDA reviewed this study but considers the results preliminary until a full report of the study has been published. The preliminary results in this study (Refs. 61 and 62 (1 study)) showed a cholesterol-reducing effect of plant sterol esters in hypercholesterolemic subjects who consumed soybean oil sterol esters as part of a low saturated fat and low cholesterol diet. In this study, 224 men and women with mild-to-moderate hypercholesterolemia instructed to follow a National Cholesterol Education Program Step I diet were randomly assigned to one of three groups: (1) control reduced-fat spread, (2) reduced-fat spread containing 1.76 g/d of plant sterol esters (1.1 g/d free plant sterols) (low intake group), or (3) reduced-fat spread containing 3.52 g/d of plant sterol esters (2.2 g/d free plant sterols) (high-intake test group). All subjects consumed 14 g/d of spread in two 7 g servings/day, with food. Subjects in the low- and high-intake groups who consumed "80 percent of scheduled servings had decreases in serum total cholesterol of 5.2 and 6.6 percent, and LDL cholesterol of 7.6 and 8.1 percent, respectively, versus control ($p<0.001$). The difference between the two test groups with regard to serum total and LDL cholesterol levels was not statistically significant. HDL cholesterol responses did not differ among the groups. These preliminary results indicate that a plant sterol ester-containing reduced-fat spread, in a diet low in saturated fat and cholesterol, can reduce cholesterol.

(b) *Hypercholesterolemics (serum cholesterol < 300 mg/dL): "typical" or "usual" diets.* Four studies (Refs. 57, 58, 67, and 74) show a relationship between consumption of plant sterols and reduced blood cholesterol in hypercholesterolemic subjects consuming diets within the range of a typical American diet. A fifth study (Refs. 63 and 64 (1 study)) shows inconclusive results.

Jones et al. (Ref. 58) conducted a controlled feeding crossover study in which diets were based on a fixed-food North American diet formulated to meet Canadian recommended nutrient intakes. This study reported significantly lower plasma total cholesterol (9.1 percent, $p < 0.005$) and LDL cholesterol (13.2 percent, $p < 0.02$) in male subjects consuming 2.94 g/d vegetable oil sterol esters (1.84 g/d free plant sterols delivered in 23 g of margarine each day; daily margarine doses were divided into three equal

portions and added to each meal) for 21 days compared to 21 days on control margarine. Plasma HDL cholesterol did not differ across groups and there was no significant weight change shown by the subjects while consuming any of the margarine mixtures.

Hendriks et al. (Ref. 57) reported the effects of feeding three different levels of vegetable oil sterol esters (1.33, 2.58, and 5.18 g/d corresponding to 0.83, 1.61, and 3.24 g/d free plant sterols, respectively) incorporated in spreads (25 g/d of spread replaced an equivalent amount of the spread(s) habitually used; one-half was consumed at lunch, one-half at dinner) in apparently healthy normocholesterolemic and mildly hypercholesterolemic subjects using a randomized, double-blind placebo-controlled balanced incomplete Latin square design with five treatments and four periods. The vegetable oil sterols were esterified to sunflower oil and the degree of esterification was 82 percent. Blood total and LDL cholesterol levels were reduced compared to the control spread ($p < 0.001$) after 3.5 weeks. Blood total cholesterol decreased by 4.9, 5.9, and 6.8 percent for daily consumption of 1.33, 2.58, and 5.18 g/d plant sterol esters, respectively. For LDL cholesterol these decreases were 6.7, 8.5, and 9.9 percent. No significant differences in cholesterol-lowering effect between the three levels of plant sterol esters could be detected. There were no effects on HDL cholesterol. The subjects' body weight differed after daily consumption of 2.58 and 5.18 g plant sterol esters by 0.3 kilogram (kg) ($p < 0.01$), but this small difference in body weight probably did not affect the study findings.

Another study by Jones et al. (Ref. 74) investigated the effects of a mixture of plant sterols and plant stanols. The plant stanol compound sitostanol made up about 20 percent of the mixture by weight. The remaining sterol component of the mixture was composed mostly of the plant sterols sitosterol and campesterol from tall oil (derived from pine wood). The investigators evaluated the cholesterol-lowering properties of this nonesterified plant sterol/stanol mixture in a controlled feeding regimen based on a "prudent," fixed-food North American diet formulated to meet Canadian recommended nutrient intakes. Thirty-two hypercholesterolemic men were fed either a diet of prepared foods alone or the same diet plus 1.7 g per d of the plant sterol/stanol mixture (in 30 g/d of margarine, consumed during 3 meals) for 30 days in a parallel study design. The plant sterol/stanol mixture had no statistically significant effect on plasma total cholesterol concentrations. However, LDL cholesterol concentrations on day 30 had decreased by 8.9 percent ($p < 0.01$) and 24.4 percent ($p < 0.001$) with the control and plant sterol/stanol-enriched diets, respectively. On day 30, LDL cholesterol concentrations were significantly lower ($p < 0.05$) by 15.5 percent in the group consuming the plant sterol/stanol mixture compared to the control group. HDL cholesterol concentrations did not change significantly during the study.

Weststrate and Meijer (Ref. 67) evaluated the effects of different plant sterols on plasma total and LDL cholesterol in normocholesterolemic and mildly hypercholesterolemic subjects consuming their usual diets with the addition of a test or placebo margarine. A randomized double-blind placebo-controlled balanced incomplete Latin square design with five treatments and four periods of 3.5 weeks was utilized to compare the effect of margarines (30 g/d) with added sterol esters from soybean oil (4.8 g/d; 3 g/d free plant sterol), sheanut oil (2.9 g/d) or ricebran oil (1.6 g/d) or with plant stanol esters (4.6 g/d; 2.7 g/d free plant stanols) to a placebo margarine. The sterol esters from soybean oil were mainly esters from sitosterol, campesterol, and stigmasterol. Plasma total and LDL cholesterol concentrations were significantly reduced, by 8.3 and 13.0 percent ($p < 0.05$), respectively, compared to control, in the soybean oil sterol ester margarine group. Similar reductions were reported in the plant stanol ester margarine group (see discussion of this study in section III. C.2.b of this document). Sterols from sheanut oil and rice bran oil did not have a significant effect on cholesterol levels. No effects on HDL cholesterol concentrations were reported in either the control or any of the test groups. The cholesterol-lowering effects of ingestion of plant sterol/stanol esters on blood cholesterol did not differ between normocholesterolemic and mildly hypercholesterolemic subjects. The authors concluded that both the margarine with plant stanol esters and the margarine with sterol esters from soybean oil were effective in lowering blood total and LDL cholesterol levels without affecting HDL cholesterol concentrations. The authors further suggested that incorporating such substances in edible fat-containing products may substantially reduce the risk of cardiovascular disease in the population.

Two reports of apparently the same study (Refs. 63 and 64) gave inconclusive results regarding the relationship between plant sterol consumption and blood cholesterol levels. Interpretation of this study is complicated by design issues such as concerns about sample size and level of plant sterol administered, but both reports are discussed here and summarized in table 1 of this document because they provide information to assist in determining the minimum level of plant sterol esters necessary to provide a health benefit.

Miettinen and Vanhanen (Refs. 63 and 64 (1 study)) reported the effect of small amounts of sitosterol (700 mg/d free sterols) and sitostanol (700 mg/d free stanols) dissolved in 50 g rapeseed oil (RSO) mayonnaise on serum cholesterol in 31 subjects with hypercholesterolemia for 9 weeks. Subjects did not change their diets except for replacing 50 g/d of dietary fat with the 50 g/d of RSO mayonnaise. It appears that these authors later conducted another 9-week phase of the study using sitostanol esters (1.36 g/d plant stanol esters or 800 mg/d free stanols) dissolved in 50 g RSO mayonnaise. The results of this later phase were reported in the Miettinen reference (Ref. 63), together with the earlier results. The Vanhanen reference (Ref. 64) reports only the earlier results for sitosterol and sitostanol. The Vanhanen reference (Ref. 64) reports reduced serum total cholesterol concentrations (8.5 percent) during the RSO mayonnaise run-in period (stabilization period before the intervention begins) compared to values before the run-in period when combining all subjects. Continuation of RSO mayonnaise in the RSO mayonnaise control group (n=8) during the experimental period had no further effect on blood cholesterol (Refs. 63 and 64). ("N" refers to the number of subjects.) Neither sitosterol (n=9) nor sitostanol (n=7) significantly altered serum total cholesterol or LDL cholesterol concentrations compared to the RSO control group (n=8) during the experimental period (Refs. 63 and 64). Sitostanol ester (n=7), however, significantly reduced serum total and LDL cholesterol levels compared to the RSO control group (Ref. 63). Furthermore, serum total cholesterol was significantly reduced by 4 percent ($p < 0.05$) during the experimental period in an analysis, which compared the combined plant sterol/stanol groups (sitostanol, sitosterol, and sitostanol ester groups; n=23) to the RSO control group (n=8) (Ref. 63). HDL cholesterol did not change in the plant sterol group compared to the RSO control group (Ref. 63).

The agency notes that it is difficult to decipher from the descriptions in these

reports the amount of plant sterol that was consumed and the level of cholesterol-lowering that was observed. For the sitosterol group, as an example, the method section states that 722 mg/d of sitosterol was added to the RSO mayonnaise, yet the abstract mentions that the RSO mayonnaise contained an additional 625 mg/d of sitosterol (Ref. 64). The results section of the Miettinen reference (Ref. 63) notes that in the combined plant sterol/stanol groups, total and LDL cholesterol levels were slightly but significantly decreased up to 4 percent, yet the abstract states that serum total cholesterol was reduced by about 5 percent in the combined plant sterol/stanol groups. Therefore, FDA considers the results in these reports inconclusive because of inconsistencies in the descriptions of methods and results.

(c) *Normocholesterolemics: "typical" or "usual" diets.* The results of three studies (Refs. 51, 65, and 75) support a cholesterol-lowering effect of plant sterols in subjects with normal cholesterol values.

Ayesh et al. (Ref. 51), in a controlled feeding study, reported significantly lower serum total cholesterol (18 percent, $p < 0.0001$) and LDL cholesterol (23 percent, $p < 0.0001$) in subjects consuming 13.8 g/d vegetable oil sterol esters (8.6 g/d free plant sterols delivered in 40 g of margarine each day consumed with breakfast and dinner under supervision) for 21 days in males and 28 days in females, compared to subjects consuming a control margarine. These results were calculated as the difference from baseline to days 21 for male and 28 for female; analysis of covariance was adjusted for gender. There was no significant difference in effect on HDL cholesterol between control and plant sterol groups.

In a double-blind crossover study, Sierksma et al. (Ref. 75) showed that daily consumption of 25 g of a spread enriched with free soybean oil sterols (0.8 g/d) for 3 weeks lowered plasma total and LDL cholesterol concentrations respectively by 3.8 percent ($p < 0.05$) and 6 percent ($p < 0.05$) compared with a placebo spread. No effect on plasma HDL cholesterol was found. Subjects followed their usual diets, except that they replaced their usual spread with the test or placebo spread. The investigators also tested sheanut-oil sterols (3.3 g/d) in 25 g of spread and found that the sheanut-oil spread did not lower plasma total and LDL cholesterol levels. The sheanut-oil sterols were primarily phenolic acid esters of 4,4-dimethyl sterols, whereas the soybean-oil product contained 4-desmethyl sterols (the class of sterols containing no methyl group at the carbon 4 atom). The structure of 4-desmethyl sterols is more similar to cholesterol than the structure of 4,4-dimethyl sterols. The investigators stated that soybean-oil sterol structural similarity to cholesterol may offer increased competition with cholesterol for incorporation in mixed micelles, the most likely mechanism for the blood cholesterol-lowering action of plant sterols.

Pelletier et al. (Ref. 65) reported reductions in blood total cholesterol (10 percent, $p < 0.001$) and LDL cholesterol (15 percent, $p < 0.001$), compared to a control period, in subjects consuming 740 mg/d of soybean oil sterols (nonesterified) in 50 g/d of butter for 4 weeks. These results were obtained in a crossover experiment in 12 normocholesterolemic men consuming a controlled, but "normal" diet. The total fat intake as a percent of energy was 36.4 percent during both the control and the plant sterol-feeding period. The cholesterol intake during the control period was 436 mg/d; it was 410 mg/d during the plant sterol-feeding period. The diets were designed to have a plant sterol to cholesterol ratio of 2.0, which has repeatedly been shown to affect cholesterol levels in various animal models. There was no significant difference in effect on HDL cholesterol between control and plant sterol groups.

(d) *Other studies: research synthesis study.* FDA considered the results of a March 25, 2000, research synthesis study by Law (Ref. 100) of the effect of plant sterols and stanols on serum cholesterol concentrations. While evaluation of research synthesis studies, including meta-analyses, is of interest, the appropriateness of such analytical techniques in establishing substance/disease relationships has not been determined. There are ongoing efforts to identify criteria and critical factors to consider in both conducting and using such analyses, but standardization of this methodology is still emerging. Therefore, this research synthesis study was considered as supporting evidence but did not weigh heavily within the body of evidence on the relationship between plant sterol/stanol esters and CHD.

Law performed a research synthesis analysis of the effect of plant sterols and stanols on serum cholesterol concentrations by pooling data from randomized trials identified by a Medline search using the term "plant sterols." Law obtained additional data for analysis from other studies cited in papers and review articles. A total of 14 studies that employed either a parallel or crossover design were incorporated in the analysis, consisting of 20 dose comparisons of either plant sterols or plant stanols to a control vehicle. The data described the effects on serum LDL cholesterol concentrations obtained from using spreads (or in some cases, mayonnaise, olive oil, or butter) with and without added plant sterols or stanols. Studies that included children with familial hypercholesterolemia were excluded from the research synthesis analysis. Law included in the research synthesis analysis study populations with severe hypercholesterolemia (mean serum total cholesterol greater than 300 mg/dL) and study populations with previous myocardial infarction or noninsulin dependent diabetes mellitus, as well as study populations with mildly and moderately hypercholesterolemic and/or normal cholesterol concentrations.

Based on the placebo-adjusted reduction in serum LDL cholesterol, the analysis indicated that 2 g of plant sterol (equivalent to 3.2 g/d of plant sterol esters) or plant stanol (equivalent to 3.4 g/d of plant stanol esters) added to a daily intake of spread (or mayonnaise, olive oil, or butter) reduces serum concentrations of LDL cholesterol by an average of 20.9 mg/dL (0.54 millimole per liter (mmol/l)) in people aged 50 to 59 ($p=0.005$), 16.6 mg/dL (0.43 mmol/l) in those aged 40 to 49 ($p=0.005$), and 12.8 mg/dL (0.33 mmol/l) in those aged 30 to 39 ($p=0.005$). The results indicated that the reduction in the concentration of LDL cholesterol at each dose is significantly greater in older people versus younger people. The reductions in blood total cholesterol concentrations were similar to the LDL cholesterol reductions and there was little change in serum concentrations of HDL cholesterol. The results of this analysis also suggested that doses greater than about 2 g of plant sterol (3.2 g/d of plant sterol esters) or stanol (3.4 g/d of plant stanol esters) per day would not result in further reduction in LDL cholesterol (Ref. 100).

Observational studies and randomized trials concerning the relationship between serum cholesterol and the risk of heart disease (Ref. 101) indicate that for people aged 50 to 59, a reduction in LDL cholesterol of about 19.4 mg/dL (0.5 mmol/l) translates into a 25 percent reduction in the risk of heart disease after about 2 years. Studies administering plant sterols and stanols have demonstrated the potential to provide this protection. According to Law, the cholesterol-lowering capacity of plant sterols and stanols is even larger than the effect that could be expected to occur if people ate less animal fat (or saturated fat) (Ref. 100).

(e) *Summary.* In one preliminary report of hypercholesterolemic subjects consuming a low saturated fat and low cholesterol diet (Refs. 61 and 62 (1 study)), plant sterol ester intake was associated with statistically significant decreases in serum total and LDL cholesterol levels. Levels of HDL cholesterol did not change during plant sterol consumption compared to controls. Levels of plant sterol ester found to be effective in lowering serum total and LDL cholesterol levels, in the context of a diet low in saturated fat and cholesterol, were reported to be 1.76 and 3.52 g/d (1.1 and 2.2 g/d of free plant sterol) (Refs. 61 and 62 (1 study)).

In four (Refs. 57, 58, 67, and 74) of five (Refs. 57, 58, 67, 74, and 63 and 64 (1 study)) studies of hypercholesterolemic subjects consuming "usual" diets that were generally high in total fat, saturated fat and cholesterol, plant sterol intake was associated with statistically significant decreases in blood total and/or LDL cholesterol levels. Levels of HDL cholesterol were found to be unchanged by consumption of diets containing plant sterol (Refs. 57, 58, 67, 74, and 63 and 64 (1 study)). Levels of plant sterol ester found to be effective in lowering blood total and/or LDL cholesterol levels, in the context of a usual diet, ranged in these studies from 1.33 (Ref. 57) to 5.18 g/d (Ref. 57) (equivalent to 0.83 to 3.24 g/d of free plant sterol).

The results of one study in hypercholesterolemic subjects consuming "usual" diets (Refs. 63 and 64 (1 study)) are inconclusive; this may be due to lack of statistical power (e.g., sample size too small to detect the hypothesized difference between groups) or too low a dose of plant sterols to provide an effect. As previously discussed, the descriptions of methods and results also were inconsistent and difficult to interpret. These investigators report no effect of 700 mg/d of plant sterol (equivalent to 1.12 g/d of plant sterol esters) on blood cholesterol levels. However, when the results of three test groups (700 mg/d plant sterol, 700 mg/d plant stanol, 1.36 mg/d plant stanol ester) were pooled and compared to a control group, a statistically significant effect on reducing serum total cholesterol emerged, perhaps because the increased number of subjects in this pooled analysis artificially increased the ability to detect a difference.

In three of three studies (Refs. 51, 65, and 75) of healthy adults with normal blood cholesterol levels consuming a "usual" diet, plant sterol intake was associated with statistically significant decreases in both blood total and LDL cholesterol levels. HDL cholesterol levels were not significantly affected by plant sterol intake. Levels of plant sterol found to be effective in lowering blood total and LDL cholesterol ranged in these studies from 0.74 (Ref. 65) to 8.6 g/d (equivalent to 1.2 to 13.8 g/d of plant sterol esters) (Ref. 51).

Based on these studies, FDA finds there is scientific evidence for a consistent, clinically significant effect of plant sterol esters on blood total and LDL cholesterol. The cholesterol-lowering effect of plant sterol esters is consistent in both mildly and moderately hypercholesterolemic populations and in populations with normal cholesterol concentrations. The cholesterol-lowering effect of plant sterol esters has been reported in addition to the effects of a low saturated fat and low cholesterol diet. It has been consistently reported that plant sterols do not affect HDL cholesterol levels. These conclusions are drawn from the review of the well controlled clinical studies and are supported by the research synthesis study of Law (Ref. 100).

2. Studies Evaluating the Effects of Plant Stanol Esters on Blood Cholesterol

As discussed in section III.B.1.b of this document, FDA reviewed 24 studies (Refs. 58, 63 and 64 (1 study), 67, 74, 77 through 80, 81 and 82 (1 study), and 83 through 97) on plant stanols, including both free and esterified forms. Of these, 15 met the selection criteria listed in section III.B.2. of this document (Refs. 58, 63 and 64 (1 study), 67, 74, 77, 78, 80, 81 and 82 (1 study), 88 through 92, 94, and 97). These studies are summarized in table 2 at the end of this document and discussed below. The nine remaining studies (Refs. 79, 83 through 87, 93, 95, and 96) failed to meet the selection criteria because of insufficient information to evaluate the design and method of the study or because the populations studied were not considered representative of the general U.S. adult population. For example, some of the studies were performed in children with type II or familial hypercholesterolemia; others used adult subjects with mean serum total cholesterol levels > 300 mg/dL or subjects with preexisting disease (e.g., diabetes). As supporting evidence, the results of a community intervention study (Ref. 102) and a research synthesis study (Ref. 100) that included a number of the plant stanol ester studies submitted in the petition are discussed in section III.C.2.d of this document.

Studies typically report the amount of free plant stanol consumed, rather than the levels of stanol esters administered. Where possible, we report both the amount of plant stanol ester and the equivalent free stanol.

(a) *Hypercholesterolemics (serum cholesterol < 300 mg/dL): low saturated fat and cholesterol diets.* Two studies (Refs. 77 and 80) showed a relationship between consumption of plant stanol esters and reduced blood cholesterol in hypercholesterolemic subjects who consumed plant stanol esters as part of a low saturated fat and low cholesterol diet.

Andersson et al. (Ref. 80) randomized subjects to receive one of three test diets: Either a low fat margarine containing 3.4 g/d plant stanol esters (2 g/d of plant stanols) with a controlled, low saturated fat, low cholesterol diet; a control low fat margarine containing no plant stanol esters with a controlled, low saturated fat, low cholesterol diet; or to continue their normal diet with the addition of the margarine containing 3.4 g/d plant stanol esters (2 g/d of plant stanols). Serum total and LDL cholesterol were reduced in all three groups after 8 weeks. The group consuming the margarine containing plant stanol esters with the low saturated fat, low cholesterol diet showed 12 percent ($p < 0.0035$) and 15 percent ($p < 0.0158$) reductions in serum total and LDL cholesterol levels, respectively, compared to the group that consumed a control low fat margarine with a controlled, low saturated fat, low cholesterol diet. The serum total and LDL cholesterol reductions were reported to be 4 percent ($p < 0.0059$) and 6 percent ($p < 0.0034$), respectively, for the group consuming the margarine containing plant stanol esters with the low saturated fat, low cholesterol diet compared to the group consuming the margarine containing plant stanol esters with a normal diet. Although a normal diet and control margarine group was not included, this study suggests that 3.4 g/d of plant stanol esters in conjunction with a normal or controlled, low saturated fat, low cholesterol diet can significantly lower serum cholesterol levels. There was no change in HDL cholesterol levels in the normal diet, plant stanol ester margarine group. The study results suggest that the reduction in serum cholesterol levels is significantly greater when the plant stanol esters are consumed as part of a diet low in saturated fat and cholesterol. HDL cholesterol was decreased, however, in subjects in both low saturated fat, low cholesterol diet groups, and this result was statistically significant in the group that consumed the plant stanol ester margarine in conjunction with this diet.

Hallikainen et al. (Ref. 77) randomly assigned 55 mildly hypercholesterolemic subjects, after a 4-week high fat diet (36 to 38 percent of energy from fat), to one of three low fat margarine groups: a 3.9 g/d (2.31 g/d of free plant stanols) wood stanol ester-containing margarine, a 3.9 g/d (2.16 g/d of free plant stanols) vegetable oil stanol ester-containing margarine, or a control margarine group. The groups consumed the margarines for 8 weeks as part of a diet resembling that of the National Heart, Lung, and Blood Institute's National Cholesterol Education Program Step II diet (a diet in which saturated fat intake is less than 7 percent of calories and cholesterol is less than 200 mg/d) (Ref. 99). During the experimental period, the serum total cholesterol reduction was significantly greater in the wood stanol ester-containing margarine (10.6 percent, $p < 0.001$) and vegetable oil stanol ester-containing margarine (8.1 percent, $p < 0.05$) groups than in the control group, but no significant differences were found between the wood stanol ester-containing margarine and vegetable oil stanol ester-containing margarine groups. The LDL cholesterol reduction was significantly greater in the wood stanol ester-containing margarine (13.7 percent $p < 0.01$) group than in the control group. For the vegetable oil stanol ester-containing margarine group, the LDL cholesterol reduction was 8.6 percent greater than in the control, but the difference was not statistically significant ($p= 0.072$). However, there were no significant differences reported between the wood stanol ester-containing margarine and vegetable oil stanol ester-containing margarine groups for LDL cholesterol. HDL cholesterol concentrations did not change during the study. The authors state, "* * * that plant stanols can reduce serum cholesterol concentrations, even in conjunction with a markedly low dietary cholesterol intake, indicates that plant stanols must inhibit not only the absorption of dietary cholesterol but also that of biliary cholesterol."

The results of another study (Ref. 97) did not show a relationship between consumption of plant stanols and blood cholesterol in hypercholesterolemic subjects who consumed plant stanols as part of a low saturated fat and low cholesterol diet. In this study, Denke (Ref. 97) tested the cholesterol-lowering effects of dietary supplementation with plant stanols (3 g/d suspended in safflower oil and packed into gelatin capsules) in 33 men with moderate hypercholesterolemia who were consuming a Step 1 diet. Plant stanol consumption did not significantly lower plasma total cholesterol or LDL cholesterol compared with the Step 1 diet alone. HDL cholesterol levels were also unchanged. The authors state that although previous reports suggested that low dose plant stanol consumption is an effective means of reducing plasma cholesterol concentrations, its effectiveness may be attenuated when the diet is low in cholesterol. The agency notes that, unlike several of the studies submitted with the petition, this study was not a randomized, placebo-controlled, double-blind study, but rather a fixed sequence design. One result of this design was that during the plant stanol dietary supplement phase the subjects consumed an additional 12 g of fat that they did not consume in other phases because each dietary supplement contained 1g of safflower oil and subjects were instructed to consume 4 capsules per meal (subjects were to consume a total of 12 capsules (3000 mg) in three divided doses during three meals). The agency does not give as much weight to this study as it does the studies in which subjects were randomly assigned to placebo or plant stanol arms of a study with all else being equal among the participants.

(b) *Hypercholesterolemics (serum cholesterol < 300 mg/dL): "typical" or "usual" diets.* Eight studies (Refs. 63 and 64 (1 study), 67, 78, 81 and 82 (1 study), 88 through 90, and 94) show a relationship between consumption of plant stanols and reduced blood total and LDL cholesterol in hypercholesterolemic subjects consuming diets within the range of a typical American diet. Two studies (Refs. 58 and 74) show a relationship between consumption of plant stanols and reduced LDL cholesterol, but not blood total cholesterol, in the same category of subjects consuming diets within the range of a typical American diet.

Hallikainen et al. (Ref. 88) conducted a single-blind, crossover study in which 22 hypercholesterolemic subjects consumed margarine containing four different doses of plant stanol esters, including 1.4, 2.7, 4.1, and 5.4 g/d (0.8, 1.6, 2.4, and 3.2 g/d of free plant stanols) for 4 weeks each. These test margarine phases were compared to a control margarine phase, also 4 weeks long. All subjects followed the same standardized diet throughout the study, and the order of the margarine phases was randomized. Serum total cholesterol concentration decreased (calculated in reference to control) by 2.8 percent for the 1.4 g/d dose ($p=0.384$), 6.8 percent for the 2.7 g/d dose ($p< 0.001$), 10.3 percent for the 4.1 g/d dose ($p<0.001$) and 11.3 percent ($p< 0.001$) for the 5.4 g/d dose of plant stanol esters. The respective decreases for LDL cholesterol were 1.7 percent ($p=0.892$), 5.6 percent ($p< 0.05$), 9.7 percent ($p<0.001$) and 10.4 percent ($p<0.001$). Although decreases were numerically greater with 4.1 and 5.4 g doses than with the 2.7 g dose, these differences were not statistically significant ($p=0.054$-0.516). This study demonstrates that at least 2.7 g/d of plant stanol esters can significantly reduce both serum total cholesterol and LDL cholesterol levels by at least 5.6 percent compared to control. No statistically significant changes in HDL cholesterol were observed with any of the plant stanol ester margarines.

Gylling and Miettinen (Ref. 78) reported the serum cholesterol-lowering effects of feeding different campestanol/sitostanol mixtures in margarine or butter in 23 postmenopausal women using a double-blind crossover design. The participants were randomly allocated to study periods where they consumed 25 g/d of plant stanol-containing rapeseed oil margarine with either 5.4 g sitostanol ester-rich (3.18 g of free plant stanols; wood-derived plant stanol esters with a campestanol to sitostanol ratio 1:11) plant stanol esters or 5.7 g campestanol ester-rich (3.16 g of free plant stanols; vegetable oil-derived plant stanol esters with a campestanol to sitostanol ratio 1:2) plant stanol esters. After 6 weeks, subjects consumed the other margarine for an additional 6 weeks. Following an 8 week home diet wash-out period, 21 of the subjects were randomly assigned to consume either 25 g of butter or 4.1 g/d plant stanol esters (2.43 g/d of free plant stanols with a campestanol to sitostanol ratio 1:1) in 25 g of butter for an additional 5 weeks. Throughout the study, subjects consumed their usual diets, except that they were instructed to substitute the 25 g/d of butter or margarine consumed as part of the study for 25 g of their normal daily fat intake. Both the wood and vegetable stanol ester margarines lowered serum total cholesterol by 4 and 6 percent, respectively, compared to baseline ($p < 0.05$ for both). LDL cholesterol was reduced by 8 and 10 percent with the wood and vegetable stanol ester margarines, respectively, versus baseline ($p < 0.05$ for both). Furthermore, HDL cholesterol was increased by 6 and 5 percent ($p < 0.05$) with the wood and vegetable stanol ester margarines, respectively, versus baseline, so the LDL/HDL cholesterol ratio was reduced by 15 percent ($p <$

0.05 for both). The two plant stanol mixtures in margarine appeared equally effective in reducing serum cholesterol. Butter alone increased serum total and LDL cholesterol by 4 percent ($p < 0.05$ for total cholesterol, not statistically significant for LDL cholesterol). Although the plant stanol ester butter did not significantly reduce serum total and LDL cholesterol compared to baseline, the plant stanol ester butter was found to decrease serum total cholesterol by 8 percent and LDL cholesterol by 12 percent ($p < 0.05$ for both) compared to butter alone. There was no significant change in HDL cholesterol between the two butter groups. The study reported that plant stanol esters are able to decrease serum total and LDL cholesterol in a saturated environment, i.e., when plant stanol ester is consumed in butter, a high saturated-fat food, and compared to the effects of butter without plant stanol esters. The observation that the plant stanol ester butter did not reduce blood cholesterol levels compared to baseline suggests that plant stanol esters do not completely counteract the impact of a high saturated-fat diet on blood cholesterol levels.

Nguyen et al. (Ref. 90) examined the blood cholesterol-lowering effects in subjects consuming either a European spread containing 5.1 g/d plant stanol esters (3 g/d free plant stanols), a U.S.-reformulated spread containing 5.1 g/d plant stanol esters (3 g/d free plant stanols), a U.S.-reformulated spread containing 3.4 g/d plant stanol esters (2 g/d of free plant stanols), or a U.S.-reformulated spread without plant stanol esters for 8 weeks. The subjects consumed a total of 24 g of spread in three 8 g servings a day, but made no other dietary changes. Serum total cholesterol ($p < 0.001$) and LDL cholesterol ($p < 0.02$) levels were significantly reduced in all three test groups compared with the placebo group at all time points during the ingredient phase. The U.S. spread containing 5.1 g/d plant stanol esters lowered serum total and LDL cholesterol by 6.4 and 10.1 percent, respectively, when compared to baseline ($p < 0.001$). Subjects consuming the 5.1 g/d plant stanol esters European spread achieved a 4.7 percent reduction in serum total cholesterol and a 5.2 percent reduction in LDL cholesterol compared to baseline ($p < 0.001$). The 3.4 g/d plant stanol ester U.S. spread group showed a 4.1 percent reduction in both serum total and LDL cholesterol levels compared to baseline ($p < 0.001$). HDL cholesterol levels were unchanged throughout the study.

Weststrate and Meijer (Ref. 67) evaluated the effects of different plant sterols and stanols on plasma total and LDL cholesterol in normocholesterolemic and mildly hypercholesterolemic subjects. The subjects consumed their usual diets with the addition of a test or placebo margarine. A randomized double-blind placebo-controlled balanced incomplete Latin square design with five treatments and four periods of 3.5 weeks was utilized to compare the effect of margarines (30 g/d) with added plant stanol esters (4.6 g/d; 2.7 g/d free plant stanols), or with added plant sterol esters from sheanut oil (2.9 g/d), ricebran oil (1.6 g/d), or soybean oil (4.8 g/d; 3 g/d free plant sterol) to a placebo margarine. Plasma total and LDL cholesterol concentrations were significantly reduced by 7.3 and 13.0 percent ($p < 0.05$), respectively, compared to control, in the plant stanol ester margarine group. Similar reductions were reported in the soybean oil sterol ester margarine group (see discussion of this study in section III.C.1.b of this document). No effect on HDL cholesterol concentrations was reported during the study.

In a long term study conducted in Finland (Ref. 89), 153 mildly hypercholesterolemic subjects were instructed to consume 24 g/d of canola oil margarine or the same margarine with added plant stanol esters for a targeted consumption of 5.1 g/d plant stanol esters (3 g/d free plant stanols), without other dietary changes. At the end of 6 months, those consuming plant stanol esters were randomly assigned either to continue the test margarine with a targeted intake of 5.1 g/d plant stanol esters or to switch to a targeted intake of 3.4 g/d plant stanol esters (2 g/d free plant stanols) for an additional 6 months. The control group also continued for another 6 months. Based on measured margarine consumption, average plant stanol ester intakes were 4.4 g/d (in the 5.1 g/d target group) and 3.1 g/d (in the 3.4 g/d target group). The mean 1 year reduction in serum total cholesterol was 10.2 percent in the 4.4 g/d plant stanol ester group, as compared with an increase of 0.1 percent in the control group. The difference in the change in serum total cholesterol concentration between the two groups was −24 mg/dL ($p < 0.01$). The respective reductions in LDL cholesterol were 14.1 percent in the 4.4 g/d plant stanol ester group and 1.1 percent in the control group. The differences in the change in LDL cholesterol concentration between the two groups was −21 mg/dL ($p < 0.001$). Significant reductions in serum total and LDL cholesterol were also reported after consuming plant stanol esters for 6 months. Unlike the group consuming 4.4 g/d of plant stanol esters for 12 months, where continued reductions in serum total and LDL cholesterol were observed from 6 to 12 months, the reduction in plant stanol ester intake to 3.1 g/d at 6 months was not followed by any further decrease in the serum total and LDL cholesterol concentrations. Serum HDL cholesterol concentrations were not affected by plant stanol esters.

Vanhanen et al. (Ref. 94) reported the hypocholesterolemic effects of 1.36 g/d of plant stanol esters (800 mg/d of free plant stanols) in RSO mayonnaise for 9 weeks followed by 6 weeks of consumption of 3.4 g/d of plant stanol esters (2 g/d of free plant stanols) in RSO mayonnaise compared to a group receiving RSO mayonnaise alone. Subjects consumed their usual diets, except that they were instructed to substitute the RSO mayonnaise for 50 g/d of their normal daily fat intake. After 9 weeks of consumption of the lower dose plant stanol ester mayonnaise, the changes in serum levels of total and LDL cholesterol were −4.1 percent ($p < 0.05$) and −10.3 percent (not statistically significant), respectively, as compared to the control. Greater reductions in both serum total and LDL cholesterol were observed after consumption of 3.4 g/d of plant stanol esters for an additional 6 weeks ($p < 0.05$). The changes in serum levels of total and LDL cholesterol were −9.3 percent and −15.2 percent, respectively, for subjects consuming 3.4 g/d of plant stanol esters as compared to control. Plant stanol ester consumption in RSO mayonnaise did not change HDL cholesterol levels compared to control RSO mayonnaise.

Blomqvist et al. (Ref. 81) and Vanhanen et al. (Ref. 82) separately reported the results of another study showing plasma cholesterol-lowering effects of plant stanol esters dissolved in RSO mayonnaise. After subjects replaced 50 g of their daily fat intake by 50 g of RSO mayonnaise for 4 weeks, they were randomized into two groups, one that continued with the original RSO mayonnaise (control group) and the other with RSO mayonnaise in which 5.8 g of plant stanol ester was dissolved (3.4 g/d of free plant stanols in 50 g of mayonnaise preparation). After 6 weeks on the plant stanol ester-enriched diet, plasma total and LDL cholesterol were reduced from 225 ± 27 (control group) to 2 ± 34 mg/dL (plant stanol ester group) ($p < 0.001$) and from 134 ± 18 (control group) to 124 ± 32 mg/dL (plant stanol ester) ($p < 0.01$), respectively (Ref. 81). In the report by

Blomqvist (Ref. 81), HDL cholesterol was reported to be significantly lower in the plant stanol ester group compared to the control group. Using the same data, with the exception that the number of control subjects utilized in the analysis was 33 rather than 32 as in the Blomqvist report, HDL cholesterol was reported to be unchanged in the report by Vanhanen (Ref. 82). The agency does not give as much weight to this study because the two reports lacked sufficient detail on the reason for the varying number of control subjects.

Two reports of apparently the same study (Refs. 63 and 64) gave inconclusive results regarding the relationship between plant stanol ester consumption and blood cholesterol levels. Interpretation of this study is complicated by design issues such as concerns about sample size and level of plant sterol/stanol administered, but both reports are discussed here and summarized in table 2 of this document because they provide information to assist in determining the minimum level of plant stanol esters necessary to provide a health benefit.

Miettinen and Vanhanen (Refs. 63 and 64 (1 study)) reported the effect of small amounts of sitosterol (700 mg/d free sterols) and sitostanol (700 mg/d free stanols) dissolved in 50 g RSO mayonnaise on serum cholesterol in 31 subjects with hypercholesterolemia for 9 weeks. Subjects did not change their diets except for replacing 50 g/d of dietary fat with the 50 g/d of RSO mayonnaise. It appears that these authors later conducted another 9-week phase of the study using sitostanol esters (1.36 g/d plant stanol esters or 800 mg/d free stanols) dissolved in 50 g RSO mayonnaise. The results of this later phase were reported in the Miettinen reference (Ref. 63), together with the earlier results. The Vanhanen reference (Ref. 64) reports only the earlier results for sitosterol and sitostanol. The Vanhanen reference (Ref. 64) reports reduced serum total cholesterol (8.5 percent) concentrations during the RSO mayonnaise run-in period compared to values before the run-in period when combining all subjects. Continuation of RSO mayonnaise in the RSO mayonnaise control group (n=8) during the experimental period had no further effect on blood cholesterol (Refs. 63 and 64). Free sitostanol (n=7) did not significantly alter serum total cholesterol or LDL cholesterol compared to the RSO control group during the experimental period (Refs. 63 and 64). HDL cholesterol also did not change in the free sitostanol group (Ref. 63). Serum total and LDL cholesterol were significantly reduced in the sitostanol ester group (n=7), however (Ref. 63). The mean change in serum total cholesterol from baseline was −7.4 mg/dL in the sitostanol ester group, compared to +4.6 mg/dL in the control group ($p < 0.05$). The mean change in LDL cholesterol from baseline was -7.7 mg/dL in the sitostanol ester group compared to +3.1 mg/dL in the control group ($p < 0.05$). A statistically significant increase in HDL cholesterol from baseline, however, was reported in the sitostanol ester-treated group (Ref. 63).

The agency notes that it is difficult to decipher from the descriptions in these reports the amount of plant stanol ester that was consumed and the level of cholesterol-lowering that was observed. For the sitostanol ester group, as an example, the experimental design section states that 800 mg/d of sitostanol transesterified with RSO fatty acids was added to the RSO mayonnaise, yet table 1 of this document shows that the amount of sitostanol ester in the RSO mayonnaise was 830 mg (Ref. 63). Since the conversion factor to obtain the stanol ester equivalent of a given amount of free stanol is 1.7, the amounts of sitostanol and sitostanol ester given in the experimental design section and table 1 cannot both be correct. Based on information in the results section of the Miettinen reference (Ref. 63), serum total cholesterol reduction in the sitostanol ester group can be calculated to be approximately 18 percent as compared to control, yet the abstract of the Vanhanen reference mentions that sitostanol ester reduced serum total cholesterol by 7 percent (Ref. 63). Therefore, FDA considers the results in these reports inconclusive because of inconsistencies in the descriptions of methods and results.

Two studies (Refs. 58 and 74) show a relationship between consumption of plant stanols and reduced LDL cholesterol, but not blood total cholesterol, in subjects consuming a diet within the range of a typical American diet, although the diet was a controlled feeding regimen formulated to meet Canadian recommended nutrient intakes.

Jones et al. (Ref. 58) reported the effects of consuming 2.94 g/d of plant sterol esters in 23 g of margarine, 3.31 g/d of plant stanol esters in 23 g of margarine (1.84 g/d free plant stanols; daily margarine doses were divided into three equal portions and added to each meal) and 23 g/d of control margarine for 21 days each, using a controlled feeding crossover study design. During the experimental period, subjects consumed a fixed-food North American diet formulated to meet Canadian recommended nutrient intakes. The results from consumption of the plant sterol ester margarine are discussed in section III.C.1.b of this document. Plasma LDL cholesterol levels were reduced by 6.4 percent ($p < 0.02$) in the plant stanol ester group compared to the control group. Plasma total cholesterol was not significantly reduced in the plant stanol ester group. Plasma HDL cholesterol did not differ across groups, and there was no significant weight change shown by the subjects while consuming any of the margarine mixtures.

Jones et al. (Ref. 74) evaluated the effects of a mixture of plant stanols and plant sterols. The plant stanol compound sitostanol made up about 20 percent of the mixture by weight. The remaining sterol component of the mixture was mostly composed of the plant sterols sitosterol and campesterol. These investigators evaluated the cholesterol-lowering properties of this nonesterified plant sterol/stanol mixture in a controlled feeding regimen based on a "prudent," fixed-food North American diet formulated to meet Canadian recommended nutrient intakes. Thirty-two hypercholesterolemic men were fed either a diet of prepared foods alone or the same diet plus 1.7 g/d of the plant sterol/stanol mixture (in 30 g/d of margarine, consumed during 3 meals) for 30 days in a parallel study design. The plant sterol/stanol mixture had no statistically significant effect on plasma total cholesterol concentrations. However, LDL cholesterol concentrations on day 30 had decreased by 8.9 percent ($p < 0.01$) and 24.4 percent ($p < 0.001$) with the control and plant sterol/stanol-enriched diets, respectively. On day 30, LDL cholesterol concentrations were significantly lower ($p < 0.05$) by 15.5 percent in the group consuming the plant sterol/stanol mixture compared to the control group. HDL cholesterol concentrations did not change significantly during the study.

(c) *Normocholesterolemics: "typical" or "usual" diets.* Two studies (Refs. 91 and 92) show a relationship between consumption of plant stanols and reduced blood cholesterol in subjects with normal cholesterol concentrations consuming a typical American diet.

