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ORIGINAL SUBMISSION

*CARDIA*BEAT™ (VEGETABLE OILS/FATS/FISH OILS CONTAINING TRANSESTERIFIED PHYTOSTEROL ESTERS) GRAS NOTIFICATION

GENERALLY RECOGNIZED AS SAFE (GRAS) EXEMPTION CLAIM

Volume 1 of 1

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

Prepared by: Enzymotec Ltd
P.O. Box 6,
Migdal HaEmeq
Israel, 23106

June 8, 2006

Enzymotec
Delivering Lipids

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OFFICE OF
FOOD ADDITIVE SAFETY

June 6, 2006

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

Re: GRAS Notification

Dear Sir or Madam:

In accordance with proposed 21 CFR §170.36 [Notice of a claim for exemption based on a Generally Recognized As Safe (GRAS) determination] published in the Federal Register (62 FR 18939-18964), I am submitting in triplicate, as the notifier, Enzymotec, Ltd, P.O. Box 6, Migdal HaEmeq, Israel 23106 a GRAS notification of CardiaBeat™ for use in foods, a GRAS panel report setting forth the basis for the GRAS determination, and *curricula vitae* of the members of the GRAS panel for review by the agency.

Sincerely,

Ms. Iris Meiri-Bendek
Regulatory Affairs Manager

Enclosures

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Enzymotec ltd. P.O. Box 6 Migdal HaEmeq 23106 Israel -Tel: 972 4 6545112 -Fax: 972 4 6443799 www.enzymotec.com

I GENERALLY RECOGNIZED AS SAFE (GRAS) EXEMPTION CLAIM FOR VEGETABLE OIL-BASED CARDIABEAT™

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

CardiaBeat™, phytosterol ester containing fatty acids derived from vegetable oils, as defined in the report in Appendix I entitled, "**EXPERT PANEL CONSENSUS STATEMENT regarding THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF *CARDIABEAT*™ (VEGETABLE OILS/FATS/FISH OILS CONTAINING TRANSESTERIFIED PLANT PHYTOSTEROL ESTERS**", dated October 3rd, 2005, has been determined to be Generally Recognized As Safe (GRAS), consistent with Section 201(s) of the *Federal Food, Drug, and Cosmetic Act*. It should be noted, that the expert panel reviewed the entire Enzymotec Ltd. **Cardia**Beat product line, which includes phytosterol esters derived from both vegetable and fish oil sources. The current FDA GRAS Notification addresses only the **Cardia**Beat products derived from vegetables oil sources, and therefore the information pertaining to fish oil fatty acids, EPA and DHA, contained in the expert panel report are for this notification considered to be extraneous. This determination is based on scientific procedures as described in the following sections, under the conditions of its intended use in food, among experts qualified by their relevant national and international experience and scientific training and expertise to evaluate the safety of food ingredients. Therefore, the use of **Cardia**Beat™ derived from vegetable oil in food as described below is exempt from the requirement of premarket approval.

Signed,

June, 8, 2006
Date

Ms. Iris Meiri-Bendek
Regulatory Affairs
Enzymotec, Ltd
P.O. Box 6, Migdal HaEmeq
Israel 23106

B. Name and Address of Notifier

Ms. Iris Meiri-Bendek
Regulatory Affairs
Enzymotec, Ltd
P.O. Box 6, Migdal HaEmeq
Israel 23106

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C. Common Name of the Notified Substance

CardiaBeat™ (Phytosterol/Plant sterol esters of vegetable oil fatty acids in a matrix of vegetable oil glycerides)

D. Conditions of Intended Use in Food

The individual proposed food-uses and use-levels for regular and high grade **Cardia**Beat™ derived from vegetable oils, and the resulting use levels of phytosterol esters, are presented in Table 1. The use-level employed for regular and high grade vegetable oil-based **Cardia**Beat™ is based on an Interim Final Rule (IFR), which authorized the use, on food labels and in food labeling for spreads and dressings for salad, of health claims relating to the relationship between phytosterol esters and a reduced risk of Coronary Heart Disease (CHD) (FDA, 2000). This claim requires a phytosterol ester content of at least 0.65 g/serving, providing a minimum of 1.3 g/day. This use level, which is equivalent to 2.6 and 1.1 g/serving of regular and high grade vegetable oil-based **Cardia**Beat™, respectively, was applied to all food categories for which regular and high grade vegetable oil-based **Cardia**Beat™ are proposed for use.