Plat and Mensink (Ref. 92) examined the effects of two plant stanol ester preparations in healthy subjects with normal serum cholesterol levels. During a 4 week run-in period, 112 subjects consumed a rapeseed oil margarine (20 g/d) and shortening (10 g/d). For the next 8 weeks, 42 subjects continued with these products, while the other

subjects received margarine (20 g/d) and shortening (10 g/d) with a vegetable oil-based stanol ester mixture (6.8 g/d plant stanol esters or 3.8 g/d free plant stanols) or pine wood-based stanol ester mixture (6.8 g/d plant stanol ester or 4 g/d plant stanol). Subjects did not change their diets except for replacing 30 g/d of dietary fat with the 30 g/d of test margarine and shortening. In the vegetable oil plant stanol ester group, the mean change in serum total cholesterol from baseline was −16.6 mg/dL, compared to −1.6 mg/dL in the control group ($p < 0.001$). In the pine wood stanol ester group, the mean change in serum total cholesterol from baseline was −16.3 mg/dL compared to −1.6 mg/dL in the control group ($p < 0.001$). Compared to consumption of a control margarine and shortening, consumption of 6.8 g/d of vegetable oil-based stanol esters lowered LDL cholesterol by 14.6 ± 8.0 percent ($p < 0.001$). Consumption of 6.8 g/d of the pine wood-based stanol esters showed a comparable decrease of 12.8 ± 11.2 percent ($p < 0.001$) in comparison to control margarine consumption. Decreases in LDL cholesterol were not significantly different between the two experimental groups ($p = 0.793$). Serum HDL cholesterol did not change during the study.

Niinikoski et al. (Ref. 91) randomly assigned 24 subjects with normal serum cholesterol levels to use either a plant stanol ester margarine (5.1 g/d plant stanol esters; 3 g/d of free plant stanols) or ordinary rapeseed oil margarine (control) for 5 weeks. Subjects followed their normal diets, except for substituting the test or control margarine for normal dietary fat intake. During the study period the mean plus/minus standard deviation for serum total cholesterol decreased more in the plant stanol ester spread group (-31 plus/minus 19.4) compared to the ordinary rapeseed oil spread group (-11.6 plus/minus 19.4) ($p < 0.05$). Serum non-HDL (LDL plus very low density lipoprotein) cholesterol also decreased more in the plant stanol ester group (-31 plus/minus 23) compared to the control group (-11.6 plus/minus 19.4) ($p < 0.05$), but the plant stanol ester spread did not influence HDL cholesterol concentration ($p = 0.71$ between groups).

(d) *Other studies: research synthesis study.* As discussed in section III.C.1.d of this document, the agency considered the results of a March 25, 2000, research synthesis study (Ref. 100) of the effect of plant sterols and plant stanols on serum cholesterol concentrations as supporting evidence on the relationship between plant sterol/stanol esters and CHD. In this research synthesis study, the combined effect of plant sterols and stanols on serum cholesterol concentrations was analyzed by pooling data from 14 randomized trials that employed either a parallel or crossover design, consisting of 20 dose comparisons of either plant sterols or plant stanols to a control vehicle. The data described the effects on serum LDL cholesterol concentrations obtained from using spreads (or, in some cases, mayonnaise, olive oil, or butter) with and without added plant sterols or stanols.

Based on the placebo-adjusted reduction in serum LDL cholesterol, the analysis indicated that 2 g of plant sterol (equivalent to 3.2 g/d of plant sterol esters) or plant stanol (equivalent to 3.4 g/d of plant stanol esters) added to a daily intake of spread (or mayonnaise, olive oil, or butter) reduces serum concentrations of LDL cholesterol by an average of 20.9 mg/dL in people aged 50 to 59 ($p=0.005$), 16.6 mg/dL in those aged 40 to 49 ($p=0.005$), and 12.8 mg/dL in those aged 30 to 39 ($p=0.005$). The results indicated that the reduction in the concentration of LDL cholesterol at each dose is significantly greater in older people versus younger people. Reductions in blood total cholesterol concentrations were similar to the LDL cholesterol reductions and there was little change in serum concentrations of HDL cholesterol. The results of this analysis also suggested that doses greater than about 2 g of plant sterol (3.2 g/d of plant sterol esters) or stanol (3.4 g/d of plant stanol esters) per day would not result in further reduction in LDL cholesterol.

Observational studies and randomized trials concerning the relationship between serum cholesterol and the risk of heart disease (Ref. 101) indicate that for people aged 50 to 59, a reduction in LDL cholesterol of about 19.4 mg/dL (0.5 mmol/l) translates into a 25 percent reduction in the risk of heart disease after about 2 years. Studies administering plant sterols and stanols have demonstrated the potential to provide this protection. According to Law, the cholesterol-lowering capacity of plant sterols and stanols is even larger than the effect that could be expected to occur if people ate less animal fat (or saturated fat) (Ref. 100).

Community Intervention Study

The plant stanol ester petitioner also submitted a community intervention study by Puska et al. (Ref. 102) that described the relationship between consumption of plant stanol ester-containing margarine and serum total cholesterol concentrations in North Karelia, Finland. FDA considered this study as supporting evidence for the relationship between plant stanol esters and CHD. In the early 1970's, Finland had the highest cardiovascular-related mortality in the world. Since 1972, active prevention programs carried out in the framework of the North Karelia Project have reduced these high rates. A central target of these programs was promotion of dietary changes to reduce population cholesterol levels. In spite of great success in the 1970's and 1980's, cholesterol levels at the end of the 1980's remained, by international standards, relatively high in North Karelia, especially in rural areas. The Village Cholesterol Competition was introduced as an innovative method to promote further cholesterol reduction in the population. Puska et al. (Ref. 102) describe two competitions (1991 and 1997) in which serum cholesterol values of subjects ages 20 to 70 years in participating villages were measured twice during a 2 month period. The village with the greatest mean reduction in serum cholesterol was awarded a monetary prize. The 1991 competition is not relevant to this interim rule because plant stanol ester-containing spreads were not available at the time. However, the 1997 competition is relevant because plant stanol ester-containing spreads had become available and, as discussed below, were consumed by a significant number of participants. Subjects were asked to complete a questionnaire about demographic factors, risk factors, dietary changes, and physical activity. The questionnaire included specific questions on changes in use of milk, fat spreads, fat used for baking, and food preparation. Participating villages were responsible for arranging intervention activities and blood cholesterol measurements.

Sixteen villages, with a total of 1,333 participants, were included in the results. There were 8 weeks between the initial and final blood cholesterol measurements. Approximately 24 percent of the participants changed their fat spread on bread to recommended alternatives (e.g., from butter to margarine), but 57 percent did not make any changes in their choice of spread. Use of plant stanol ester-containing spread increased nearly fivefold, whereas use of butter, butter-vegetable oil mixture and normal vegetable margarine use declined. Approximately 200 participants began to use plant stanol ester spread during the competition as their fat spread on bread.

The winning village had an average serum total cholesterol reduction of 16 percent ($p < 0.001$). Results for each village were calculated as the mean percent reduction in individual

cholesterol levels. The mean reduction in serum total cholesterol of all participating villages was 9 percent ($p < 0.001$). In 14 of 16 villages, the reduction between the initial and final blood cholesterol measurements was statistically significant ($p < 0.05$). The investigators observed that the greater the self-reported daily use of the plant stanol ester spread, the greater the serum cholesterol reduction. Furthermore, of those who reported using more than 5 teaspoonfuls per day of plant stanol ester-containing spread, an average serum total cholesterol reduction of 21.3 percent was achieved.

(e) *Summary.* In two (Refs. 77 and 80) of three (Refs. 77, 80, and 97) studies of hypercholesterolemic subjects consuming low saturated fat and low cholesterol diets, plant stanol ester intake was associated with statistically significant decreases in total and LDL cholesterol levels when compared to a control group. Levels of HDL cholesterol were found to be unchanged (Refs. 77, 80, and 97).

Levels of plant stanol esters found to be effective in lowering total and LDL cholesterol levels, in the context of a diet low in saturated fat and cholesterol, were 3.4 g (Ref. 80) and 3.9 g (Ref. 77) (equivalent to 2 and 2.31 g of free plant stanols, respectively). Other results from one of these studies (Ref. 77) reported a statistically significant effect of 3.9 g/d of vegetable oil stanol esters (2.16 g/d of free plant stanols) on blood total cholesterol, but not LDL cholesterol. Dietary supplementation with 3 g of plant stanols per day (equivalent to 5.1 g/d of plant stanol esters) to hypercholesterolemic subjects consuming a low saturated fat and low cholesterol diet (Ref. 97) did not significantly lower plasma total or LDL cholesterol.

In 10 of 10 studies of hypercholesterolemic subjects consuming "usual" diets (Refs. 58, 63 and 64 (1 study), 67, 74, 78, 81 and 82 (1 study), 88 through 90, and 94), plant stanol ester intake was associated with statistically significant decreases in blood total and/or LDL cholesterol levels. In seven (Refs. 58, 67, 74, 88 through 90, and 94) of these ten studies, HDL cholesterol levels were not significantly affected by plant stanol dietary treatment. In 2 studies (Refs. 63 and 64 (1 study) and 78) of the 10 studies, plant stanol esters were reported to increase the levels of HDL cholesterol from baseline levels. Two separate published reports of another study (Refs. 81 and 82) were inconsistent in their description of effects on HDL cholesterol. One publication (Ref. 81) reported HDL cholesterol to be significantly lower in the plant stanol ester group compared to a control group, but the other publication reported that the difference in HDL cholesterol between the two groups was not significant (Ref. 82). This incongruity may be due to the difference in the number of control subjects utilized in the analysis between the two publications. The agency notes that the majority of studies do not report a statistically significant change in HDL cholesterol in the plant stanol ester groups compared to the control groups.

Levels of plant stanol esters found to be effective in lowering total and/or LDL cholesterol levels in hypercholesterolemic subjects consuming a "usual" diet ranged from 1.36 to 5.8 g/d (equivalent to 0.8 to 3.4 g/d of free plant stanols) (Refs. 58, 63 and 64 (1 study), 67, 74, 78, 81 and 82 (1 study), 88 through 90, and 94). In the study by Hallikainen et al. (Ref. 88), 1.4 g/d plant stanol ester (0.8 g/d of free plant stanol) did not significantly reduce serum cholesterol levels, but intakes of 2.7, 4.1, and 5.4 g/d of plant stanol esters (1.6, 2.4, and 3.2 g/d of free plant stanols, respectively) were found to significantly reduce both serum total and LDL cholesterol levels. In another of the 10 studies described above (Ref. 94), subjects consuming a higher dose (3.4 g/d, equivalent to 2 g/d of free plant stanols) of plant stanol esters showed statistically significant reductions in both blood total and LDL cholesterol, but a lower dose of plant stanol esters (1.36 g/d, equivalent to 0.8 g/d of free plant stanols) showed reductions in blood total, but not in LDL cholesterol. The results of the study by Miettinen and Vanhanen (Refs. 63 and 64) are inconclusive. This may be due to lack of statistical power (e.g., sample size too small to detect the hypothesized difference between groups) or too low a dose of plant stanols to provide an effect. As previously discussed, the descriptions of methods and results also were inconsistent and difficult to interpret. Although these investigators reported (Ref. 63) a statistically significant effect of 1.36 g/d plant stanol esters (equivalent to 0.8 g/d of free plant stanols) on reducing serum total and LDL cholesterol compared to a control group, there was no effect of 700 mg/d of the free plant stanols (equivalent to 1.19 g/d of plant stanol esters) on blood cholesterol levels.

Two studies (Refs. 91 and 92) examined the effects of plant stanol esters in healthy adults with normal cholesterol levels consuming a "usual" diet. Both of these studies demonstrated significant decreases in blood total and LDL cholesterol or non-HDL cholesterol levels when compared to controls. Levels of plant stanol esters found to be effective were 6.8 g/d (vegetable oil stanol esters; 3.8 g/d of free plant stanols) (Ref. 92), 6.8 g/d (pine wood stanol esters; 4 g/d of free plant stanols) (Ref. 92), and 5.1 g/d (source unreported; approximately 3 g/d of free plant stanols) (Ref. 91). HDL cholesterol levels were not significantly affected by plant stanol consumption in these reports.

Based on these studies, FDA finds there is scientific evidence for a consistent, clinically significant effect of plant stanol esters on blood total and LDL cholesterol. The cholesterol-lowering effect of plant stanol esters is consistent in both mildly and moderately hypercholesterolemic populations and in populations with normal cholesterol concentrations. The cholesterol-lowering effect of plant stanol esters has been reported in addition to the effects of a low saturated fat and low cholesterol diet. Most studies also report that plant stanols do not affect HDL cholesterol levels. These conclusions are drawn from the review of the well controlled clinical studies and are supported by the research synthesis study of Law (Ref. 100) and the community intervention trial of Puska et al. (Ref. 102).

IV. Decision to Authorize a Health Claim Relating Plant Sterol/Stanol Esters to Reduction in Risk of CHD

A. Relationship Between Plant Sterol Esters and CHD

The plant sterol esters petition provided information on pertinent human studies that evaluated the effects on serum total cholesterol and LDL cholesterol levels from dietary intervention with plant sterols or plant sterol esters in subjects with normal to mildly or moderately elevated serum cholesterol levels. FDA reviewed the information in the petition as well as other pertinent studies identified by the agency's literature search.

FDA concludes that, based on the totality of publicly available scientific evidence, there is significant scientific agreement to support a relationship between consumption of plant sterol esters and the risk of CHD. The evidence that plant sterol esters affect the risk of CHD is provided by studies that measured the effect of plant sterol ester consumption on the two major risk factors for CHD, serum total and LDL cholesterol.

In most intervention trials in subjects with mildly to moderately elevated cholesterol levels (total cholesterol <300 mg/dL), plant sterol esters were found to

reduce blood total and/or LDL cholesterol levels to a significant degree (Refs. 57, 58, 61 and 62 (1 study), 67, and 74). Moreover, HDL cholesterol levels were unchanged (Refs. 57, 58, 61 and 62 (1 study), 67, and 74). Results in normocholesterolemic subjects (Refs. 51, 65, and 75) were similar to the results in mildly to moderately hypercholesterolemic subjects.

Most of the studies in subjects with mildly to moderately elevated cholesterol levels used "usual" diets in either a controlled feeding (Refs. 58 and 74) or free-living (Refs. 57, 63 and 64 (1 study), and 67) situation, but one study used a low saturated fat, low cholesterol diet during the study (Refs. 61 and 62 (1 study)). All three of the studies in subjects with normal blood cholesterol levels used "usual" diets in either a controlled feeding (Refs. 51 and 65) or free-living (Ref. 75) situation. Plant sterol esters have been reported to lower blood cholesterol levels in subjects with mildly to moderately elevated cholesterol consuming either a "usual" diet or low saturated fat, low cholesterol diet and in subjects with normal blood cholesterol levels consuming "usual" diets. Therefore, the evidence suggests that the blood cholesterol-lowering response occurs regardless of the type of background diet subjects consume.

Plant sterols (esterified or free) were tested in either a spread, margarine, or butter carrier and produced fairly consistent results regardless of the food carrier and apparent differences in processing techniques. Given the variability of amounts and of food carriers in which plant sterols and plant sterol esters were provided in the diets studied, the response of blood cholesterol levels to plant sterols appears to be consistent and substantial, except for plant sterols from sheanut oil and ricebran oil (Refs. 67 and 75).

Based on the totality of the publicly available scientific evidence, the agency concludes that there is significant scientific agreement that plant sterol esters from certain sources will help reduce serum cholesterol and that such reductions may reduce the risk of CHD. Section 101.83(c)(2)(ii)(A)(*1*) (discussed in section V.C of this document) specifies the plant sterol esters that have been demonstrated to have a relationship to the risk of CHD. In the majority of clinical studies evaluating plant sterols or plant sterol esters, blood total and LDL cholesterol were the lipid fractions shown to be the most affected by plant sterol intervention. As discussed in section I of this document, reviews by Federal agencies and other scientific bodies have concluded that there is substantial epidemiologic and clinical evidence that high blood levels of total cholesterol and LDL cholesterol represent major contributors to CHD and that dietary factors that decrease blood total cholesterol and LDL cholesterol will affect the risk of CHD (56 FR 60727 at 60728, and Refs. 18 through 21).

Given all of this evidence, the agency is authorizing a health claim on the relationship between plant sterol esters and reduced risk of CHD.

B. Relationship Between Plant Stanol Esters and CHD

The plant stanol esters petition provided information on pertinent human studies that evaluated the effects on serum total cholesterol and LDL cholesterol levels from dietary intervention with plant stanols or plant stanol esters in subjects with normal to mildly or moderately elevated serum cholesterol levels. FDA reviewed the information in the plant stanol esters petition as well as other pertinent studies from the plant sterol esters petition and from the studies identified by the agency's literature search.

FDA concludes that, based on the totality of publicly available scientific evidence, there is significant scientific agreement to support a relationship between consumption of plant stanol esters and the risk of CHD. The evidence that plant stanol esters affect the risk of CHD is provided by studies that measured the effect of plant stanol ester consumption on the two major risk factors for CHD, serum total and LDL cholesterol.

In most intervention trials in subjects with mildly to moderately elevated cholesterol levels (total cholesterol <300 mg/dL), plant stanol esters were found to reduce blood total and/or LDL cholesterol levels to a significant degree (Refs. 58, 63 and 64 (1 study), 67, 74, 77, 78, 80, 81 and 82 (1 study), 88 through 90, and 94). Moreover, HDL cholesterol levels were unchanged in most intervention studies (Refs. 58, 67, 74, 77, 80, 88 through 90, and 94). Results in normocholesterolemic subjects (Refs. 91 and 92) were similar to the results in mildly to moderately hypercholesterolemic subjects.

Most of the studies in subjects with mildly to moderately elevated cholesterol levels used "usual" diets in either a controlled feeding (Refs. 58 and 74) or free-living (Refs. 63 and 64 (1 study), 67, 78, 81 and 82 (1 study), 88 through 90, and 94) situation, but three studies used a low saturated fat, low cholesterol diet during the study (Refs. 77, 80 and 97). Both of the studies in subjects with normal blood cholesterol levels (Refs. 91 and 92) used "usual" diets in a free-living situation. Plant stanol esters have been reported to lower blood cholesterol levels in subjects with mildly to moderately elevated cholesterol consuming either a "usual" diet or low saturated fat, low cholesterol diet and in subjects with normal blood cholesterol levels consuming "usual" diets. Therefore, the evidence suggests that the blood cholesterol-lowering response occurs regardless of the type of background diet subjects consume.

Plant stanol esters were tested in either a spread, margarine, butter, mayonnaise or shortening carrier and produced fairly consistent results regardless of the food carrier and apparent differences in processing techniques. Given the variability of amounts and food carriers in which plant stanol esters were provided in the diets studied, the response of blood cholesterol levels appears to be consistent and substantial.

Based on the totality of the publicly available scientific evidence, the agency concludes that there is significant scientific agreement that plant stanol esters will help reduce blood cholesterol and that such reductions may reduce the risk of CHD. Section 101.83(c)(2)(ii)(B)(*1*) (discussed in section V.C of this document) specifies the plant stanol esters that have been demonstrated to have a relationship to the risk of CHD. In the majority of clinical studies evaluating plant stanol esters, blood total and LDL cholesterol were the lipid fractions shown to be the most affected by plant stanol intervention. As discussed in section I of this document, reviews by Federal agencies and other scientific bodies have concluded that there is substantial epidemiologic and clinical evidence that high blood levels of total cholesterol and LDL cholesterol represent major contributors to CHD and that dietary factors that decrease blood total cholesterol and LDL cholesterol will affect the risk of CHD (56 FR 60727 at 60728, and Refs. 18 through 21).

Given all of this evidence, the agency is authorizing a health claim on the relationship between plant stanol esters and reduced risk of CHD.

V. Description and Rationale for Components of Health Claim

A. Relationship Between Plant Sterol/Stanol Esters and CHD and the Significance of the Relationship

New section 101.83(a) describes the relationship between diets containing plant sterol/stanol esters and the risk of CHD. In §101.83(a)(1), the agency recounts that CHD is the most common and serious form of CVD, and that CHD

refers to diseases of the heart muscle and supporting blood vessels. This paragraph also notes that high blood total and LDL cholesterol levels are associated with increased risk of developing CHD and identifies the levels of total cholesterol and LDL cholesterol that would put an individual at high risk of developing CHD, as well as those blood cholesterol levels that are associated with borderline high risk. This information will assist consumers in understanding the seriousness of CHD.

In §101.83(a)(2), the agency recounts that populations with a low incidence of CHD tend to have low blood total and LDL cholesterol levels. This paragraph states that these populations also tend to have dietary patterns that are low in total fat, saturated fat, and cholesterol, and high in plant foods that contain fiber and other components. This information is consistent with that provided in the regulations authorizing health claims for fiber-containing fruits, vegetables, and grain products and CHD (§101.77), soluble fiber from certain foods and CHD (§101.81), and soy protein and CHD (§101.82). The agency believes that this information provides a basis for a better understanding of the numerous factors that contribute to the risk of CHD, including the relationship of plant sterol/stanol esters and diets low in saturated fat and cholesterol to the risk of CHD.

Section 101.83(a)(3) states that diets that include plant sterol/stanol esters may reduce the risk of CHD.

Section 101.83(b) describes the significance of the diet-disease relationship. In §101.83(b)(1), the agency recounts that CHD remains a major public health concern in the United States because the disease accounts for more deaths than any other disease or group of diseases. The regulation states that early management of modifiable CHD risk factors, such as high blood total and LDL cholesterol levels, is a major public health goal that can assist in reducing the risk of CHD. This information is consistent with the evidence that lowering blood total and LDL cholesterol levels reduces the risk of CHD (56 FR 60727, 58 FR 2739, and Refs. 18 through 21 and 50). Section 101.83(b)(2) states that including plant sterol/stanol esters in the diet helps to lower blood total and LDL cholesterol levels. FDA concludes that this statement is scientifically valid based on the evidence that it has reviewed on this diet-disease relationship.

B. Nature of the Claim

In new §101.83(c)(1), FDA is providing that the general requirements for health claims in §101.14 must be met, except that the disqualifying level for total fat per 50 g in §101.14(a)(4) does not apply to spreads and dressings for salad, and the minimum nutrient contribution requirement in §101.14(e)(6) does not apply to dressings for salad. FDA has decided to except these plant sterol/stanol ester products from the specified requirements in §101.14(a)(4) and (e)(6) because it has determined that permitting the health claim on such products will help consumers develop a dietary approach that will result in significantly lower blood cholesterol levels and an accompanying reduction in the risk of heart disease. The basis for this decision is discussed in more detail in section V.D of this document. The agency is requesting comments on this decision.

In §101.83(c)(2)(i), FDA is authorizing a health claim on the relationship between diets that contain plant sterol/stanol esters and the risk of CHD. The agency is authorizing this health claim based on its review of the scientific evidence on this substance-disease relationship, which shows that diets that contain plant sterol/stanol esters help to reduce total and LDL cholesterol (Refs. 51, 57, 58, 61 and 62 (1 study), 63 and 64 (1 study), 65, 67, 74, 75, 77, 78, 80, 81 and 82 (1 study), 88 through 92, and 94). This result is significant for the risk of heart disease because elevated levels of total and LDL cholesterol are associated with increased risk of CHD (Refs. 18 through 21).

In §101.83(c)(2)(i)(A), FDA is requiring, consistent with other health claims to reduce the risk of CHD, that the claim state that plant sterol/stanol esters should be consumed as part of a diet low in saturated fat and cholesterol. The agency acknowledges that most of the scientific evidence for an effect of plant sterol/stanol esters on blood cholesterol levels was provided by studies that used "usual" diets (Refs. 51, 57, 58, 63 and 64 (1 study), 65, 67, 74, 75, 78, 81 and 82 (1 study), 88 through 92, and 94). Some studies used low fat, low cholesterol diets and also found a cholesterol-lowering effect of plant sterol/stanol esters (Refs. 61 and 62 (1 study), 77, and 80). The results were consistent across studies, regardless of the background diet used. However, not all studies reported whether reductions in cholesterol were achieved as compared to baseline. The results of one study that investigated the effects of plant stanol esters added to butter (Ref. 78) suggest that plant stanol esters may not be able to fully counteract the impact of a high saturated fat diet on blood cholesterol levels. In that study, plant stanol esters added to butter significantly reduced both serum total cholesterol and LDL cholesterol compared to control (butter alone), but there was no significant reduction in either serum total or LDL cholesterol compared to baseline. Since there must be a cholesterol reduction compared to baseline in order for risk of CHD to decrease, it would be misleading for the claim to imply that plant sterol/stanol esters affect the risk of CHD regardless of diet, when that may not be the case.

In addition, as more fully discussed in section V.A of this document, CHD is a major public health concern in the United States, and the totality of the scientific evidence provides strong and consistent support that diets high in saturated fat and cholesterol are associated with elevated levels of blood total and LDL cholesterol and, thus, CHD (56 FR 60727 at 60737). The majority of Americans consume amounts of total fat and saturated fat that exceed the recommendations made in the Dietary Guidelines for Americans (Ref. 103). For example, from 1994 to 1996 only about one-third of Americans age 2 and older consumed no more than 30 percent of calories from total fat and only about one-third consumed less than 10 percent calories from saturated fat (Ref. 104). Dietary guidelines from both government and private scientific bodies conclude that the majority of the American population would benefit from decreased consumption of dietary saturated fat and cholesterol (Refs. 18 through 21). Thus, the agency finds that it will be more helpful to Americans' efforts to maintain healthy dietary practices if claims about the effect of plant sterol/stanol esters on the risk of CHD also recommend a diet low in saturated fat and cholesterol.

Moreover, the agency finds that for the public to understand fully, in the context of the total daily diet, the significance of consumption of plant sterol/stanol esters on the risk of CHD (see section 403(r)(3)(B)(iii) of the act), information about the total diet must be included as part of the claim. Therefore, the agency believes the plant sterol/stanol-containing food product bearing the health claim should provide information on consuming plant sterol/stanol esters in the context of a healthy diet. In fact, as evidenced by the requirement in section 403(r)(3)(B)(iii) of the act that health claims be stated so that the public may understand the significance of the information in the context of "a total daily diet," Congress intended FDA to consider the role of substances in food in a way that will enhance the chances of consumers constructing diets that are balanced and

healthful overall (Ref. 105). Therefore, the agency finds that the health claim that is the subject of this interim rule should be consistent with the Dietary Guidelines for Americans, 2000 (Ref. 103) guideline for fat and saturated fat intake, which states, "Choose a diet that is low in saturated fat and cholesterol and moderate in total fat."

In §101.83(c)(2)(i)(B), the agency is requiring, consistent with other health claims, that the relationship be qualified with the terms "may" or "might." These terms are used to make clear that not all persons can necessarily expect to benefit from these dietary changes (see 56 FR 60727 at 60740 and 58 FR 2552 at 2573) or to experience the same degree of blood cholesterol reduction. The requirement that the claim use the term "may" or "might" to relate the ability of plant sterol/stanol esters to reduce the risk of CHD is also intended to reflect the multifactorial nature of the disease.

In §101.83(c)(2)(i)(C), the agency is requiring, consistent with other authorized health claims, that the terms "coronary heart disease" or "heart disease" be used in specifying the disease. These terms are commonly used in dietary guidance materials, and therefore they should be readily understandable to the consumer (see 56 FR 60727 at 60740 and 58 FR 2552 at 2573).

In §101.83(c)(2)(i)(D), the agency is requiring that the claim specify the substance as "plant sterol esters" or "plant stanol esters," except that if the sole source of plant sterols or stanols is vegetable oil, the claim may use the term "vegetable oil sterol esters" or "vegetable oil stanol esters," as appropriate.

Section 101.83(c)(2)(i)(E), consistent with other authorized health claims, requires that the claim not attribute any degree of risk reduction of CHD to consumption of diets that contain plant sterol/stanol esters. Also consistent with other authorized claims, §101.83(c)(2)(i)(F) requires that the claim not imply that consumption of diets that contain plant sterol/stanol esters is the only recognized means of reducing CHD risk.

Investigators have estimated the size of the reduction in risk of heart disease produced by a given reduction in blood cholesterol concentration according to age and the time needed to attain the full reduction in risk (Ref. 101), but these data are population estimates and do not reflect individual risk reduction potential. Moreover, population risk reduction estimates from plant sterol/stanol ester consumption cannot be determined because the data do not reveal a consistent level of blood cholesterol reduction for a given plant sterol/stanol ester intake level. Therefore, the plant sterol/stanol ester studies that the agency reviewed do not provide a basis for determining the percent reduction in risk of CHD likely to be realized from consuming plant sterol/stanol esters, and therefore claims of a particular degree of risk reduction would be misleading.

Section 101.83(c)(2)(i)(G) requires that the claim specify the daily dietary intake of plant sterol or stanol esters needed to reduce the risk of CHD and the contribution one serving of the product makes to achieving the specified daily dietary intake. This requirement is consistent with requirements set forth in §§101.81 and 101.82.

Section 101.83(c)(2)(i)(G)(*1*) specifies the daily dietary intake of plant sterol esters needed to reduce the risk of CHD.

In the studies the agency reviewed that show a statistically significant effect of plant sterols on total and LDL cholesterol, the amounts fed ranged from 0.74 to 8.6 g/d of free plant sterols, which is equivalent to approximately 1.2 to 13.8 g/d of plant sterol esters (Refs. 51, 57, 58, 61 and 62 (1 study), 65, 67, and 75). (Without the high outlier of 8.6 g/d of free plant sterol ester consumed in one study (Ref. 51), the range is 0.74 g/d to 3.24 g/d of free plant sterols (Refs. 57, 58, 61 and 62 (1 study), 65, 67, and 75.)) In proposing 1 g/d of free plant sterols (1.6 g/d plant sterol esters) as the daily dietary intake level associated with reduced risk of CHD, the plant sterol ester petitioner asserted (Ref. 1, page 41) that intakes above 1 g/d have consistently been shown to lower blood total and LDL cholesterol, citing the studies by Maki et al. (Refs. 61 and 62 (1 study), Hendriks et al. (Ref. 57), and Weststrate and Meijer (Ref. 67), but that intakes below this level have not. As support for the latter statement, the petitioner cited the reports by Miettinen and Vanhanen (Refs. 63 and 64 (1 study)), which found no statistically significant blood cholesterol reduction from consumption of 0.7 of plant sterols (equivalent to 1.12 g/d of plant sterol esters).

Although the agency agrees with the plant sterol ester petitioner that free plant sterol consumption of greater than 1 g/d (1.6 g/d of plant sterol esters) has consistently been shown to lower total and LDL cholesterol levels (Refs. 51, 57, 58, 61 and 62 (1 study), and 67), the agency reviewed the studies to determine whether there is a lower level at which consumption of plant sterols has consistently shown cholesterol-lowering effects. There were three studies (Refs. 57, 65, and 75) that found a statistically significant reduction in cholesterol with free plant sterol consumption less than 1 g/d. Hendriks et al. (Ref. 57) reported the effects of feeding three different levels of plant sterol esters, including 1.33 g/d (equivalent to 0.83 g/d free plant sterols). At that intake level, blood total cholesterol decreased by 4.9 percent ($p < 0.001$), and LDL cholesterol decreased by 6.7 percent ($p < 0.001$), compared to a control spread. Sierksma et al (Ref. 75) reported that daily consumption of 0.8 g/d of free soybean oil sterols lowered plasma total and LDL cholesterol concentrations by 3.8 percent ($p < 0.05$) and 6 percent ($p < 0.05$), respectively, compared to a control spread. Pelletier et al. (Ref. 65) reported a 10 percent reduction in blood total cholesterol ($p < 0.001$) and a 15 percent reduction in LDL cholesterol ($p < 0.001$), compared to a control group, in subjects consuming 0.74 g/d of soybean sterols (nonesterified) in 50 g/d of butter for 4 weeks.

For the purpose of setting the daily dietary intake level to be used in the plant sterol esters and risk of CHD health claim, the agency is placing greater emphasis on studies that incorporated plant sterol esters into foods that will be permitted to bear the claim. Therefore, the study by Pelletier et al. (Ref. 65), in which 0.74 g/d of free plant sterols were incorporated into butter, rather than a vegetable-based spread, is less relevant in determining a useful daily intake level. (Butter would not be able to bear the claim because it exceeds the disqualifying levels for cholesterol and saturated fat on a 50 gram basis.) The daily intake level utilized in the study by Pelletier et al. (Ref. 65) is also very close to that used in the study by Miettinen and Vanhanen (Refs. 63 and 64 (1 study)) which found that 0.7 g/d of free plant sterols did not result in statistically significant reductions of blood total and LDL cholesterol. For the purpose of setting a daily intake level, FDA therefore focused instead on the intakes consumed in the Sierksma et al. report (Ref. 75), 0.8 g/d of free plant sterols (equivalent to 1.3 g/d of plant sterol esters), and the Hendriks et al. report (Ref. 57), 0.83 g/d of free plant sterols (1.33 g/d of plant sterol esters). These two intake levels are almost identical, and both resulted in statistically significant reductions in blood total and LDL cholesterol. As previously noted, all other studies with higher intakes of plant sterols also resulted in statistically significant reductions of both blood total and LDL cholesterol (Refs. 51, 57,

58, 61 and 62 (1 study), and 67). The agency therefore finds that consumption of at least 0.8 g/d of free plant sterols, or 1.3 g/d of plant sterol esters, has consistently been shown to lower blood total and LDL cholesterol. Accordingly, FDA is providing in §101.83(c)(2)(i)(G)(*1*) that the daily intake of plant sterol esters associated with reduced risk of CHD is 1.3 g or more of plant sterol esters per day. The agency is asking for comments on this determination.

Section 101.83(c)(2)(i)(G)(*2*) specifies the daily dietary intake of plant stanol esters needed to reduce the risk of CHD. In the studies the agency reviewed that show a statistically significant effect of plant stanols on blood total and LDL cholesterol, the amounts fed ranged from 0.8 to 4 g/d of free plant stanols, which is equivalent to approximately 1.36 to 6.8 g/d of plant stanol esters (Refs. 63 and 64 (1 study), 67, 77, 78, 80, 81 and 82 (1 study), 88 through 92, and 94). In proposing 3.4 g/d of plant stanol esters (2 g/d free plant stanols) as the daily dietary intake level associated with reduced risk of CHD, the plant stanol ester petitioner asserted (Ref. 6, page 12) that intakes of at least 3.4 g/d of plant stanol esters have been shown to significantly reduce blood total and LDL cholesterol, citing the studies by Miettinen et al. (Ref. 89) and Nguyen (Ref. 90).

Although the agency agrees with the plant stanol ester petitioner that plant stanol ester consumption of approximately 3.4 g/d has been shown to significantly lower total and LDL cholesterol levels in several studies (Refs. 80, 89, 90, and 94), FDA notes that two other studies (Refs. 77 and 97) with an intake level of plant stanol esters greater than 3.4 g/d did not report significant reductions in blood total and LDL cholesterol levels. The study by Denke (Ref. 97) did not find reductions in either total or LDL cholesterol after consumption of a total daily intake of 3 g/d of free plant stanols (equivalent to 5.1 g/d of plant stanol esters). Unlike most of the other studies that the agency reviewed, however, the Denke study (Ref. 97) was not a randomized, placebo-controlled, double-blind study, but rather a fixed sequence design. One result of this design was that during the plant stanol dietary supplement phase the subjects consumed an additional 12 g of fat that they did not consume in other phases; this makes comparisons between phases difficult, and therefore FDA gives less weight to this study.

In a report by Hallikainen et al. (Ref. 77), total cholesterol, but not LDL cholesterol, was significantly reduced after consumption of 3.9 g/d plant stanol esters from a vegetable oil source; this same study reported statistically significant reductions in both blood total and LDL cholesterol from a daily intake of 3.9 g/d of plant stanol esters from a wood-derived source. After evaluating the relative effectiveness of the vegetable oil and wood-derived plant stanol esters, however, the authors of this study concluded that the cholesterol-lowering effects of plant stanol esters from these two sources did not differ significantly. Pointing out that there were no significant differences in absolute or percentage changes in cholesterol concentrations between the vegetable oil and wood-derived plant stanol ester groups and that the percentage reduction in LDL cholesterol for the vegetable oil stanol esters compared to control was "almost significant" ($p = 0.072$), these authors concluded that both wood-derived stanol esters and vegetable oil stanol esters reduce serum cholesterol concentrations "with apparently equal efficacy." Another study supports this conclusion. Plat et al. (Ref. 92) compared the reductions in blood total and LDL cholesterol in subjects who consumed 6.8 g/d of wood-derived stanol esters with the blood total and LDL cholesterol reductions in subjects who consumed an equal amount of vegetable oil stanol esters. Again, no statistically significant differences were found; in numerical terms, the cholesterol reductions associated with the vegetable oil stanol esters were slightly greater.

In light of the strong evidence (four studies) that 3.4 g/d of plant stanol esters significantly lowers both total and LDL cholesterol, FDA concludes that intakes of 3.4 g/d or more of plant stanol esters can be expected to significantly lower both total and LDL cholesterol. As explained above, the agency is giving less weight to the Denke study (Ref. 97), in which the intake of plant stanols was equivalent to 5.1 g/d of plant stanol esters, than to the four studies at the 3.4 g/d intake (Refs. 80, 89, 90, and 94) because of a weakness in the design of the Denke study. Although the failure of the Hallikainen study (Ref. 77) to show a statistically significant reduction in LDL cholesterol at 3.9 g/d of vegetable oil stanol esters raises a question about whether the source of the plant stanol esters affects the daily intake level necessary to achieve a benefit, it appears that this was an anomalous result, as explained above. Two studies (Refs. 77 and 92) have concluded that plant stanol esters from vegetable oil and plant stanol esters from wood sources have equal effectiveness in lowering both total and LDL cholesterol.

FDA also reviewed the studies to determine whether there is a level lower than 3.4 g/d at which consumption of plant stanol esters has consistently shown cholesterol-lowering effects. The lowest level at which a study found statistically significant reductions in both total and LDL cholesterol was 1.36 g/d of plant stanol esters (Refs. 63 and 64 (1 study)). However, another study at the same level reported a statistically significant reduction in serum total but not LDL cholesterol (Ref. 58). Further, a study by Hallikainen et al. (Ref. 88) at a slightly higher level reported that 1.4 g/d of plant stanol esters did not significantly reduce serum total or LDL cholesterol levels. The same study (Ref. 88) reported that 2.7 g/d of plant stanol ester significantly reduced serum total and LDL cholesterol levels. However, Jones et al. (Ref. 58) found significant LDL cholesterol, but not total cholesterol, reductions with intake of 3.31 g/d plant stanol esters (Ref. 58). Thus, the agency was unable to find an intake level lower than 3.4 g/d that consistently showed cholesterol-lowering effects for both total and LDL cholesterol.

Except as previously noted for the studies by Denke (Ref. 97) and Hallikainen (Ref. 77), all the studies with intakes of 3.4 g/d or more of plant stanol esters resulted in statistically significant reductions of both total and LDL cholesterol levels (Refs. 67, 77, 78, 80, 81 and 82 (1 study), 88 through 92, and 94). The agency agrees with the petitioner that a total daily intake of at least 3.4 g/d of plant stanol esters (equivalent to 2 g/d of free plant stanols) represents an amount that has been shown to be effective in reducing blood cholesterol. Accordingly, FDA is providing in §101.83(c)(2)(i)(G)(*2*) that the daily intake of plant stanol esters associated with reduced risk of CHD is 3.4 g or more of plant stanol esters per day. The agency is asking for comments on this determination.

In §101.83(c)(2)(i)(H), FDA is requiring the claim to state that the daily dietary intake of plant sterol/stanol esters should be consumed in two servings eaten at different times. In the studies showing a statistically significant effect of plant sterols or plant sterol esters on blood total and LDL cholesterol levels, subjects were provided with and instructed to consume the daily intake of plant sterols or plant sterol esters in two (Refs. 51, 57, 61 and 62 (1 study), and 67) or three (Refs. 58 and 74) servings at different times of the day, or subjects were provided with the plant sterol-

containing food and asked to replace from 25 to 50 g of their typical dietary fat intake with an equal amount of the test food over the course of the day's dietary intake, usually during meals (Refs. 63 and 64 (1 study), 65, and 75). The agency concludes that, to be consistent with the conditions of the studies on which the claim is based, the daily intake of plant sterol esters should be consumed in at least two servings eaten at different times during the day with other foods. For the reasons given in section V.D.1.a of this document, FDA is specifying two servings as the target number of servings.

Similarly, in the studies showing a statistically significant effect of plant stanols or plant stanol esters on blood total and LDL cholesterol levels, subjects were provided with and instructed to consume the daily intake of plant stanols or plant stanol esters in two (Ref. 67) or three (Refs. 58, 74, 80, and 88 through 92) servings at different times of the day, or subjects were provided with the plant stanol-containing food and asked to replace from 25 to 50 g of their typical dietary fat intake with an equal amount of the test food over the course of the day's dietary intake, usually during meals (Refs. 63 and 64 (1 study), 77, 78, 81 and 82 (1 study), and 94). The agency concludes that, to be consistent with the conditions of the studies on which the claim is based, the daily intake of plant stanol esters should be consumed in at least two servings eaten at different times during the day with other foods. For the reasons given in section V.D.1.b of this document, FDA is specifying two servings as the target number of servings.

C. Nature of the Substance

Section 101.83(c)(2)(ii)(A)(*1*) specifies the plant sterol esters that have been demonstrated to have a relationship to the risk of CHD. Plant sterols can be classified on structural and biosynthetical grounds into 4-desmethyl sterols, 4-monomethyl sterols, and 4,4-dimethyl sterols. Plant sterols of the 4-desmethyl sterol class are the plant sterols that have demonstrated the blood cholesterol-lowering effect (Refs. 51, 57, 58, 63 and 64 (1 study), 65, 67, and 75). The major 4-desmethyl sterols are beta-sitosterol, campesterol and stigmasterol (Ref. 106).

Most of the studies that the agency reviewed used vegetable oil sterols, particularly those derived from soybean oil, as the source of beta-sitosterol, campesterol, and stigmasterol. These three 4-desmethyl sterols are also the predominant sterols in corn and canola oil. According to the plant sterol ester petitioner, the typical sterol composition of plant sterol esters is as follows: beta-sitosterol contributes from 30 to 65 percent (by weight) of the sterols, campesterol contributes from 10 to 40 percent of the sterols, and stigmasterol contributes from 6 to 30 percent of the sterols, with other sterols making up no more than 9 percent of the total (Ref. 1, appendix E). The composition of the vegetable oils used as sterol sources in most of the studies that demonstrated a cholesterol-lowering effect was similar (Refs. 51, 57, 58, 65, 67, and 75).

Ricebran oil and sheanut oil principally contain the methylated sterols of the 4,4-dimethyl sterol class. Studies investigating the effects of sterols from ricebran oil and sheanut oil on blood cholesterol levels have not found a cholesterol-lowering effect (Refs. 67 and 75). The structure of the 4-desmethyl sterols is more similar to cholesterol than the structure of 4,4-dimethyl sterols. Because of this structural similarity, it has been suggested that the 4-desmethyl sterols may offer more opportunity for competition with cholesterol for incorporation into mixed micelles, one of the putative mechanisms for the blood cholesterol-lowering action of sterols (Ref. 75).

In studies that found a significant effect on blood cholesterol levels and reported the sterol composition of the plant sterol esters tested, the total amount of the major 4-desmethyl sterols (beta-sitosterol, campesterol and stigmasterol) provided to the subjects during the experimental period ranged from 76 to 98 percent (Refs. 51, 57, 58, 65, 67, and 75), with only 1 study at 76 percent (Ref. 65). The rest of the studies clustered toward the high end of the range, between 89 to 98 percent (Refs. 51, 57, 58, 67, and 75). The agency believes there are a number of likely sources of variability in the sterol composition of the plant sterol ester mixtures, including variability in analytical determinations, processing, seasonal changes, and variety of the crop used. FDA does not have data on the extent of variability in sterol composition but has concluded that it is necessary to provide for some such variability. Given the distribution of the sterol composition percentages in the studies that showed significant effects on blood cholesterol levels and the possible variability of plant sterols in the finished product, FDA has decided to require that the combined percentage of beta-sitosterol, campesterol, and stigmasterol in the plant sterol component of plant sterol esters be 80 percent or higher as a condition of eligibility to bear the health claim. The agency requests comments on the variability of the level of beta-sitosterol, campesterol, and stigmasterol in plant sterols, particularly with respect to the variability of these levels in the plant sterol component of plant sterol ester products used in studies that reported significant cholesterol-lowering effects.

The agency is specifying that only edible oils may be used as the source oils for plant sterols. The agency is also specifying that food-grade fatty acids must be used to esterify the plant sterols. Although the agency is not specifying further the type of fatty acid, such as chain length and degree of unsaturation, FDA expects that the fatty acids will primarily be monounsaturated or polyunsaturated fatty acids to avoid increases in saturated fatty acid content of the final food products.

Section 101.83(c)(2)(ii)(A)(*1*) provides that the plant sterol substance that is the subject of the health claim for reduced risk of CHD is plant sterol esters prepared by esterifying a mixture of plant sterols from edible oils with food-grade fatty acids. Consistent with information in the petition and the sterol composition of test substances used in the studies that showed a cholesterol-lowering effect, §101.83(c)(2)(ii)(A)(*1*) further provides that the plant sterol mixture shall contain at least 80 percent beta-sitosterol, campesterol, and stigmasterol (combined weight). The agency is requesting comments on these requirements.

Section 101.83(c)(2)(ii)(A)(*2*) sets out FDA's decision that plant sterol esters, when evaluated for compliance purposes by the agency, will be measured by a method that is based upon a standard triglyceride or cholesterol determination that uses sample saponification followed by hexane extraction and includes an internal standard. The extract is analyzed by gas chromatography. The method, found in appendix F of the plant sterol esters petition (Ref. 1) and titled, "Determination of the Sterol Content in Margarines, Halvarines, Dressings, Fat Blends and Sterol Fatty Acid Ester Concentrates By Capillary Gas Chromatography," developed by Unilever United States, Inc., dated February 1, 2000, describes a gas chromatographic procedure for determination of the total sterol content in margarines, halvarines (low fat spreads), dressings, fats or fat blends and in sterol ester concentrates. The method is designed for total sterol levels of approximately 10 percent in margarines, fat and fat blends, 8 percent

in halvarines, from 3 to 10 percent in dressings, and approximately 60 percent in sterol ester concentrates. An internal standard is added for quantification. The sample is saponified and the unsaponifiable portion is extracted with heptane. The extract is then analyzed by gas chromatography using a nonpolar stationary phase capillary column with beta-cholestanol as an internal standard. The petitioner has submitted data that demonstrate the precision and inter-analyst reproducibility of the method (Ref. 1, appendix F). Specific sterols have been identified based on gas chromatography/mass spectrometry (GC/MS) analysis and comparison of data in the mass spectral library of the National Institute of Standards and Technology (NIST) (Ref. 4). The method has neither been subjected to validation through the Association of Official Analytical Chemist's (AOAC's) collaborative study or peer-verified method validation procedures, nor is it published in the open literature. FDA is requesting comments on the suitability of the plant sterol ester petitioner's method for assuring that foods bearing the health claim contain the qualifying levels of plant sterol esters. In this document, FDA is incorporating the plant sterol ester petitioner's method by reference in accordance with 5 U.S.C. 552(a) and 1 CFR part 51. Copies of the method may be obtained from the Center for Food Safety and Applied Nutrition's Office of Nutritional Products, Labeling, and Dietary Supplements, Division of Nutrition Science and Policy, 200 C St. SW., rm. 2831, Washington, DC 20204, and may be examined at the Center for Food Safety and Applied Nutrition's Library, 200 C St. SW., rm. 3321, Washington, DC, or at the Office of the Federal Register, 800 North Capital St. NW., suite 700, Washington, DC.

Section 101.83(c)(2)(ii)(B)(*1*) specifies the plant stanol esters that have been demonstrated to have a relationship to the risk of CHD. Sitostanol and campestanol, the saturated (at the 5 position) derivatives of beta-sitosterol, campesterol, and stigmasterol, are the plant stanols that have demonstrated the blood cholesterol-lowering effect (Refs. 58, 63 and 64 (1 study), 67, 77, 78, 81 and 82 (1 study), 88 through 92, and 94). Like the sterols from which they derive, sitostanol and campestanol are in the 4-desmethyl sterol class, and as such are similar in structure to cholesterol. Sitostanol is formed by the hydrogenation of beta-sitosterol, and also by the complete hydrogenation of stigmasterol (stigmasterol has two double bonds that are saturated during the hydrogenation process, whereas sitostanol has one double bond that is saturated during the hydrogenation process). Campestanol is formed by the hydrogenation of campesterol.

Most of the studies that the agency reviewed used vegetable oil stanols or wood-derived plant stanols as the source of sitostanol and campestanol. According to the plant stanol ester petitioner, the stanols in plant stanol esters are derived from hydrogenated plant sterol mixtures or extracted from plant sources (Ref. 8, page 18). In studies that found a significant effect on blood cholesterol levels and reported the stanol composition of the plant stanol esters tested, the combined percentage of sitostanol and campestanol ranged from 64 to 100 percent by weight (Refs. 58, 63 and 64 (1 study), 67, 77, 78, 88, 90, and 92), with only one study at 64 percent (Refs. 63 and 64 (1 study). The rest of the studies clustered toward the high end of the range, between 89 and 100 percent (Refs. 58, 67, 77, 78, 88, 90, and 92).

The agency believes there are a number of likely sources of variability in the stanol composition of the plant stanol ester mixtures, including variability in analytical determinations, processing, seasonal changes, and variety of the crop used. FDA does not have data on the extent of variability in stanol composition but has concluded that it is necessary to provide for some such variability. Given the distribution of the stanol composition percentages in the studies that showed significant effects on blood cholesterol levels and the possible variability of plant stanols in the finished product, FDA has decided to require that the combined percentage of sitostanol and campestanol in the plant stanol component of plant stanol esters be 80 percent or higher as a condition of eligibility to bear the health claim. The agency requests comments on the variability of the level of sitostanol and campestanol in plant stanols, particularly with respect to the variability of these levels in the plant stanol component of plant stanol ester products used in studies that reported significant cholesterol-lowering effects.

The agency is specifying the source material for plant stanols, which may be either plant-derived oils or wood. The plant stanol ester petitioner's GRAS determination, and consequently the agency's safe and lawful conclusion in section II.B.3.b.i of this document, apply only to plant stanols derived from edible oils or from byproducts of the kraft paper pulping process (Ref. 46). Therefore, FDA is providing that plant-derived oils used as the source for plant stanols must be edible oils. If wood is used as the source material, the plant stanols must be derived from byproducts of the kraft paper pulping process. The agency is also specifying that food-grade fatty acids must be used to esterify the plant stanols. Although the agency is not specifying further the type of fatty acid, such as chain length and degree of unsaturation, FDA expects that the fatty acids will primarily be monounsaturated or polyunsaturated fatty acids to avoid increases in saturated fatty acid content of the final food products.

Section 101.83(c)(2)(ii)(B)(*1*) provides that the plant stanol substance that is the subject of the health claim for reduced risk of CHD is plant stanol esters prepared by esterifying a mixture of plant stanols derived from edible oils or byproducts of the kraft paper pulping process with food-grade fatty acids. Consistent with the stanol composition of test substances used in the studies that showed a cholesterol-lowering effect, §101.83(c)(2)(ii)(B)(*1*) further provides that the plant stanol mixture shall contain at least 80 percent sitostanol and campestanol (combined weight). The agency is requesting comments on these requirements.

Section 101.83(c)(2)(ii)(B)(*2*) sets out FDA's decision that plant stanol esters, when evaluated for compliance purposes by the agency, will be measured using a standard cholesterol determination that uses sample saponification, followed by heptane extraction, derivatization to trimethylsilyl ethers and analyzed by gas chromatography.

The plant stanol ester petition (Refs. 8, 11, and 14) provided the following four analytical methods developed by McNeil Consumer Healthcare dated February 15, 2000, for use in different food matrices. The method titled "Determination of Stanols and Sterols in Benecol® [3] Tub Spread" describes a procedure for determination of stanols and sterols in tub spreads containing 6 to 18 percent stanol esters. The primary analytes are sitostanol, campestanol, sitosterol and campesterol. Samples are saponified directly with alcoholic potassium hydroxide. Stanols and sterols remain in the unsaponified fraction and are extracted with hexane. The extracted stanols and sterols are then derivatized to trimethylsilyl ethers and analyzed by gas chromatography. The internal standard utilized is cholestanol.

[3] Benecol®" is the plant stanol ester petitioner's brand of plant stanol ester-containing food products.

The method titled "Determination of Stanols and Sterols in Benecol Snack Bars" is suitable for the determination of stanols and sterols in snack bars containing 2.5 to 7.5 percent stanol esters. The method titled "Determination of Stanols and Sterols in Benecol® Dressing" is suitable for determination of stanols and sterols in dressing for salad containing 3 to 8 percent stanol esters. Both the dressing for salad and snack bar procedures are similar to that described above for Benecol® tub spread.

The method titled "Determination of Stanols and Sterols in Benecol® Softgels" describes a procedure for determination of stanols and sterols in softgels (gelatin capsules with liquid center) containing from 464 to 696 nanograms of stanol esters. The primary analytes are sitostanol, campestanol, sitosterol and campesterol. Stanol ester centers are washed from the gelatin shell and directly saponified with alcoholic potassium hydroxide. Stanols and sterols remain in the unsaponified fraction and are extracted with hexane. The extracted stanols and sterols are then derivatized to trimethylsilyl ethers and analyzed by gas chromatography. The internal standard utilized is cholestanol.

The methods described above separate the major plant stanols in food products from their sterol derivatives. The petitioner has submitted data that show that these analytical methods are linear over a specified range, accurate, precise and reproducible (Refs. 8, 11, and 13). Gas chromatography/mass spectrometry studies were used to confirm the identity of the major stanols (Ref. 14). The data obtained from GC/MS studies with the plant stanol ester raw material and with chemical standards were compared with published spectra and confirmed the purity and identity of the major stanols, sitostanol and campestanol. The method has neither been subjected to validation through the AOAC's collaborative study or peer-verified method validation procedures, nor is it published in the open literature. FDA is requesting comments on the suitability of the plant stanol ester petitioner's methods for assuring that foods bearing the health claim contain the qualifying levels of plant stanol esters. In this document, FDA is incorporating the plant stanol ester petitioner's methods by reference in accordance with 5 U.S.C. 552(a) and 1 CFR part 51. Copies of the methods may be obtained from the Center for Food Safety and Applied Nutrition's Office of Nutritional Products, Labeling, and Dietary Supplements, Division of Nutrition Science and Policy, 200 C St. SW., rm. 2831, Washington, DC 20204, or may be examined at the Center for Food Safety and Applied Nutrition's Library, 200 C St. SW., rm. 3321, Washington, DC, and at the Office of the Federal Register, 800 North Capital St. NW., suite 700, Washington, DC.

D. Nature of the Food Eligible to Bear the Claim

1. Eligible Types of Foods and Qualifying Level of Plant Sterol/Stanol Esters Per Serving

a. *Plant sterol esters.* Section 101.83(c)(2)(iii)(A)(*1*) provides that the types of foods eligible to bear the plant sterol esters and risk of CHD health claim are spreads and dressings for salad. Section 101.83(c)(2)(iii)(A)(*1*) requires that any food bearing the health claim contain at least 0.65 g of plant sterol esters per reference amount customarily consumed (RACC) (i.e., per standardized serving). See §101.12 for an explanation of how RACC's are determined and a list of RACC's for commonly consumed foods. As discussed in section V.B of this document, the daily dietary intake level of plant sterol esters that has been associated with reduced risk of CHD is approximately 1.3 g or more per day.

The petitioner suggested that the qualifying level for foods to bear a health claim be 1.6 g per RACC, the same as the target daily intake level associated with reduced risk of CHD. The petitioner stated that the RACC's for spreads and dressings for salad, 1 and 2 tablespoons (tbsp), respectively, are similar to the mean daily intakes of spreads and dressings for salad identified in the U.S. Department of Agriculture (USDA) 1994/96 Continuing Surveys of Food Intakes by Individuals (Ref. 1, appendix G), which were 11.4 and 40 g/d, respectively. The petitioner reasoned that the qualifying level per RACC should be the same as the target daily intake level to assure that people who consume only one serving a day of spread or dressings will still be able to obtain the health benefits of the target daily intake level.

Although FDA recognizes that, based on the plant sterol ester petitioner's data, U.S. mean consumption for users of such products is only one serving of spread or dressing for salad a day, the agency is persuaded by the evidence from the studies supporting the claim that the daily amount should be consumed in at least two servings eaten at different times (see discussion of §101.83(c)(2)(i)(H) in section V.B of this document).

The agency has generally made the assumption that a daily food consumption pattern includes three meals and a snack (see 58 FR 2302 at 2379, January 6, 1993). Because of the wide variety of types of foods that could contain qualifying levels of soy protein in the soy protein/CHD health claim (§101.82) or soluble fiber in the soluble fiber/CHD health claim (§101.81), the agency concluded that the assumption of four servings/day of such foods was reasonable. Therefore, the daily qualifying level for soluble fiber substances and soy protein foods was based on consumption of four servings/day of such products. In contrast, however, there is not a wide variety of foods that contain plant sterol esters in significant quantities, and therefore the agency believes that it would be difficult for many consumers to eat four servings a day of such foods. The agency also has concluded that a recommendation for four servings of plant sterol ester-containing foods per day would not be an appropriate dietary recommendation because such foods are necessarily fat-based.

FDA believes that a recommendation for plant sterol-containing products to be consumed over two servings per day is reasonable in light of the composition of these products (i.e., their fat content) and the limited number of available products. Therefore, the agency is requiring that a food bearing a health claim for plant sterol esters and risk of CHD contain at least 0.65 g of plant sterol esters per reference amount customarily consumed (1.3 g divided by two servings per day). The agency is requesting comments on this decision.

The plant sterol ester petitioner requested that the claim be permitted for spreads and dressings for salad. The petitioner did not request authorization to use the health claim in the labeling of any other type of conventional food nor in the labeling of dietary supplements. The agency concluded in section II.B.3.a that the petitioner satisfied the requirement of §101.14(b)(3)(ii) to demonstrate that the use of plant sterol esters in spreads and dressings for salad at the levels necessary to justify a claim is safe and lawful. Furthermore, the petitioner submitted analytical methods for measurement of plant sterol esters in spreads and dressings for salad. Therefore, the agency is providing that the foods eligible to bear the health claim are spreads and dressings for salad. If comments on this interim final rule submit supporting data establishing that the use of plant sterol esters in other food products is safe and lawful and provide a validated analytical method that permits accurate determination of the amount of plant

sterol esters in these foods, FDA will consider broadening the categories of foods eligible to bear the claim in the final rule.

b. *Plant stanol esters.* Section 101.83(c)(2)(iii)(A)(*2*) provides that the types of foods eligible to bear the plant stanol esters and risk of CHD health claim are spreads, dressing for salad, snack bars, and dietary supplements in softgel form. Section 101.83(c)(2)(iii)(A)(*2*) requires that any food bearing the health claim contain at least 1.7 g of plant stanol esters per reference amount customarily consumed. As discussed in section V.B of this document, the daily dietary intake level of plant stanol esters that has been associated with reduced risk of CHD is 3.4 g or more per day.

The plant stanol ester petitioner suggested that the qualifying level for foods to bear a health claim be 0.85 g per RACC. The petitioner explained that this level was derived by dividing the target daily intake level of 3.4 g plant stanol esters by four daily servings.

As discussed in section V.B of this document, analysis of the studies supporting the claim has persuaded FDA that the daily intake of plant stanol esters should be consumed in at least two servings eaten at different times. Moreover, as with plant sterol esters (see section V.D.1.a of this document), FDA believes that two servings of plant stanol esters per day is a more appropriate baseline than four. There is not a wide variety of foods that contain plant stanol esters in significant quantities, and therefore it would be difficult for many consumers to eat four servings a day of such foods. The agency also has concluded that a recommendation for four servings of plant sterol ester-containing foods per day would not be an appropriate dietary recommendation because such foods, like foods containing plant sterol esters, are necessarily fat-based.

As with plant sterol esters, the agency believes that a recommendation for the daily intake of plant stanol esters to be consumed over two servings per day is reasonable in light of the composition of products containing plant stanol esters (i.e., their fat content) and the limited number of available products. Therefore, the agency is requiring that a food bearing a health claim for plant stanol esters and risk of CHD contain at least 1.7 g of plant stanol esters per reference amount customarily consumed (3.4 g divided by two servings per day). The agency is requesting comments on this decision.

The plant stanol ester petitioner requested that the claim be authorized for use on conventional foods and dietary supplements. The agency concluded in section II.B.3.b of this document that the petitioner satisfied the requirement of §101.14(b)(3)(ii) to demonstrate that the use of plant stanol esters in conventional foods or dietary supplements at the levels necessary to justify the claim is safe and lawful. The petitioner also submitted analytical methods for measurement of plant stanol esters in spreads, dressings for salad, snack bars, and dietary supplements in softgel (gelatin capsules with liquid center) form; however, the petitioner did not submit an analytical method suitable for measurement of plant stanol esters in other foods. Without such a method, FDA would have no way to verify that foods bearing the health claim contain the qualifying level of plant stanol esters per RACC, and false claims could be made that would mislead consumers. Therefore, the agency concludes that only foods for which a suitable method is available should be authorized to bear the health claim. Accordingly, FDA is providing that the foods eligible to bear the health claim are spreads, dressings for salad, snack bars, and dietary supplements in softgel form. If comments on this interim final rule provide a validated analytical method that permits accurate determination of the amount of plant stanol esters in other foods, FDA will consider broadening the categories of foods eligible to bear the claim in the final rule.

2. Fat Content Requirements

a. *Low fat.* In §101.83(c)(2)(iii)(B), the agency is requiring, consistent with other authorized heart disease health claims, that foods bearing the health claim meet the requirements for "low saturated fat" and "low cholesterol" (see §101.62(c)(2) and (d)(2) (21 CFR 101.62(c)(2) and (d)(2)). As discussed elsewhere in this document and in the preamble to the final rule on fiber-containing fruits, vegetables, and grain products and CHD (58 FR 2552 at 2573), the scientific evidence linking diets low in saturated fat and cholesterol to reduced risk of CHD is strong. Therefore, FDA has consistently required foods that make claims about reducing the risk of CHD to be low in saturated fat and cholesterol.

With few exceptions, as noted below, FDA has also required that foods bearing the previously authorized CHD health claims meet the requirements for "low fat" (see §101.62(b)(2)). In the dietary lipid and CVD proposed rule, FDA proposed that in order for a food to bear the health claim, the food must meet the requirements for a "low" claim relative to total fat content (56 FR 60727 at 60739). The agency noted that, while total fat is not directly related to increased risk for CHD, it may have significant indirect effects. The agency mentioned that low fat diets facilitate reductions in the intake of saturated fat and cholesterol to recommended levels. Furthermore, the agency noted that obesity is a major risk factor for CHD, and dietary fats, which have more than twice as many calories per gram as proteins and carbohydrates, are major contributors to total calorie intakes. For many adults, maintenance of desirable body weight is more readily achieved with moderation of intake of total fat. The agency also concluded that this approach would be most consistent with the U.S. Dietary Guidelines, 4th edition (Ref. 107) and other dietary guidance that recommended diets low in saturated fat, total fat, and cholesterol. In the dietary saturated fat and cholesterol and CHD final rule (58 FR 2739 at 2742), FDA required most foods bearing the claim to meet the requirements for "low fat," but allowed for the exception that fish and game meats could instead meet the less demanding requirements for "extra lean," because these foods are appropriately included in a diet low in fat, saturated fat, and cholesterol. The agency also waived the requirement for "low fat" on products consisting of or derived from whole soybeans in the soy protein final rule (64 FR 57700 at 57718), as long as those products contained no additional fat not derived from the soybeans. FDA noted that products derived from whole soybeans are useful sources of soy protein that, like fish and game meats that are "extra lean," can be appropriately incorporated in a diet that is low in fat, saturated fat, and cholesterol.

The recently distributed Dietary Guidelines for Americans, 2000 (Ref. 103) modify the previous guideline for total fat intake. The new guideline states, "Choose a diet that is low in saturated fat and cholesterol and moderate in total fat." This new guideline also states, "Some kinds of fat, especially saturated fats, increase the risk for coronary heart disease by raising the blood cholesterol. In contrast, unsaturated fats (found mainly in vegetable oils) do not increase blood cholesterol." This modification in the dietary guidelines, from the recommendation to choose a diet low in total fat in the 4th edition of the U.S. Dietary Guidelines (Ref. 107) to the recommendation to choose a diet moderate in total fat in the Dietary Guidelines for Americans, 2000 (Ref. 103) is based on current scientific

evidence of the role of diet in CHD, which does not support assigning first priority to a diet low in total fat (Ref. 108). The agency's reliance on dietary guidelines in this rulemaking and in previous health claim regulations is based on provisions of the 1990 amendments that direct FDA to issue health claim regulations that take into account the role of the nutrients in food in a way that will enhance the chances of consumers maintaining healthy dietary practices (see section 403(r)(3)(A) and (r)(3)(B) of the act (21 U.S.C. 343(r)(3)(A) and (r)(3)(B)), along with legislative history that mentions the role of health claims in encouraging Americans to eat balanced, healthful diets that meet federal government recommendations (Ref. 105).

The agency finds that not imposing a "low fat" requirement is consistent with the emphasis in the new Dietary Guidelines for Americans, 2000 (Ref. 103) on diets moderate in total fat. Inasmuch as fats are currently the only technically feasible carriers of plant sterol/stanol esters, requiring foods bearing the health claim to be "low fat" would greatly limit the number of foods that could use this health claim. Such a requirement would lessen the public health benefits of the rule. On the other hand, there are a number of foods, such as spreads and dressings for salad, that can be formulated to contain plant stanol or sterol esters while still qualifying as "low saturated fat" and "low cholesterol." Given the strength of the evidence supporting the cholesterol-lowering effects of plant sterol/stanol esters, the agency is requiring that foods bearing this health claim meet the nutrient content requirements in §101.62 for "low saturated fat" and "low cholesterol," but not the requirements for "low fat."

b. *Disqualifying levels.* The plant sterol ester and plant stanol ester petitioners requested an exception for certain food products from the disqualifying nutrient level for total fat per 50 g of food in the general health claim regulations (§101.14(a)(4)). The plant sterol ester petitioner requested an exception for spreads and dressings for salad, and the plant stanol ester petitioner requested an exception for all foods with small serving sizes (less than or equal to 2 tbsp or 30 g per RACC). Section 403(r)(3)(A)(ii) of the act provides that a health claim may only be made for a food that:

> does not contain, as determined by the Secretary by regulation, any nutrient in an amount which increases to persons in the general population the risk of a disease or health-related condition which is diet related, taking into account the significance of the food in the total daily diet, except that the Secretary may by regulation permit such a claim based on a finding that such a claim would assist consumers in maintaining healthy dietary practices and based on a requirement that the label contain a disclosure * * *.

Accordingly, if FDA finds that such a claim will assist consumers in maintaining healthy dietary practices, the agency may issue a regulation permitting the claim, provided that the regulation requires the label of foods that bear the claim to identify the nutrient that exceeds the disqualifying level. The general requirements for health claims, §101.14(a)(4) and (e)(3), implement this provision of the act. Section 101.14(a)(4) defines the disqualifying levels of total fat, saturated fat, cholesterol, and sodium for different types of foods. The disqualifying level for total fat is 13 g per RACC, per labeled serving size, and, for foods with a RACC of 30 g or less or 2 tbsp or less (i.e., foods with a small serving size), per 50 g. All three criteria apply; i.e., if a food with a small serving size contains more than 13 g of total fat per 50 g, it is considered to exceed the disqualifying level for total fat even if it contains less than 13 g of total fat per RACC and per labeled serving size. Section 101.14(e)(3) provides that the nutrient content of foods that bear a health claim must be within the disqualifying levels in §101.14(a)(4), unless: (1) FDA has established alternative disqualifying levels in the regulation authorizing the claim; or (2) FDA has permitted the claim based on a finding that it will assist consumers in maintaining healthy dietary practices, and the label of foods bearing the claim bears the required disclosure statement about the nutrient that exceeds the disqualifying level.

FDA first considered the plant sterol ester petitioner's request for an exception limited to spreads and dressings for salad. As noted above, foods with reference amounts of 30 g or 2 tbsp or less must contain no more than 13 g of total fat per 50 g of food product to avoid disqualification (§101.14(a)(4)). Reference amounts customarily consumed for spreads and dressings for salad are 1 tbsp and 30 g, respectively. Many spreads and dressings for salad contain total fat levels above the 13 g total fat per 50 g food disqualifying level. Spreads and dressings for salad, however, are appropriate vehicles for plant sterol/stanol esters because such substances are soluble in these fat-based foods.

In the proposed rule entitled "Food Labeling: Nutrient Content Claims, General Principles; Health Claims, General Requirements and Other Specific Requirements for Individual Health Claims" (60 FR 66206, December 21, 1995; hereinafter the 1995 proposed rule), the agency proposed four factors as being important to a decision as to whether to grant an exception from a disqualifying level (60 FR 66206 at 66222). The agency applied these four factors in its consideration of whether to grant an exception from the per 50 g disqualifying level of total fat for spreads and dressings for salad.

The first factor is whether the disease that is the subject of the petition is of such public health significance, and the role of the diet so critical, that the use of a disqualifying level is not appropriate. CHD is of the highest public health significance, and the role of the diet is critical to reducing the risk of CHD. The National Heart, Lung and Blood Institute in its report, "Morbidity and Mortality: 1998 Chartbook on Cardiovascular, Lung and Blood Diseases," published in 1998, estimated that the prevalence of CHD in the United States was 12 million (Ref. 109). Furthermore, it was estimated that 2,130,000 hospitalizations and 9,941,000 visits to physicians' offices were the result of CHD in the United States in 1995 (Ref. 109). CHD is the leading cause of premature, permanent disability in the U.S. labor force, accounting for 19 percent of disability allowances by the Social Security Administration. CHD has a significant effect on U.S. health care costs. For 1999, total direct costs related to CHD were estimated at $53.1 billion and indirect costs from lost productivity associated with morbidity (illness and disability) and mortality (premature deaths) at $46.7 billion (Ref. 22). The agency notes that since plant sterol/stanol esters have been shown to significantly reduce blood cholesterol levels, and thereby help reduce the risk of CHD, an exception from the disqualifying level appears appropriate when considering the disease that is the subject of the claim.

The second factor is whether, absent an exception from the disqualifying levels, the availability of foods that qualify for a health claim would be adequate to address the public health concern that is the subject of the health claim. If only a limited number of food products qualify to bear the claim because of the disqualifying levels, the agency would consider providing an exception. Without an exception from the disqualifying level for total fat, all currently marketed spreads and dressings for salad containing plant sterol/stanol esters would be ineligible to bear the health claim, and the number

of foods eligible for this health claim would be limited to such an extent that the public health value of the claim would be undermined. The agency therefore concludes that the second factor also supports granting an exception.

The third factor in the 1995 proposed rule was whether there is "evidence that the population to which the health claim is targeted is not at risk for the disease or health-related condition associated with the disqualifying nutrient" (60 FR 66206 at 66222). The agency stated that the current disqualifying nutrients—total fat, saturated fat, cholesterol and sodium—are associated with diseases or health-related conditions that pose risks to the general population, but that there may be some categories of foods that are targeted to specific subpopulations that are not at particular risk for the disease or health-related condition associated with the disqualifying nutrient (toddlers, for example). Because the target population for this health claim is the general population, not a specific subpopulation that is not at risk for CHD, FDA concludes that the third factor does not weigh in favor of granting an exception from the disqualifying levels for total fat.

The final factor is whether there are any other public health reasons for providing for disclosure of the total fat level rather than disqualification. In this regard, the agency notes that the scientific evidence indicates that plant sterol/stanol esters could contribute significantly to reducing the risk of CHD in the United States. As reviewed in section III.C of this document, a number of well controlled randomized trials have found that plant sterol/stanol esters reduce cholesterol levels in amounts that can be easily consumed by the average adult when incorporated into spreads or dressings for salad. The agency has determined that permitting the health claim on plant sterol/stanol ester-containing spreads and dressings for salad will help consumers develop a dietary approach that will result in significantly lower cholesterol levels and an accompanying reduction in the risk of heart disease.

Another public health reason for providing for disclosure of the total fat level rather than disqualification concerns the change in expert opinion on total fat intake, the risk of CHD, and general health. Although diets high in saturated fat and cholesterol are implicated in CHD, current scientific evidence does not indicate that diets high in unsaturated fat are associated with CHD (Refs. 103 and 108). Furthermore, the 2000 Dietary Guidelines Advisory Committee concluded that the scientific evidence on dietary fat and health supports assigning first priority to reducing saturated fat and cholesterol intake, not total fat intake (Ref. 108). In fact, the new guideline for fat intake in the Dietary Guidelines for Americans, 2000 (Ref. 103) states, "Choose a diet that is low in saturated fat and cholesterol and moderate in total fat."

Based on the agency's analysis of the four factors identified in the 1995 proposed rule (60 FR 66206 at 66222) and consistent with the new Dietary Guidelines for Americans, 2000 (Ref. 103), the agency has determined that, despite the fact that spreads and dressings for salad that contain plant stanol/sterol esters may also contain a disqualifying level of total fat per 50 g, a health claim for plant sterol/stanol esters on such foods will assist consumers in maintaining healthy dietary practices. Therefore, the agency is providing in §101.83(c)(2)(iii)(C) a limited exception to the per 50 g disqualifying nutrient level for total fat in §101.14(a)(4) for spreads and dressings for salad that contain plant sterol/stanol esters. The agency is requesting comment on this decision. All foods bearing the health claim for plant sterol/stanol esters and risk of CHD must, however, meet the requirements for "low saturated fat" and "low cholesterol" (see §101.83(c)(2)(iii)(B)). Likewise, all foods bearing the claim must meet the 13 g limit for total fat per RACC and per labeled serving size.

In accordance with §101.14(e)(3), FDA is also providing that spreads and dressings for salad that take advantage of the exception to the disqualifying level must bear a disclosure statement that complies with §101.13(h) (21 CFR 101.13(h)). This statement must identify the disqualifying nutrient and refer the consumer to more information about the nutrient, as follows: "See nutrition information for fat content." This statement must be included on the label of spreads and dressings for salad that bear a health claim for plant sterol/stanol esters and risk of CHD and that contain more than 13 g of total fat per 50 g of product. Requirements for the format and placement of the disclosure statement are found in §101.13(h)(4).

FDA considered the plant stanol ester petitioner's request that the exception to the disqualifying level for total fat per 50 g apply to all foods with small serving sizes. The agency has decided not to grant this request. There is a wide variety of foods that are consumed in small serving sizes, and the agency is not aware of any public health rationale that would justify applying the exception to all possible foods that are consumed in small serving sizes. Nor did the plant stanol ester petitioner provide such a rationale. The petitioner first argued generally that the benefits of cholesterol reduction through consumption of plant stanol esters would outweigh any negative dietary consequences of consuming foods that would not qualify for the health claim absent an exception from the disqualifying level for total fat (Ref. 8, page 25). The petitioner then argued more specifically that foods containing plant stanol esters replace other fat-containing foods in the diet (Ref. 8, page 25): "Benecol foods are promoted as foods to be used in place of other similar foods. In the case of spreads, for example, Benecol spreads can be used as an alternative to butter, margarine or other spreads and, therefore, will not increase the overall level of fat in the diet while providing the cholesterol-lowering benefits of plant stanol esters."

This rationale would not apply to all foods with small serving sizes, however, because not all such foods are used in place of other foods. This rationale provided by the petitioner applies to spreads and dressings for salad, but not necessarily to other foods with small serving sizes. FDA also does not agree that the health benefits of plant stanol esters outweigh the negative consequences of consuming high fat foods to such an extent that an unlimited exception to the disqualifying level for total fat should be permitted for all foods with small serving sizes. The agency further concludes that such a broad exception is not necessary because the availability of spreads and dressings for salad that qualify for the health claim will be sufficient so that consumers will be able to eat a sufficient quantity of plant sterol/stanol esters to receive the cholesterol-lowering benefits those substances provide. It is also likely that there are other types of foods that can be formulated to fall within the limits for total fat in §101.14(a)(4).

Despite FDA's reluctance to grant broad exceptions to the disqualifying levels, the agency is willing to consider additional exceptions on a limited, case-by-case basis. Manufacturers of products other than spreads and dressings for salad that exceed the disqualifying level of total fat may submit comments with supporting information or petition the agency for an exception from disqualification in accordance with §101.14(e)(3) if they wish to make the health claim that is the subject of this interim final rule.

3. Minimum Nutrient Contribution Requirement

The plant sterol ester and plant stanol ester petitioners requested an exception for certain food products containing plant sterol/stanol esters from the minimum nutrient contribution requirement in the general health claim regulations (§101.14(e)(6)). The plant sterol ester petitioner requested an exception for dressings for salad, and the plant stanol ester petitioner requested a general exception for all foods. Section 101.14(e)(6) specifies that conventional foods bearing a health claim must contain 10 percent or more of the Reference Daily Intake or the Daily Reference Value for vitamin A, vitamin C, iron, calcium, protein, or fiber per reference amount customarily consumed before any nutrient addition, except as otherwise provided in individual regulations authorizing particular health claims. Dietary supplements are not subject to this requirement. As explained in the 1993 health claims final rule (58 FR 2478), FDA concluded that such a requirement is necessary to ensure that the value of health claims will not be trivialized or compromised by their use on foods of little or no nutritional value (58 FR 2478 at 2521). FDA adopted this requirement in response to Congress' intent that health claims be used to help Americans maintain a balanced and healthful diet (Ref. 105) (58 FR 2478 at 2489 and 2521).

The agency concludes that, with respect to dressings for salad, the minimum nutrient content requirements of §101.14(e)(6), while important, are outweighed by the public health importance of communicating the cholesterol-lowering benefits from consumption of plant sterol/stanol esters. The agency believes that the value of health claims will not be trivialized or compromised by their use on dressings for salad because dressings for salad often are consumed with foods rich in nutrients and fiber. Salads, for example, are usually rich in vegetables that provide important nutrients at significant levels, e.g., tomatoes—vitamins A and C; carrots—vitamin A; spinach—vitamin A and calcium.

In recognition of the usefulness of plant sterol/stanol esters in reducing blood cholesterol and the nutritional value of salad, FDA has determined that there is sufficient public health evidence to support providing an exception from §101.14(e)(6) for plant sterol/stanol ester-containing dressings for salad. However, the agency has decided not to grant the plant stanol ester petitioner's request for a general exception from the minimum nutrient content requirement. The basis for the plant stanol ester petitioner's request for such an exception is that the cholesterol-lowering benefits of plant stanol ester-containing foods do not depend upon the presence of 10 percent or more of the Reference Daily Intake or the Daily Reference Value for vitamin A, vitamin C, iron, calcium, protein, or fiber. The agency, however, concludes that this rationale is not sufficient to justify an exception for all possible foods that would require an exception from the minimum nutrient contribution requirement in order to use the health claim. FDA believes that case-by-case consideration of the justification for an exception is necessary to ensure that the goals of the minimum nutrient contribution requirement are not undermined.

Accordingly, in §101.83(c)(2)(iii)(D), the agency is providing that dressings for salad bearing the health claim are excepted from the minimum nutrient requirement of §101.14(e)(6), but that other foods must comply with this requirement to be eligible to bear a health claim about plant sterol/stanol esters and the risk of CHD. The agency is requesting comment on this decision.

Manufacturers of foods that do not meet the minimum nutrient contribution requirement may submit comments with supporting information or petition the agency to request an exception from this requirement if they wish to use the health claim that is the subject of this interim final rule.

E. Optional Information

FDA is providing in §101.83(d)(1) that the claim may state that the development of heart disease depends on many factors and, consistent with other authorized CHD health claims, may list the risk factors for heart disease. The risk factors are those currently listed in §§101.75(d)(1), 101.77(d)(1), 101.81(d)(1), and 101.82(d)(1). The claim may also provide additional information about the benefits of exercise and management of body weight to help lower the risk of heart disease.

In §101.83(d)(2), consistent with §§101.75(d)(2), 101.77(d)(2), 101.81(d)(2), and 101.82(d)(2), FDA is providing that the claim may state that the relationship between diets that include plant sterol/stanol esters and reduced risk of heart disease is through the intermediate link of "blood cholesterol" or "blood total cholesterol" and "LDL cholesterol." The relationship between plant sterol/stanol esters and reduced blood total cholesterol and LDL cholesterol is supported by the scientific evidence summarized in this interim final rule.

In §101.83(d)(3), the agency is providing that, consistent with §§101.75(d)(3), 101.77(d)(3), 101.81(d)(3), and 101.82(d)(3), the claim may include information from §101.83(a) and (b). These paragraphs summarize information about the relationship between diets that include plant sterol/stanol esters and the risk of CHD and about the significance of that relationship. This information helps to convey the seriousness of CHD and the role that a diet that includes plant sterol/stanol esters can play to help reduce the risk of CHD.

In §101.83(d)(4), the agency is providing that the claim may include information on the relationship between saturated fat and cholesterol in the diet and the risk of CHD. This information helps to convey the importance of keeping saturated fat and cholesterol intake low to reduce the risk of CHD.

In §101.83(d)(5), the agency is providing that the claim may state that diets that include plant sterol/stanol esters and are low in saturated fat and cholesterol are part of a dietary pattern that is consistent with current dietary guidelines for Americans.

In §101.83(d)(6), the agency is providing that the claim may state that individuals with elevated blood total and LDL cholesterol should consult their physicians for medical advice and treatment. If the claim defines high or normal blood total and LDL cholesterol levels, then the claim shall state that individuals with high blood cholesterol should consult their physicians for medical advice and treatment.

In §101.83(d)(7), the agency is providing that the claim may include information on the number of people in the United States who have heart disease. The sources of this information shall be identified, and it shall be current information from the National Center for Health Statistics, the National Institutes of Health, or "Nutrition and Your Health: Dietary Guidelines for Americans, 2000," USDA and Department of Health and Human Services (DHHS), Government Printing Office (GPO) (Ref. 103).

The optional information provided in §101.83(d)(4) through (d)(7) is consistent with optional information set forth in §§101.75, 101.77, 101.81, and 101.82. The intent of this information is to help consumers understand the seriousness of CHD in the United States and the role of diets that include plant sterol/stanol esters and are low in saturated fat and cholesterol in reducing the risk of CHD.

F. Model Health Claims

In §101.83(e), FDA is providing model health claims to illustrate the requirements of §101.83. FDA emphasizes that these model health claims are illustrative only. These model claims illustrate the required, and some of the optional, elements of the interim final rule. Because the agency is authorizing a claim about the relationship between plant sterol/stanol esters and CHD, not approving specific claim wording, manufacturers will be free to design their own claim so long as it is consistent with §101.83(c) and (d).

In §101.83(e)(1)(i) and (e)(1)(ii), the model claims illustrate all of the required elements of the health claim for plant sterol esters. The first claim states, "Foods containing at least 0.65 grams per serving of plant sterol esters, eaten twice a day with meals for a daily total intake of at least 1.3 grams, as part of a diet low in saturated fat and cholesterol, may reduce the risk of heart disease. A serving of [name of the food] supplies grams of vegetable oil sterol esters." The second claim states, "Diets low in saturated fat and cholesterol that include two servings of foods that provide a daily total of at least 1.3 grams of vegetable oil sterol esters in two meals may reduce the risk of heart disease. A serving of [name of the food] supplies grams of vegetable oil sterol esters."

In §101.83(e)(2)(i) and (e)(2)(ii), the model claims illustrate all of the required elements of the health claim for plant stanol esters. The first claim states, "Foods containing at least 1.7 grams per serving of plant stanol esters, eaten twice a day with meals for a total daily intake of at least 3.4 grams, as part of a diet low in saturated fat and cholesterol, may reduce the risk of heart disease. A serving of [name of the food] supplies grams of plant stanol esters." The second claim states, "Diets low in saturated fat and cholesterol that include two servings of foods that provide a daily total of at least 3.4 grams of vegetable oil stanol esters in two meals may reduce the risk of heart disease. A serving of [name of the food supplies grams of vegetable oil stanol esters."