Additionally, phytosterols have been granted GRAS status in several food categories as detailed in Table 2. As a result, the intake of phytosterols esters has been estimated based on the proposed food uses for **Cardia**Beat™, as well as the approved GRAS use levels. Since the original intake analysis was conducted, 3 additional GRAS notifications (GRN 000176, 000177, and 000181) have been submitted to the FDA, to which the FDA has responded with no questions. The majority of the proposed food-uses contained in these GRAS notifications were either already proposed for use with **Cardia**Beat™ and/or other GRAS notifications, or contained phytosterol at a low use-level and therefore the estimated intake of phytosterols resulting from all proposed food uses for **Cardia**Beat™ and all food uses identified in GRAS notifications did not change significantly. Both levels are reported in the current GRAS notification.

Table 1 Summary of the Individual Proposed Food-Uses and Use-Levels for Regular and High Grade Vegetable Oil-based CardiaBeat™ Products and the Corresponding use-Levels of Phytosterol Esters in the United States

Food Category	Proposed Food-Use	Regular Grade CardiaBeat™ Use-Level (g/RACC)[a]	High Grade CardiaBeat™ Use-Level (g/RACC)[a]	Phytosterol ester Use-Level (g/RACC)[a]
Baked Goods and Baking Mixes	Cakes	2.6	1.1	0.65
	Cookies	2.6	1.1	0.65
	Grain-Based Crackers	2.6	1.1	0.65
	French Toast, Pancakes, and Waffles	2.6	1.1	0.65
	Pastries	2.6	1.1	0.65

	Pies	2.6	1.1	0.65
	Quick Breads	2.6	1.1	0.65
	Yeast Breads and Rolls	2.6	1.1	0.65
Fats and Oils	Butter	2.6	1.1	0.65
	Fat-Based Sauces	2.6	1.1	0.65
	Margarine and Margarine-Like Spreads	2.6	1.1	0.65
	Mayonnaise and Mayonnaise-Type Dressings	2.6	1.1	0.65
	Oils (including vegetable shortening)	2.6	1.1	0.65
	Salad Dressings (regular and low calorie)	2.6	1.1	0.65
Frozen Dairy Desserts and Mixes	Ice Cream and Frozen Milk Desserts	2.6	1.1	0.65
	Frozen Yogurt	2.6	1.1	0.65
Gelatins, Puddings, and Fillings	Puddings, Custards and Other Milk Desserts	2.6	1.1	0.65
Grain Products and Pastas	Frozen Grain-Based Meals	2.6	1.1	0.65
	Grain Mixtures	2.6	1.1	0.65
	Pastas	2.6	1.1	0.65
	Grain-based Patties	2.6	1.1	0.65
	Rice and Other Cereal Grains	2.6	1.1	0.65
Gravies and Sauces	White Sauces and Milk Gravies	2.6	1.1	0.65
Hard Candy	Hard Candy	2.6	1.1	0.65
Milk	Milk	2.6	1.1	0.65
Milk Products	Cheese (Natural and Cream)	2.6	1.1	0.65
	Cheese (Processed and Spreads)	2.6	1.1	0.65
	Cheese (Imitation)	2.6	1.1	0.65
	Cheese Mixtures	2.6	1.1	0.65
	Creams and Cream Substitutes	2.6	1.1	0.65
	Evaporated, Condensed, and Dry Milks	2.6	1.1	0.65
	Flavored Milk and Milk Drinks	2.6	1.1	0.65
	Milk-Based Meal Replacements	2.6	1.1	0.65
	Sour Cream	2.6	1.1	0.65
	Milk, Fluid, Imitation	2.6	1.1	0.65
	Yogurt	2.6	1.1	0.65
Soft Candy	Candies and Chocolate	2.6	1.1	0.65
Soups and Soup Mixes	Grain Based Soups	2.6	1.1	0.65
	Cheese Soups	2.6	1.1	0.65
Snack Foods	Grain-Based Salty Snacks	2.6	1.1	0.65

[a] RACC – Reference Amounts Customarily Consumed Per Eating Occasion (21 CFR §101.12). When a range of values is reported for a proposed food-use, particular foods within that food-use may differ with respect to their RACC.

Table 2 Summary of the Food-Uses and Use Levels for Phytosterols and Phytosterol Esters that are Generally Recognized as Safe (GRAS) in the United States

Food Category	Food-Use	Use Level (%)
Baked Goods	White Breads, Rolls, Buns, and Comparable Non-Standardized White Bread Products (GRN 000112, 000176)	1.3*
Beverages and Beverage Bases	Health Drinks (GRN 000061, 000176)	0.41
	Egg Nog Mixes (GRN 000181†)	2.0
Breakfast Cereals	Ready-to-Eat Breakfast Cereals (GRN 000176)	0.73 – 2.67
Confection	Hard and Soft Candy (GRN 000176)	2.67 – 20.0
Coffee and Tea	Ground Roasted Coffee (GRN 000177)	0.42
Dairy Product Analogs	Cream Analogs (GRN 000176)	2.67
	Ice Cream Substitutes (GRN 000176)	0.17
	Soy Milk (GRN 000176)	0.16
Egg Products	Imitation Egg Products (GRN 000181†)	2.0
	Milk and Egg Dips (GRN 000181†)	2.0
Fats and Oils	Salad Dressing (GRN 000061, *000112, 000181†)	3.33
	Mayonnaise (GRN 000112, 000176)	4.33*
	Vegetable Oil (for home use for baking, frying, salad dressings) (GRN 000053, 000176)	13.3
	Vegetable Oil Spread (GRN 000039, 000061, 000112, 000176)	12.0
Gelatins, Puddings, and Fillings	Puddings (GRN 000176)	0.32
Grain Products and Pastas	Health Bars (GRN 000061, 000112, 000176)	2.5
	Pasta and Noodles (GRN 000176, 000181†)	0.29
Gravies and Sauces	Sauces (GRN 000176)	0.32 - 1.33
Milk Products	Cream Cheese and Cream Cheese-Like Products (GRN 000112, 000176)	2.17*
	Ice Cream and Non-standardized Ice Cream Products (GRN 000112)	0.54*
	Milk-based juice beverages (GRN 000112)	0.27*
	Yogurt and Yogurt-type Products (GRN 000061, 000112, 000176)	0.44 (regular) 0.83 (frozen)
Plant Protein Products	Meat Analogs	0.73
Processed Fruits and Fruit Juices	Fruit Juice (GRN 000176)	0.42
Processed Vegetables and Vegetable Juices	Vegetable Juice (GRN 000176)	0.42
Snack Foods	Salty Snack (GRN 000176)	1.33

Table 2 Summary of the Food-Uses and Use Levels for Phytosterols and Phytosterol Esters that are Generally Recognized as Safe (GRAS) in the United States		
Food Category	**Food-Use**	**Use Level (%)**
Soups and Soup Mixes	Processed Soups (GRN 000176)	0.16

* Use-levels not specified in GRAS notices. A use-level of 0.65 g/RACC, corresponding to the requirements for health claims regarding phytosterol esters and risk of coronary heart disease (21 CFR 101.83), was assumed.
† For GRN 000181, food codes for Egg Products were selected based on 9CFR590.1