The plant stanol ester petitioner proposed three model health claims that included the following statements, respectively: "5 g of plant stanol esters per day is more effective in reducing cholesterol and may further reduce the risk of heart disease," "5 g plant stanol esters may be more beneficial in reducing the risk of heart disease," and "5 g plant stanol esters per day has been shown to further lower LDL (bad) cholesterol and may further reduce the risk of heart disease." The agency reviewed the scientific evidence to determine whether the data supported these statements, starting with four studies (Refs. 88 through 90, and 94) that reported the blood cholesterol-lowering effects from two or more consumption levels of plant stanol esters.

Hallikainen et al. (Ref. 88) conducted a single-blind, crossover study in which 22 hypercholesterolemic subjects consumed margarine containing four different doses of plant stanol esters, including 1.4, 2.7, 4.1, and 5.4 g/d (0.8, 1.6, 2.4, and 3.2 g/d of free plant stanols), for 4 weeks each. These test margarine phases were compared to a control margarine phase, also 4 weeks long. Serum total cholesterol concentration decreased (calculated in reference to control) by 2.8 percent ($p=0.384$), 6.8 percent ($p< 0.001$), 10.3 percent ($p<0.001$) and 11.3 percent ($p< 0.001$) by doses from 1.4 to 5.4 g plant stanol esters. The respective decreases for LDL cholesterol were 1.7 percent ($p=0.892$), 5.6 percent (< 0.05), 9.7 percent ($p<0.001$) and 10.4 percent ($p<0.001$). Although serum total and LDL cholesterol decreases were numerically greater with the 4.1 and 5.4 g doses than with the 2.7 g dose, these differences were not statistically significant ($p=0.054$-0.516).

Nguyen et al. (Ref. 90) examined the blood cholesterol-lowering effects in subjects consuming either a U.S.-reformulated spread containing 5.1 g/d plant stanol esters (3 g/d free plant stanols), a U.S.-reformulated spread containing 3.4 g per d plant stanol esters (2 g/d of free plant stanols), or a U.S.-reformulated spread without plant stanol esters for 8 weeks. Serum total cholesterol ($p < 0.001$) and LDL cholesterol ($p <0.02$) levels were significantly reduced in the 5.1 and 3.4 g/d plant stanol ester groups compared with the placebo group. The U.S. spread containing 5.1 g/d plant stanol esters lowered serum total and LDL cholesterol by 6.4 and 10.1 percent, respectively, when compared to baseline ($p <0.001$). The 3.4 g/d plant stanol ester U.S. spread group showed a 4.1 percent reduction in both serum total and LDL cholesterol levels compared to baselinese 105 ($p < 0.001$). The reduction in the LDL cholesterol level was found to be significantly greater in the 5.1 g/d plant stanol ester group compared to the 3.4 g/d plant stanol ester group ($p < 0.001$). The authors did not report a statistical analysis comparing serum total cholesterol concentrations between the two consumption levels of plant stanol esters.

Miettinen et al. (Ref. 89) instructed 153 mildly hypercholesterolemic subjects to consume 24 g/d of canola oil margarine or the same margarine with added plant stanol esters for a targeted consumption of 5.1 g/d plant stanol esters (3 g/d free plant stanols), without other dietary changes. At the end of 6 months, those consuming plant stanol esters were randomly assigned either to continue the test margarine with a targeted intake of 5.1 g/d plant stanol esters or to switch to a targeted intake of 3.4 g/d plant stanol esters (2 g/d free plant stanols) for an additional 6 months. Based on measured margarine consumption, average plant stanol ester intakes were 4.4 g/d (in the 5.1 g/d target group) and 3.1 g/d (in the 3.4 g/d target group). Significant reductions in serum total and LDL cholesterol were reported after consuming 4.4 or 3.1 g/d of plant stanol esters compared to the control group ($p < 0.01$). Moreover, a statistically significant difference was observed between the 6th and 12th months in the serum total cholesterol ($p= 0.047$) and LDL cholesterol ($p= 0.017$) curves between the 4.4 and 3.1 g/d plant stanol ester groups, representing a greater serum total cholesterol and LDL cholesterol reduction in the 4.4 g/d plant stanol ester group compared to the 3.1 g/d plant stanol ester group. The authors state, however, "Despite the finding that the decreasing trends between the 6th and 12th months in the total and LDL cholesterol concentrations in the group consuming 2.6 g of sitostanol were slightly different from the increasing trends in the group consuming 1.8 g, for practical purposes the two doses produced similar cholesterol-lowering effects."

Vanhanen et al. (Ref. 94) reported the hypocholesterolemic effects of 1.36 g/d of plant stanol esters (800 mg/d of free plant stanols) RSO mayonnaise for 9 weeks followed by 6 weeks of consumption of 3.4 g/d of plant stanol esters (2 g/d of free plant stanols) in RSO mayonnaise compared to a group receiving RSO mayonnaise alone. After 9 weeks of consumption of the lower dose (1.36 g/d) plant stanol ester mayonnaise, the changes in serum levels of total and LDL cholesterol were -4.1 percent ($p < 0.05$) and -10.3 percent (not statistically significant), respectively, as compared to the control. Greater reductions in both serum total and LDL cholesterol were observed after consumption of 3.4 g/d of plant stanol esters for an additional 6 weeks ($p < 0.05$). The changes in serum levels of total and LDL cholesterol were -9.3 percent and -15.2 percent,

respectively, for subjects consuming 3.4 g/d of plant stanol esters as compared to control. These investigators commented:

[T]he reductions in the serum cholesterol level by SaE [sitostanol ester] were dose-dependent, indicating that the low dose, less than 1 g of sitostanol/day, reduced LDL-cholesterol insufficiently (8.5%). Accordingly, the higher dose, about 2 g/d, appears to be large enough for a reasonable (about 15%) lowering of serum LDL cholesterol. Preliminary studies with even higher doses, 3 g/d, does not appear to increase the cholesterol-lowering effect, even though cholesterol absorption efficiency decreases by almost two-thirds in men with non-insulin-dependent diabetes mellitus at least * * *.

In only one (Ref. 90) of the four studies (Refs. 88 through 90, and 94) described above did the investigators report a statistically significant greater reduction in blood total and LDL cholesterol from consumption of 5 g or more of plant stanol ester compared to a lower consumption level of plant stanol ester. Another study (Ref. 88) found no statistically significant difference between the cholesterol-lowering effects of 5.4 g/d plant stanol esters and two lower intake levels (2.7 and 4.1 g/d). The remaining two studies (Refs. 89 and 94) involved maximum intakes of less than 5 g/d, but in both studies the authors expressed the opinion that higher intakes did not appear to increase the cholesterol-lowering effect for practical purposes. In addition to these multiple-dose studies, FDA reviewed six single-dose studies (Refs. 67, 77, 78, 81 and 82 (1 study), 91, and 92) that reported statistically significant blood cholesterol-lowering effects from daily intake levels greater than 3.4 g/d of plant stanol esters. The agency compared these studies to the studies that found statistically significant blood cholesterol-lowering effects at intakes of plant stanol esters at or close to the 3.4 g/d level. Considering all the studies described above that reported the cholesterol-lowering effectiveness of total daily intake levels greater than 3.4 g/d of plant stanol esters (Refs. 67, 77, 78, 81 and 82 (1 study), 88 through 92, and 94), the blood cholesterol-lowering effect for total cholesterol ranged from 7.1 percent from 5.8 g/d of plant stanol esters (Refs. 81 and 82 (1 study)) to 11.3 percent from 5.4 g/d of plant stanol esters (Ref. 88), and for LDL cholesterol the range was from 7.5 percent from 5.8 g/d of plant stanol esters (Refs. 81 and 82 (1 study)) to 15 percent from 4.4 g/d of plant stanol esters (Ref. 89). These cholesterol-lowering results are similar to those observed in studies that utilized a daily total intake at or close to 3.4 g/d of plant stanol esters (Refs. 58, 80, 89, 90, and 94). In these lower daily intake studies, the blood total cholesterol reduction ranged from 9.3 percent (Ref. 94) to 12 percent (Ref. 80) for 3.4 g/d of plant stanol esters. Similarly, for LDL cholesterol the reductions associated with these lower daily intake levels ranged from 6.4 percent for 3.31 g/d of plant stanol esters (Ref. 58) to 15 percent for 3.4 g/d of plant stanol esters (Refs. 80 and 94). Thus, comparison of the blood cholesterol-lowering ranges between the higher and the lower daily intake levels of plant stanol esters suggests that there is no increased benefit from daily intake levels greater than 3.4 g/d.

Furthermore, the results of a research synthesis analysis (Ref. 100) suggest that intakes greater than about 3.4 g/d of plant stanol esters (2 g/d of plant stanol) would not result in further reduction in LDL cholesterol. This analysis found that a continuous dose response exists up to the 3.4 g/d level, but at higher daily intake levels of plant stanol esters, no further reduction in LDL cholesterol was apparent. Another recent analysis of the dose responsiveness to plant stanol esters, using a compilation of data from published studies, indicates a curvilinear dose response for both blood total and LDL cholesterol, with a clear leveling-off at an intake of about 3.74 g/d plant stanol esters (2.2 g/d free plant stanols) (Ref. 110).

The agency therefore concludes that the weight of the evidence does not support the comparative claims requested by the plant stanol esters petitioner and that such claims would be misleading to consumers. Therefore, FDA is not including the petitioner's requested comparative claims in the model health claims in §101.83 and is not authorizing the plant sterol/stanol esters and risk of CHD health claim to include any statements claiming that 5 g per day of plant stanol esters is more effective than 3.4 g per day of plant stanol esters in reducing blood total or LDL cholesterol or in reducing the risk of heart disease.

VI. Issuance of an Interim Final Rule, Immediate Effective Date, and Opportunity for Public Comment

FDA is issuing this rule as an interim final rule, effective immediately, with an opportunity for public comment. Section 403(r)(7) of the act authorizes FDA (by delegation from the Secretary of Health and Human Services (the Secretary)) to make proposed regulations issued under section 403(r) of the act effective upon publication pending consideration of public comment and publication of a final regulation, if the agency determines that such action is necessary for public health reasons. This authority enables the Secretary to act promptly on petitions that provide information that is necessary to: (1) Enable consumers to develop and maintain healthy dietary practices, (2) enable consumers to be informed promptly and effectively of important new knowledge regarding nutritional and health benefits of food, or (3) ensure that scientifically sound nutritional and health information is provided to consumers as soon as possible. Proposed regulations made effective upon publication under this authority are deemed to be final agency action for purposes of judicial review. The legislative history indicates that such regulations should be issued as interim final rules (H. Conf. Rept. No. 105 399, at 98 (1997)).

Both the plant sterol ester petitioner and the plant stanol ester petitioner have submitted requests for the agency to consider making any proposed regulation on the petitioned health claims effective upon publication in an interim final rule (Refs. 6 and 16).

The plant stanol ester petitioner's request states that all three of the criteria in section 403(r)(7)(A) of the act are met:

As the petition makes clear, regular consumption of plant stanol esters as part of a healthy dietary pattern provides substantial health benefits. The health claim will, for the first time, provide consumers with important health information on the package label regarding the role of plant stanol esters in lowering cholesterol and reducing the risk of heart disease—information which should be made available to consumers at the earliest possible time. The health claim will provide consumers with scientifically sound information on the nutritional and health benefits of foods containing plant stanol ester, and will enable consumers to develop and maintain healthy dietary practices that include the incorporation of plant stanol esters into their diets.

The plant sterol ester petitioner's request also states that all three of the criteria in section 403(r)(7)(A) of the act are met, and its rationale for meeting the criteria is similar to that of the plant stanol ester petitioner. The plant sterol ester petitioner also points out that if firms are required to wait until publication of a final rule to use the petitioned health claim, consumers will likely not read it on labeling until May 2001 or later. The petitioner further states, if FDA permits the claim to be used upon publication of the proposed rule, however, the claim could appear on labeling almost a year earlier, providing a significant period of time during which consumers could

effectively use the information to make healthier dietary choices.

The agency has considered the requests to make any proposed rule for plant sterol/stanol esters and CHD effective upon publication and concurs that the standard in section 403(r)(7)(A) of the act is met. The agency agrees with the plant sterol ester and plant stanol ester petitioners that authorizing the health claim immediately will help consumers develop and maintain healthy dietary practices. As discussed above, FDA has concluded that there is significant scientific agreement that plant sterol/stanol esters reduce blood total and LDL cholesterol levels. The reported reductions in blood total and LDL cholesterol levels are significant and may have a profound impact on population risk of CHD if consumption of plant stanol esters becomes widespread. The agency has determined that issuance of an interim final rule is necessary to enable consumers to be informed promptly and effectively of this important new knowledge regarding the nutritional and health benefits of plant sterol/stanol esters. The agency has also determined that issuance of an interim final rule is necessary to ensure that scientifically sound nutritional and health information is provided to consumers as soon as possible.

FDA invites public comment on this interim final rule. The agency will consider modifications to this interim final rule based on comments made during the comment period. Interested persons may submit to the Dockets Management Branch (address above) written comments regarding this interim final rule by November 22, 2000. Two copies of any comments are to be submitted, except that individuals may submit one copy. Comments are to be identified with the docket number found in brackets in the heading of this document. Received comments may be seen in the Dockets Management Branch between 9 a.m. and 4 p.m., Monday through Friday.

These regulations are effective September 8, 2000. The agency will address comments and confirm or amend the interim rule in a final rule.

VII. Environmental Impact

The agency has determined under 21 CFR 25.30(k) that this action is of a type that does not individually or cumulatively have a significant effect on the human environment. Therefore, neither an environmental assessment nor an environmental impact statement is required.

VIII. Analysis of Economic Impacts

A. Benefit-Cost Analysis

FDA has examined the economic implications of this interim final rule as required by Executive Order 12866. Executive Order 12866 directs agencies to assess all costs and benefits of available regulatory alternatives and, when regulation is necessary, to select regulatory approaches that maximize net benefits (including potential economic, environmental, public health and safety, and other advantages; distributive impacts; and equity). Executive Order 12866 classifies a rule as significant if it meets any one of a number of specified conditions, including having an annual effect on the economy of $100 million or adversely affecting in a material way a sector of the economy, competition, or jobs. A regulation is also considered a significant regulatory action if it raises novel legal or policy issues. FDA has determined that this interim final rule is not a significant regulatory action as defined by Executive Order 12866.

The authorization of health claims about the relationship between plant sterol/stanol esters and coronary heart disease leads to costs and benefits only to those food manufacturers who choose to use the claim. This interim final rule would not require that any labels be redesigned or that any products be reformulated. Therefore, this rule will not generate any direct compliance costs. No firm will choose to bear the cost of redesigning labels unless it believes that the claim will lead to increased sales of its product sufficient to justify that cost. The benefit of this rule is to provide new information in the market regarding the relationship between plant sterol/stanol esters and the risk of coronary heart disease. FDA authorization for this health claim will provide consumers with the assurance that this information is truthful, not misleading, and scientifically valid.

B. Small Entity Analysis

FDA has examined the economic implications of this interim final rule as required by the Regulatory Flexibility Act (5 U.S.C. 601 612). If a rule has a significant economic impact on a substantial number of small entities, the Regulatory Flexibility Act requires the agency to analyze regulatory options that would minimize the economic impact of the rule on small entities.

As previously explained, this interim final rule will not generate any direct compliance costs. Small businesses will incur costs only if they choose to take advantage of the marketing opportunity presented by this interim final rule. No small entity, however, will choose to bear the cost of redesigning labels unless it believes that the claim will lead to increased sales of its product sufficient to justify those costs.

Accordingly, FDA certifies that this interim final rule will not have a significant economic impact on a substantial number of small entities. Therefore, under the Regulatory Flexibility Act, no further analysis is required.

C. Unfunded Mandates Reform Act of 1995

Title II of the Unfunded Mandates Reform Act of 1995 (Public Law 104 4) requires cost-benefit and other analyses before any rulemaking if the rule would include a "Federal mandate that may result in the expenditure by State, local, and tribal governments, in the aggregate, or by the private sector, of $100,000,000 or more (adjusted annually for inflation) in any 1 year." FDA has determined that this interim final rule does not constitute a significant regulatory action under the Unfunded Mandates Reform Act.

IX. Paperwork Reduction Act

FDA concludes that the labeling provisions of this interim final rule are not subject to review by the Office of Management and Budget because they do not constitute a "collection of information" under the Paperwork Reduction Act of 1995 (44 U.S.C. 3501 3520). Rather, the food labeling health claim on the association between plant sterol/stanol esters and coronary heart disease is a "public disclosure of information originally supplied by the Federal government to the recipient for the purpose of disclosure to the public" (5 CFR 1320.3(c)(2)).

X. Federalism

FDA has analyzed this interim final rule in accordance with the principles set forth in Executive Order 13132. FDA has determined that the rule does not contain policies that have substantial direct effects on the states, on the relationship between the National Government and the States, or on the distribution of power and responsibilities among the various levels of government. Accordingly, the agency has concluded that the interim final rule does not contain policies that have federalism implications as defined in the order and consequently, a federalism summary impact statement is not required.

XI. References

The following references have been placed on display in the Dockets

Management Branch (address above) and may be seen by interested persons between 9 a.m. and 4 p.m., Monday through Friday.

1. Lipton, "Petition for Health Claim—Vegetable Oil Sterol Esters and Coronary Heart Disease," Item CP1, Docket 00P 1275, Dockets Management Branch, February 1, 2000.

2. Letter from Daniel R. Dwyer, Kleinfeld, Kaplan and Becker, to Sharon A. Ross, FDA, Item MT1, Docket 00P 1275, Dockets Management Branch, March 31, 2000.

3. Letter from Daniel R. Dwyer, Kleinfeld, Kaplan and Becker, to Sharon A. Ross, FDA, Item MT2, Docket 00P 1275, Dockets Management Branch, May 3, 2000.

4. Letter from Daniel R. Dwyer, Kleinfeld, Kaplan and Becker, to Lynn A. Larsen, FDA, June 30, 2000.

5. Letter from Lynn A. Larsen, FDA, to Nancy Schnell, Lipton, May 11, 2000.

6. Letter from Daniel R. Dwyer, Kleinfeld, Kaplan and Becker, to Lynn A. Larsen, FDA, June 26, 2000.

7. Letter from Nancy L. Schnell, Lipton, to Christine J. Lewis, FDA, August 2, 2000.

8. McNeil Consumer Healthcare, "Petition for Health Claim—Plant Stanol Esters and Coronary Heart Disease," Item CP1, Docket 00P 1276, Dockets Management Branch, February 15, 2000.

9. Letter from G. A. Leveille, McNeil Consumer Healthcare, to Sharon Ross, FDA, Item MM2, Docket 00P 1276, Dockets Management Branch, February 28, 2000.

10. Letter from Gilbert A. Leveille, McNeil Consumer Healthcare, to Sharon Ross, FDA, Item MM3, Docket 00P 1276, Dockets Management Branch, March 21, 2000.

11. Letter from Gilbert A. Leveille, McNeil Consumer Healthcare, to Sharon Ross, FDA, Item MM4, Docket 00P 1276, Dockets Management Branch, April 3, 2000.

12. Letter from Gilbert A. Leveille, McNeil Consumer Healthcare, to Sharon Ross, FDA, Item MM5, Docket 00P 1276, Dockets Management Branch, May 1, 2000.

13. Letter from Gilbert A. Leveille, McNeil Consumer Healthcare, to Sharon A. Ross, FDA, June 23, 2000.

14. Letter from Gilbert A. Leveille, McNeil Consumer Healthcare, to Lynn Larsen, FDA, July 18, 2000.

15. Letter from Lynn A. Larsen, FDA, to Dr. Gilbert A. Leveille, McNeil Consumer Healthcare, May 25, 2000.

16. Letter from Mark A. Sievers, Johnson & Johnson (parent company to McNeil Consumer Healthcare), to Lynn A. Larsen, FDA, June 14, 2000.

17. Letter from Gilbert A. Leveille, McNeil Consumer Healthcare, to Lynn Larsen, FDA, July 17, 2000.

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45. Letter from Daniel R. Dwyer, Kleinfeld, Kaplan and Becker, to George H. Pauli, FDA, September 24, 1999.

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47. Letter from John C. Young, McNeil Consumer Healthcare, to Alan Rulis, FDA, July 21, 1999.

48. Letter from John C. Young, McNeil Consumer Healthcare, to Alan Rulis, FDA, October 13, 1999.

49. McNeil Consumer Healthcare, "New Dietary Ingredient Notification—Plant Stanol Esters," Docket 95S 0316, Dockets Management Branch, August 19, 1999.

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List of Subjects in 21 CFR Part 101

Food labeling, Incorporation by reference, Nutrition, Reporting and recordkeeping requirements.

Therefore, under the Federal Food, Drug, and Cosmetic Act and under authority delegated to the Commissioner of Food and Drugs, 21 CFR part 101 is amended as follows:

PART 101—FOOD LABELING

1. The authority citation for 21 CFR part 101 continues to read as follows:

Authority: 15 U.S.C. 1453, 1454, 1455, 21 U.S.C. 321, 331, 342, 343, 348, 371.

2. Section 101.83 is added to subpart E to read as follows:

§101.83 Health claims: plant sterol/stanol esters and risk of coronary heart disease (CHD).

(a) *Relationship between diets that include plant sterol/stanol esters and the risk of CHD.* (1) Cardiovascular disease means diseases of the heart and circulatory system. Coronary heart disease (CHD) is one of the most common and serious forms of cardiovascular disease and refers to diseases of the heart muscle and supporting blood vessels. High blood total cholesterol and low density lipoprotein (LDL) cholesterol levels are associated with increased risk of developing coronary heart disease. High CHD rates occur among people with high total cholesterol levels of 240 milligrams per deciliter (mg/dL) (6.21 millimole per liter (mmol/l)) or above and LDL cholesterol levels of 160 mg/dL (4.13 mmol/l) or above. Borderline high risk blood cholesterol levels range from 200 to 239 mg/dL (5.17 to 6.18 mmol/l) for total cholesterol, and 130 to 159 mg/dL (3.36 to 4.11 mmol/l) of LDL cholesterol.

(2) Populations with a low incidence of CHD tend to have relatively low blood total cholesterol and LDL cholesterol levels. These populations also tend to have dietary patterns that are not only low in total fat, especially saturated fat and cholesterol, but are also relatively high in plant foods that contain dietary fiber and other components.

(3) Scientific evidence demonstrates that diets that include plant sterol/stanol esters may reduce the risk of CHD.

(b) *Significance of the relationship between diets that include plant sterol/stanol esters and the risk of CHD.* (1) CHD is a major public health concern in the United States. It accounts for more deaths than any other disease or group of diseases. Early management of risk factors for CHD is a major public health goal that can assist in reducing risk of CHD. High blood total and LDL cholesterol are major modifiable risk factors in the development of CHD.

(2) The scientific evidence establishes that including plant sterol/stanol esters in the diet helps to lower blood total and LDL cholesterol levels.

(c) *Requirements—(1) General.* All requirements set forth in §101.14 shall

be met, except §101.14(a)(4) with respect to the disqualifying level for total fat per 50 grams (g) in dressings for salad and spreads and §101.14(e)(6) with respect to dressings for salad.

(2) *Specific requirements*—(i) *Nature of the claim.* A health claim associating diets that include plant sterol/stanol esters with reduced risk of heart disease may be made on the label or labeling of a food described in paragraph (c)(2)(iii) of this section, provided that:

(A) The claim states that plant sterol/stanol esters should be consumed as part of a diet low in saturated fat and cholesterol;

(B) The claim states that diets that include plant sterol/stanol esters "may" or "might" reduce the risk of heart disease;

(C) In specifying the disease, the claim uses the following terms: "heart disease" or "coronary heart disease";

(D) In specifying the substance, the claim uses the term "plant sterol esters" or "plant stanol esters," except that if the sole source of the plant sterols or stanols is vegetable oil, the claim may use the term "vegetable oil sterol esters" or "vegetable oil stanol esters";

(E) The claim does not attribute any degree of risk reduction for CHD to diets that include plant sterol/stanol esters;

(F) The claim does not imply that consumption of diets that include plant sterol/stanol esters is the only recognized means of achieving a reduced risk of CHD; and

(G) The claim specifies the daily dietary intake of plant sterol or stanol esters that is necessary to reduce the risk of CHD and the contribution one serving of the product makes to the specified daily dietary intake level. Daily dietary intake levels of plant sterol and stanol esters that have been associated with reduced risk of are:

(*1*) 1.3 g or more per day of plant sterol esters.

(*2*) 3.4 g or more per day of plant stanol esters.

(H) The claim specifies that the daily dietary intake of plant sterol or stanol esters should be consumed in two servings eaten at different times of the day with other foods.

(ii) *Nature of the substance*—(A) *Plant sterol esters.* (*1*) Plant sterol esters prepared by esterifying a mixture of plant sterols from edible oils with food-grade fatty acids. The plant sterol mixture shall contain at least 80 percent beta-sitosterol, campesterol, and stigmasterol (combined weight).

(*2*) FDA will measure plant sterol esters by the method entitled "Determination of the Sterol Content in Margarines, Halvarines, Dressings, Fat Blends and Sterol Fatty Acid Ester Concentrates by Capillary Gas Chromatography," developed by Unilever United States, Inc., dated February 1, 2000, the method, which is incorporated by reference in accordance with 5 U.S.C. 552(a) and 1 CFR part 51, may be obtained from the Center for Food Safety and Applied Nutrition, Office of Nutritional Products, Labeling, and Dietary Supplements, Division of Nutrition Science and Policy, 200 C St. SW., rm. 2831, Washington, DC 20204, and may be examined at the Center for Food Safety and Applied Nutrition's Library, 200 C St. SW., rm. 3321, Washington, DC, or at the Office of the Federal Register, 800 North Capitol St. NW., suite 700, Washington, DC.

(B) *Plant stanol esters.* (*1*) Plant stanol esters prepared by esterifying a mixture of plant stanols derived from edible oils or byproducts of the kraft paper pulping process with food-grade fatty acids. The plant stanol mixture shall contain at least 80 percent sitostanol and campestanol (combined weight).

(*2*) FDA will measure plant stanol esters by the following methods developed by McNeil Consumer Heathcare dated February 15, 2000: "Determination of Stanols and Sterols in Benecol Tub Spread"; "Determination of Stanols and Sterols in Benecol Dressing"; "Determination of Stanols and Sterols in Benecol Snack Bars"; or "Determination of Stanols and Sterols in Benecol Softgels." These methods are incorporated by reference in accordance with 5 U.S.C. 552(a) and 1 CFR part 51. Copies may be obtained from the Center for Food Safety and Applied Nutrition, Office of Nutritional Products, Labeling, and Dietary Supplements, Division of Nutrition Science and Policy, 200 C St., SW., rm. 2831, Washington, DC, 20204, or may be examined at the Center for Food Safety and Applied Nutrition's Library, 200 C St., SW., rm. 3321, Washington, DC, and at the Office of the Federal Register, 800 North Capitol St. NW., suite 700, Washington, DC.

(iii) *Nature of the food eligible to bear the claim.* (A) The food product shall contain:

(*1*) At least 0.65 g of plant sterol esters that comply with paragraph (c)(2)(ii)(A)(*1*) of this section per reference amount customarily consumed of the food products eligible to bear the health claim, specifically spreads and dressings for salad, or

(*2*) At least 1.7 g of plant stanol esters that comply with paragraph (c)(2)(ii)(B)(*1*) of this section per reference amount customarily consumed of the food products eligible to bear the health claim, specifically spreads, dressings for salad, snack bars, and dietary supplements in softgel form.

(B) The food shall meet the nutrient content requirements in §101.62 for a "low saturated fat" and "low cholesterol" food; and

(C) The food must meet the limit for total fat in §101.14(a)(4), except that spreads and dressings for salad are not required to meet the limit for total fat per 50 g if the label of the food bears a disclosure statement that complies with §101.13(h); and

(D) The food must meet the minimum nutrient contribution requirement in §101.14(e)(6) unless it is a dressing for salad.

(d) *Optional information.* (1) The claim may state that the development of heart disease depends on many factors and may identify one or more of the following risk factors for heart disease about which there is general scientific agreement: A family history of CHD; elevated blood total and LDL cholesterol; excess body weight; high blood pressure; cigarette smoking; diabetes; and physical inactivity. The claim may also provide additional information about the benefits of exercise and management of body weight to help lower the risk of heart disease.

(2) The claim may state that the relationship between intake of diets that include plant sterol/stanol esters and reduced risk of heart disease is through the intermediate link of "blood cholesterol" or "blood total and LDL cholesterol."

(3) The claim may include information from paragraphs (a) and (b) of this section, which summarize the relationship between diets that include plant sterol/stanol esters and the risk of CHD and the significance of the relationship.

(4) The claim may include information from the following paragraph on the relationship between saturated fat and cholesterol in the diet and the risk of CHD: The scientific evidence establishes that diets high in saturated fat and cholesterol are associated with increased levels of blood total and LDL cholesterol and, thus, with increased risk of CHD. Intakes of saturated fat exceed recommended levels in the diets of many people in the United States. One of the major public health recommendations relative to CHD risk is to consume less than 10 percent of calories from saturated fat and an average of 30 percent or less of total calories from all fat. Recommended daily cholesterol intakes are 300 mg or less per day. Scientific evidence demonstrates that diets low in saturated fat and cholesterol are associated with

lower blood total and LDL cholesterol levels.

(5) The claim may state that diets that include plant sterol or stanol esters and are low in saturated fat and cholesterol are consistent with "Nutrition and Your Health: Dietary Guidelines for Americans," U.S. Department of Agriculture (USDA) and Department of Health and Human Services (DHHS), Government Printing Office (GPO).

(6) The claim may state that individuals with elevated blood total and LDL cholesterol should consult their physicians for medical advice and treatment. If the claim defines high or normal blood total and LDL cholesterol levels, then the claim shall state that individuals with high blood cholesterol should consult their physicians for medical advice and treatment.

(7) The claim may include information on the number of people in the United States who have heart disease. The sources of this information shall be identified, and it shall be current information from the National Center for Health Statistics, the National Institutes of Health, or "Nutrition and Your Health: Dietary Guidelines for Americans," U.S. Department of Agriculture (USDA) and Department of Health and Human Services (DHHS), Government Printing Office (GPO).

(e) *Model health claim.* The following model health claims may be used in food labeling to describe the relationship between diets that include plant sterol or stanol esters and reduced risk of heart disease:

(1) *For plant sterol esters:* (i) Foods containing at least 0.65 g per serving of plant sterol esters, eaten twice a day with meals for a daily total intake of at least 1.3 g, as part of a diet low in saturated fat and cholesterol, may reduce the risk of heart disease. A serving of [name of the food] supplies grams of vegetable oil sterol esters.

(ii) Diets low in saturated fat and cholesterol that include two servings of foods that provide a daily total of at least 1.3 g of vegetable oil sterol esters in two meals may reduce the risk of heart disease. A serving of [name of the food] supplies grams of vegetable oil sterol esters.

(2) *For plant stanol esters:* (i) Foods containing at least 1.7 g per serving of plant stanol esters, eaten twice a day with meals for a total daily intake of at least 3.4 g, as part of a diet low in saturated fat and cholesterol, may reduce the risk of heart disease. A serving of [name of the food] supplies grams of plant stanol esters.

(ii) Diets low in saturated fat and cholesterol that include two servings of foods that provide a daily total of at least 3.4 g of vegetable oil stanol esters in two meals may reduce the risk of heart disease. A serving of [name of the food] supplies grams of vegetable oil stanol esters.

Dated: August 30, 2000.

Margaret Dotzel,

Associate Commissioner for Policy.

TABLES 1 AND 2 TO PREAMBLE:

Note: These tables will not appear in the Code of Federal Regulations.

TABLE 1.—PLANT STEROL ESTERS AND CHD (STUDIES ARE LISTED IN REVERSE CHRONOLOGICAL ORDER)

Study	Design	Population	Vegetable oil sterols: dose/form	Duration	Dietary intakes	Results
Jones PJ, 2000 (Ref. 58)	Randomized double-blind crossover balanced Latin square design.	N=15 (M) hypercholesterolemic subjects; plasma total cholesterol concentrations ranging from 232 mg/dL to 387 mg/dL. *Means at day 0:* (1) Control group 250±9 mg/dL (2) Phytosterol ester group: 247±7 mg/dL (3) Phytostanol ester group 247±7 mg/dL	(1) *Control;* (2) Phytosterol esters *2.94 g/d* (1.84 g/d free); (3) Phytostanol esters 3.13 g/d (1.84 g/d free) —in 23 g of margarine (margarine consumed 3X/d with meals). *Sterol source:* vegetable oil.	Run-in period NR; 21 days duration on each phase: margarine control, phytosterol ester margarine, and phytostanol ester margarine; each phase followed by a 5-week washout.	Subjects consumed a fixed intake North American solid foods diet in a controlled feeding situation; diets formulated to meet Canadian recommended nutrient intakes. *Dietary intake during study:* Total fat (% TE): 35 Saturated fat (% TE): 10 Cholesterol (mg/d): NR	Percent change in cholesterol compared to control at day 21: *Total-C* phytosterol esters: −9.1† phytostanol esters: −5.5 *LDL–C* phytosterol esters: −13.2* phytostanol esters: −6.4* *HDL–C* phytosterol esters: 0 phytostanol esters: 0 †P < 0.005, *P <0.02, relative to control
Maki KC, submitted for publication (Refs. 61 and 62)	Randomized, double-blind, three-arm parallel controlled study.	N= 224 randomized; N= 193 completed study (M/F) (control N= 83; low PSE N= 75; high PSE N= 35) mild to moderate hypercholesterolemics (mean baseline total cholesterol: 240 mg/dL).	(1) *Control;* (2) Low phytosterol esters (PSE) group: *1.76 g/d* (1.1 g/d free); (3) High phytosterol esters group: *3.52 g/d* (2.2 g/d free) —in 14 g/d of reduced fat (40%) spread (two 7 g servings/d, with food). *Sterol source:* soybean oil.	4 week run-in period, followed by 5 week treatment period.	Run-in diet: NCEP Step I diet and a conventional 50% fat spread; background diet: NCEP Step I diet and a reduced-fat (40%) spread. *Dietary intake, end of study:* *Total Fat (% TE)* control: 29.5±0.8 low PSE: 29.1±0.9 high PSE: 28.8±1.4 Saturated Fat (%TE) control: 9.1±0.4 low PSE: 8.6±0.4 high PSE: 9.1±0.6 Cholesterol (mg/d) control: 182±13 low PSE: 203±16 high PSE: 194±19	Percent change in cholesterol at end of 5 weeks, relative to control: *Total-C* low PSE group: −5.2%* high PSE group: −6.6%* *LDL–C* low PSE group: −7.6%* high PSE group: −8.1%* *HDL–C* low PSE group: 0.8% high PSE group: 1.6% *P <0.001

Ayesh R, 1999 (Ref. 51)	Randomized placebo-controlled dietary study.	N=21 (10 M/ 11F) healthy population; inclusion criteria at baseline for total serum cholesterol concentration: 158 to 255 mg/dL (mean 187±25 mg/dL).	(1) *Control*; (2) Phytosterol ester *13.8 g/d* (8.6 g/d free) —in 40 g/d of margarine; consumed with breakfast and dinner under supervision. *Sterol source:* vegetable oil.	Run-in duration: 21 days M and 28 days F; treatment duration: 21 days M and 28 days F.	Controlled diet based on a typical British diet; breakfast and dinner consumed under supervision, but lunch and snacks were provided and consumed unsupervised outside the unit. *Dietary intake during study:* Total fat (% TE): 40% Saturated fat (% TE): NR Cholesterol (mg/day): 460	Percent change in cholesterol at end of 21/28 days, relative to control: *Total-C:* −18%* *LDL–C:* −23%* *HDL–C:* −7% *(P<0.0001)
Hendriks HFJ, 1999 (Ref. 57)	Randomized, double-blind, crossover, balanced incomplete Latin square design; 5 spreads, 4 periods.	N= 100 (42 M/ 58 F), but 80 subjects for each spread (incomplete Latin square design= 5 spreads in four periods); normocholesterolemic and mildly cholesterolemic volunteers; inclusion criteria at baseline for total serum cholesterol concentration: < 290 mg/dL (baseline total cholesterol: mean 197±38 mg/dL, range: 105 to 287 mg/dL).	(1) Butter (*control*); (2) Spread (*control*); (3) Plant sterol ester *1.33 g/d* (0.83 g/d free); (4) Plant sterol ester *2.58 g/d* (1.61 g/d free); (5) Plant sterol ester *5.18 g/d* (3.24 g/d free) —in 25 g/d of spread (or butter); spreads replaced an equivalent amount of the spread(s) habitually used; ½ at lunch, ½ at dinner. *Sterol source:* soybean and other vegetable oil.	No run-in period; each subject consumed 4 spreads for a period of 3.5 weeks each; wash-out period NR.	Consumption of habitual Dutch diet (self-selected diets on study). *Dietary intake, end of study:* *Total fat (% TE)* control:*33.9±5.6* 1.33 g/d PSE: 32.9±5.2 2.58 g/d PSE: 33.3±5.5 5.18 g/d PSE: 33.9±5.5 *Saturated fat (% TE)* control: 13.5±2.9 1.33 g/d PSE: 13.4±2.5 2.58 g/d PSE: 13.3±2.7 5.18 g/d PSE: 13.5±2.86 *Cholesterol (mg/d)* control: 245±58.5 1.33 g/d PSE: 245±68.6 2.58 g/d PSE: 248±61 5.18 g/d PSE: 261±63	Percent change in cholesterol at end of 3.5 weeks, relative to control spread: *Total-C* 1.33 g/d PSE: −4.9* 2.58 g/d PSE: −5.9* 5.18 g/d PSE: −6.8* *LDL–C* 1.33 g/d PSE: −6.7* 2.58 g/d PSE: −8.5* 5.18 g/d PSE: −9.9* *HDL–C* 1.33 g/d PSE: −0.3 2.58 g/d PSE: −1.3 5.18 g/d PSE: −1.5 *(P < 0.0001)

TABLE 1.—PLANT STEROL ESTERS AND CHD (STUDIES ARE LISTED IN REVERSE CHRONOLOGICAL ORDER)—Continued

Study	Design	Population	Vegetable oil sterols: dose/form	Duration	Dietary intakes	Results
Jones PJH, 1999 (Ref. 74)	Randomized double-blind placebo-controlled, parallel study.	N=32 (M) hypercholesterolemic subjects (N= 16 control group, N=16 phytosterol group); inclusion criteria serum total cholesterol concentrations between 252 to 387 mg/dL; mean cholesterol at baseline, mg/dL: control group 263.5 ± 50, phytosterol group 260.5 ± 44.5.	(1) *Control;* (2) Sitostanol-containing phytosterols (20% sitostanol, remaining plant sterols are sitosterol, campesterol) *1.7 g/d* —in 30 g/d of margarine consumed during 3 meals; sterols/stanols not esterified. *Sterol source:* tall oil (derived from pine wood).	No run-in period; experimental period: 30 days; 20 days followup after experimental period.	Controlled feeding regimen for all subjects; a 'prudent,' fixed-food North American diet formulated to meet Canadian recommended nutrient intakes. *Dietary intake during study:* Total fat (% TE): 35% Saturated fat (% TE): 11% Cholesterol (mg/d): NR	*Day 30 cholesterol (mg/dL):* *Total-C* control: 236±56 sitostanol-containing phytosterols: 210±36 *LDL–C* control: 176±52 sitostanol-containing phytosterols: 130±36 ($p < 0.05$ relative to control group) *HDL–C* control: 23±7 sitostanol-containing phytosterols: 26±7 *Day 0 to day 30, percent change:* *LDL–C* control: −8.9%, $P < 0.01$ sitostanol-containing phytosterols: −24.4%, $P < 0.001$ sitostanol-containing phytosterols: −15.5%, $P < 0.05$, relative to control

Sierksma A, 1999 (Ref. 75)	Balanced, double-blind crossover design.	N=76, 75, or 74 healthy volunteers (39 M/37 F); baseline plasma total cholesterol levels < 310 mg/dL.	(1) *Control* (Flora spread); (2) Soybean sterols: *0.8 g/d* (non-esterified); (3) Sheanut oil sterols (esterified): *3.3 g/d* —in 25 g /d spread. *Sterol source:* soybean oil or sheanut oil.	Run-in period: 1 week on control spread; experimental period: 3 weeks each experimental period, 9 weeks total; no wash-out period (balanced design with period by group random allocation).	Volunteers maintained normal dietary patterns during study; spreads were meant to replace all or part of the volunteers' habitual spread or butter used for spreading, but not to be used for baking or frying. *Dietary intake during study:* *Total fat (% TE)* control: 38.3 soybean sterols: 38.3 sheanut sterols: 38.4 *Saturated fat (% TE)* control: 13.9 soybean sterols: 13.8 sheanut sterols: 14.3* *Cholesterol (mg/d)* control: 246 soybean sterols: 247 sheanut sterols: 242 *P < 0.05	Cholesterol (mg/dL): mean (95% CI) *Total-C* control: 196 (193, 199) soybean sterols: 188 (186, 191)* sheanut sterols: 194 (191, 197) *LDL–C* control: 122 (119, 124) soybean sterols: 114 (112, 116)* sheanut sterols: 119 (116, 122) *HDL–C* control: 50 (49, 50) soybean sterols: 50 (49, 51) sheanut sterols: 50 (49, 51) P < 0.05, relative to control *Percent change, relative to control:* *Total–C* soybean sterols: −3.8%* *LDL–C* soybean sterols: −6%* *HDL–C: 0* * P < 0.05