CardiaBeat™ products comprise a family of structurally related edible oils that contain between approximately 20 and 60% phytosterol fatty acid esters, depending on the grade, and smaller amounts of diglycerides (DAG). The different oils in this family arise from use of different commercial vegetable oils, such as canola, soybean, sunflower, olive and palm oils, and hard oil, such as cocoa butter, which contain higher levels of saturated fatty acids and hence have higher melting temperatures. Within each group, there are a number of different grades that differ in terms of processing and refining conditions, contain different levels of free fatty acids, and are suitable for different food uses. The regular grade **Cardia**Beat™ contains more of the component oil and less of the phytosterol fatty acid esters, while the high grade **Cardia**Beat™ contains more of the phytosterol fatty acid esters. All the oil types are proposed for use in **Cardia**Beat™; however, each oil type will be restricted to food uses best suited to its functional properties. The consumption estimates for **Cardia**Beat™ were determined from all proposed uses combined to determine population exposures to phytosterol fatty acid esters and diglyceride components that occur in vegetable oil-based forms of **Cardia**Beat™.

The consumption of regular and high grades of vegetable oil-based **Cardia**Beat™ from all proposed food-uses, and the resulting intake of phytosterol esters from all proposed and approved food-uses of phytosterol esters, was estimated using the USDA CSFII 1994-1996 and the 1998 Supplemental Children's Survey (USDA CSFII 1998). On an all-user basis, the mean and 90th percentile intakes of regular grade vegetable oil-based **Cardia**Beat™ by the total U.S. population from all proposed food-uses were estimated to be 20.3 and 33.6 g/person/day, respectively, equivalent to 0.39 and 0.76 g/kg body weight/day, respectively. The mean and 90th percentile all-user intakes of high grade vegetable oil-based **Cardia**Beat™ by the total U.S. population from all proposed food-uses were estimated to be 8.4 and 14.0 g/person/day, respectively, equivalent to 0.16 and 0.32 g/kg body weight/day, respectively. Based on the composition of regular and high grade **Cardia**Beat™, the 90th percentile all-user consumption of DAG is expected to range between 4.27 and 5.75 g/person/day, equivalent to 0.061 to 0.082 mg/kg body weight/day.

Consumption of all of the proposed food-uses of vegetable oil-based **Cardia**Beat™ would provide all-user mean and 90th percentile phytosterol ester intakes of 5.06 and 8.40 g/person/day, respectively (0.10 to 0.19 mg/kg body weight/day, respectively). When the approved food uses were added to the proposed food uses of vegetable oil-based

CardiaBeat™, the all-user mean and 90th percentile intakes of phytosterol esters were determined to be 6.63 and 12.14 g/person/day (0.14 and 0.26 g/kg body weight/day), respectively. Following an update to the approved food uses from recently published GRN 000176, 000177, and 000181, the all-user mean and 90th percentile intakes of phytosterol esters were determined to be 7.34 and 12.89 g/person/day (0.15 and 0.27 g/kg body weight/day), respectively. Based on the specifications for vegetable oil-based **Cardia**Beat™, the 90th percentile all-user intake of phytosterols alone (in their free form), resulting from both the proposed food uses of vegetable oil-based **Cardia**Beat™ and the approved GRAS uses of phytosterol esters, is expected to be approximately 9.02 g phytosterol/person/day, equivalent to 0.18 g/kg body weight/day.

E. Basis for the GRAS Determination

The determination that vegetable oil-based **Cardia**Beat™, as defined in Appendix I, is GRAS on the basis of scientific procedures (see Appendix I entitled, "**EXPERT PANEL CONSENSUS STATEMENT regarding THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF *CARDIABEAT™* (VEGETABLE OILS/FATS/FISH OILS CONTAINING TRANSESTERIFIED PLANT PHYTOSTEROL ESTERS**") pursuant to 21 CFR § 170.30. This determination is based on the views of experts who are qualified by scientific training and experience to evaluate the safety of **Cardia**Beat™, as a component of food.

The safety of vegetable oil-based **Cardia**Beat™, is based on animal and clinical studies conducted employing vegetable oil-based **Cardia**Beat™, as well as studies in which the individual components, phytosterols and diglycerides, were employed. The animal studies performed with phytosterols and/or diglycerides included mutagenicity/genotoxicity, acute, subchronic, and a single generation reproductive toxicity study. Human clinical trials also are included in support of the efficacy of vegetable oil-based **Cardia**Beat™.