TABLE 1.—PLANT STEROL ESTERS AND CHD (STUDIES ARE LISTED IN REVERSE CHRONOLOGICAL ORDER)—Continued

Study	Design	Population	Vegetable oil sterols: dose/form	Duration	Dietary intakes	Results
Weststrate JA, 1998 (Ref. 67)	Randomized double-blind crossover, balanced incomplete Latin square design with 5 margarines, 4 periods of 3.5 weeks.	N= 95 (100 enrolled= 50 M/ 50 F) but approximately 80 subjects for each margarine (incomplete Latin square design= 5 margarines in four periods); normocholesterolemic and mildly hypercholesterolemic subjects; inclusion criteria at baseline for total plasma cholesterol concentration: < 310 mg/dL (baseline total cholesterol: mean 207±41 mg/dL).	(1) *Control* (Flora spread); (2) Plant stanol esters *4.6 g/d* (2.7 g/d free); (3) Soybean sterol esters *4.8 g/d* (3 g/d free); (4) Ricebran sterols *1.6 g/d* (5) Sheanut sterols *2.9 g/d*; —in 30 g/d of margarine, consumption at lunch and dinner; margarine replaced margarines habitually used. *Sterol source:* soybean, ricebran and sheanut.	Run-in of 5 days; each subject consumed 4 margarines for a period of 3.5 weeks each; wash-out period between experimental periods- NR.	Volunteers were requested to retain their normal dietary pattern. *Dietary intake during study:* *Total fat (% TE)* control: 42 plant stanol esters: 41.8 soybean sterol esters: 41.5 ricebran sterols: 41.4 sheanut sterols: 41.3 *Saturated fat (%TE)* control: 15.9 plant stanol esters: 16.2 soybean sterol esters: 15.3 ricebran sterols: 15.4 sheanut sterols: 16.9 *Cholesterol (mg/d)* control: 233; plant stanol esters: 243 soybean sterol esters: 226 ricebran sterols: 233 sheanut sterols: 227	Percent change in cholesterol at the end of 3.5 weeks, relative to control spread: *Total-C* plant stanol esters: −7.3* soybean sterol esters: −8.3* ricebran sterols: −1.1 sheanut sterols: −0.7 *LDL–C* plant stanol esters: −13* soybean sterol esters: −13* ricebran sterols: −1.5 sheanut sterols: −0.9 *HDL–C* plant stanol esters: 0.1 soybean sterol esters: 0.6 ricebran sterols: −1.3 sheanut sterols: −1.2 *P <0.05
Pelletier X, 1995 (Ref. 65)	Randomized, crossover design (blinding NR).	N= 12 normolipidic healthy men (baseline cholesterol levels NR).	(1) *Group 1:* 4 weeks normal diet followed by 4 weeks plant sterol-enriched diet *0.740 g/d*; (2) *Group 2:* 4 weeks plant sterol-enriched diet *0.740 g/d* followed by 4 weeks normal diet —in 50 g/d of butter; plant sterols are not esterified. *Sterol source:* soybean oil.	1 week run-in period and two experimental periods of 4 weeks each; wash-out period NR.	Subjects on a controlled diet, but diet is a "normal" diet. *Dietary intake, during study:* *Total fat (% TE)* Period 1: 36.4±7.1 Period 2: 36.4±6.9 *Saturated fat (% TE)* Control: NR Plant Sterol: NR *Cholesterol (mg/d)* *Control: 436* Plant Sterol: 410	Percent change in cholesterol at end of 4 weeks, plant sterol-enriched butter relative to control butter: *Total-C* −10%* *LDL–C* −15%* *HDL–C* +4.6% P < 0.001

Miettinen, TA, 1994 (Ref. 63) (same as or partial study of Vanhanen HT, 1992 (Ref. 64))	Randomized, placebo-controlled, double-blind study.	N= 31 (22 M/ 9 F) (control N= 8; sitosterol N= 9; sitostanol N= 7; sitostanol ester N= 7); hypercholesterolemic subjects; inclusion criteria at baseline for total serum cholesterol concentration: >232 mg/dL.	(1) Rapeseed oil (RSO) *control*; (2) Sitosterol *0.7 g/d*; (3) Sitostanol *0.7 g/d*; (4) Sitostanol ester *1.36 g/d* (0.8 g/d free) —in 50 g/d of RSO mayonnaise. *Sterol source:* NR.	6 week run-in period; 9 week study period.	No diet changes other than replacing 50 g of typical daily fat by 50 g of RSO mayonnaise. *Dietary intake at end of study for all subjects:* *Total fat (g/d)* 114±9 Saturated fat (% of total fat) 12.4±0.7% Cholesterol (mg/d) 326±28	Change in cholesterol from end of run-in period to end of 9 week study period (mg/dL): *Total-C* RSO control: +4.6±4.3 sitosterol: −7.7±5.0 sitostanol: −0.4±5.4 sitostanol ester: −7.4±3.1† *LDL–C* RSO control: +3.1±4.3 sitosterol: −7.0±4.3 sitostanol: −1.2±4.6 sitostanol ester: −7.7±3.1*† *HDL–C* RSO control: +2.3±1.2 sitosterol: +0.00±1.5 sitostanol: +2.3±1.5 sitostanol ester: +2.3±0.8* *P < 0.05, relative to run-in †P < 0.05, relative to RSO control
Vanhanen HT, 1992 (Ref. 64) (same as or partial study of Miettinen TA, 1994 (Ref. 63))	Placebo-controlled, randomized, double-blind study.	N=24 (M and F) (control group n= 8; sitosterol group n= 9; sitostanol group n=7) hypercholesterolemic individuals (serum cholesterol> 232 mg/dL).	(1) Rapeseed oil *control*; (2) Sitosterol: *0.625 or 0.722 g/d*; (3) Sitostanol:*0.630 g/d* —in 50 g/d of rapeseed oil mayonnaise; plant sterols/stanols are not esterified. *Sterol source:* rapeseed oil.	6 week run-in on rapeseed oil spread; 9 week period.	On average 50 g of visible dietary fat as butter, margarine, milk fat, sausages and cheeses was replaced by the fat spread. *Dietary intake during study:* Total fat: NR Saturated fat: NR Cholesterol: NR	*Percent change in cholesterol at end of 9 week study period, relative to control:* *Total-C* sitosterol group: −7.6 (NS) sitostanol group: −9.7 (NS) *Cholesterol at end of study* (mg/dL): *Total–C* control: 239±10 sitosterol group: 221±13 sitostanol group: 216±9 all NS *LDL–C:* NR *HDL–C:* NR

Table 1. Plant Sterol Esters and CHD continued

Acronyms and Abbreviations Used in Table

d day
d deciliter
CI confidence interval
F female
g gram
HDL C serum high density lipoprotein cholesterol level
LDL C serum low in density lipoprotein cholesterol level
M male
mg miligram
N number
NCEP National Cholesterol Education Program
NR not reported
NS not statistically significant
% percent
P probability of type 1 error
PSE phytosterol ester
TE total energy
Total-C serum total cholesterol level
RSO rapseed oil (or canola oil)
X times

TABLE 2.—PLANT STANOL ESTERS AND CHD (STUDIES ARE LISTED IN REVERSE CHRONOLOGICAL ORDER)

Study	Design	Population	Plant stanol: dose/form	Duration	Dietary intakes	Results
Hallikainen MA, 2000 (Ref. 88)	Randomized single-blind, crossover design (dose-dependent study).	N= 22 (M/F) hypercholesterolemic subjects; inclusion criteria: serum total cholesterol concentrations ranging from 193.5 to 329 mg/dL (mean at baseline: 266 50 mg/dL).	(1) *Control*; (2) Plant stanol esters *1.4 g/d*, (0.8 g/d free); (3) Plant stanol esters *2.7 g/d* (1.6 g/d free); (4) Plant stanol esters *4.1 g/d* (2.4 g/d free); (5) Plant stanol esters *5.4 g/d* (3.2 g/d free) —in 25 g of margarine taken in two to three portions with meals. *Stanol source:* NR. All subjects followed the same dosage order; the order of dose periods was randomly determined as follows: 2.4, 3.2, 1.6, 0 (control) and 0.8 g/d.	Run-in duration: 1 week period; 5 test periods of 4 weeks each; no washout between periods.	Subjects followed a standardized background diet throughout the study. *Dietary intake during study:* *Total fat (% TE)* control: 34.3±4.9 1.4 g/d: 33.4±4.9 2.7 g/d: 33.4±4.3 4.1 g/d: 32.5±5.4 5.4 g/d: 33.5±4.2 *Saturated fat (% TE)* control: 10.3±2.2 1.4 g/d: 9.4±1.9 2.7 g/d: 9.3±1.3 4.1 g/d: 8.5±2.1 5.4 g/d: 9.3±2.2 *Cholesterol (mg/d)* control: 158 1.4 g/d: 179 2.7 g/d: 155 4.1 g/d: 153 5.4 g/d: 177	*Cholesterol after test (mg/dL):* *Total-C* control: 252±40 1.4 g/d: 245±45 2.7 g/d: 235±38* 4.1 g/d: 225±36* 5.4 g/d: 223±30* *LDL–C* control: 171±37 1.4 g/d: 168±39 2.7 g/d: 161±34† 4.1 g/d: 153±29* 5.4 g/d: 151±27* *HDL–C* control: 58±12 1.4 g/d: 58±12 2.7 g/d: 59±12 4.1 g/d: 58±14 5.4 g/d: 58±12 *Percent change, relative to control:* Total-C 1.4 g/d: −2.8% 2.7 g/d: −6.8% * 4.1 g/d: −10.3% * 5.4 g/d: −11.3% * *LDL–C* 1.4 g/d: −1.7% 2.7 g/d: −5.6%† 4.1 g/d: −9.7% * 5.4 g/d: −10.4% * *†P 20< 0.001 or †P < 0.05 vs control
Jones PJ, 2000 (Ref. 58)	Randomized double-blind crossover balanced Latin square design.	N=15 (M) hypercholesterolemic subjects; plasma total cholesterol concentrations ranging from 232 mg/dL to 387 mg/dL. *Means at day 0:* (1) Control group 250±9 mg/dL (2) Phytosterol ester group: 247±7 mg/dL (3) Phytostanol ester group 247±7 mg/dL	(1)*Control*; (2) Phytosterol esters*2.94 g/d* (1.84 g/d free); (3) Phytostanol esters*3.31 g/d* (1.84 g/d free) —in 23 g of margarine (margarine consumed 3X/d with meals). *Stanol source:* vegetable oil.	Run-in period NR; 21 days duration on each phase: margarine control, phytosterol ester margarine, and phytostanol ester margarine; each phase followed by a 5-week washout.	Subjects consumed a fixed intake North American solid foods diet in a controlled feeding situation; diets formulated to meet Canadian recommended nutrient intakes. *Dietary intake during study:* Total fat (% TE): 35 Saturated fat (% TE): 10 Cholesterol (mg/d): NR	Percent change in cholesterol from control at day 21: *Total-C* phytosterol esters: −9.1‡ phytostanol esters: −5.5 *LDL–C* phytosterol esters: −13.2 * phytostanol esters: −6.4* *HDL–C* phytosterol esters: 0 phytostanol esters: 0 ‡P <0.005, *P <0.02, relative to control

TABLE 2.—PLANT STANOL ESTERS AND CHD (STUDIES ARE LISTED IN REVERSE CHRONOLOGICAL ORDER)—Continued

Study	Design	Population	Plant stanol: dose/form	Duration	Dietary intakes	Results
Plat J, 2000 (Ref. 92)	Randomized double-blind, placebo-controlled study.	N= 112 (41 M/71 F) non-hypercholesterolemic subjects (control N= 42, pine wood stanol esters N= 34, vegetable oil stanol esters N= 36); inclusion criteria: serum total cholesterol concentrations < 252 mg/dL.	(1) *Control*; (2) Pine wood stanol esters *6.8 g/d* (4 g/d free); (3) Vegetable oil stanol esters *6.8 g/d* (3.8 g/d free) —in 20 g of rapeseed oil margarine plus 10 g of rapeseed oil shortening per day. *Stanol source:* pine wood based or vegetable oil.	Run-in duration: 4 weeks; experimental period: 8 weeks.	Subjects consumed usual habitual diet with the exception that 30 g of test margarine and shortening replaced 30 g of daily fat intake. *Dietary intake during study:* *Total fat (% TE)* control: 39.2±4.2 wood stanol esters: 39.6±3.8 vegetable stanol esters: 40.1±4.1 *Saturated fat (% TE)* control: 14.3±2.0 wood stanol esters: 13.5±1.6 vegetable stanol esters: 13.6±2.2 Cholesterol (mg/d) control: 221.5 wood stanol esters: 238.5 vegetable stanol esters: 239.5	*Change in cholesterol from run-in to experimental period (mg/dL):* Total-C control: −1.6±15.5 wood stanol esters: −16.3±15.1* vegetable stanol esters: −16.6±10.8* *LDL–C* control: −2.3±14.3 wood stanol esters: −15.9±13.9* vegetable stanol esters: −16.6±10.1* *HDL–C* control: 0.4±6.2 wood stanol esters: 0.4±5.0 vegetable stanol esters: 0.0±4.3 *Percent change, relative to control:* *Total-C* wood stanol esters: −8.1±7.5%* vegetable stanol esters: −8.6 ±5.1%* *LDL–C* wood stanol esters: −12.8±11.2%* vegetable stanol esters: −14.6 ±8.0%* * P < 0.001 relative to control

Andersson A, 1999 (Ref. 80)	Randomized double-blind study.	N= 61 (28 M/33 F) moderately hypercholesterolemic subjects ((1) test diet+control margarine: N= 21 (2) test diet+test margarine: N= 19 (3) usual diet+test margarine: N= 21); inclusion criteria: serum total cholesterol levels at screening >194 mg/dL; mean serum cholesterol at baseline: 264±44; exclusion criteria: serum cholesterol > 330 mg/dL at screening.	(1) Controlled lipid-lowering diet (test diet) + low fat margarine (*control* margarine); (2) Controlled lipid-lowering diet (test diet) + a low fat *3.4 g/d* stanol ester (2g/d free)-containing margarine (test margarine); (3) Usual diet (control diet)+ a low fat *3.4 g/d* stanol ester (2g/d free)-containing margarine (test margarine) —in 25 g/d (use 3X per day) of low fat (40% fat) margarine made from low erucic acid rapeseed (canola) oil. *Stanol source:* NR.	Run-in period: 4 weeks; experimental period: 8 weeks.	Subjects consumed either usual diet (control diet) or controlled feeding lipid lowering diet (test diet) during study. *Calculated /food analysis nutrient composition of test diet:* Total fat (%TE): 35 Saturated fat (%TE): 8 Cholesterol(mg/d): 171 *Estimated (dietary records) nutrient composition of control diet:* Total fat (%TE): 31.8±4.6 Saturated fat (%TE): 11.9±2.2 Cholesterol (mg/d): 279±104	*Percent change in cholesterol from baseline:* *Total-C* test diet+control margarine: −8* test diet+test margarine: −15* control diet+test margarine: −9* *LDL–C* test diet+control margarine: −12* test diet+test margarine: −19* control diet+test margarine: −12* *HDL–C* test diet+control margarine: −4 test diet+test margarine: −7 † control diet+test margarine: 0 *P < 0.0001; †P <0.0005, relative to baseline *Percent change (P value) for differences between test diet+test margarine relative to test diet+control margarine:* *Total-C:* −12% (P < −0.0035) *LDL–C:* −15% (P < −0.0158) *HDL–C:* 0% (P < 0.1226) *Percent change (P value) for differences between test diet+test margarine relative to usual diet+ test margarine:* Total-C: −4% (P < 0.0059) LDL–C: −6% (P < 0.0034) HDL–C: −6% (P < 0.0–3)

TABLE 2.—PLANT STANOL ESTERS AND CHD (STUDIES ARE LISTED IN REVERSE CHRONOLOGICAL ORDER)—Continued

Study	Design	Population	Plant stanol: dose/form	Duration	Dietary intakes	Results
Gylling H, 1999 (Ref. 78)	Margarine study: randomized double-blind crossover study; after the margarine period the same women were randomized to the Butter study, which is a randomized double-blind crossover study.	N=23 during margarine period, N= 21 during butter period; moderately hypercholesterolemic postmenopausal women; inclusion criteria: serum cholesterol between 213 and 310 mg/dL.	(1) Sitostanol ester margarine *5.4 g/d* (3.18 g/day free) (wood oil); (2) Campestanol ester margarine *5.7 g/d* (3.16 g/d free) (vegetable oil); (3) Butter *control*; (4) Sitostanol ester butter *4.1 g/d* (2.43 g/d free) (wood oil) —in 25 g of margarine or butter. *Stanol source:* wood or vegetable oil.	Run-in period: 1 week; the margarine interventions lasted 6 weeks, the butter interventions lasted 5 weeks; a washout period of 8 weeks separated the margarine and butter studies.	Subjects were advised to replace 25 g of their normal dietary fat with stanol ester margarine or butter with or without stanol esters. *Dietary intake during study:* *Total fat (g/d)* margarine period: 93±6 butter period: 97±6 *Saturated fat* margarine period: NR butter period: NR *Cholesterol (mg/d)* margarine period: 262±19 butter period: 323±19	*Cholesterol at end of period (mg/dL):* *Total-C* run-in home diet: 235±6 sitostanol ester margarine: 224±7* campestanol ester margarine: 221±7* butter control: 245±8* sitostanol ester butter: 228±7 † *LDL–C* run-in home diet: 154±5 sitostanol ester margarine: 140±5* campestanol ester margarine: 139±7* butter control: 161±7 sitostanol ester butter: 143±6† *HDL–C* run-in home diet: 60±3.5 sitostanol ester margarine: 63±4* campestanol ester margarine: 63±3* butter control: 63±4* sitostanol ester butter: 63±4 *Percent change from butter control:* *Total-C* sitostanol ester butter: −8%† *LDL–C* sitostanol ester butter: −12%† *Significantly different from run-in home diet, P < 0.05; †Significantly different from butter, P < 0.05

Hallikainen MA, 1999 (Ref. 77)	Randomized double-blind, placebo-controlled, parallel study.	N= 55 (M/F); hypercholesterolemic subjects ((1)control margarine N= 6 M, 11 F, (2) wood stanol ester-containing margarine (WSEM) N= 8 M, 10 F, (3) vegetable oil stanol ester-containing margarine (VOSEM) N= 6 M, 14 F); inclusion criteria serum total cholesterol concentrations between 2– to 290 mg/dL; mean cholesterol at baseline, mg/dL: control group 229±25 WSEM group 246±29; VOSEM group 238±31.	(1) *Control margarine*; (2) WSEM *3.9 g/d* (2.31 g/d free); (3) VOSEM *3.9 g/d* (2.16 g/d free) —in 25 g low-erucic acid RSO-based low fat (40% or 35% fat) margarine per day. *Stanol source:* wood or vegetable.	Run-in period: 4 week; experimental period: 8 weeks.	Subjects consumed the margarines as part of a diet resembling that of the National Cholesterol Education Program's Step II diet. *Dietary intake during study:* *Total fat (%TE)* control: 26.5±3.1 WSEM: 26.4±3.3 VOSEM: 25.6±3.9 *Saturated fat (%TE)* control: 7.3±1.6 WSEM: 7.0±1.4 VOSEM: 6.8±1.7 *Cholesterol (mg/day)* control: 135 WSEM: 164 VOSEM: 139	*Change in cholesterol from week 0 to week 8 (mg/dL):* *Total-C* control: −18.6±19 WSEM: −46.8±23.6* VOSEM: −38±22.8† *LDL–C* control: −17.4±22.8 WSEM: −41±17‡ VOSEM: −31±19.4 HDL–C control: 0.4±5.8 WSEM: −1.2±6.6 VOSEM: −1.9±7 *Percent change, relative to control:* *Total-C* WSEM: −10.6%* VOSEM: −8.1%† *LDL–C* WSEM: 13.7%‡ VOSEM: 8.6% Significantly different from control group: *P < 0.001, †P < 0.05, ‡P < 0.01
Jones PJH, 1999 (Ref. 74)	Randomized double-blind placebo-controlled, parallel study.	N=32(M) hypercholesterolemic subjects (N= 16 control group, N=16 phytosterol group); inclusion criteria serum total cholesterol concentrations between 252 to 387 mg/dL; mean cholesterol at baseline, mg/dL: control group 263.5±50, phytosterol group 260.5 ±44.5.	*(1) Control*; (2) Sitostanol-containing phytosterols (20% sitostanol, remaining plant sterols are sitosterol, campesterol) 1.7 g/d —in 30 g/d of margarine consumed during 3 meals; sterols/stanols not esterified. *Sterol source:* tall oil (derived from pine wood)	No run-in period; experimental period: 30 days; 20 days followup after experimental period.	Controlled feeding regimen for all subjects, a 'prudent,' fixed-food North American diet formulated to meet Canadian recommended nutrient intakes *Dietary intake during study:* Total fat (% TE): 35% Saturated fat (% TE): 11% Cholesterol (mg/d): NR	*Day 30 cholesterol (mg/dL):* *Total-C* control: 236±56 sitostanol-containing phytosterols: 210±36 *LDL–C* control: 176±52 sitostanol-containing phytosterols: 130±36 (p < 0.05 relative to control group) *HDL–C* control: 23±7 sitostanol-containing phytosterols: 26±7 *Day 0 to day 30 (% change):* *LDL–C* control: −8.9%, P < 0.01 sitostanol-containing phytosterols: −24.4%, P < 0.001 sitostanol-containing phytosterols: −15.5%, P <0.05, relative to control

TABLE 2.—PLANT STANOL ESTERS AND CHD (STUDIES ARE LISTED IN REVERSE CHRONOLOGICAL ORDER)—Continued

Study	Design	Population	Plant stanol: dose/form	Duration	Dietary intakes	Results
Nguyen TT, 1999 (Ref. 90)	Multicenter, randomized double-blind, placebo-controlled parallel study.	N= 298 (51% M/ 49% F) mildly hypercholesterolemic subjects; ((1) control N= 76, (2) EU 3G N=74, (3) US 3G N= 71, (4) US 2G N= 77); inclusion criteria serum total cholesterol concentrations between 200 to 280 mg/dL; mean baseline total cholesterol: 233±20 mg/dL.	(1) *Control:* US reformulation of vegetable oil spread; (2) EU 3G: *5.1 g/d* stanol esters (3g/d free) European formulation of vegetable oil spread; (3) US 3G: *5.1 g/d* stanol esters (3 g/d free) US reformulation of vegetable oil spread; (4) US 2G: *3.4 g/d* stanol esters (2 g/d free) US reformulation of vegetable oil spread —in 24 g/d spread (three 8 g servings a day). *Stanol source:* wood.	Run-in period: 4 weeks; experimental period: 8 weeks.	Usual dietary habits maintained, but some subjects on a NCEP Step I diet, so background diets varied, but diet composition reported not to differ among the four groups. *Dietary intake during study:* Total fat (% TE): 32.8 (6.8) Saturated fat (% TE): 9.8 (3.0) Cholesterol (mg/d): 234 (147)	*Percent change in cholesterol from baseline to week 8:* *Total-C* control: 0.5* EU 3G: −4.7* US 3G: −6.4* US 2G: −4.1* *LDL–C* control: 0.1* EU 3G: −5.2* US 3G: −10.1* US 2G: −4.1* *HDL–C* control: 2.0 EU 3G: 0.0 US 3G: 0.0 US 2G: 0.0 *P < 0.001, relative to baseline Total-C (P < 0.001) and LDL–C (P <0.02) levels were significantly reduced in all 3 active-ingredient groups compared with the placebo group at all time points during the ingredient phase. (see figures in paper for values)

Weststrate JA, 1998 (Ref. 67)	Randomized double-blind crossover balanced incomplete Latin square design with 5 margarines, 4 periods of 3.5 weeks.	N= 95 (100 enrolled= 50 M/ 50 F) but approximately 80 subjects for each margarine (incomplete Latin square design= 5 margarines in four periods); normocholesterolemic and mildly hypercholesterolemic subjects; inclusion criteria at baseline for total plasma cholesterol concentration: < 310 mg/dL (baseline total cholesterol: mean 207 ±41mg/dL).	(1) *Control* (Flora spread); (2) Plant stanol esters *4.6 g/d* (2.7 g/d free); (3) Soybean sterol esters *4.8 g/d* (3 g/d free); (4) Ricebran sterols *1.6 g/d* free; (5) Sheanut sterols *2.9 g/d* free —in 30 g/d of margarine, consumption at lunch and dinner; margarines replaced margarines habitually used. *Stanol source:* wood.	Run-in of 5 days; each subject consumed 4 margarines for a period of 3.5 weeks each; wash-out period between experimental periods- NR.	Volunteers were requested to retain their normal dietary pattern. *Dietary intake during study:* *Total fat (% TE)* control: 42 plant stanol esters: 41.8 soybean sterol esters: 41.5 ricebran sterols: 41.4 sheanut sterols: 41.3 *Saturated fat (%TE)* control: 15.9 plant stanol esters: 16.2 soybean sterol esters: 15.3 ricebran sterols: 15.4 sheanut sterols: 16.9 *Cholesterol (mg/d)* control: 233 plant stanol esters: 243 soybean sterol esters: 226 ricebran sterols: 233 sheanut sterols: 227	Percent change in cholesterol at end of 3.5 weeks, relative to control: *Total-C* plant stanol esters: −7.3* soybean sterol esters: −8.3* ricebran sterols: −1.1 sheanut sterols: −0.7 *LDL–C* plant stanol esters: −13* soybean sterol esters: −13* ricebran sterols: −1.5 sheanut sterols: −0.9 *HDL–C* plant stanol esters: 0.1 soybean sterol esters: 0.6 ricebran sterols: −1.3 sheanut sterols: −1.2 *P <0.05
Niinikoski H, 1997 (Ref. 91)	Randomized double-blind, placebo-controlled study.	N=24 (M/F) normocholesterolemic subjects (N=12 (4 M/8 F) control, N=12 (4 M/8 F) sitostanol ester); baseline serum total cholesterol: 197±38.7 mg/dL.	*(1) Control*; (2) Sitostanol ester 5.1 g/d (3 g/d free); —in 24 g of a RSO based margarine to be used on bread, in food preparation and in baking in three 8 g portions over the day. *Stanol source:* NR.	No run-in period; experimental period: 5 weeks.	Subjects were advised to replace normal dietary fat for 5 weeks with the study margarine; the amount and quality of ingested fat were planned to be equal in both groups. *Dietary intake during study:* Total fat: NR Saturated fat: NR Cholesterol: NR	*Cholesterol change from baseline to 5 weeks (mg/dL):* Total-C control: −11.6±19.4 sitostanol ester: −31±19.4* *Non-HDL–C* control: −11.6±19.4 sitostanol ester: −31±23* *HDL–C* control: −1.5 ±6.6 sitostanol ester: −2.3±4.6 *P <0.05, relative to control

TABLE 2.—PLANT STANOL ESTERS AND CHD (STUDIES ARE LISTED IN REVERSE CHRONOLOGICAL ORDER)—Continued

Study	Design	Population	Plant stanol: dose/form	Duration	Dietary intakes	Results
Denke MA., 1995 (Ref. 97)	Fixed sequence design with three sequential experimental periods.	N= 33 (M) moderate hypercholesterolemic subjects; total cholesterol concentration after run-in period: 239±29.	(1) *Control* (Step 1 Diet alone); (2) Plant stanol *3 g/d* + Step 1 Diet; (3) *Washout* (Step 1 Diet alone) —plant stanol was suspended in safflower oil and packed into gelatin capsules, each capsule containing 250 mg sitostanol and 1 g of safflower oil; subjects instructed to consume 4 capsules per meal (subjects were to consume a total of 12 capsules (3 g) in three divided doses during three meals); plant stanols not esterified. *Stanol source:* tall oil.	1 month run-in on Step I Diet; experimental periods: 3 months in duration; washout period: 1 month.	Subjects were instructed to follow a cholesterol-lowering diet in which dietary cholesterol was restricted to < 200 mg/d (Step I Diet). *Dietary intake (self-reported intake):* Total fat (%TE): 30 Saturated fatty acids (%TE): 10 Cholesterol (mg/d): 188	Cholesterol, at end of each period (mg/dL): *Total–C* control: 239±29 plant stanol + Step I Diet: 238±31 washout: 244±29 *LDL–C* control: 175±26 plant stanol + Step I Diet: 172±31 washout: 181±30 *HDL–C* control: 39±11 plant stanol + Step I Diet: 41±12 washout: 39±11 *NS* differences between any period.

Miettinen TA, 1995 (Ref. 89)	Randomized double-blind, placebo-controlled study.	N= 153 (42% M/ 58% F) (N= 51 control margarine, N=102 test margarine) mild hypercholesterolemic subjects; inclusion criteria: serum cholesterol concentration ±216 mg/dL.	*(1) Control margarine;* (2) Sitostanol ester *5.1 g/d* (3 g/d free) for 1 year; (3) Sitostanol ester *5.1 g/d* (3 g/d free) for 6 months, followed by sitostanol ester 3.4 g/d (2 g/d free) for next 6 months —in 24 g/d margarine. Actual intake of sitostanol ester for 5.1 g/d: *4.4 g/d* for 3.4 g/d: *3.1 g/d.* *Stanol source:* wood.	Run-in period: 6 weeks; experimental period: 1 year; after 6 months the sitostanol-ester group was randomly reassigned either to continue their intake of 4.4 g/d of sitostanol ester (N= 51) or to reduce their intake to 3.1 g/d (N= 51); subjects were not informed of this change in sitostanol ester intake.	During the study subjects were advised to replace 24 g per day of their normal dietary fat with a margarine containing RSO, according to careful instructions from a qualified nurse, otherwise typical ad libitum diet during study. *Dietary intake during study:* *Total fat (%TE)* control: 34.9±0.9 4.4 g/d stanol ester: 35.7±0.8 3.1g/d stanol ester: 34.8±0.9 *Saturated fat (%TE)* control: 13.9±0.5 4.4 g/d stanol ester: 14.4±0.4 3.1 g/d stanol ester: 14.3±0.7 *Cholesterol (mg/d)* control: 314±27 4.4 g/d stanol ester: 340±37 3.1 g/d stanol ester: 308±20	Cholesterol concentration at 1 year (mg/dL): *Total-C* control: 237±4 4.4 g/d stanol ester: 210±4* 3.1 g/d stanol ester: 214±4* *LDL–C* control: 157±4 4.4 g/d stanol ester: 134±3* 3.1 g/d stanol ester: 138±3* *HDL–C* control: 54±2 4.4 g/d stanol ester: 53±1 3.1 g/d stanol ester: 58±2 *P < 0.001, relative to baseline *Mean change after 1 year (mg/dL):* *Total-C* control: −1 4.4 g/d stanol ester: −25* (difference −24 (95% CI: −17 to −32)) *LDL–C* control: −3 4.4 g/d stanol ester: −24* (difference −21 (95% CI: −14 to −29)) *HDL–C* control: 0.0 4.4 g/d stanol ester: 0.4 *P < 0.001, relative to control

TABLE 2.—PLANT STANOL ESTERS AND CHD (STUDIES ARE LISTED IN REVERSE CHRONOLOGICAL ORDER)—Continued

Study	Design	Population	Plant stanol: dose/form	Duration	Dietary intakes	Results
Miettinen, T A, 1994 (Ref. 63) (same as or partial study of Vanhanen HT, 1992 (Ref. 64))	Randomized placebo-controlled, double-blind study.	N= 31 (22 M/ 9 F) (control N= 8; sitosterol N= 9; sitostanol N= 7; sitostanol ester N= 7); hypercholesterolemic subjects; inclusion criteria at baseline for total serum cholesterol concentration: > 232 mg/dL.	(1) RSO *control*; (2) Sitosterol *0.7 g/d*; (3) Sitostanol *0.7 g/d*; (4) Sitostanol ester *1.36 g/d* (0.8 g/d free) —in 50 g/d of RSO mayonnaise. *Stanol source:* NR.	6 week run-in period; 9 week study period.	No diet changes other than replacing 50 g of typical daily fat by 50 g of RSO mayonnaise. *Dietary intake at end of study for all subjects:* *Total fat (g/d)* 114±9 *Saturated fat* (% of total fat) 12.4±0.7% *Cholesterol* (mg/d) 326±28	Change in cholesterol from end of run-in period to end of 9 week study period (mg/dL): *Total-C* RSO control: 4.6±4.3 sitosterol: −7.7±5.0 sitostanol: −0.4±5.4 sitostanol ester: −7.4±3.1† *LDL–C* RSO control: 3.1±4.3 sitosterol: −7.0±4.3 sitostanol: −1.2±4.6 sitostanol ester: −7.7±3.1*† *HDL–C* RSO control: 2.3±1.2 sitosterol: 0.00±1.5 sitostanol: 2.3±1.5 sitostanol ester: 2.3±0.8* *P < 0.05, relative to run-in †P < 0.05, relative to RSO control

Vanhanen HT, 1994 (Ref. 94)	Randomized double-blind, placebo-controlled study.	N= 15 (11M/ 4 F) mildly hypercholesterolemic subjects (N= 8 control group, N= 7 sitostanol group); serum cholesterol selection criteria > 232 mg/dL.	(1) *Control* (RSO mayonnaise); (2) Sitostanol ester *1.36 g/d* (0.8 g/d free); (3) Sitostanol ester *3.4 g/d* (2 g/d free) —in 50 g/d of RSO mayonnaise. *Stanol source:* NR.	Run-in period: 6 weeks; experimental period: 15 weeks; lower dose sitostanol for 9 weeks, followed by higher dose sitostanol for 6 weeks.	Subjects replaced 50 g of their usual dietary fat by 50 g of RSO mayonnaise, otherwise usual diet. *Dietary intake during run-in period (reported to be similar to the experimental period):* *Total fat (g/d):* control group: 124 sitostanol group: 118 *Saturated fat:* control group: NR sitostanol group: NR *Cholesterol (mg/day):* control group: 321 sitostanol group: 265	*Cholesterol change from baseline (mg/dL):* *Total-C* control: 5±5 1.36 g/d: −7.4±3.1‡ control: 8.1±5.4 3.4 g/d: −11.2 3.5*‡ *LDL–C* control: 3.1±4.6 1.36 g/d: −7.7±3.1* control: 5.8±5.4 3.4 g/d: −15.1±2.7*‡ *HDL–C* control: 2.3±1.2 1.36 g/d: 2.3±0.8 control: 0.8±1.9 3.4 g/d: 2.7±1.5 *Percent change, relative to control:* *Total-C* 1.36 g/d: −4.1%‡ 3.4 g/d: −9.3%‡ *TLDL–C* 1.36 g/d: −10.3% 3.4 g/d: −15.2%‡ *HDL–C* 1.36 g/d: 0.5% 3.4 g/d: 0% *P < 0.05, relative to baseline ‡P < 0.05, relative to control
Blomqvist SM, 1993 (Ref. 81) (same as Vanhanen HT, 1993 (Ref. 82))	Randomized double-blind, placebo-controlled study.	N= 67 (47 M/ 20 F) moderately hypercholesterolemic subjects (N= 66 in Tables: control N=32; sitostanol ester N=34); plasma cholesterol concentration at baseline: 246 † 33 mg/dL.	(1) *Control* (RSO mayonnaise); (2) Sitostanol ester *5.8 g/d* (3.4 g/d free) —in 50 g RSO mayonnaise. *Stanol source:* NR.	Run-in period: 4 weeks; experimental period: 6 weeks.	Subjects replaced 50 g of daily fat intake with 50 g of RSO mayonnaise; a second 7-day diet record performed during the experimental period indicated that diet composition was similar to that during the run-in period. *Dietary intake during the standardization period (run-in):* Total fat (% TE): 37 Saturated fat (% TE): 12 Cholesterol (mg/d): 270	Cholesterol after 6 weeks (mg/dL): *Total-C* control: 225±27 sitostanol ester: 2–±34* *LDL–C* control: 134±18 sitostanol ester: 124±32† *HDL–C* control: 53±11 sitostanol ester: 51±12* †P < 0.01; * P < 0.001, relative to control

TABLE 2.—PLANT STANOL ESTERS AND CHD (STUDIES ARE LISTED IN REVERSE CHRONOLOGICAL ORDER)—Continued

Study	Design	Population	Plant stanol: dose/form	Duration	Dietary intakes	Results
Vanhanen HT, 1993 (Ref. 82) (same as Blomqvist SM, 1993 (Ref. 81))	PRandomized double-blind, placebo-controlled study.	N= 67 (47 M/ 20 F) moderately hypercholesterolemic subjects; (control N=33; sitostanol ester N=34); serum cholesterol selection criteria > 232 mg/dL.	(1) *Control* (RSO mayonnaise); (2) Sitostanol ester *5.8 g/d* (3.4 g/d free) —in 50 g RSO mayonnaise. *Stanol source:* NR.	Run-in period: 4 weeks; experimental period: 6 weeks.	Subjects replaced 50 g of daily fat intake with 50 g of RSO mayonnaise; a second 7-day diet record performed during the experimental period indicated that diet composition was similar to that during the run-in period. *Dietary intake during the standardization period (run-in):* Total fat (% TE): 37 Saturated fat (% TE): 12 Cholesterol (mg/d): 270	Cholesterol change from baseline period, mg/dL (cholesterol concentration at 6 weeks in mg/dL): *Total-C* control: −2.7±2.3 (225) itostanol ester: −17.0±2.3* (2–) *LDL–C* control: −1.5±2.7 (142) sitostanol ester: −14.3±2.3* (130) *HDL–C* control: −1.2±0.8 (53) sitostanol ester: −1.2±0.8 (52) *P < 0.05, relative to control
Vanhanen HT, 1992 (Ref. 64) (same as or partial study of Miettinen, TA, 1994 (Ref. 63))	Placebo-controlled, randomized double blind study.	N=24 (M and F) (control group N= 8; sitosterol group N= 9; sitostanol group N=7) hypercholesterolemic individuals (serum cholesterol > 232 mg/dL).	(1) RSO *control;* (2) Sitosterol: *0.625 or 0.722 g/d;* (3) Sitostanol: *0.630 g/d* —in 50 g/d of RSO mayonnaise; plant sterols/stanols are not esterified. *Stanol source:* rapeseed oil.	6 week run-in on RSO spread; 9 week period.	On average 50 g of visible dietary fat as butter, margarine, milk fat, sausages and cheeses was replaced by the fat spread. *Dietary intake during study:* Total fat: NR Saturated fat: NR Cholesterol: NR	*Percent change in cholesterol at end of 9 week study period, relative to control:* *Total-C* sitosterol group: −7.6(NS) sitostanol group: −9.7(NS) *At end of study* (mg/dL): *Total-C* control: 239±10 sitosterol group: 221±13 sitostanol group: 216±9 all NS *LDL–C:* NR *HDL–C:* NR

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Table 2.—Plant Stanol Esters and CHD—continued

Acronyms and Abbreviations Used in Table

d day
dl deciliter
CI confidence interval
EU European
EU 3G European, 3 grams
F female
g gram
HDL C serum high density lipoprotein cholesterol level
LDL C serum low density lipoprotein cholesterol level
M male
mg milligram
N number
NCEP National Cholesterol Education Program
NR not reported
NS not statistically significant
% percent
P probability of type I error
TE total energy
Total C serum total cholesterol level
RSO rapeseed oil (or canola oil)
US United States
US 2G United States, 2 grams
US 3G United States, 3 grams
VOSEM vegetable oil stanol ester-containing margarine
WSEM wood stanol ester-containing margarine
X times

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9

U. S. Department of Health and Human Services
U. S. Food and Drug Administration
Center for Food Safety and Applied Nutrition
Office of Nutritional Products, Labeling, and Dietary Supplements
February 14, 2003

FDA Letter Regarding Enforcement Discretion With Respect to Expanded Use of an Interim Health Claim Rule About Plant Sterol/Stanol Esters and Reduced Risk of Coronary Heart Disease

Fred L. Shinnick, Ph.D.
Manager, Regulatory and Scientific Affairs
Cargill Health & Food Technologies
15407 McGinty Road W. MS110
Wayzata, Minnesota 55391

Dear Dr. Shinnick:

This letter is in response to your letter, dated January 6, 2003, requesting that FDA issue a letter stating its intention not to enforce certain requirements in the interim final rule (IFR) authorizing a health claim for plant sterol/stanol esters and reduced risk of coronary heart disease (CHD) (21 CFR 101.83). Citing new scientific evidence and comments submitted to FDA in the plant sterol/stanol esters health claim rulemaking, you requested that FDA exercise enforcement discretion until such time as a final rule is issued that extends the authorized health claim to all the forms and sources of phytosterols,[1] and product forms, that may effectively reduce blood cholesterol levels. You stated that the scientific literature now supports expanding the health claim to free forms of plant sterols and stanols; and to a wider range of products, including low-fat products. You further stated that current science shows that the lowest effective daily intake of free phytosterols is 800 mg/day with the minimum addition of 400 mg free phytosterols per serving, and that the phytosterol substance should consist of at least 80 percent sitosterol, campesterol, stigmasterol, sitostanol, and campestanol (combined weight).

In response to two health claim petitions, FDA published the IFR (65 FR 54686, September 8, 2000), which authorized the use, on food labels and in food labeling, of health claims on the relationship between plant sterol/stanol esters and reduced risk of CHD, pending consideration of public comment and publication of a final regulation. The IFR authorizes the use of a health claim relating plant sterol/stanol esters and reduced risk of CHD on labeling of (1) spreads and dressings for salad containing at least 0.65 g plant sterol esters per serving, and of (2) spreads, dressings for salad, snack bars, and dietary supplements in softgel form containing at least 1.7 g plant stanol esters per serving. Specific eligibility requirements for the use of the claim are listed in §101.83(c).

We received many comments in response to the IFR and to a notice reopening the comment period (66 FR 50825; October 5, 2001). These comments have brought to FDA's attention substantial additional scientific evidence regarding the cholesterol-lowering efficacy of phytosterols that has been published in peer-reviewed scientific journals since issuance of the IFR. The IFR authorized a health claim for only plant sterol esters and plant stanol esters, the substances that were the subjects of the two health claim petitions. Comments and supporting scientific evidence now suggest that currently available scientific support extends to a broader range of phytosterol substances. As you pointed out in your letter, FDA has received notifications regarding Generally Recognized as Safe (GRAS) determinations for various phytosterols used as food ingredients at levels necessary to justify the health claim. The agency has not objected to these notifications.

Publication of the phytosterol health claim final rule is an "A List" priority in CFSAN's FY 2003 Program Priorities. Pending completion of the final rule, FDA believes that it would be appropriate to consider the exercise of enforcement discretion with regard to use of the health claim on a wider range of foods.

Based on preliminary review of the comments and additional scientific evidence, FDA intends to consider the exercise of enforcement discretion, pending publication of the final rule, with respect to certain requirements of the health claim. The agency will consider exercising enforcement discretion with regard to the use of a claim about reduced risk of CHD in the labeling of a phytosterol-containing food,[(2)] including foods other than those specified in §101.83(c)(2)(iii)(A), if: (1) the food contains at least 400 mg per reference amount customarily consumed (RACC) of phytosterols; (2) mixtures of phytosterol substances (i.e., mixtures of sterols and stanols) contain at least 80 percent beta-sitosterol, campesterol, stigmasterol, sitostanol, and campestanol (combined weight); (3) the food meets the requirements of §101.83(c)(2)(iii)(B)-(D); (4) products containing phytosterols, including mixtures of sterols and stanols or free forms, use a collective term in lieu of the terms required by §101.83(c)(2)(i)(D) in the health claim to describe the substance (e.g., "plant sterols" or "phytosterol"); (5) the claim specifies that the daily dietary intake of phytosterols that may reduce the risk of CHD is 800 milligrams (mg) or more per day, expressed as the weight of free phytosterol; (6) vegetable oils for home use that exceed the total fat disqualifying level bear the health claim along with a disclosure statement that complies with §101.13(h); and (7) the use of the claim otherwise complies with §101.83.

FDA is developing a final rule on this health claim and intends to publish it as expeditiously as possible. The agency cautions manufacturers that the final rule may differ from the broadened criteria listed above and that manufacturers would then be required to change their labels to conform to the final rule.

In the interim (i.e., before publication of the final rule), the agency intends to develop and promptly issue guidance that will contain FDA's enforcement discretion criteria for health claims for phytosterols and reduced risk of CHD. In issuing this guidance, FDA will adhere to the Agency's Good Guidance Practices (GGPs), 21 CFR 10.115.

Sincerely,

Christine L. Taylor, Ph.D.
Director
Office of Nutritional Products, Labeling and Dietary Supplements
Center for Food Safety and Applied Nutrition

Footnotes:

1. The term "phytosterols' is used as a collective term for plant sterols, and their hydrogenated stanol forms, whether used in the free sterol form or esterified with fatty acids. Phytosterol is a term commonly used by manufacturers and distributors of these substances.
2. The term "food" includes dietary supplements. See 21 U.S.C. 321(ff) (last sentence).

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Pages 000136 - 000139 have been removed in accordance with copyright laws. Please see appended bibliography list of the references that have been removed from this request.

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Intertek Automotive Research
5404 Bandera Rd., San Antonio, TX 78238

LAB CODE: NA

CLIENT CODE: ASN-FPA-002314

Report Date: 3/14/2008

CONDUCTED FOR: Arboris, LLC
P.O. Box 2008
Savannah, GA 31402

Determination of PAH content by GCMS

		RUN 1	RUN 2	RUN 3		AVERAGE
COMPND 1 NAME	PHENANTHRENE	0.00	0.44	0.65		0.37
COMPND 2 NAME	ANTHRACENE	0.00	0.00	0.00		0.00
COMPND 3 NAME	FLUORANTHENE	0.00	0.00	0.00		0.00
COMPND 4 NAME	PYRENE	0.27	0.21	0.00		0.16
COMPND 5 NAME	BENZ(A)ANTHRENE	0.00	0.00	0.00		0.00
COMPND 6 NAME	CHRYSENE	0.00	0.00	0.00		0.00
					light PAHs	**0.53**
COMPND 7 NAME	BENZO(B)FLUORANTHENE	0.00	0.00	0.00		0.00
COMPND 8 NAME	BENZO(K)FLUORANTHENE	0.00	0.00	0.00		0.00
COMPND 9 NAME	BENZO(A)PYRENE	0.00	0.00	0.00		0.00
COMPND 10 NAME	PERYLENE	0.00	0.00	0.00		0.00
COMPND 11 NAME	INDENO(123CD)PYRENE	0.00	0.00	0.00		0.00
COMPND 12 NAME	DIBENZ(AH)ANTHRACENE	0.00	0.00	0.00		0.00
COMPND 13 NAME	BENZO(GHI)PERYLENE	1.30	1.29	1.19		1.26
COMPND 14 NAME	ANTHANTHRENE	0.00	0.00	0.00		0.00
COMPND 15 NAME	CORONENE	0.00	0.00	0.00		0.00
					heavy PAHs	**1.26**

Remarks: None

L. Orzech
Senior Chemist

000141

Intertek Automotive Research
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LAB CODE: NA

CLIENT CODE: ASN-FPA-002317

Report Date: 3/14/2008

CONDUCTED FOR: Arboris, LLC
P.O. Box 2008
Savannah, GA 31402

Determination of PAH content by GCMS

		RUN 1	RUN 2	RUN 3		AVERAGE
COMPND 1 NAME	PHENANTHRENE	0.00	0.00	0.00		0.00
COMPND 2 NAME	ANTHRACENE	0.00	0.00	0.00		0.00
COMPND 3 NAME	FLUORANTHENE	0.88	0.74	0.51		0.71
COMPND 4 NAME	PYRENE	0.41	0.65	0.35		0.47
COMPND 5 NAME	BENZ(A)ANTHRENE	0.10	0.03	0.57		0.23
COMPND 6 NAME	CHRYSENE	0.25	0.00	0.00		0.08
					light PAHs	**1.50**
COMPND 7 NAME	BENZO(B)FLUORANTHENE	0.00	0.07	0.00		0.02
COMPND 8 NAME	BENZO(K)FLUORANTHENE	0.00	0.00	0.00		0.00
COMPND 9 NAME	BENZO(A)PYRENE	0.00	0.00	0.00		0.00
COMPND 10 NAME	PERYLENE	0.09	0.07	0.05		0.07
COMPND 11 NAME	INDENO(123CD)PYRENE	0.00	0.11	0.00		0.04
COMPND 12 NAME	DIBENZ(AH)ANTHRACENE	0.00	0.00	0.00		0.00
COMPND 13 NAME	BENZO(GHI)PERYLENE	0.00	0.72	0.20		0.31
COMPND 14 NAME	ANTHANTHRENE	0.20	0.31	0.36		0.29
COMPND 15 NAME	CORONENE	0.29	0.00	0.00		0.10
					heavy PAHs	**0.82**

Remarks: None

L. Orzech
Senior Chemist

000142

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LAB CODE: 7S0947MA1

CLIENT CODE: ASN-FPA-002738

Report Date: 3/14/2008

CONDUCTED FOR: Arboris, LLC
P.O. Box 2008
Savannah, GA 31402

Determination of PAH content by GCMS

		RUN 1	RUN 2	RUN 3		AVERAGE
COMPND 1 NAME	PHENANTHRENE	0.42	0.62	0.48		0.51
COMPND 2 NAME	ANTHRACENE	0.00	0.08	0.00		0.03
COMPND 3 NAME	FLUORANTHENE	0.03	0.24	0.00		0.09
COMPND 4 NAME	PYRENE	0.20	0.43	0.00		0.21
COMPND 5 NAME	BENZ(A)ANTHRENE	0.70	0.55	0.00		0.42
COMPND 6 NAME	CHRYSENE	0.00	0.22	0.59		0.27
					light PAHs	**1.52**
COMPND 7 NAME	BENZO(B)FLUORANTHENE	1.52	1.04	0.81		1.12
COMPND 8 NAME	BENZO(K)FLUORANTHENE	1.08	1.05	0.03		0.72
COMPND 9 NAME	BENZO(A)PYRENE	0.00	0.00	0.04		0.01
COMPND 10 NAME	PERYLENE	0.85	1.03	0.50		0.79
COMPND 11 NAME	INDENO(123CD)PYRENE	0.00	0.00	0.00		0.00
COMPND 12 NAME	DIBENZ(AH)ANTHRACENE	0.00	0.00	0.00		0.00
COMPND 13 NAME	BENZO(GHI)PERYLENE	1.02	0.93	0.56		0.84
COMPND 14 NAME	ANTHANTHRENE	0.03	0.00	0.32		0.12
COMPND 15 NAME	CORONENE	0.00	0.00	0.00		0.00
					heavy PAHs	**3.60**

Remarks: None

L. Orzech
Senior Chemist

000143

Intertek Automotive Research
5404 Bandera Rd., San Antonio, TX 78238

LAB CODE: 7S0954MA1

CLIENT CODE: ASN-FPA-002744

Report Date: 3/14/2008

CONDUCTED FOR: Arboris, LLC
P.O. Box 2008
Savannah, GA 31402

Determination of PAH content by GCMS

		RUN 1	RUN 2	RUN 3		AVERAGE
COMPND 1 NAME	PHENANTHRENE	0.76	0.55	0.39		0.57
COMPND 2 NAME	ANTHRACENE	0.98	1.18	0.62		0.93
COMPND 3 NAME	FLUORANTHENE	0.15	0.31	0.24		0.23
COMPND 4 NAME	PYRENE	0.18	0.28	0.38		0.28
COMPND 5 NAME	BENZ(A)ANTHRENE	0.00	0.00	0.64		0.21
COMPND 6 NAME	CHRYSENE	0.00	0.00	0.00		0.00
					light PAHs	**2.22**
COMPND 7 NAME	BENZO(B)FLUORANTHENE	0.00	0.00	0.47		0.16
COMPND 8 NAME	BENZO(K)FLUORANTHENE	0.00	0.00	0.00		0.00
COMPND 9 NAME	BENZO(A)PYRENE	0.00	0.00	0.00		0.00
COMPND 10 NAME	PERYLENE	0.00	0.00	0.00		0.00
COMPND 11 NAME	INDENO(123CD)PYRENE	0.00	0.00	0.00		0.00
COMPND 12 NAME	DIBENZ(AH)ANTHRACENE	0.09	0.00	0.00		0.03
COMPND 13 NAME	BENZO(GHI)PERYLENE	0.21	0.05	0.00		0.09
COMPND 14 NAME	ANTHANTHRENE	0.00	0.00	0.00		0.00
COMPND 15 NAME	CORONENE	0.00	0.00	0.00		0.00
					heavy PAHs	**0.28**

Remarks: None

L. Orzech
Senior Chemist

000144

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SILLIKER, Inc.
Ohio Laboratory
2057 Builders Place, Columbus, OH 43204
Tel. 614/ 486 0150 Fax. 614/ 486 0151

CERTIFICATE OF ANALYSIS

COA No:	OHI-32285552-0
Supersedes:	None
COA Date	4/7/08
Page 1 of 1	

COPY TO:
Mr. Jesse Boyer
Quality Assurance Supervisor
Arboris LLC
1780 Tamarak Road
Newark, OH 43055

ORIGINAL TO:
Mr. Randy Clouston
Quality Control Supervisor
Arboris LLC
1780 Tamarack Road
Newark, OH 43055

Received From:	Newark, OH
Received Date:	4/2/08
P.O.# / ID:	N2008-309
Location of Test: (except where noted)	Columbus, OH

Analytical Results

Desc. 1: Phytosterols
Desc. 2: A6M0129
Laboratory ID: 312670791
Condition Rec'd: NORMAL
Temp Rec'd (°C): 12.3

Analyte	Result	Units	Method Reference	Test Date	Loc.
Enterobacteriaceae - Petrifilm	<10	/g	CMMEF, 4th ed.	4/3/08	
Total Microbial Count - USP	<10	/g	USP30, NF25, 2007	4/4/08	
Yeast and Mold - USP			USP30, NF25, 2007	4/7/08	
Yeast	<10	/g			
Mold	<10	/g			

Desc. 1: Phytosterols
Desc. 2: C8M0104
Laboratory ID: 312670794
Condition Rec'd: NORMAL
Temp Rec'd (°C): 12.3

Analyte	Result	Units	Method Reference	Test Date	Loc.
Enterobacteriaceae - Petrifilm	<10	/g	CMMEF, 4th ed.	4/3/08	
Total Microbial Count - USP	<10	/g	USP30, NF25, 2007	4/4/08	
Yeast and Mold - USP			USP30, NF25, 2007	4/7/08	
Yeast	<10	/g			
Mold	<10	/g			

Amitha Miele — Laboratory Director

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CTS Agri-Food Laboratory
Malledijk 18
P.O.Box 200
3200 AE Spijkenisse
Dir Tel (0181) 694500
Dir Fax (0181) 694506

Arboris, LLC

Attn. Mr. J. Boyer
P.O.Box 2008
GA 31402 Savannah
United States of America

ANALYTICAL REPORT 200710000854

The analysis of the sample said to be : STEROL

Received : 11/10/07 From : Arboris, LLC
Sample packing : Plastic Bottle
Sample sealed : Sealed

Identification of the order as indicated to us :

Parcel : Pine tree sterols
Reference : ASN-FPA-002795 (Lot J7M0104)

The sample as detailed has been analysed in accordance with the instructions received, and showed following results :

Organophosphorous pesticide residues
[0034-GC]

Chlorthion	Less than 20 ppb
Disulfoton	Less than 20 ppb
Malathion	Less than 20 ppb
Parathion (-methyl)	Less than 20 ppb
Parathion (-ethyl)	Less than 20 ppb
Sulfotepp	Less than 20 ppb
Fenthion	Less than 20 ppb
Diazinon	Less than 20 ppb
Dimethoate	Less than 20 ppb
Fenitrothion	Less than 20 ppb
Bromophos (-methyl)	Less than 20 ppb
Bromophos (-ethyl)	Less than 20 ppb
Chlorfenvinphos	Less than 20 ppb
Chlorpyriphos (-methyl)	Less than 20 ppb
Chlorpyriphos (-ethyl)	Less than 20 ppb
Pirimiphos (-methyl)	Less than 20 ppb
Methidation	Less than 20 ppb
Ethion	Less than 20 ppb

Chlorinated pesticide residues
[0034-GC]

Tecnazen	Less than 10 ppb
HCB	Less than 10 ppb
Alpha-HCH	Less than 10 ppb
Beta-HCH	Less than 15 ppb
Delta-HCH	Less than 10 ppb
Lindan	Less than 15 ppb

See next page

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R.C. Rotterdam No. 24226722 Member of the SGS Group (Société Générale de Surveillance)
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ANALYTICAL REPORT 200710000854

Quintozen	Less than 10 ppb
Heptachlor	Less than 10 ppb
Alpha-Chlordan	Less than 10 ppb
Gamma-Chlordan	Less than 10 ppb
Aldrin	Less than 10 ppb
Dieldrin	Less than 10 ppb
Endrin	Less than 10 ppb
Methoxychlor	Less than 10 ppb
p,p-DDE	Less than 10 ppb
o,p-DDE	Less than 10 ppb
p,p-DDD	Less than 10 ppb
o,p-DDD	Less than 10 ppb
p,p-DDT	Less than 10 ppb
o,p-DDT	Less than 10 ppb

Polycyclic Aromatic Hydrocarbons
[0030-LC]

-Phenanthrene	Less than 1.0 µg/kg
-Anthracene	Less than 0.5 µg/kg
-Fluoranthene	Less than 0.5 µg/kg
-Pyrene	Less than 0.5 µg/kg
-1,2-benzo-anthracene	0.58 µg/kg
-Chrysene	Less than 0.5 µg/kg
-Benzo[b]fluoranthene	Less than 0.5 µg/kg
-Perylene	Less than 0.5 µg/kg
-Benzo[k]fluoranthene	Less than 0.5 µg/kg
-Benzo[a]pyrene	0.24 µg/kg
-Dibenzo[a,h]anthracene	1.3 µg/kg
-Benzo[g,h,i]perylene	Less than 0.5 µg/kg
-Indeno[1,2,3,cd]pyrene	Less than 0.5 µg/kg
-Anthantrene	Less than 0.5 µg/kg
-Coronene	Less than 0.5 µg/kg

Test comment:
Total Light PAH's sum of Anthracene,Phenanthrene, Fluoranthene, Pyrene, Chrysene and 1,2-Benzo-anthracene.
Total PAH's sum of total light and total heavy PAH's

Residual Solvents
[0033-GC]

Methanol	Less than 0.1 mg/kg
Ethanol	Less than 0.1 mg/kg
Acetone	Less than 0.1 mg/kg
Propanol (1-)	Less than 0.1 mg/kg
Propanol (2-)	Less than 0.1 mg/kg
Butanone	Less than 0.1 mg/kg
Hexane (n-)	Less than 0.1 mg/kg
Butanol (n-)	Less than 0.1 mg/kg
Isooctane (2,2,4-Trimethylpentane)	Less than 0.1 mg/kg
Heptane	Less than 0.1 mg/kg
Remark	No extra peaks higher than 1 mg/kg observed

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ANALYTICAL REPORT 200710000854

Dioxine
[Test performed within the SGS Group]

See attached certificate

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SGS CTS Agri-Food Laboratory

A. Hoogland, Laboratory Manager
Spijkenisse, October 30, 2007

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CTS Agri-Food Laboratory
Malledijk 18
P.O.Box 200
3200 AE Spijkenisse
Dir Tel (0181) 694500
Dir Fax (0181) 694506

Arboris, LLC

Attn. Mr. J. Boyer
P.O.Box 2008
GA 31402 Savannah
United States of America

ANALYTICAL REPORT 200710000855

The analysis of the sample said to be : STEROL

Received : 11/10/07 From : Arboris, LLC
Sample packing : Plastic Bottle
Sample sealed : Sealed

Identification of the order as indicated to us :

Parcel : Pine tree sterols
Reference : ASN-FPA-002799 (Lot J7M0105)

The sample as detailed has been analysed in accordance with the instructions received, and showed following results :

Organophosphorous pesticide residues
[0034-GC]

Chlorthion	Less than 20 ppb
Disulfoton	Less than 20 ppb
Malathion	Less than 20 ppb
Parathion (-methyl)	Less than 20 ppb
Parathion (-ethyl)	Less than 20 ppb
Sulfotepp	Less than 20 ppb
Fenthion	Less than 20 ppb
Diazinon	Less than 20 ppb
Dimethoate	Less than 20 ppb
Fenitrothion	Less than 20 ppb
Bromophos (-methyl)	Less than 20 ppb
Bromophos (-ethyl)	Less than 20 ppb
Chlorfenvinphos	Less than 20 ppb
Chlorpyriphos (-methyl)	Less than 20 ppb
Chlorpyriphos (-ethyl)	Less than 20 ppb
Pirimiphos (-methyl)	Less than 20 ppb
Methidation	Less than 20 ppb
Ethion	Less than 20 ppb

Chlorinated pesticide residues
[0034-GC]

Tecnazen	Less than 10 ppb
HCB	Less than 10 ppb
Alpha-HCH	Less than 10 ppb
Beta-HCH	Less than 15 ppb
Delta-HCH	Less than 10 ppb
Lindan	Less than 15 ppb

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ANALYTICAL REPORT 200710000855

Quintozen	Less than 10 ppb
Heptachlor	Less than 10 ppb
Alpha-Chlordan	Less than 10 ppb
Gamma-Chlordan	Less than 10 ppb
Aldrin	Less than 10 ppb
Dieldrin	Less than 10 ppb
Endrin	Less than 10 ppb
Methoxychlor	Less than 10 ppb
p,p-DDE	Less than 10 ppb
o,p-DDE	Less than 10 ppb
p,p-DDD	Less than 10 ppb
o,p-DDD	Less than 10 ppb
p,p-DDT	Less than 10 ppb
o,p-DDT	Less than 10 ppb

Polycyclic Aromatic Hydrocarbons
[0030-LC]

-Phenanthrene	1.3 µg/kg
-Anthracene	Less than 0.5 µg/kg
-Fluoranthene	Less than 0.5 µg/kg
-Pyrene	Less than 0.5 µg/kg
-1,2-benzo-anthracene	Less than 0.5 µg/kg
-Chrysene	Less than 0.5 µg/kg
-Benzo[b]fluoranthene	1.2 µg/kg
-Perylene	Less than 0.5 µg/kg
-Benzo[k]fluoranthene	Less than 0.5 µg/kg
-Benzo[a]pyrene	0.32 µg/kg
-Dibenzo[a,h]anthracene	1.2 µg/kg
-Benzo[g,h,i]perylene	0.54 µg/kg
-Indeno[1,2,3,cd]pyrene	Less than 0.5 µg/kg
-Anthantrene	Less than 0.5 µg/kg
-Coronene	Less than 0.5 µg/kg

Test comment:
Total Light PAH's sum of Anthracene,Phenanthrene, Fluoranthene, Pyrene, Chrysene and 1,2-Benzo-anthracene.
Total PAH's sum of total light and total heavy PAH's

Residual Solvents
[0033-GC]

Methanol	Less than 0.1 mg/kg
Ethanol	Less than 0.1 mg/kg
Acetone	Less than 0.1 mg/kg
Propanol (1-)	Less than 0.1 mg/kg
Propanol (2-)	Less than 0.1 mg/kg
Butanone	Less than 0.1 mg/kg
Hexane (n-)	Less than 0.1 mg/kg
Butanol (n-)	Less than 0.1 mg/kg
Isooctane (2,2,4-Trimethylpentane)	Less than 0.1 mg/kg
Heptane	Less than 0.1 mg/kg
Remark	No extra peaks higher than 1 mg/kg observed

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ANALYTICAL REPORT 200710000855

Dioxine
[Test performed within the SGS Group]

See attached certificate

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SGS CTS Agri-Food Laboratory

A. Hoogland, Laboratory Manager
Spijkenisse, October 30, 2007

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CTS Agri-Food Laboratory
Malledijk 18
P.O.Box 200
3200 AE Spijkenisse
Dir Tel (0181) 694500
Dir Fax (0181) 694506

Arboris, LLC

Attn. Mr. J. Boyer
P.O.Box 2008
GA 31402 Savannah
United States of America

ANALYTICAL REPORT 200710000856

The analysis of the sample said to be : STEROL

Received : 11/10/07 From : Arboris, LLC
Sample packing : Plastic Bottle
Sample sealed : Sealed

Identification of the order as indicated to us :

Parcel : Pine tree sterols
Reference : ASN-FPA-002800 (Lot J7M0106)

The sample as detailed has been analysed in accordance with the instructions received, and showed following results :

Organophosphorous pesticide residues
[0034-GC]

Chlorthion	Less than 20 ppb
Disulfoton	Less than 20 ppb
Malathion	Less than 20 ppb
Parathion (-methyl)	Less than 20 ppb
Parathion (-ethyl)	Less than 20 ppb
Sulfotepp	Less than 20 ppb
Fenthion	Less than 20 ppb
Diazinon	Less than 20 ppb
Dimethoate	Less than 20 ppb
Fenitrothion	Less than 20 ppb
Bromophos (-methyl)	Less than 20 ppb
Bromophos (-ethyl)	Less than 20 ppb
Chlorfenvinphos	Less than 20 ppb
Chlorpyriphos (-methyl)	Less than 20 ppb
Chlorpyriphos (-ethyl)	Less than 20 ppb
Pirimiphos (-methyl)	Less than 20 ppb
Methidation	Less than 20 ppb
Ethion	Less than 20 ppb

Chlorinated pesticide residues
[0034-GC]

Tecnazen	Less than 10 ppb
HCB	Less than 10 ppb
Alpha-HCH	Less than 10 ppb
Beta-HCH	Less than 15 ppb
Delta-HCH	Less than 10 ppb
Lindan	Less than 15 ppb

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ANALYTICAL REPORT 200710000856

Quintozen	Less than 10 ppb
Heptachlor	Less than 10 ppb
Alpha-Chlordan	Less than 10 ppb
Gamma-Chlordan	Less than 10 ppb
Aldrin	Less than 10 ppb
Dieldrin	Less than 10 ppb
Endrin	Less than 10 ppb
Methoxychlor	Less than 10 ppb
p,p-DDE	Less than 10 ppb
o,p-DDE	Less than 10 ppb
p,p-DDD	Less than 10 ppb
o,p-DDD	Less than 10 ppb
p,p-DDT	Less than 10 ppb
o,p-DDT	Less than 10 ppb

Polycyclic Aromatic Hydrocarbons
[0030-LC]

-Phenanthrene	1.2 µg/kg
-Anthracene	Less than 0.5 µg/kg
-Fluoranthene	Less than 0.5 µg/kg
-Pyrene	Less than 0.5 µg/kg
-1,2-benzo-anthracene	Less than 0.5 µg/kg
-Chrysene	1.8 µg/kg
-Benzo[b]fluoranthene	Less than 0.5 µg/kg
-Perylene	Less than 0.5 µg/kg
-Benzo[k]fluoranthene	Less than 0.5 µg/kg
-Benzo[a]pyrene	0.22 µg/kg
-Dibenzo[a,h]anthracene	1.2 µg/kg
-Benzo[g,h,i]perylene	Less than 0.5 µg/kg
-Indeno[1,2,3,cd]pyrene	0.61 µg/kg
-Anthantrene	Less than 0.5 µg/kg
-Coronene	Less than 0.5 µg/kg

Test comment:
Total Light PAH's sum of Anthracene,Phenanthrene, Fluoranthene, Pyrene, Chrysene and 1,2-Benzo-anthracene.
Total PAH's sum of total light and total heavy PAH's

Residual Solvents
[0033-GC]

Methanol	Less than 0.1 mg/kg
Ethanol	Less than 0.1 mg/kg
Acetone	Less than 0.1 mg/kg
Propanol (1-)	Less than 0.1 mg/kg
Propanol (2-)	Less than 0.1 mg/kg
Butanone	Less than 0.1 mg/kg
Hexane (n-)	Less than 0.1 mg/kg
Butanol (n-)	Less than 0.1 mg/kg
Isooctane (2,2,4-Trimethylpentane)	Less than 0.1 mg/kg
Heptane	Less than 0.1 mg/kg
Remark	No extra peaks higher than 1 mg/kg observed

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Member of the SGS Group (Société Générale de Surveillance)

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ANALYTICAL REPORT 200710000856

Dioxine
[Test performed within the SGS Group]

See attached certificate

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SGS CTS Agri-Food Laboratory

A. Hoogland, Laboratory Manager
Spijkenisse, October 30, 2007

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CTS Agri-Food Laboratory
Malledijk 18
P.O.Box 200
3200 AE Spijkenisse
Dir Tel (0181) 694500
Dir Fax (0181) 694506

Arboris, LLC

Attn. Mr. J. Boyer
P.O.Box 2008
GA 31402 Savannah
United States of America

ANALYTICAL REPORT 200710000857

The analysis of the sample said to be : STEROL

Received : 11/10/07 From : Arboris, LLC
Sample packing : Plastic Bottle
Sample sealed : Sealed

Identification of the order as indicated to us :

Parcel : Pine tree sterols
Reference : ASN-FPA-002803 (Lot J7M0107)

The sample as detailed has been analysed in accordance with the instructions received, and showed following results :

Organophosphorous pesticide residues
[0034-GC]

Chlorthion	Less than 20 ppb
Disulfoton	Less than 20 ppb
Malathion	Less than 20 ppb
Parathion (-methyl)	Less than 20 ppb
Parathion (-ethyl)	Less than 20 ppb
Sulfotepp	Less than 20 ppb
Fenthion	Less than 20 ppb
Diazinon	Less than 20 ppb
Dimethoate	Less than 20 ppb
Fenitrothion	Less than 20 ppb
Bromophos (-methyl)	Less than 20 ppb
Bromophos (-ethyl)	Less than 20 ppb
Chlorfenvinphos	Less than 20 ppb
Chlorpyriphos (-methyl)	Less than 20 ppb
Chlorpyriphos (-ethyl)	Less than 20 ppb
Pirimiphos (-methyl)	Less than 20 ppb
Methidation	Less than 20 ppb
Ethion	Less than 20 ppb

Chlorinated pesticide residues
[0034-GC]

Tecnazen	Less than 10 ppb
HCB	Less than 10 ppb
Alpha-HCH	Less than 10 ppb
Beta-HCH	Less than 15 ppb
Delta-HCH	Less than 10 ppb
Lindan	Less than 15 ppb

See next page

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Member of the SGS Group (Société Générale de Surveillance)
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Quintozen	Less than 10 ppb
Heptachlor	Less than 10 ppb
Alpha-Chlordan	Less than 10 ppb
Gamma-Chlordan	Less than 10 ppb
Aldrin	Less than 10 ppb
Dieldrin	Less than 10 ppb
Endrin	Less than 10 ppb
Methoxychlor	Less than 10 ppb
p,p-DDE	Less than 10 ppb
o,p-DDE	Less than 10 ppb
p,p-DDD	Less than 10 ppb
o,p-DDD	Less than 10 ppb
p,p-DDT	Less than 10 ppb
o,p-DDT	Less than 10 ppb

Polycyclic Aromatic Hydrocarbons
[0030-LC]

-Phenanthrene	Less than 1.0 µg/kg
-Anthracene	Less than 0.5 µg/kg
-Fluoranthene	0.53 µg/kg
-Pyrene	Less than 0.5 µg/kg
-1,2-benzo-anthracene	Less than 0.5 µg/kg
-Chrysene	Less than 0.5 µg/kg
-Benzo[b]fluoranthene	Less than 0.5 µg/kg
-Perylene	Less than 0.5 µg/kg
-Benzo[k]fluoranthene	Less than 0.5 µg/kg
-Benzo[a]pyrene	0.28 µg/kg
-Dibenzo[a,h]anthracene	0.99 µg/kg
-Benzo[g,h,i]perylene	Less than 0.5 µg/kg
-Indeno[1,2,3,cd]pyrene	0.50 µg/kg
-Anthantrene	Less than 0.5 µg/kg
-Coronene	Less than 0.5 µg/kg

Test comment:
Total Light PAH's sum of Anthracene,Phenanthrene, Fluoranthene, Pyrene, Chrysene and 1,2-Benzo-anthracene.
Total PAH's sum of total light and total heavy PAH's

Residual Solvents
[0033-GC]

Methanol	Less than 0.1 mg/kg
Ethanol	Less than 0.1 mg/kg
Acetone	Less than 0.1 mg/kg
Propanol (1-)	Less than 0.1 mg/kg
Propanol (2-)	Less than 0.1 mg/kg
Butanone	Less than 0.1 mg/kg
Hexane (n-)	Less than 0.1 mg/kg
Butanol (n-)	Less than 0.1 mg/kg
Isooctane (2,2,4-Trimethylpentane)	Less than 0.1 mg/kg
Heptane	Less than 0.1 mg/kg
Remark	No extra peaks higher than 1 mg/kg observed

See next page

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ANALYTICAL REPORT 200710000857

Dioxine
[Test performed within the SGS Group]

See attached certificate

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SGS CTS Agri-Food Laboratory

A. Hoogland, Laboratory Manager
Spijkenisse, October 30, 2007

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SGS CTS/AGRI-FOOD Laboratory
Puntweg 6
3208 LD Spijkenisse
Nederland

ANALYTICAL REPORT **IAC0708216**

Number of samples:	3
Kind of sample:	sterol
Identification of the samples::	200708000535-1 200708000536-1 200708000537-1
Date of receipt:	10/08/2007
Start date of analysis:	14/08/2007
Asked analysis:	Dioxin & dioxin-like PCBs

Analytical results:

2,3,7,8-substituted PCDF's & PCDD's (ng WHO-TEQ/kg) : see p. 2-4/13
(HRGC/HRMS ; ECO/AV/IAC/005)

Determination of dioxin-like PCBs (ng WHO-TEQ/kg) : see p. 5-7/13
(HRGC/HRMS ; ECO/AV/IAC/016)

Recovery extraction standards: see p. 8-13/13

Remark: the recoveries marked with (**) do not meet the quality criteria of the analytical method.

ANTWERP, 28/08/2007

I.A.C.
A Division of SGS Belgium NV

Marc Van Ryckeghem
Division Manager

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The analytical report can only be used within the specific context of the order and is only valid for the samples analysed.
The measurement uncertainty has been determined and is available in the laboratory. On request, the data will be transmitted.

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ANALYTICAL REPORT: IAC0708216

Determination of 2,3,7,8-substituted PCDF and PCDD

Product: sterol
Sample: 200708000535-1

Your reference: Lot H7M0103

Compound name	Concentration (ng/kg)	WHO-TEQ (ng/kg) Lowerbound	WHO-TEQ (ng/kg) Mediumbound	WHO-TEQ (ng/kg) Upperbound	WHO-TEQ (ng/kg) Limit of Reporting
2,3,7,8-TCDF	< 0,052	0	0,0026	0,0052	0,0052
2,3,7,8-TCDD	< 0,058	0	0,029	0,058	0,058
1,2,3,7,8-PeCDF	< 0,060	0	0,0015	0,0030	0,0030
2,3,4,7,8-PeCDF	< 0,060	0	0,015	0,030	0,030
1,2,3,7,8-PeCDD	< 0,089	0	0,044	0,089	0,089
1,2,3,4,7,8-HxCDF	0,10	0,010	0,010	0,010	0,0046
1,2,3,6,7,8-HxCDF	< 0,041	0	0,0020	0,0041	0,0041
2,3,4,6,7,8-HxCDF	< 0,043	0	0,0022	0,0043	0,0043
1,2,3,7,8,9-HxCDF	0,043	0,0043	0,0043	0,0043	0,0035
1,2,3,4,7,8-HxCDD	< 0,058	0	0,0029	0,0058	0,0058
1,2,3,6,7,8-HxCDD	< 0,046	0	0,0023	0,0046	0,0046
1,2,3,7,8,9-HxCDD	< 0,053	0	0,0027	0,0053	0,0053
1,2,3,4,6,7,8-HpCDF	< 1,0	0	0,0051	0,010	0,010
1,2,3,4,7,8,9-HpCDF	< 0,38	0	0,0019	0,0038	0,0038
1,2,3,4,6,7,8-HpCDD	0,75	0,0075	0,0075	0,0075	0,0038
OCDF	< 1,5	0	0,000076	0,00015	0,00015
OCDD	< 2,5	0	0,00012	0,00025	0,00025
total (100 %)		0,022	0,13	0,25	0,24
total (88 % d.w.)		0,019	0,11	**0,22**	0,21

The TEQ values have been calculated using the WHO toxicity equivalence factors (TEF) according to Martin Van den Berg et al. (Environmental Health Perspectives (1998), 106: 775-792). Lowerbound concentration : For the calculation of the total concentration, the values, which were lower than the limit of reporting, were regarded as zero. Mediumbound concentration : For the calculation of the total mediumbound concentration, the values, which were lower than the limit of reporting, were regarded as the half of the limit of reporting. Upperbound concentration : For the calculation of the total upperbound concentration, the values, which were lower than the limit of reporting, were regarded as the value of the limit of reporting. The measurement uncertainty has been determined and is available in the laboratory. On request, the data will be transmitted. The RSD of the control sample is less than 10%.

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ANALYTICAL REPORT: IAC0708216

Determination of 2,3,7,8-substituted PCDF and PCDD

Product: sterol
Sample: 200708000536-1

Your reference: Lot H7M0104

Compound name	Concentration (ng/kg)	WHO-TEQ (ng/kg) Lowerbound	WHO-TEQ (ng/kg) Mediumbound	WHO-TEQ (ng/kg) Upperbound	WHO-TEQ (ng/kg) Limit of Reporting
2,3,7,8-TCDF	< 0,18	0	0,0088	0,018	0,018
2,3,7,8-TCDD	< 0,048	0	0,024	0,048	0,048
1,2,3,7,8-PeCDF	< 0,052	0	0,0013	0,0026	0,0026
2,3,4,7,8-PeCDF	< 0,052	0	0,013	0,026	0,026
1,2,3,7,8-PeCDD	< 0,074	0	0,037	0,074	0,074
1,2,3,4,7,8-HxCDF	0,068	0,0068	0,0068	0,0068	0,0038
1,2,3,6,7,8-HxCDF	< 0,034	0	0,0017	0,0034	0,0034
2,3,4,6,7,8-HxCDF	< 0,036	0	0,0018	0,0036	0,0036
1,2,3,7,8,9-HxCDF	< 0,030	0	0,0015	0,0030	0,0030
1,2,3,4,7,8-HxCDD	< 0,049	0	0,0025	0,0049	0,0049
1,2,3,6,7,8-HxCDD	< 0,038	0	0,0019	0,0038	0,0038
1,2,3,7,8,9-HxCDD	0,048	0,0048	0,0048	0,0048	0,0044
1,2,3,4,6,7,8-HpCDF	< 0,70	0	0,0035	0,0070	0,0070
1,2,3,4,7,8,9-HpCDF	< 0,31	0	0,0016	0,0031	0,0031
1,2,3,4,6,7,8-HpCDD	0,66	0,0066	0,0066	0,0066	0,0031
OCDF	< 1,9	0	0,000094	0,00019	0,00019
OCDD	< 3,4	0	0,00017	0,00034	0,00034
total (100 %)		0,018	0,12	0,22	0,21
total (88 % d.w.)		0,016	0,11	**0,19**	0,18

The TEQ values have been calculated using the WHO toxicity equivalence factors (TEF) according to Martin Van den Berg et al. (Environmental Health Perspectives (1998), 106: 775-792). Lowerbound concentration : For the calculation of the total concentration, the values, which were lower than the limit of reporting, were regarded as zero. Mediumbound concentration : For the calculation of the total mediumbound concentration, the values, which were lower than the limit of reporting, were regarded as the half of the limit of reporting. Upperbound concentration : For the calculation of the total upperbound concentration, the values, which were lower than the limit of reporting, were regarded as the value of the limit of reporting. The measurement uncertainty has been determined and is available in the laboratory. On request, the data will be transmitted. The RSD of the control sample is less than 10%.

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ANALYTICAL REPORT: IAC0708216

Determination of 2,3,7,8-substituted PCDF and PCDD

Product: sterol
Sample: 200708000537-1

Your reference: Lot H7M0105

Compound name	Concentration (ng/kg)	WHO-TEQ (ng/kg) *Lowerbound*	WHO-TEQ (ng/kg) *Mediumbound*	WHO-TEQ (ng/kg) *Upperbound*	WHO-TEQ (ng/kg) *Limit of Reporting*
2,3,7,8-TCDF	< 0,14	0	0,0072	0,014	0,014
2,3,7,8-TCDD	< 0,048	0	0,024	0,048	0,048
1,2,3,7,8-PeCDF	0,061	0,0030	0,0030	0,0030	0,0026
2,3,4,7,8-PeCDF	< 0,052	0	0,013	0,026	0,026
1,2,3,7,8-PeCDD	< 0,075	0	0,037	0,075	0,075
1,2,3,4,7,8-HxCDF	< 0,046	0	0,0023	0,0046	0,0046
1,2,3,6,7,8-HxCDF	< 0,034	0	0,0017	0,0034	0,0034
2,3,4,6,7,8-HxCDF	< 0,037	0	0,0018	0,0037	0,0037
1,2,3,7,8,9-HxCDF	< 0,030	0	0,0015	0,0030	0,0030
1,2,3,4,7,8-HxCDD	< 0,049	0	0,0025	0,0049	0,0049
1,2,3,6,7,8-HxCDD	< 0,038	0	0,0019	0,0038	0,0038
1,2,3,7,8,9-HxCDD	< 0,044	0	0,0022	0,0044	0,0044
1,2,3,4,6,7,8-HpCDF	< 0,84	0	0,0042	0,0084	0,0084
1,2,3,4,7,8,9-HpCDF	< 0,32	0	0,0016	0,0032	0,0032
1,2,3,4,6,7,8-HpCDD	0,61	0,0061	0,0061	0,0061	0,0032
OCDF	< 1,4	0	0,000068	0,00014	0,00014
OCDD	< 3,3	0	0,00016	0,00033	0,00033
total (100 %)		0,0092	0,11	0,21	0,21
total (88 % d.w.)		0,0081	0,097	**0,18**	0,18

The TEQ values have been calculated using the WHO toxicity equivalence factors (TEF) according to Martin Van den Berg et al. (Environmental Health Perspectives (1998), 106: 775-792). Lowerbound concentration : For the calculation of the total concentration, the values, which were lower than the limit of reporting, were regarded as zero. Mediumbound concentration : For the calculation of the total mediumbound concentration, the values, which were lower than the limit of reporting, were regarded as the half of the limit of reporting. Upperbound concentration : For the calculation of the total upperbound concentration, the values, which were lower than the limit of reporting, were regarded as the value of the limit of reporting. The measurement uncertainty has been determined and is available in the laboratory. On request, the data will be transmitted. The RSD of the control sample is less than 10%.

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ANALYTICAL REPORT: IAC0708216

Determination of dioxin-like PCBs

Product: sterol
Sample: 200708000535-1

Your reference: Lot H7M0103

Compound name	Concentration (ng/kg)	WHO-TEQ (ng/kg) Lowerbound	WHO-TEQ (ng/kg) Mediumbound	WHO-TEQ (ng/kg) Upperbound	WHO-TEQ (ng/kg) Limit of Reporting
Non-ortho PCBs					
3,4,4',5-TeCB (PCB #81)	< 2,5	0	0,00013	0,00025	0,00025
3,3',4,4'-TeCB (PCB #77)	< 5,1	0	0,00025	0,00051	0,00051
3,3',4,4',5-PeCB (PCB #126)	< 1,3	0	0,063	0,13	0,13
3,3',4,4',5,5'-HxCB (PCB #169)	< 1,3	0	0,0063	0,013	0,013
Mono-ortho PCBs					
2',3,4,4',5-PeCB (PCB #123)	< 5,1	0	0,00025	0,00051	0,00051
2,3',4,4',5-PeCB (PCB #118)	< 51	0	0,0025	0,0051	0,0051
2,3,4,4',5-PeCB (PCB #114)	< 5,1	0	0,0013	0,0025	0,0025
2,3,3',4,4'-PeCB (PCB #105)	< 25	0	0,0013	0,0025	0,0025
2,3',4,4',5,5'-HxCB (PCB #167)	< 25	0	0,00013	0,00025	0,00025
2,3,3',4,4',5-HxCB (PCB #156)	< 25	0	0,0063	0,013	0,013
2,3,3',4,4',5'-HxCB (PCB #157)	< 5,1	0	0,0013	0,0025	0,0025
2,3,3',4,4',5,5'-HpCB (PCB #189)	< 5,1	0	0,00025	0,00051	0,00051
total (100 %)		0	0,083	0,17	0,17
total (88 % d.w.)		0	0,073	**0,15**	0,15

The TEQ values have been calculated using the WHO toxicity equivalence factors (TEF) according to Martin Van den Berg et al. (Environmental Health Perspectives (1998), 106: 775-792). Lowerbound concentration : For the calculation of the total concentration, the values, which were lower than the limit of reporting, were regarded as zero. Mediumbound concentration : For the calculation of the total mediumbound concentration, the values, which were lower than the limit of reporting, were regarded as the half of the limit of reporting. Upperbound concentration : For the calculation of the total upperbound concentration, the values, which were lower than the limit of reporting, were regarded as the value of the limit of reporting. The measurement uncertainty has been determined and is available in the laboratory. On request, the data will be transmitted. The RSD of the control sample is less than 10%.

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ANALYTICAL REPORT: IAC0708216

Determination of dioxin-like PCBs

Product: sterol
Sample: 200708000536-1

Your reference: Lot H7M0104

Compound name	Concentration (ng/kg)	WHO-TEQ (ng/kg) Lowerbound	WHO-TEQ (ng/kg) Mediumbound	WHO-TEQ (ng/kg) Upperbound	WHO-TEQ (ng/kg) Limit of Reporting
Non-ortho PCBs					
3,4,4',5-TeCB (PCB #81)	< 2,5	0	0,00013	0,00025	0,00025
3,3',4,4'-TeCB (PCB #77)	< 5,0	0	0,00025	0,00050	0,00050
3,3',4,4',5-PeCB (PCB #126)	< 1,3	0	0,063	0,13	0,13
3,3',4,4',5,5'-HxCB (PCB #169)	< 1,3	0	0,0063	0,013	0,013
Mono-ortho PCBs					
2',3,4,4',5-PeCB (PCB #123)	< 5,0	0	0,00025	0,00050	0,00050
2,3',4,4',5-PeCB (PCB #118)	< 50	0	0,0025	0,0050	0,0050
2,3,4,4',5-PeCB (PCB #114)	< 5,0	0	0,0013	0,0025	0,0025
2,3,3',4,4'-PeCB (PCB #105)	< 25	0	0,0013	0,0025	0,0025
2,3',4,4',5,5'-HxCB (PCB #167)	< 25	0	0,00013	0,00025	0,00025
2,3,3',4,4',5-HxCB (PCB #156)	< 25	0	0,0063	0,013	0,013
2,3,3',4,4',5'-HxCB (PCB #157)	< 5,0	0	0,0013	0,0025	0,0025
2,3,3',4,4',5,5'-HpCB (PCB #189)	< 5,0	0	0,00025	0,00050	0,00050
total (100 %)		0	0,083	0,17	0,17
total (88 % d.w.)		0	0,073	**0,15**	0,15

The TEQ values have been calculated using the *WHO toxicity equivalence factors* (TEF) according to Martin Van den Berg et al. (Environmental Health Perspectives (1998), 106: 775-792). Lowerbound concentration : For the calculation of the total concentration, the values, which were lower than the limit of reporting, were regarded as zero. Mediumbound concentration : For the calculation of the total mediumbound concentration, the values, which were lower than the limit of reporting, were regarded as the half of the limit of reporting. Upperbound concentration : For the calculation of the total upperbound concentration, the values, which were lower than the limit of reporting, were regarded as the value of the limit of reporting. The measurement uncertainty has been determined and is available in the laboratory. On request, the data will be transmitted. The RSD of the control sample is less than 10%.

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ANALYTICAL REPORT: IAC0708216

Determination of dioxin-like PCBs

Product: sterol
Sample: 200708000537-1

Your reference: Lot H7M0105

Compound name	Concentration (ng/kg)	WHO-TEQ (ng/kg) Lowerbound	WHO-TEQ (ng/kg) Mediumbound	WHO-TEQ (ng/kg) Upperbound	WHO-TEQ (ng/kg) Limit of Reporting
Non-ortho PCBs					
3,4,4',5-TeCB (PCB #81)	< 2,5	0	0,00013	0,00025	0,00025
3,3',4,4'-TeCB (PCB #77)	< 5,1	0	0,00025	0,00051	0,00051
3,3',4,4',5-PeCB (PCB #126)	< 1,3	0	0,063	0,13	0,13
3,3',4,4',5,5'-HxCB (PCB #169)	< 1,3	0	0,0063	0,013	0,013
Mono-ortho PCBs					
2',3,4,4',5-PeCB (PCB #123)	< 5,1	0	0,00025	0,00051	0,00051
2,3',4,4',5-PeCB (PCB #118)	< 51	0	0,0025	0,0051	0,0051
2,3,4,4',5-PeCB (PCB #114)	< 5,1	0	0,0013	0,0025	0,0025
2,3,3',4,4'-PeCB (PCB #105)	< 25	0	0,0013	0,0025	0,0025
2,3',4,4',5,5'-HxCB (PCB #167)	< 25	0	0,00013	0,00025	0,00025
2,3,3',4,4',5-HxCB (PCB #156)	< 25	0	0,0063	0,013	0,013
2,3,3',4,4',5'-HxCB (PCB #157)	< 5,1	0	0,0013	0,0025	0,0025
2,3,3',4,4',5,5'-HpCB (PCB #189)	< 5,1	0	0,00025	0,00051	0,00051
total (100 %)		0	0,083	0,17	0,17
total (88 % d.w.)		0	0,073	**0,15**	0,15

The TEQ values have been calculated using the WHO toxicity equivalence factors (TEF) according to Martin Van den Berg et al. (Environmental Health Perspectives (1998), 106: 775-792). Lowerbound concentration : For the calculation of the total concentration, the values, which were lower than the limit of reporting, were regarded as zero. Mediumbound concentration : For the calculation of the total mediumbound concentration, the values, which were lower than the limit of reporting, were regarded as the half of the limit of reporting. Upperbound concentration : For the calculation of the total upperbound concentration, the values, which were lower than the limit of reporting, were regarded as the value of the limit of reporting. The measurement uncertainty has been determined and is available in the laboratory. On request, the data will be transmitted. The RSD of the control sample is less than 10%.

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ANALYTICAL REPORT: IAC0708216

Recovery extraction standards

	sterol 200708000535-1
13C-2,3,7,8-TCDD (%)	53,1 (**)
13C-1,2,3,7,8-PeCDD (%)	53,7 (**)
13C-1,2,3,4,7,8-HxCDD (%)	59,7 (**)
13C-1,2,3,6,7,8-HxCDD (%)	55,6 (**)
13C-1,2,3,4,6,7,8-HpCDD (%)	81,3
13C-OCDD (%)	105
13C-2,3,7,8-TCDF (%)	51,8 (**)
13C-1,2,3,7,8-PeCDF (%)	46,3 (**)
13C-2,3,4,7,8-PeCDF (%)	49,1 (**)
13C-1,2,3,4,7,8-HxCDF (%)	44,8 (**)
13C-1,2,3,6,7,8-HxCDF (%)	40,3 (**)
13C-2,3,4,6,7,8-HxCDF (%)	56,8 (**)
13C-1,2,3,7,8,9-HxCDF (%)	55,5 (**)
13C-1,2,3,4,6,7,8-HpCDF (%)	45,9 (**)
13C-1,2,3,4,7,8,9-HpCDF (%)	61,3
13C-OCDF (%)	79,7

(**) does not meet the quality criteria of the analytical method

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ANALYTICAL REPORT: IAC0708216

Recovery extraction standards

	sterol 200708000536-1
13C-2,3,7,8-TCDD (%)	56,1 (**)
13C-1,2,3,7,8-PeCDD (%)	54,0 (**)
13C-1,2,3,4,7,8-HxCDD (%)	50,4 (**)
13C-1,2,3,6,7,8-HxCDD (%)	62,1
13C-1,2,3,4,6,7,8-HpCDD (%)	65,9
13C-OCDD (%)	51,6 (**)
13C-2,3,7,8-TCDF (%)	56,9 (**)
13C-1,2,3,7,8-PeCDF (%)	47,5 (**)
13C-2,3,4,7,8-PeCDF (%)	53,0 (**)
13C-1,2,3,4,7,8-HxCDF (%)	45,4 (**)
13C-1,2,3,6,7,8-HxCDF (%)	46,7 (**)
13C-2,3,4,6,7,8-HxCDF (%)	51,7 (**)
13C-1,2,3,7,8,9-HxCDF (%)	47,6 (**)
13C-1,2,3,4,6,7,8-HpCDF (%)	42,0 (**)
13C-1,2,3,4,7,8,9-HpCDF (%)	45,5 (**)
13C-OCDF (%)	38,1 (**)

(**) does not meet the quality criteria of the analytical method

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ANALYTICAL REPORT: IAC0708216

Recovery extraction standards

	sterol 200708000537-1
13C-2,3,7,8-TCDD (%)	55,9 (**)
13C-1,2,3,7,8-PeCDD (%)	55,4 (**)
13C-1,2,3,4,7,8-HxCDD (%)	53,1 (**)
13C-1,2,3,6,7,8-HxCDD (%)	62,3
13C-1,2,3,4,6,7,8-HpCDD (%)	65,4
13C-OCDD (%)	54,2 (**)
13C-2,3,7,8-TCDF (%)	57,4 (**)
13C-1,2,3,7,8-PeCDF (%)	49,3 (**)
13C-2,3,4,7,8-PeCDF (%)	53,1 (**)
13C-1,2,3,4,7,8-HxCDF (%)	45,9 (**)
13C-1,2,3,6,7,8-HxCDF (%)	46,8 (**)
13C-2,3,4,6,7,8-HxCDF (%)	52,4 (**)
13C-1,2,3,7,8,9-HxCDF (%)	49,8 (**)
13C-1,2,3,4,6,7,8-HpCDF (%)	42,9 (**)
13C-1,2,3,4,7,8,9-HpCDF (%)	48,6 (**)
13C-OCDF (%)	39,3 (**)

(**) does not meet the quality criteria of the analytical method

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ANALYTICAL REPORT: IAC0708216

Recovery extraction standards

	sterol 200708000535-1
Non-ortho PCBs	
13C-PCB 81 (%)	32,1
13C-PCB 77 (%)	41,0
13C-PCB 126 (%)	86,7
13C-PCB 169 (%)	76,7
Mono-ortho PCBs	
13C-PCB 114 (%)	78,5
13C-PCB 105 (%)	68,1
13C-PCB 123 (%)	73,9
13C-PCB 167 (%)	44,3
13C-PCB 156 (%)	60,9
13C-PCB 157 (%)	80,0
13C-PCB 118 (%)	88,0
13C-PCB 189 (%)	62,7

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ANALYTICAL REPORT: IAC0708216

Recovery extraction standards

	sterol 200708000536-1
Non-ortho PCBs	
13C-PCB 81 (%)	48,8
13C-PCB 77 (%)	45,7
13C-PCB 126 (%)	75,7
13C-PCB 169 (%)	81,2
Mono-ortho PCBs	
13C-PCB 114 (%)	63,2
13C-PCB 105 (%)	62,0
13C-PCB 123 (%)	67,1
13C-PCB 167 (%)	40,2
13C-PCB 156 (%)	61,0
13C-PCB 157 (%)	77,4
13C-PCB 118 (%)	70,4
13C-PCB 189 (%)	61,3

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ANALYTICAL REPORT: IAC0708216

Recovery extraction standards

	sterol 200708000537-1
Non-ortho PCBs	
13C-PCB 81 (%)	49,1
13C-PCB 77 (%)	44,0
13C-PCB 126 (%)	76,6
13C-PCB 169 (%)	76,8
Mono-ortho PCBs	
13C-PCB 114 (%)	51,5
13C-PCB 105 (%)	66,2
13C-PCB 123 (%)	62,5
13C-PCB 167 (%)	47,6
13C-PCB 156 (%)	59,4
13C-PCB 157 (%)	76,8
13C-PCB 118 (%)	64,2
13C-PCB 189 (%)	67,4

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ANALYSERAPPORT **IAC0705324**

Aantal monsters:	3
Aard monsters:	sterolen
Identificatie monsters:	200705001195-1, 20070501196-1, 200705001197-1
Datum van ontvangst:	22/05/2007
Startdatum analyse:	23/05/2007
Gevraagde analyse:	Dioxines & CoPCB's

Analyseresultaten:

2,3,7,8-substituted PCDF's & PCDD's (ng-WHO-TEQ/kg) : zie p. 2-4/13
(HRGC/HRMS ; ECO/AV/IAC/005)

Bepaling van Coplanaire PCB's (ng-WHO-TEQ/kg) : zie p. 5-7/13
(HRGC/HRMS ; ECO/AV/IAC/016)

Recovery extractie standaarden: zie p. 8-13/13

Opmerking: (**) recoveries voldoen niet aan de kwaliteitscriteria van de analysemethode.

ANTWERPEN, 25/06/2007

I.A.C.
Een Divisie van SGS Belgium NV

Marc Van Ryckeghem
Division Manager

Alle rapporten worden opgesteld op naam en voor rekening van de opdrachtgever, die uitdrukkelijk aanvaardt dat deze rapporten slechts een momentopname vertegenwoordigen en steeds in hun geheel en in de context ervan dienen te worden voorgelegd en/of vermeld.
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Het analyserapport kan enkel en alleen aangewend worden binnen de specifieke context van de opdracht en is enkel geldig voor de geanalyseerde monsters.

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ANALYSERAPPORT: IAC0705324

Bepaling van 2,3,7,8-gesubstitueerde PCDF's en PCDD's

Product: sterol
Monsterkenmerk: 200705001195-1

Uw referentie: -

Component	Concentratie (ng/kg)	WHO-TEQ (ng/kg) *Ondergrens*	WHO-TEQ (ng/kg) *Middelwaarde*	WHO-TEQ (ng/kg) *Bovengrens*	WHO-TEQ (ng/kg) *rapportagegrens*
2,3,7,8-TCDF	0,075	0,0075	0,0075	0,0075	0,0045
2,3,7,8-TCDD	< 0,047	0	0,024	0,047	0,047
1,2,3,7,8-PeCDF	0,065	0,0032	0,0032	0,0032	0,0026
2,3,4,7,8-PeCDF	< 0,051	0	0,013	0,026	0,026
1,2,3,7,8-PeCDD	< 0,074	0	0,037	0,074	0,074
1,2,3,4,7,8-HxCDF	0,065	0,0065	0,0065	0,0065	0,0037
1,2,3,6,7,8-HxCDF	0,042	0,0042	0,0042	0,0042	0,0034
2,3,4,6,7,8-HxCDF	< 0,036	0	0,0018	0,0036	0,0036
1,2,3,7,8,9-HxCDF	< 0,030	0	0,0015	0,0030	0,0030
1,2,3,4,7,8-HxCDD	< 0,049	0	0,0024	0,0049	0,0049
1,2,3,6,7,8-HxCDD	0,057	0,0057	0,0057	0,0057	0,0037
1,2,3,7,8,9-HxCDD	< 0,044	0	0,0022	0,0044	0,0044
1,2,3,4,6,7,8-HpCDF	0,43	0,0043	0,0043	0,0043	0,0031
1,2,3,4,7,8,9-HpCDF	< 0,31	0	0,0016	0,0031	0,0031
1,2,3,4,6,7,8-HpCDD	0,74	0,0074	0,0074	0,0074	0,0031
OCDF	2,5	0,00025	0,00025	0,00025	0,00012
OCDD	19	0,0019	0,0019	0,0019	0,00012
Totaal (100 %)		0,041	0,12	0,21	0,19
Totaal (88 % d.s.)		0,036	0,11	**0,18**	0,17

Voor de berekening van de TEQ-waarden werden de WHO toxiciteits equivalent factoren gehanteerd volgens Martin Van den Berg et al. (Environmental Health Perspectives (1998), 106: 775-792). Bij de berekening van het totaal zijn de waarden kleiner dan de rapportagegrens gelijkgesteld aan nul ; zie kolom "Ondergrens" (Lowerbound - principe).
Bij de berekening van het totaal zijn de waarden kleiner dan de rapportagegrens gelijkgesteld aan de helft van de rapportagegrens; zie kolom "Middelwaarde" (Mediumbound - principe). Bij de berekening van het totaal zijn de waarden kleiner dan de rapportagegrens gelijkgesteld aan de rapportagegrens ; zie kolom "Bovengrens" (Upperbound - principe).
De meetonzekerheid werd bepaald en is beschikbaar in het laboratorium. Op eenvoudig verzoek kunnen deze gegevens overgemaakt worden. De RSD van het controlestaal is kleiner dan 10%.

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ANALYSERAPPORT: IAC0705324

Bepaling van 2,3,7,8-gesubstitueerde PCDF's en PCDD's

Product: sterol
Monsterkenmerk: 200705001196-1

Uw referentie: -

Component	Concentratie (ng/kg)	WHO-TEQ (ng/kg) Ondergrens	WHO-TEQ (ng/kg) Middelwaarde	WHO-TEQ (ng/kg) Bovengrens	WHO-TEQ (ng/kg) rapportagegrens
2,3,7,8-TCDF	0,087	0,0087	0,0087	0,0087	0,0047
2,3,7,8-TCDD	< 0,050	0	0,025	0,050	0,050
1,2,3,7,8-PeCDF	0,11	0,0054	0,0054	0,0054	0,0027
2,3,4,7,8-PeCDF	0,058	0,029	0,029	0,029	0,027
1,2,3,7,8-PeCDD	0,079	0,079	0,079	0,079	0,077
1,2,3,4,7,8-HxCDF	0,15	0,015	0,015	0,015	0,0039
1,2,3,6,7,8-HxCDF	0,050	0,0050	0,0050	0,0050	0,0035
2,3,4,6,7,8-HxCDF	0,039	0,0039	0,0039	0,0039	0,0038
1,2,3,7,8,9-HxCDF	0,058	0,0058	0,0058	0,0058	0,0031
1,2,3,4,7,8-HxCDD	0,071	0,0071	0,0071	0,0071	0,0051
1,2,3,6,7,8-HxCDD	0,40	0,040	0,040	0,040	0,0039
1,2,3,7,8,9-HxCDD	0,089	0,0089	0,0089	0,0089	0,0046
1,2,3,4,6,7,8-HpCDF	0,80	0,0080	0,0080	0,0080	0,0033
1,2,3,4,7,8,9-HpCDF	< 0,33	0	0,0016	0,0033	0,0033
1,2,3,4,6,7,8-HpCDD	3,1	0,031	0,031	0,031	0,0033
OCDF	2,9	0,00029	0,00029	0,00029	0,00013
OCDD	23	0,0023	0,0023	0,0023	0,00013
Totaal (100 %)		0,25	0,28	0,30	0,20
Totaal (88 % d.s.)		0,22	0,25	**0,26**	0,18

Voor de berekening van de TEQ-waarden werden de WHO toxiciteits equivalent factoren gehanteerd volgens Martin Van den Berg et al. (Environmental Health Perspectives (1998), 106: 775-792). Bij de berekening van het totaal zijn de waarden kleiner dan de rapportagegrens gelijkgesteld aan nul ; zie kolom "Ondergrens" (Lowerbound - principe).
Bij de berekening van het totaal zijn de waarden kleiner dan de rapportagegrens gelijkgesteld aan de helft van de rapportagegrens; zie kolom "Middelwaarde" (Mediumbound - principe). Bij de berekening van het totaal zijn de waarden kleiner dan de rapportagegrens gelijkgesteld aan de rapportagegrens ; zie kolom "Bovengrens" (Upperbound - principe).
De meetonzekerheid werd bepaald en is beschikbaar in het laboratorium. Op eenvoudig verzoek kunnen deze gegevens overgemaakt worden. De RSD van het controlestaal is kleiner dan 10%.

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ANALYSERAPPORT: IAC0705324

Bepaling van 2,3,7,8-gesubstitueerde PCDF's en PCDD's

Product: sterol
Monsterkenmerk: 200705001197-1

Uw referentie: -

Component	Concentratie (ng/kg)	WHO-TEQ (ng/kg) Ondergrens	WHO-TEQ (ng/kg) Middelwaarde	WHO-TEQ (ng/kg) Bovengrens	WHO-TEQ (ng/kg) rapportagegrens
2,3,7,8-TCDF	0,045	0,0045	0,0045	0,0045	0,0045
2,3,7,8-TCDD	< 0,047	0	0,024	0,047	0,047
1,2,3,7,8-PeCDF	0,060	0,0030	0,0030	0,0030	0,0026
2,3,4,7,8-PeCDF	< 0,051	0	0,013	0,026	0,026
1,2,3,7,8-PeCDD	< 0,074	0	0,037	0,074	0,074
1,2,3,4,7,8-HxCDF	0,067	0,0067	0,0067	0,0067	0,0037
1,2,3,6,7,8-HxCDF	0,050	0,0050	0,0050	0,0050	0,0034
2,3,4,6,7,8-HxCDF	0,040	0,0040	0,0040	0,0040	0,0036
1,2,3,7,8,9-HxCDF	< 0,030	0	0,0015	0,0030	0,0030
1,2,3,4,7,8-HxCDD	< 0,049	0	0,0024	0,0049	0,0049
1,2,3,6,7,8-HxCDD	0,085	0,0085	0,0085	0,0085	0,0037
1,2,3,7,8,9-HxCDD	0,092	0,0092	0,0092	0,0092	0,0044
1,2,3,4,6,7,8-HpCDF	< 0,31	0	0,0016	0,0031	0,0031
1,2,3,4,7,8,9-HpCDF	< 0,31	0	0,0016	0,0031	0,0031
1,2,3,4,6,7,8-HpCDD	1,6	0,016	0,016	0,016	0,0031
OCDF	< 1,2	0	0,000062	0,00012	0,00012
OCDD	17	0,0017	0,0017	0,0017	0,00012
Totaal (100 %)		0,058	0,14	0,22	0,19
Totaal (88 % d.s.)		0,051	0,12	**0,19**	0,17

Voor de berekening van de TEQ-waarden werden de WHO toxiciteits equivalent factoren gehanteerd volgens Martin Van den Berg et al. (Environmental Health Perspectives (1998), 106: 775-792). Bij de berekening van het totaal zijn de waarden kleiner dan de rapportagegrens gelijkgesteld aan nul ; zie kolom "Ondergrens" (Lowerbound - principe).
Bij de berekening van het totaal zijn de waarden kleiner dan de rapportagegrens gelijkgesteld aan de helft van de rapportagegrens; zie kolom "Middelwaarde" (Mediumbound - principe). Bij de berekening van het totaal zijn de waarden kleiner dan de rapportagegrens gelijkgesteld aan de rapportagegrens ; zie kolom "Bovengrens" (Upperbound - principe).
De meetonzekerheid werd bepaald en is beschikbaar in het laboratorium. Op eenvoudig verzoek kunnen deze gegevens overgemaakt worden. De RSD van het controlestaal is kleiner dan 10%.

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ANALYSERAPPORT: IAC0705324

Bepaling van Coplanaire PCB's

Product: sterol
Monsterkenmerk: 200705001195-1

Uw referentie: -

Component	Concentratie (ng/kg)	WHO-TEQ (ng/kg) Ondergrens	WHO-TEQ (ng/kg) Middelwaarde	WHO-TEQ (ng/kg) Bovengrens	WHO-TEQ (ng/kg) rapportagegrens
Non-ortho PCBs					
3,4,4',5-TeCB (PCB #81)	< 2,5	0	0,00012	0,00025	0,00025
3,3',4,4'-TeCB (PCB #77)	< 5,0	0	0,00025	0,00050	0,00050
3,3',4,4',5-PeCB (PCB #126)	< 1,2	0	0,062	0,12	0,12
3,3',4,4',5,5'-HxCB (PCB #169)	< 1,2	0	0,0062	0,012	0,012
Mono-ortho PCBs					
2',3,4,4',5-PeCB (PCB #123)	< 5,0	0	0,00025	0,00050	0,00050
2,3',4,4',5-PeCB (PCB #118)	< 50	0	0,0025	0,0050	0,0050
2,3,4,4',5-PeCB (PCB #114)	< 5,0	0	0,0012	0,0025	0,0025
2,3,3',4,4'-PeCB (PCB #105)	< 25	0	0,0012	0,0025	0,0025
2,3',4,4',5,5'-HxCB (PCB #167)	< 25	0	0,00012	0,00025	0,00025
2,3,3',4,4',5-HxCB (PCB #156)	< 25	0	0,0062	0,012	0,012
2,3,3',4,4',5'-HxCB (PCB #157)	< 5,0	0	0,0012	0,0025	0,0025
2,3,3',4,4',5,5'-HpCB (PCB #189)	< 5,0	0	0,00025	0,00050	0,00050
Totaal (100 %)		0	0,082	0,16	0,16
Totaal (88 % d.s.)		0	0,072	**0,14**	0,14

Voor de berekening van de TEQ-waarden werden de WHO toxiciteits equivalent factoren gehanteerd volgens Martin Van den Berg et al. (Environmental Health Perspectives (1998), 106: 775-792). Bij de berekening van het totaal zijn de waarden kleiner dan de rapportagegrens gelijkgesteld aan nul ; zie kolom "Ondergrens" (Lowerbound - principe).
Bij de berekening van het totaal zijn de waarden kleiner dan de rapportagegrens gelijkgesteld aan de helft van de rapportagegrens; zie kolom "Middelwaarde" (Mediumbound - principe). Bij de berekening van het totaal zijn de waarden kleiner dan de rapportagegrens gelijkgesteld aan de rapportagegrens ; zie kolom "Bovengrens" (Upperbound - principe).
De meetonzekerheid werd bepaald en is beschikbaar in het laboratorium. Op eenvoudig verzoek kunnen deze gegevens overgemaakt worden. De RSD van het controlestaal is kleiner dan 10%.

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ANALYSERAPPORT: IAC0705324

Bepaling van Coplanaire PCB's

Product: sterol
Monsterkenmerk: 200705001196-1

Uw referentie: -

Component	Concentratie (ng/kg)	WHO-TEQ (ng/kg) Ondergrens	WHO-TEQ (ng/kg) Middelwaarde	WHO-TEQ (ng/kg) Bovengrens	WHO-TEQ (ng/kg) rapportagegrens
Non-ortho PCBs					
3,4,4',5-TeCB (PCB #81)	< 2,6	0	0,00013	0,00026	0,00026
3,3',4,4'-TeCB (PCB #77)	< 5,2	0	0,00026	0,00052	0,00052
3,3',4,4',5-PeCB (PCB #126)	< 1,3	0	0,066	0,13	0,13
3,3',4,4',5,5'-HxCB (PCB #169)	< 1,3	0	0,0066	0,013	0,013
Mono-ortho PCBs					
2',3,4,4',5-PeCB (PCB #123)	< 5,2	0	0,00026	0,00052	0,00052
2,3',4,4',5-PeCB (PCB #118)	< 52	0	0,0026	0,0052	0,0052
2,3,4,4',5-PeCB (PCB #114)	< 5,2	0	0,0013	0,0026	0,0026
2,3,3',4,4'-PeCB (PCB #105)	< 26	0	0,0013	0,0026	0,0026
2,3',4,4',5,5'-HxCB (PCB #167)	< 26	0	0,00013	0,00026	0,00026
2,3,3',4,4',5-HxCB (PCB #156)	< 26	0	0,0066	0,013	0,013
2,3,3',4,4',5'-HxCB (PCB #157)	< 5,2	0	0,0013	0,0026	0,0026
2,3,3',4,4',5,5'-HpCB (PCB #189)	< 5,2	0	0,00026	0,00052	0,00052
Totaal (100 %)		0	0,086	0,17	0,17
Totaal (88 % d.s.)		0	0,076	**0,15**	0,15

Voor de berekening van de TEQ-waarden werden de WHO toxiciteits equivalent factoren gehanteerd volgens Martin Van den Berg et al. (Environmental Health Perspectives (1998), 106: 775-792). Bij de berekening van het totaal zijn de waarden kleiner dan de rapportagegrens gelijkgesteld aan nul ; zie kolom "Ondergrens" (Lowerbound - principe).
Bij de berekening van het totaal zijn de waarden kleiner dan de rapportagegrens gelijkgesteld aan de helft van de rapportagegrens; zie kolom "Middelwaarde" (Mediumbound - principe). Bij de berekening van het totaal zijn de waarden kleiner dan de rapportagegrens gelijkgesteld aan de rapportagegrens ; zie kolom "Bovengrens" (Upperbound - principe).
De meetonzekerheid werd bepaald en is beschikbaar in het laboratorium. Op eenvoudig verzoek kunnen deze gegevens overgemaakt worden. De RSD van het controlestaal is kleiner dan 10%.

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ANALYSERAPPORT: IAC0705324

Bepaling van Coplanaire PCB's

Product: sterol
Monsterkenmerk: 200705001197-1

Uw referentie: -

Component	Concentratie (ng/kg)	WHO-TEQ (ng/kg) Ondergrens	WHO-TEQ (ng/kg) Middelwaarde	WHO-TEQ (ng/kg) Bovengrens	WHO-TEQ (ng/kg) rapportagegrens
Non-ortho PCBs					
3,4,4',5-TeCB (PCB #81)	< 2,5	0	0,00012	0,00025	0,00025
3,3',4,4'-TeCB (PCB #77)	< 5,0	0	0,00025	0,00050	0,00050
3,3',4,4',5-PeCB (PCB #126)	< 1,2	0	0,062	0,12	0,12
3,3',4,4',5,5'-HxCB (PCB #169)	< 1,2	0	0,0062	0,012	0,012
Mono-ortho PCBs					
2',3,4,4',5-PeCB (PCB #123)	< 5,0	0	0,00025	0,00050	0,00050
2,3',4,4',5-PeCB (PCB #118)	< 50	0	0,0025	0,0050	0,0050
2,3,4,4',5-PeCB (PCB #114)	< 5,0	0	0,0012	0,0025	0,0025
2,3,3',4,4'-PeCB (PCB #105)	< 25	0	0,0012	0,0025	0,0025
2,3',4,4',5,5'-HxCB (PCB #167)	< 25	0	0,00012	0,00025	0,00025
2,3,3',4,4',5-HxCB (PCB #156)	< 25	0	0,0062	0,012	0,012
2,3,3',4,4',5'-HxCB (PCB #157)	< 5,0	0	0,0012	0,0025	0,0025
2,3,3',4,4',5,5'-HpCB (PCB #189)	< 5,0	0	0,00025	0,00050	0,00050
Totaal (100 %)		0	0,082	0,16	0,16
Totaal (88 % d.s.)		0	0,072	**0,14**	0,14

Voor de berekening van de TEQ-waarden werden de WHO toxiciteits equivalent factoren gehanteerd volgens Martin Van den Berg et al. (Environmental Health Perspectives (1998), 106: 775-792). Bij de berekening van het totaal zijn de waarden kleiner dan de rapportagegrens gelijkgesteld aan nul ; zie kolom "Ondergrens" (Lowerbound - principe).
Bij de berekening van het totaal zijn de waarden kleiner dan de rapportagegrens gelijkgesteld aan de helft van de rapportagegrens; zie kolom "Middelwaarde" (Mediumbound - principe). Bij de berekening van het totaal zijn de waarden kleiner dan de rapportagegrens gelijkgesteld aan de rapportagegrens ; zie kolom "Bovengrens" (Upperbound - principe).
De meetonzekerheid werd bepaald en is beschikbaar in het laboratorium. Op eenvoudig verzoek kunnen deze gegevens overgemaakt worden. De RSD van het controlestaal is kleiner dan 10%.

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ANALYSERAPPORT: IAC0705324

Recovery extractie standaarden

	sterol 200705001195-1
13C-2,3,7,8-TCDD (%)	37,9 (**)
13C-1,2,3,7,8-PeCDD (%)	35,8 (**)
13C-1,2,3,4,7,8-HxCDD (%)	38,1 (**)
13C-1,2,3,6,7,8-HxCDD (%)	36,4 (**)
13C-1,2,3,4,6,7,8-HpCDD (%)	51,3 (**)
13C-OCDD (%)	44,2 (**)
13C-2,3,7,8-TCDF (%)	35,3 (**)
13C-1,2,3,7,8-PeCDF (%)	31,2 (**)
13C-2,3,4,7,8-PeCDF (%)	37,6 (**)
13C-1,2,3,4,7,8-HxCDF (%)	35,7 (**)
13C-1,2,3,6,7,8-HxCDF (%)	34,4 (**)
13C-2,3,4,6,7,8-HxCDF (%)	44,5 (**)
13C-1,2,3,7,8,9-HxCDF (%)	39,7 (**)
13C-1,2,3,4,6,7,8-HpCDF (%)	35,2 (**)
13C-1,2,3,4,7,8,9-HpCDF (%)	39,1 (**)
13C-OCDF (%)	33,7 (**)

(**) recoveries voldoen niet aan de kwaliteitscriteria van de analysemethode

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ANALYSERAPPORT: IAC0705324

Recovery extractie standaarden

	sterol 200705001196-1
13C-2,3,7,8-TCDD (%)	44,2 (**)
13C-1,2,3,7,8-PeCDD (%)	37,7 (**)
13C-1,2,3,4,7,8-HxCDD (%)	43,7 (**)
13C-1,2,3,6,7,8-HxCDD (%)	48,8 (**)
13C-1,2,3,4,6,7,8-HpCDD (%)	57,9 (**)
13C-OCDD (%)	49,9 (**)
13C-2,3,7,8-TCDF (%)	51,7 (**)
13C-1,2,3,7,8-PeCDF (%)	42,4 (**)
13C-2,3,4,7,8-PeCDF (%)	44,2 (**)
13C-1,2,3,4,7,8-HxCDF (%)	45,5 (**)
13C-1,2,3,6,7,8-HxCDF (%)	46,4 (**)
13C-2,3,4,6,7,8-HxCDF (%)	51,3 (**)
13C-1,2,3,7,8,9-HxCDF (%)	46,9 (**)
13C-1,2,3,4,6,7,8-HpCDF (%)	44,3 (**)
13C-1,2,3,4,7,8,9-HpCDF (%)	47,8 (**)
13C-OCDF (%)	38,8 (**)

(**) recoveries voldoen niet aan de kwaliteitscriteria van de analysemethode

SGS

ANALYSERAPPORT: IAC0705324

Recovery extractie standaarden

	sterol 200705001197-1
13C-2,3,7,8-TCDD (%)	51,6 (**)
13C-1,2,3,7,8-PeCDD (%)	45,8 (**)
13C-1,2,3,4,7,8-HxCDD (%)	53,1 (**)
13C-1,2,3,6,7,8-HxCDD (%)	56,1 (**)
13C-1,2,3,4,6,7,8-HpCDD (%)	69,9
13C-OCDD (%)	74,8
13C-2,3,7,8-TCDF (%)	60,0
13C-1,2,3,7,8-PeCDF (%)	51,2 (**)
13C-2,3,4,7,8-PeCDF (%)	53,1 (**)
13C-1,2,3,4,7,8-HxCDF (%)	59,0 (**)
13C-1,2,3,6,7,8-HxCDF (%)	56,6 (**)
13C-2,3,4,6,7,8-HxCDF (%)	60,8
13C-1,2,3,7,8,9-HxCDF (%)	57,2 (**)
13C-1,2,3,4,6,7,8-HpCDF (%)	55,7 (**)
13C-1,2,3,4,7,8,9-HpCDF (%)	63,1
13C-OCDF (%)	63,0

(**) recoveries voldoen niet aan de kwaliteitscriteria van de analysemethode

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ANALYSERAPPORT: IAC0705324

Recovery extractie standaarden

	sterol 200705001195-1
Non-ortho PCBs	
13C-PCB 81 (%)	45,2 (**)
13C-PCB 77 (%)	30,6 (**)
13C-PCB 126 (%)	60,3
13C-PCB 169 (%)	50,6 (**)
Mono-ortho PCBs	
13C-PCB 114 (%)	45,7 (**)
13C-PCB 105 (%)	43,3 (**)
13C-PCB 123 (%)	43,2 (**)
13C-PCB 167 (%)	39,3 (**)
13C-PCB 156 (%)	43,0 (**)
13C-PCB 157 (%)	43,6 (**)
13C-PCB 118 (%)	43,8 (**)
13C-PCB 189 (%)	35,6 (**)

(**) recoveries voldoen niet aan de kwaliteitscriteria van de analysemethode

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ANALYSERAPPORT: IAC0705324

Recovery extractie standaarden

	sterol 200705001196-1
Non-ortho PCBs	
13C-PCB 81 (%)	56,4 (**)
13C-PCB 77 (%)	41,0 (**)
13C-PCB 126 (%)	78,7
13C-PCB 169 (%)	54,3 (**)
Mono-ortho PCBs	
13C-PCB 114 (%)	55,1 (**)
13C-PCB 105 (%)	52,5 (**)
13C-PCB 123 (%)	52,2 (**)
13C-PCB 167 (%)	48,8 (**)
13C-PCB 156 (%)	49,9 (**)
13C-PCB 157 (%)	50,2 (**)
13C-PCB 118 (%)	52,4 (**)
13C-PCB 189 (%)	43,8 (**)

(**) recoveries voldoen niet aan de kwaliteitscriteria van de analysemethode

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ANALYSERAPPORT: IAC0705324

Recovery extractie standaarden

	sterol 200705001197-1
Non-ortho PCBs	
13C-PCB 81 (%)	62,2
13C-PCB 77 (%)	46,2 (**)
13C-PCB 126 (%)	92,4
13C-PCB 169 (%)	62,9
Mono-ortho PCBs	
13C-PCB 114 (%)	57,0 (**)
13C-PCB 105 (%)	55,1 (**)
13C-PCB 123 (%)	57,7 (**)
13C-PCB 167 (%)	57,3 (**)
13C-PCB 156 (%)	60,1
13C-PCB 157 (%)	58,5 (**)
13C-PCB 118 (%)	57,9 (**)
13C-PCB 189 (%)	53,4 (**)

(**) recoveries voldoen niet aan de kwaliteitscriteria van de analysemethode

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Nederland

ANALYSERAPPORT **IAC0710248**

Aantal monsters:	4
Aard monsters:	sterolen
Identificatie monsters:	200710000854-1, 855-1, 856-1, 857-1
Datum van ontvangst:	15/10/2007
Startdatum analyse:	18/10/2007
Gevraagde analyse:	Dioxines & dioxineachtige PCB's

Analyseresultaten:

2,3,7,8-substituted PCDF's & PCDD's (ng WHO-TEQ/kg) : zie p. 2-5/17
(HRGC/HRMS ; ECO/AV/IAC/005)

Bepaling van dioxineachtige PCB's (ng WHO-TEQ/kg) : zie p. 6-10/17
(HRGC/HRMS ; ECO/AV/IAC/016)

Recovery extractie standaarden: zie p. 11-17/17

ANTWERPEN, 29/10/2007

I.A.C.
Een Divisie van SGS Belgium NV

Marc Van Ryckeghem
Division Manager

Alle rapporten worden opgesteld op naam en voor rekening van de opdrachtgever, die uitdrukkelijk aanvaardt dat deze rapporten slechts een momentopname vertegenwoordigen en steeds in hun geheel en in de context ervan dienen te worden voorgelegd en/of vermeld.
SGS Belgium NV, opsteller van deze rapporten, kan niet aansprakelijk gesteld worden voor fouten of wijzigingen van resultaten ontstaan gedurende of n.a.v. elektronische- of faxtransmissie. Enkel en uitsluitend het origineel getekend rapport is bindend.
Het analyserapport kan enkel en alleen aangewend worden binnen de specifieke context van de opdracht en is enkel geldig voor de geanalyseerde monsters.

Registered office. Noorderlaan 87 B-2030 Antwerpen H.R. Antwerpen 141.810 BTW BE 404.882.750 Dexia 550-3560000-93
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ANALYSERAPPORT: IAC0710248

Bepaling van 2,3,7,8-gesubstitueerde PCDF's en PCDD's

Product: sterol
Monsterkenmerk: 200710000854-1

Uw referentie: -

Component	Concentratie (ng/kg)	WHO-TEQ (ng/kg) Ondergrens	WHO-TEQ (ng/kg) Middelwaarde	WHO-TEQ (ng/kg) Bovengrens	WHO-TEQ (ng/kg) rapportagegrens
2,3,7,8-TCDF	0,20	0,020	0,020	0,020	0,0045
2,3,7,8-TCDD	< 0,047	0	0,024	0,047	0,047
1,2,3,7,8-PeCDF	< 0,051	0	0,0013	0,0026	0,0026
2,3,4,7,8-PeCDF	< 0,051	0	0,013	0,026	0,026
1,2,3,7,8-PeCDD	< 0,074	0	0,037	0,074	0,074
1,2,3,4,7,8-HxCDF	< 0,057	0	0,0029	0,0057	0,0057
1,2,3,6,7,8-HxCDF	< 0,034	0	0,0017	0,0034	0,0034
2,3,4,6,7,8-HxCDF	< 0,036	0	0,0018	0,0036	0,0036
1,2,3,7,8,9-HxCDF	< 0,030	0	0,0015	0,0030	0,0030
1,2,3,4,7,8-HxCDD	< 0,049	0	0,0024	0,0049	0,0049
1,2,3,6,7,8-HxCDD	0,050	0,0050	0,0050	0,0050	0,0037
1,2,3,7,8,9-HxCDD	< 0,044	0	0,0022	0,0044	0,0044
1,2,3,4,6,7,8-HpCDF	< 0,35	0	0,0017	0,0035	0,0035
1,2,3,4,7,8,9-HpCDF	< 0,31	0	0,0016	0,0031	0,0031
1,2,3,4,6,7,8-HpCDD	0,61	0,0061	0,0061	0,0061	0,0031
OCDF	< 1,2	0	0,000062	0,00012	0,00012
OCDD	1,5	0,00015	0,00015	0,00015	0,00012
Totaal (100 %)		0,032	0,12	0,21	0,19
Totaal (88 % d.s.)		0,028	0,11	**0,18**	0,17

Voor de berekening van de TEQ-waarden werden de WHO toxiciteits equivalent factoren gehanteerd volgens Martin Van den Berg et al. (Environmental Health Perspectives (1998), 106: 775-792). Bij de berekening van het totaal zijn de waarden kleiner dan de rapportagegrens gelijkgesteld aan nul ; zie kolom "Ondergrens" (Lowerbound - principe).
Bij de berekening van het totaal zijn de waarden kleiner dan de rapportagegrens gelijkgesteld aan de helft van de rapportagegrens; zie kolom "Middelwaarde" (Mediumbound - principe). Bij de berekening van het totaal zijn de waarden kleiner dan de rapportagegrens gelijkgesteld aan de rapportagegrens ; zie kolom "Bovengrens" (Upperbound - principe).
De meetonzekerheid werd bepaald en is beschikbaar in het laboratorium. Op eenvoudig verzoek kunnen deze gegevens overgemaakt worden. De RSD van het controlestaal is kleiner dan 10%.

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ANALYSERAPPORT: IAC0710248

Bepaling van 2,3,7,8-gesubstitueerde PCDF's en PCDD's

Product: sterol
Monsterkenmerk: 200710000855-1

Uw referentie: -

Component	Concentratie (ng/kg)	WHO-TEQ (ng/kg) Ondergrens	WHO-TEQ (ng/kg) Middelwaarde	WHO-TEQ (ng/kg) Bovengrens	WHO-TEQ (ng/kg) rapportagegrens
2,3,7,8-TCDF	< 0,075	0	0,0038	0,0075	0,0075
2,3,7,8-TCDD	< 0,048	0	0,024	0,048	0,048
1,2,3,7,8-PeCDF	< 0,052	0	0,0013	0,0026	0,0026
2,3,4,7,8-PeCDF	< 0,052	0	0,013	0,026	0,026
1,2,3,7,8-PeCDD	< 0,074	0	0,037	0,074	0,074
1,2,3,4,7,8-HxCDF	< 0,038	0	0,0019	0,0038	0,0038
1,2,3,6,7,8-HxCDF	< 0,034	0	0,0017	0,0034	0,0034
2,3,4,6,7,8-HxCDF	< 0,036	0	0,0018	0,0036	0,0036
1,2,3,7,8,9-HxCDF	< 0,030	0	0,0015	0,0030	0,0030
1,2,3,4,7,8-HxCDD	< 0,049	0	0,0025	0,0049	0,0049
1,2,3,6,7,8-HxCDD	< 0,038	0	0,0019	0,0038	0,0038
1,2,3,7,8,9-HxCDD	< 0,044	0	0,0022	0,0044	0,0044
1,2,3,4,6,7,8-HpCDF	< 0,31	0	0,0016	0,0031	0,0031
1,2,3,4,7,8,9-HpCDF	< 0,31	0	0,0016	0,0031	0,0031
1,2,3,4,6,7,8-HpCDD	0,66	0,0066	0,0066	0,0066	0,0031
OCDF	< 1,3	0	0,000063	0,00013	0,00013
OCDD	< 1,3	0	0,000063	0,00013	0,00013
Totaal (100 %)		0,0066	0,10	0,20	0,19
Totaal (88 % d.s.)		0,0058	0,088	**0,18**	0,17

Voor de berekening van de TEQ-waarden werden de WHO toxiciteits equivalent factoren gehanteerd volgens Martin Van den Berg et al. (Environmental Health Perspectives (1998), 106: 775-792). Bij de berekening van het totaal zijn de waarden kleiner dan de rapportagegrens gelijkgesteld aan nul ; zie kolom "Ondergrens" (Lowerbound - principe).
Bij de berekening van het totaal zijn de waarden kleiner dan de rapportagegrens gelijkgesteld aan de helft van de rapportagegrens; zie kolom "Middelwaarde" (Mediumbound - principe). Bij de berekening van het totaal zijn de waarden kleiner dan de rapportagegrens gelijkgesteld aan de rapportagegrens ; zie kolom "Bovengrens" (Upperbound - principe).
De meetonzekerheid werd bepaald en is beschikbaar in het laboratorium. Op eenvoudig verzoek kunnen deze gegevens overgemaakt worden. De RSD van het controlestaal is kleiner dan 10%.

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ANALYSERAPPORT: IAC0710248

Bepaling van 2,3,7,8-gesubstitueerde PCDF's en PCDD's

Product: sterol
Monsterkenmerk: 200710000856-1

Uw referentie: -

Component	Concentratie (ng/kg)	WHO-TEQ (ng/kg) Ondergrens	WHO-TEQ (ng/kg) Middelwaarde	WHO-TEQ (ng/kg) Bovengrens	WHO-TEQ (ng/kg) rapportagegrens
2,3,7,8-TCDF	< 0,068	0	0,0034	0,0068	0,0068
2,3,7,8-TCDD	< 0,048	0	0,024	0,048	0,048
1,2,3,7,8-PeCDF	< 0,052	0	0,0013	0,0026	0,0026
2,3,4,7,8-PeCDF	< 0,052	0	0,013	0,026	0,026
1,2,3,7,8-PeCDD	< 0,074	0	0,037	0,074	0,074
1,2,3,4,7,8-HxCDF	< 0,040	0	0,0020	0,0040	0,0040
1,2,3,6,7,8-HxCDF	< 0,043	0	0,0021	0,0043	0,0043
2,3,4,6,7,8-HxCDF	< 0,037	0	0,0018	0,0037	0,0037
1,2,3,7,8,9-HxCDF	< 0,030	0	0,0015	0,0030	0,0030
1,2,3,4,7,8-HxCDD	< 0,049	0	0,0025	0,0049	0,0049
1,2,3,6,7,8-HxCDD	< 0,038	0	0,0019	0,0038	0,0038
1,2,3,7,8,9-HxCDD	< 0,044	0	0,0022	0,0044	0,0044
1,2,3,4,6,7,8-HpCDF	< 0,32	0	0,0016	0,0032	0,0032
1,2,3,4,7,8,9-HpCDF	< 0,32	0	0,0016	0,0032	0,0032
1,2,3,4,6,7,8-HpCDD	0,61	0,0061	0,0061	0,0061	0,0032
OCDF	< 1,3	0	0,000063	0,00013	0,00013
OCDD	1,4	0,00014	0,00014	0,00014	0,00013
Totaal (100 %)		0,0062	0,10	0,20	0,20
Totaal (88 % d.s.)		0,0055	0,088	**0,18**	0,18

Voor de berekening van de TEQ-waarden werden de WHO toxiciteits equivalent factoren gehanteerd volgens Martin Van den Berg et al. (Environmental Health Perspectives (1998), 106: 775-792). Bij de berekening van het totaal zijn de waarden kleiner dan de rapportagegrens gelijkgesteld aan nul ; zie kolom "Ondergrens" (Lowerbound - principe).
Bij de berekening van het totaal zijn de waarden kleiner dan de rapportagegrens gelijkgesteld aan de helft van de rapportagegrens; zie kolom "Middelwaarde" (Mediumbound - principe). Bij de berekening van het totaal zijn de waarden kleiner dan de rapportagegrens gelijkgesteld aan de rapportagegrens ; zie kolom "Bovengrens" (Upperbound - principe).
De meetonzekerheid werd bepaald en is beschikbaar in het laboratorium. Op eenvoudig verzoek kunnen deze gegevens overgemaakt worden. De RSD van het controlestaal is kleiner dan 10%.

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ANALYSERAPPORT: IAC0710248

Bepaling van 2,3,7,8-gesubstitueerde PCDF's en PCDD's

Product: sterol
Monsterkenmerk: 200710000857-1

Uw referentie: -

Component	Concentratie (ng/kg)	WHO-TEQ (ng/kg) Ondergrens	WHO-TEQ (ng/kg) Middelwaarde	WHO-TEQ (ng/kg) Bovengrens	WHO-TEQ (ng/kg) rapportagegrens
2,3,7,8-TCDF	< 0,050	0	0,0025	0,0050	0,0050
2,3,7,8-TCDD	< 0,048	0	0,024	0,048	0,048
1,2,3,7,8-PeCDF	< 0,052	0	0,0013	0,0026	0,0026
2,3,4,7,8-PeCDF	< 0,052	0	0,013	0,026	0,026
1,2,3,7,8-PeCDD	< 0,074	0	0,037	0,074	0,074
1,2,3,4,7,8-HxCDF	< 0,053	0	0,0026	0,0053	0,0053
1,2,3,6,7,8-HxCDF	< 0,034	0	0,0017	0,0034	0,0034
2,3,4,6,7,8-HxCDF	< 0,037	0	0,0018	0,0037	0,0037
1,2,3,7,8,9-HxCDF	< 0,030	0	0,0015	0,0030	0,0030
1,2,3,4,7,8-HxCDD	< 0,049	0	0,0025	0,0049	0,0049
1,2,3,6,7,8-HxCDD	< 0,038	0	0,0019	0,0038	0,0038
1,2,3,7,8,9-HxCDD	< 0,044	0	0,0022	0,0044	0,0044
1,2,3,4,6,7,8-HpCDF	< 0,32	0	0,0016	0,0032	0,0032
1,2,3,4,7,8,9-HpCDF	< 0,32	0	0,0016	0,0032	0,0032
1,2,3,4,6,7,8-HpCDD	0,49	0,0049	0,0049	0,0049	0,0032
OCDF	< 1,3	0	0,000064	0,00013	0,00013
OCDD	< 1,3	0	0,000063	0,00013	0,00013
Totaal (100 %)		0,0049	0,10	0,20	0,19
Totaal (88 % d.s.)		0,0043	0,088	**0,18**	0,17

Voor de berekening van de TEQ-waarden werden de WHO toxiciteits equivalent factoren gehanteerd volgens Martin Van den Berg et al. (Environmental Health Perspectives (1998), 106: 775-792). Bij de berekening van het totaal zijn de waarden kleiner dan de rapportagegrens gelijkgesteld aan nul ; zie kolom "Ondergrens" (Lowerbound - principe).
Bij de berekening van het totaal zijn de waarden kleiner dan de rapportagegrens gelijkgesteld aan de helft van de rapportagegrens; zie kolom "Middelwaarde" (Mediumbound - principe). Bij de berekening van het totaal zijn de waarden kleiner dan de rapportagegrens gelijkgesteld aan de rapportagegrens ; zie kolom "Bovengrens" (Upperbound - principe).
De meetonzekerheid werd bepaald en is beschikbaar in het laboratorium. Op eenvoudig verzoek kunnen deze gegevens overgemaakt worden. De RSD van het controlestaal is kleiner dan 10%.

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ANALYSERAPPORT: IAC0710248

Bepaling van dioxineachtige PCB's

Product: sterol
Monsterkenmerk: 200710000854-1

Uw referentie: -

Component	Concentratie (ng/kg)	WHO-TEQ (ng/kg) Ondergrens	WHO-TEQ (ng/kg) Middelwaarde	WHO-TEQ (ng/kg) Bovengrens	WHO-TEQ (ng/kg) rapportagegrens
Non-ortho PCBs					
3,4,4',5-TeCB (PCB #81)	< 2,5	0	0,00012	0,00025	0,00025
3,3',4,4'-TeCB (PCB #77)	< 5,0	0	0,00025	0,00050	0,00050
3,3',4,4',5-PeCB (PCB #126)	< 1,2	0	0,062	0,12	0,12
3,3',4,4',5,5'-HxCB (PCB #169)	< 1,2	0	0,0062	0,012	0,012
Mono-ortho PCBs					
2',3,4,4',5-PeCB (PCB #123)	< 5,0	0	0,00025	0,00050	0,00050
2,3',4,4',5-PeCB (PCB #118)	< 50	0	0,0025	0,0050	0,0050
2,3,4,4',5-PeCB (PCB #114)	< 5,0	0	0,0012	0,0025	0,0025
2,3,3',4,4'-PeCB (PCB #105)	< 25	0	0,0012	0,0025	0,0025
2,3',4,4',5,5'-HxCB (PCB #167)	< 25	0	0,00012	0,00025	0,00025
2,3,3',4,4',5-HxCB (PCB #156)	< 25	0	0,0062	0,012	0,012
2,3,3',4,4',5'-HxCB (PCB #157)	< 5,0	0	0,0012	0,0025	0,0025
2,3,3',4,4',5,5'-HpCB (PCB #189)	< 5,0	0	0,00025	0,00050	0,00050
Totaal (100 %)		0	0,082	0,16	0,16
Totaal (88 % d.s.)		0	0,072	**0,14**	0,14

Voor de berekening van de TEQ-waarden werden de WHO toxiciteits equivalent factoren gehanteerd volgens Martin Van den Berg et al. (Environmental Health Perspectives (1998), 106: 775-792). Bij de berekening van het totaal zijn de waarden kleiner dan de rapportagegrens gelijkgesteld aan nul ; zie kolom "Ondergrens" (Lowerbound - principe).
Bij de berekening van het totaal zijn de waarden kleiner dan de rapportagegrens gelijkgesteld aan de helft van de rapportagegrens; zie kolom "Middelwaarde" (Mediumbound - principe). Bij de berekening van het totaal zijn de waarden kleiner dan de rapportagegrens gelijkgesteld aan de rapportagegrens ; zie kolom "Bovengrens" (Upperbound - principe).
De meetonzekerheid werd bepaald en is beschikbaar in het laboratorium. Op eenvoudig verzoek kunnen deze gegevens overgemaakt worden. De RSD van het controlestaal is kleiner dan 10%.

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ANALYSERAPPORT: IAC0710248

Bepaling van dioxineachtige PCB's

Product: sterol
Monsterkenmerk: 200710000855-1

Uw referentie: -

Component	Concentratie (ng/kg)	WHO-TEQ (ng/kg) Ondergrens	WHO-TEQ (ng/kg) Middelwaarde	WHO-TEQ (ng/kg) Bovengrens	WHO-TEQ (ng/kg) rapportagegrens
Non-ortho PCBs					
3,4,4',5-TeCB (PCB #81)	< 2,5	0	0,00013	0,00025	0,00025
3,3',4,4'-TeCB (PCB #77)	< 5,0	0	0,00025	0,00050	0,00050
3,3',4,4',5-PeCB (PCB #126)	< 1,3	0	0,063	0,13	0,13
3,3',4,4',5,5'-HxCB (PCB #169)	< 1,3	0	0,0063	0,013	0,013
Mono-ortho PCBs					
2',3,4,4',5-PeCB (PCB #123)	< 5,0	0	0,00025	0,00050	0,00050
2,3',4,4',5-PeCB (PCB #118)	< 50	0	0,0025	0,0050	0,0050
2,3,4,4',5-PeCB (PCB #114)	< 5,0	0	0,0013	0,0025	0,0025
2,3,3',4,4'-PeCB (PCB #105)	< 25	0	0,0013	0,0025	0,0025
2,3',4,4',5,5'-HxCB (PCB #167)	< 25	0	0,00013	0,00025	0,00025
2,3,3',4,4',5-HxCB (PCB #156)	< 25	0	0,0063	0,013	0,013
2,3,3',4,4',5'-HxCB (PCB #157)	< 5,0	0	0,0013	0,0025	0,0025
2,3,3',4,4',5,5'-HpCB (PCB #189)	< 5,0	0	0,00025	0,00050	0,00050
Totaal (100 %)		0	0,083	0,17	0,17
Totaal (88 % d.s.)		0	0,073	**0,15**	0,15

Voor de berekening van de TEQ-waarden werden de WHO toxiciteits equivalent factoren gehanteerd volgens Martin Van den Berg et al. (Environmental Health Perspectives (1998), 106: 775-792). Bij de berekening van het totaal zijn de waarden kleiner dan de rapportagegrens gelijkgesteld aan nul ; zie kolom "Ondergrens" (Lowerbound - principe).
Bij de berekening van het totaal zijn de waarden kleiner dan de rapportagegrens gelijkgesteld aan de helft van de rapportagegrens; zie kolom "Middelwaarde" (Mediumbound - principe). Bij de berekening van het totaal zijn de waarden kleiner dan de rapportagegrens gelijkgesteld aan de rapportagegrens ; zie kolom "Bovengrens" (Upperbound - principe).
De meetonzekerheid werd bepaald en is beschikbaar in het laboratorium. Op eenvoudig verzoek kunnen deze gegevens overgemaakt worden. De RSD van het controlestaal is kleiner dan 10%.

SGS

ANALYSERAPPORT: IAC0710248

Bepaling van dioxineachtige PCB's

Product: sterol
Monsterkenmerk: 200710000856-1

Uw referentie: -

Component	Concentratie (ng/kg)	WHO-TEQ (ng/kg) Ondergrens	WHO-TEQ (ng/kg) Middelwaarde	WHO-TEQ (ng/kg) Bovengrens	WHO-TEQ (ng/kg) rapportagegrens
Non-ortho PCBs					
3,4,4',5-TeCB (PCB #81)	< 2,5	0	0,00013	0,00025	0,00025
3,3',4,4'-TeCB (PCB #77)	< 5,0	0	0,00025	0,00050	0,00050
3,3',4,4',5-PeCB (PCB #126)	< 1,3	0	0,063	0,13	0,13
3,3',4,4',5,5'-HxCB (PCB #169)	< 1,3	0	0,0063	0,013	0,013
Mono-ortho PCBs					
2',3,4,4',5-PeCB (PCB #123)	< 5,0	0	0,00025	0,00050	0,00050
2,3',4,4',5-PeCB (PCB #118)	< 50	0	0,0025	0,0050	0,0050
2,3,4,4',5-PeCB (PCB #114)	< 5,0	0	0,0013	0,0025	0,0025
2,3,3',4,4'-PeCB (PCB #105)	< 25	0	0,0013	0,0025	0,0025
2,3',4,4',5,5'-HxCB (PCB #167)	< 25	0	0,00013	0,00025	0,00025
2,3,3',4,4',5-HxCB (PCB #156)	< 25	0	0,0063	0,013	0,013
2,3,3',4,4',5'-HxCB (PCB #157)	< 5,0	0	0,0013	0,0025	0,0025
2,3,3',4,4',5,5'-HpCB (PCB #189)	< 5,0	0	0,00025	0,00050	0,00050
Totaal (100 %)		0	0,083	0,17	0,17
Totaal (88 % d.s.)		0	0,073	**0,15**	0,15

Voor de berekening van de TEQ-waarden werden de WHO toxiciteits equivalent factoren gehanteerd volgens Martin Van den Berg et al. (Environmental Health Perspectives (1998), 106: 775-792). Bij de berekening van het totaal zijn de waarden kleiner dan de rapportagegrens gelijkgesteld aan nul ; zie kolom "Ondergrens" (Lowerbound - principe).
Bij de berekening van het totaal zijn de waarden kleiner dan de rapportagegrens gelijkgesteld aan de helft van de rapportagegrens; zie kolom "Middelwaarde" (Mediumbound - principe). Bij de berekening van het totaal zijn de waarden kleiner dan de rapportagegrens gelijkgesteld aan de rapportagegrens ; zie kolom "Bovengrens" (Upperbound - principe).
De meetonzekerheid werd bepaald en is beschikbaar in het laboratorium. Op eenvoudig verzoek kunnen deze gegevens overgemaakt worden. De RSD van het controlestaal is kleiner dan 10%.

SGS

ANALYSERAPPORT: IAC0710248

Bepaling van dioxineachtige PCB's

Product: sterol
Monsterkenmerk: 200710000857-1

Uw referentie: -

Component	Concentratie (ng/kg)	WHO-TEQ (ng/kg) Ondergrens	WHO-TEQ (ng/kg) Middelwaarde	WHO-TEQ (ng/kg) Bovengrens	WHO-TEQ (ng/kg) rapportagegrens
Non-ortho PCBs					
3,4,4',5-TeCB (PCB #81)	< 2,5	0	0,00013	0,00025	0,00025
3,3',4,4'-TeCB (PCB #77)	< 5,0	0	0,00025	0,00050	0,00050
3,3',4,4',5-PeCB (PCB #126)	< 1,3	0	0,063	0,13	0,13
3,3',4,4',5,5'-HxCB (PCB #169)	< 1,3	0	0,0063	0,013	0,013
Mono-ortho PCBs					
2',3,4,4',5-PeCB (PCB #123)	< 5,0	0	0,00025	0,00050	0,00050
2,3',4,4',5-PeCB (PCB #118)	< 50	0	0,0025	0,0050	0,0050
2,3,4,4',5-PeCB (PCB #114)	< 5,0	0	0,0013	0,0025	0,0025
2,3,3',4,4'-PeCB (PCB #105)	< 25	0	0,0013	0,0025	0,0025
2,3',4,4',5,5'-HxCB (PCB #167)	< 25	0	0,00013	0,00025	0,00025
2,3,3',4,4',5-HxCB (PCB #156)	< 25	0	0,0063	0,013	0,013
2,3,3',4,4',5'-HxCB (PCB #157)	< 5,0	0	0,0013	0,0025	0,0025
2,3,3',4,4',5,5'-HpCB (PCB #189)	< 5,0	0	0,00025	0,00050	0,00050
Totaal (100 %)		0	0,083	0,17	0,17
Totaal (88 % d.s.)		0	0,073	**0,15**	0,15

Voor de berekening van de TEQ-waarden werden de WHO toxiciteits equivalent factoren gehanteerd volgens Martin Van den Berg et al. (Environmental Health Perspectives (1998), 106: 775-792). Bij de berekening van het totaal zijn de waarden kleiner dan de rapportagegrens gelijkgesteld aan nul ; zie kolom "Ondergrens" (Lowerbound - principe).
Bij de berekening van het totaal zijn de waarden kleiner dan de rapportagegrens gelijkgesteld aan de helft van de rapportagegrens; zie kolom "Middelwaarde" (Mediumbound - principe). Bij de berekening van het totaal zijn de waarden kleiner dan de rapportagegrens gelijkgesteld aan de rapportagegrens ; zie kolom "Bovengrens" (Upperbound - principe).
De meetonzekerheid werd bepaald en is beschikbaar in het laboratorium. Op eenvoudig verzoek kunnen deze gegevens overgemaakt worden. De RSD van het controlestaal is kleiner dan 10%.

SGS

ANALYSERAPPORT: IAC0710248

Recovery extractie standaarden

	sterol 200710000854-1
13C-2,3,7,8-TCDD (%)	55,8
13C-1,2,3,7,8-PeCDD (%)	54,6
13C-1,2,3,4,7,8-HxCDD (%)	44,9
13C-1,2,3,6,7,8-HxCDD (%)	42,2
13C-1,2,3,4,6,7,8-HpCDD (%)	63,3
13C-OCDD (%)	43,7
13C-2,3,7,8-TCDF (%)	59,4
13C-1,2,3,7,8-PeCDF (%)	52,0
13C-2,3,4,7,8-PeCDF (%)	55,1
13C-1,2,3,4,7,8-HxCDF (%)	37,6
13C-1,2,3,6,7,8-HxCDF (%)	32,9
13C-2,3,4,6,7,8-HxCDF (%)	40,3
13C-1,2,3,7,8,9-HxCDF (%)	47,2
13C-1,2,3,4,6,7,8-HpCDF (%)	45,8
13C-1,2,3,4,7,8,9-HpCDF (%)	50,2
13C-OCDF (%)	34,2

SGS

ANALYSERAPPORT: IAC0710248

Recovery extractie standaarden

	sterol 200710000855-1
13C-2,3,7,8-TCDD (%)	71,8
13C-1,2,3,7,8-PeCDD (%)	75,2
13C-1,2,3,4,7,8-HxCDD (%)	66,1
13C-1,2,3,6,7,8-HxCDD (%)	61,6
13C-1,2,3,4,6,7,8-HpCDD (%)	64,2
13C-OCDD (%)	51,2
13C-2,3,7,8-TCDF (%)	70,4
13C-1,2,3,7,8-PeCDF (%)	76,0
13C-2,3,4,7,8-PeCDF (%)	77,1
13C-1,2,3,4,7,8-HxCDF (%)	48,9
13C-1,2,3,6,7,8-HxCDF (%)	45,1
13C-2,3,4,6,7,8-HxCDF (%)	56,9
13C-1,2,3,7,8,9-HxCDF (%)	69,5
13C-1,2,3,4,6,7,8-HpCDF (%)	59,9
13C-1,2,3,4,7,8,9-HpCDF (%)	60,4
13C-OCDF (%)	42,7

SGS

ANALYSERAPPORT: IAC0710248

Recovery extractie standaarden

	sterol 200710000856-1
13C-2,3,7,8-TCDD (%)	55,1
13C-1,2,3,7,8-PeCDD (%)	54,9
13C-1,2,3,4,7,8-HxCDD (%)	51,3
13C-1,2,3,6,7,8-HxCDD (%)	49,0
13C-1,2,3,4,6,7,8-HpCDD (%)	49,7
13C-OCDD (%)	38,6
13C-2,3,7,8-TCDF (%)	58,1
13C-1,2,3,7,8-PeCDF (%)	51,9
13C-2,3,4,7,8-PeCDF (%)	58,0
13C-1,2,3,4,7,8-HxCDF (%)	35,3
13C-1,2,3,6,7,8-HxCDF (%)	32,2
13C-2,3,4,6,7,8-HxCDF (%)	46,8
13C-1,2,3,7,8,9-HxCDF (%)	55,8
13C-1,2,3,4,6,7,8-HpCDF (%)	44,0
13C-1,2,3,4,7,8,9-HpCDF (%)	45,5
13C-OCDF (%)	28,9

000326

SGS

ANALYSERAPPORT: IAC0710248

Recovery extractie standaarden

	sterol 200710000857-1
13C-2,3,7,8-TCDD (%)	70,5
13C-1,2,3,7,8-PeCDD (%)	79,4
13C-1,2,3,4,7,8-HxCDD (%)	61,1
13C-1,2,3,6,7,8-HxCDD (%)	58,7
13C-1,2,3,4,6,7,8-HpCDD (%)	69,4
13C-OCDD (%)	49,4
13C-2,3,7,8-TCDF (%)	66,9
13C-1,2,3,7,8-PeCDF (%)	74,3
13C-2,3,4,7,8-PeCDF (%)	80,4
13C-1,2,3,4,7,8-HxCDF (%)	47,2
13C-1,2,3,6,7,8-HxCDF (%)	42,9
13C-2,3,4,6,7,8-HxCDF (%)	53,9
13C-1,2,3,7,8,9-HxCDF (%)	66,6
13C-1,2,3,4,6,7,8-HpCDF (%)	62,2
13C-1,2,3,4,7,8,9-HpCDF (%)	64,4
13C-OCDF (%)	40,9

SGS

ANALYSERAPPORT: IAC0710248

Recovery extractie standaarden

	sterol 200710000854-1
Non-ortho PCBs	
13C-PCB 81 (%)	64,7
13C-PCB 77 (%)	27,9
13C-PCB 126 (%)	84,0
13C-PCB 169 (%)	71,3
Mono-ortho PCBs	
13C-PCB 114 (%)	68,8
13C-PCB 105 (%)	71,6
13C-PCB 123 (%)	63,8
13C-PCB 167 (%)	51,7
13C-PCB 156 (%)	60,7
13C-PCB 157 (%)	58,7
13C-PCB 118 (%)	69,0
13C-PCB 189 (%)	56,6

SGS

ANALYSERAPPORT: IAC0710248

Recovery extractie standaarden

	sterol 200710000855-1
Non-ortho PCBs	
13C-PCB 81 (%)	63,7
13C-PCB 77 (%)	72,8
13C-PCB 126 (%)	107
13C-PCB 169 (%)	79,5
Mono-ortho PCBs	
13C-PCB 114 (%)	90,3
13C-PCB 105 (%)	91,9
13C-PCB 123 (%)	81,2
13C-PCB 167 (%)	58,8
13C-PCB 156 (%)	71,1
13C-PCB 157 (%)	67,5
13C-PCB 118 (%)	91,5
13C-PCB 189 (%)	70,2

SGS

ANALYSERAPPORT: IAC0710248

Recovery extractie standaarden

	sterol 200710000856-1
Non-ortho PCBs	
13C-PCB 81 (%)	69,9
13C-PCB 77 (%)	61,8
13C-PCB 126 (%)	85,7
13C-PCB 169 (%)	72,8
Mono-ortho PCBs	
13C-PCB 114 (%)	72,7
13C-PCB 105 (%)	75,7
13C-PCB 123 (%)	66,8
13C-PCB 167 (%)	53,2
13C-PCB 156 (%)	63,8
13C-PCB 157 (%)	61,0
13C-PCB 118 (%)	74,3
13C-PCB 189 (%)	58,8

SGS

ANALYSERAPPORT: IAC0710248

Recovery extractie standaarden

	sterol 200710000857-1
Non-ortho PCBs	
13C-PCB 81 (%)	49,5
13C-PCB 77 (%)	44,2
13C-PCB 126 (%)	73,8
13C-PCB 169 (%)	54,7
Mono-ortho PCBs	
13C-PCB 114 (%)	64,7
13C-PCB 105 (%)	65,9
13C-PCB 123 (%)	59,3
13C-PCB 167 (%)	43,0
13C-PCB 156 (%)	50,4
13C-PCB 157 (%)	48,0
13C-PCB 118 (%)	66,0
13C-PCB 189 (%)	49,1

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The National Food Laboratory, Inc. (The NFL)
6363 CLARK AVENUE, DUBLIN, CALIFORNIA 94568-3097
(925) 828-1440 • www.TheNFL.com

November 26, 2007

Analytical Report No.: CL2567-144
PO#: ARBORIS

Sergio Maldonado cc: Jesse Boyer
Arboris LLC
1201 West Lathrop Ave Gate 16
Savannah, GA 31415

Listed below are the results of our analyses for sample(s) received on November 06, 2007.

Plant Phytosterols, Composite of 5 Lots ASN-FPA-002883

NFL ID: AD08743

Screen	Analyte	Result	Units	MDL	Method Ref.
	Arsenic	Not Detected	ppm	0.020	MN5013
	Cadmium	Not Detected	ppm	0.010	MN5013
	Copper	Not Detected	ppm	2	MN5004
	Iron	Not Detected	ppm	10	MN5004
	Lead	Not Detected	ppm	0.010	MN5013
	Mercury	Not Detected	ppm	0.020	MN5012

MDL = Method Detection Limit Tol. = US EPA tolerances, if applicable

Individual detection limits for the Multi-Residue Analysis can be found on our website: www. TheNFL.com

Results are reported based on the sample(s) as received, unless otherwise noted.

Please note that these results apply only to the sample(s) submitted for this report. Samples from a different portion of the same lot may produce different results.

Your sample(s) will be retained for thirty days from the date of this report. If we do not hear from you by that time, the sample(s) will be discarded.

Should you have any questions concerning these results, please do not hesitate to contact us.

Thank you for using the services of The National Food Laboratory.

Sincerely,

Grace Bandong, Laboratory Manager, Analytical Services - Chemistry

cc: Patrick Manning, Accounting

The National Food Laboratory, Inc. (The NFL)
6363 CLARK AVENUE, DUBLIN, CALIFORNIA 94568-3097
(925) 828-1440 • www.TheNFL.com

November 26, 2007

Analytical Report No.: CL2567-145
PO#: ARBORIS

Sergio Maldonado cc: Jesse Boyer
Arboris LLC
1201 West Lathrop Ave Gate 16
Savannah, GA 31415

Listed below are the results of our analyses for sample(s) received on November 09, 2007.

Plant Phytosterols Composite of 5, ASN-FPA-002890

NFL ID: AD09162

Screen	Analyte	Result	Units	MDL	Method Ref.
	Arsenic	Not Detected	ppm	0.020	MN5013
	Cadmium	Not Detected	ppm	0.010	MN5013
	Copper	Not Detected	ppm	2	MN5004
	Iron	Not Detected	ppm	10	MN5004
	Lead	Not Detected	ppm	0.010	MN5013
	Mercury	Not Detected	ppm	0.020	MN5012

MDL = Method Detection Limit Tol. = US EPA tolerances, if applicable

Individual detection limits for the Multi-Residue Analysis can be found on our website: www. TheNFL.com

Results are reported based on the sample(s) as received, unless otherwise noted.

Please note that these results apply only to the sample(s) submitted for this report. Samples from a different portion of the same lot may produce different results.

Your sample(s) will be retained for thirty days from the date of this report. If we do not hear from you by that time, the sample(s) will be discarded.

Should you have any questions concerning these results, please do not hesitate to contact us.

Thank you for using the services of The National Food Laboratory.

Sincerely,

Grace Bandong, Laboratory Manager, Analytical Services - Chemistry

cc: Patrick Manning, Accounting

The National Food Laboratory, Inc. (The NFL)
6363 CLARK AVENUE, DUBLIN, CALIFORNIA 94568-3097
(925) 828-1440 • www.TheNFL.com

November 28, 2007

Analytical Report No.: CL2567-146
PO#: ARBORIS

Sergio Maldonado cc: Jesse Boyer
Arboris LLC
1201 West Lathrop Ave Gate 16
Savannah, GA 31415

Listed below are the results of our analyses for sample(s) received on November 13, 2007.

Plant Phytosterols, Composite of 5 Lots ASN-FPA-002897

NFL ID: AD09450

Screen	Analyte	Result	Units	MDL	Method Ref.
	Arsenic	Not Detected	ppm	0.020	MN5013
	Cadmium	Not Detected	ppm	0.010	MN5013
	Copper	Not Detected	ppm	2	MN5004
	Iron	Not Detected	ppm	10	MN5004
	Lead	Not Detected	ppm	0.010	MN5013
	Mercury	Not Detected	ppm	0.020	MN5012

Plant Phytosterols, Composite of 5 Lots ASN-FPA-002903

NFL ID: AD09451

Screen	Analyte	Result	Units	MDL	Method Ref.
	Arsenic	Not Detected	ppm	0.020	MN5013
	Cadmium	Not Detected	ppm	0.010	MN5013
	Copper	Not Detected	ppm	2	MN5004
	Iron	Not Detected	ppm	10	MN5004
	Lead	Not Detected	ppm	0.010	MN5013
	Mercury	Not Detected	ppm	0.020	MN5012

November 28, 2007 Analytical Report No.: CL2567-146

Sergio Maldonado cc: Jesse Boyer
Arboris LLC

MDL = Method Detection Limit Tol. = US EPA tolerances, if applicable

Individual detection limits for the Multi-Residue Analysis can be found on our website: www. TheNFL.com

Results are reported based on the sample(s) as received, unless otherwise noted.

Please note that these results apply only to the sample(s) submitted for this report. Samples from a different portion of the same lot may produce different results.

Your sample(s) will be retained for thirty days from the date of this report. If we do not hear from you by that time, the sample(s) will be discarded.

Should you have any questions concerning these results, please do not hesitate to contact us.

Thank you for using the services of The National Food Laboratory.

Sincerely,

Grace Bandong, Laboratory Manager, Analytical Services - Chemistry

cc: Patrick Manning, Accounting

The National Food Laboratory, Inc. (The NFL)
6363 CLARK AVENUE, DUBLIN, CALIFORNIA 94568-3097
(925) 828-1440 • www.TheNFL.com

November 28, 2007

Analytical Report No.: CL2567-147
PO#: ARBORIS

Sergio Maldonado cc: Jesse Boyer
Arboris LLC
1201 West Lathrop Ave Gate 16
Savannah, GA 31415

Listed below are the results of our analyses for sample(s) received on November 16, 2007.

Plant Phytosterols Composite of 5 Lots, ASN-FPA-002912

NFL ID: AD10015

Screen	Analyte	Result	Units	MDL	Method Ref.
	Arsenic	Not Detected	ppm	0.020	MN5013
	Cadmium	Not Detected	ppm	0.010	MN5013
	Copper	Not Detected	ppm	2	MN5004
	Iron	Not Detected	ppm	10	MN5004
	Lead	Not Detected	ppm	0.010	MN5013
	Mercury	Not Detected	ppm	0.020	MN5012

MDL = Method Detection Limit Tol. = US EPA tolerances, if applicable

Individual detection limits for the Multi-Residue Analysis can be found on our website: www. TheNFL.com

Results are reported based on the sample(s) as received, unless otherwise noted.

Please note that these results apply only to the sample(s) submitted for this report. Samples from a different portion of the same lot may produce different results.

Your sample(s) will be retained for thirty days from the date of this report. If we do not hear from you by that time, the sample(s) will be discarded.

Should you have any questions concerning these results, please do not hesitate to contact us.

Thank you for using the services of The National Food Laboratory.

Sincerely,

Grace Bandong, Laboratory Manager, Analytical Services - Chemistry

cc: Patrick Manning, Accounting

SUBMISSION END

AM

pine tree extracts

RECEIVED
APR 30 2008
BY: (b)(6)

April 25, 2008

Office of Food Additive Safety, (HFS 200)
Center for Food Safety and Applied Nutrition
5100 Paint Branch Parkway
College Park, MD 20740

VIA: Federal Express

Attention: Moraima J. Ramos Valle

RE: GRAS Notification for Pine Tree Phytosterols - Amendment 01

Dear Dr. Ramos-Valle:

We have included a separate GRAS exemption claim as described in the April 17, 1997 proposed rule that you requested during our telephone conversation earlier this week. Per your request, we have listed all foods in which the phytosterols will be used.

If you have any questions or require additional information, please contact:

Dr. Clyde A. Takeguchi, Ph.D.
Phoenix Regulatory Associates, Ltd.
21525 Ridgetop Circle, Suite 240
Sterling, VA 20166
Phone: (703)-406-0906
Email: phoenix@phoenixrising.com.

Sincerely,

(b)(6)

Sergio Maldonado
Technology Manager
Arboris LLC

Attachment: GRAS Exemption Claim and two (2) copies

cc: Phoenix Regulatory Associates, Ltd. letter only

Sergio Maldonado, Technology Manager
Phone: 912-238-6685 ❁ Fax: 912-238-7424 ❁ Email: Sergio.maldonado@arboris-us.com
1101 West Lathrop Ave, Gate 16, Savannah, GA 31415, USA.
Mailing Address: P.O. Box 2008, Savannah, GA 31402, USA

GRAS Notification for Pine Tree Phytosterols (continued)
GRAS Exemption Claim (continued)

The data and information that are the basis for the Arboris' GRAS determination are available for FDA review. Any additional information may be obtained by contacting:

Dr. Clyde A. Takeguchi, Ph.D.
Phoenix Regulatory Associates, Ltd.
21525 Ridgetop Circle, Suite 240
Sterling, VA 20166
Phone: (703)-406-0906
Email: phoenix@phoenixrising.com.

(b)(6)

Sergio Maldonado
Technology Manager
Arboris LLC

April 25, 2008
Date

000343

April 25, 2008

RECEIVED
APR 30 2008
BY: (b)(6)

GRAS Notification for Pine Tree Phytosterols

GRAS Exemption Claim

Arboris LLC has determined that the use of Arboris® Sterol AS-2® and Arboris® Sterol AS-4™ is exempt from the premarket approval requirements of the Federal Food, Drug and Cosmetic Act because Abrobis has determined that such use is GRAS. In accordance with proposed regulation, the following information is provided:

Proposed 21 CFR § 170.36 (c)(1)(i) The name and address of the notifier:

Arboris LLC
1201 W. Lathrop Avenue, Gate 16
Savannah, GA 31415

Proposed 21 CFR § 170.36 (c)(1)(ii) The common or usual name of the notified substance:

Pine Tree Phytosterol
Trade Name: Arboris® Sterol AS-2® and Arboris® Sterol AS-4™

Proposed 21 CFR § 170.36 (c)(iii) The applicable conditions of use of the notified substance:

For the existing uses of phytosterols in foods to include: Margarine and vegetable-based spreads; yogurt and yogurt-like products; milk-based juice beverages; ice cream and non-standardized ice cream products; cream cheese and cream cheese-like products; snack bars; salad dressing; standardized and non-standardized bread products; baked foods; beverages; dairy analogs; cheese and cream; breakfast cereal; mayonnaise; pasta and noodles; sauces; salty snacks; processed soups; puddings; confections; vegetarian meat analogs; fruit/vegetable juice; vegetable oils; egg products, including egg whites and substitute egg products.

Proposed 21 CFR § 170.36 (c)(1)(iv) The basis for the GRAS determination:

This GRAS determination is based on scientific procedures.

Proposed 21 CFR § 170.36 (c)(1)(v) Availability of information:

The complete record that supports this GRAS determination has been submitted to the agency in the applicable GRAS submission.

GRAS Notification for Pine Tree Phytosterols (continued)
GRAS Exemption Claim (continued)

The data and information that are the basis for the Arboris' GRAS determination are available for FDA review. Any additional information may be obtained by contacting:

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(b)(6)

Sergio Maldonado
Technology Manager
Arboris LLC

April 25, 2008
Date

Reference List for Industry Submission, GRN 000250

Pages	*Author*	*Title*	*Publish Date*	*Publisher*	*BIB_Info*
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000066 - 000074	Doornbos, AME; Meynen, EM; Duchateau, GSMJE; van der Knaap, HCM; Trautwein, EA	Intake occasion affects the serum cholesterol lowering of a plant sterol-enriched single-dose yoghurt drink in mildly hypercholesterolaemic subjects	2006	European Journal of Clinical Nutrition	Volume 60, pgs 325-333
000136 - 000139	Goldberg, Anne C.; Ostlund, Richard E.; Bateman, Joyce H.; Schimmoeller, Linda; McPherson, Timothy B.; Spilburg, Curtis A.	Effect of Plant Stanol Tablets on Low-Density Lipoprotein Cholesterol Lowering in Patients on Statin Drugs	2006	The American Journal of Cardiology	Volume 97, pgs 376-379
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NA- Not applicable

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000184	Plat, J.; Brufau, G.; Dasselaar, M.; Mensink, R.P.	A Stanol Yogurt Drink Alone Or Combined With A Low-Dose OTC Statin Lowers Non-HDLC, But Also Lowers TGS & Elevates HDLC In Metabolic Syndrome Patients	June 21, 2006	XIV International Symposium on Atherosclerosis	Abstract Number We-P14:477, pg 452
000186 - 000201	Rozner, Shoshana; Garti, Nissim	The activity and absorption relationship of cholesterol and phytosterols	2006	Colloids and Surfaces A: Physiochem. Eng. Aspects	Volumes 282-283, pgs 435-456
000209 - 000213	Rudkowska, Iwona; Roynette, Catherine E.; Nakhasi, Dilip K.; Jones, Peter J.H.	Phytosterols mixed with medium-chain triglycerides and high-oleic canola oil decrease plasma lipids in overweight men	2006	Metabolism Clinical and Experimental	Volume 55, pgs 391-395
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000239 - 000248	European Commission Health & Consumer Protection Directorate-General Scientific Committee on Food	Opinion of the Scientific Committee on Food on Applicatons for Approval of a Variety of Plant Sterol-Enriched Foods	2003		Number 15, pgs 1-10

NA- Not applicable

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000250 - 000256	European Commission Health & Consumer Protection Directorate-General Scientific Committee on Food	Opinion of the Scientific Committee on Food on an application from ADM for approval of plant sterol-enriched foods	2003		Number 23, pgs 1- 7
000258 - 000272	European Commission Health & Consumer Protection Directorate-General Scientific Committee on Food	Opinion of the Scientific Committee on Food on an application from MultiBene for approval of plant-sterol enriched foods	2003		Number 24, pgs 1-15

NA- Not applicable