F. Availability of Information

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

Ms. Iris Meiri-Bendek
Regulatory Affairs
Enzymotec, Ltd
P.O. Box 6, Migdal HaEmeq
Israel 23106
Email: irisb@enzymotec.com

Should the U.S. Food and Drug Administration (FDA) have any questions or additional information requests regarding this notification, Enzymotec, Ltd. will supply these data and information.

II. Detailed Information About the Identity of the Substance

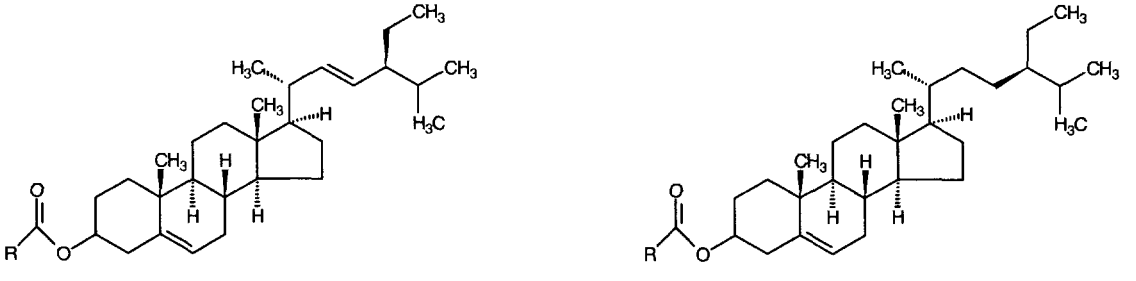
A. Identity

CardiaBeat™ are a family of oils that result from transesterification of different vegetable oils/fats with vegetable oil phytosterols to form a mixture containing phytosterol esters and containing a significant amount of diglycerides derived from the original triglyceride fraction of the original oil or fat. The empirical formulas, molecular weights, and structures of the component phytosterol esters contained in vegetable oil-based **Cardia**Beat™ are presented below.

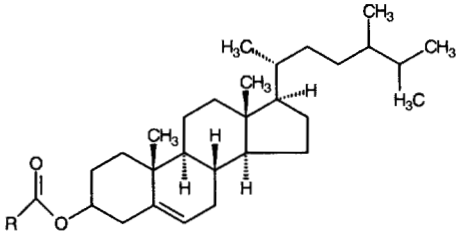
Chemical Abstracts Service:	campesterol	474-62-4
	stigmasterol	83-48-7
	β-sitosterol	5779-62-4
	brassicasterol	474-67-9
Empirical formula:	campesterol	$C_{28}H_{48}O$
	stigmasterol	$C_{29}H_{52}O$
	β-sitosterol	$C_{29}H_{50}O$
	brassicasterol	$C_{28}H_{46}O$
Formula Weight:	campesterol	MW 400.69
	stigmasterol	MW 416.73
	β-sitosterol	MW 414.72
	brassicasterol	MW 398.67

The structural formula of the phytosterol esters contained in vegetable oil-based **Cardia**Beat™ are presented in Figure 1.

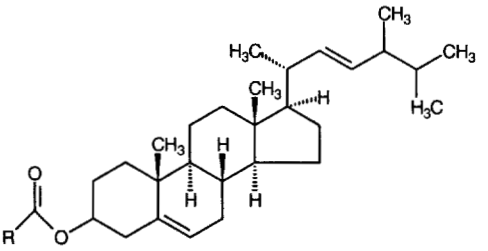
Figure 1 Structural Formula's for the Predominant Phytosterol Esters Contained in Vegetable Oil-Based CardiaBeat™



Stigmasterol ester β-Sitosterol ester



Campesterol ester



Brassicasterol ester

Where R = fatty acid moiety such as oleic acid (C18:1) or linoleic acid (C18:2)

B. Method of Manufacture

The manufacturing process for the formation of vegetable oil-based **Cardia**Beat™ products involves a transesterification reaction between the triglycerides in the source oil and the added soy phytosterol fraction using an enzymatic process, which involves the addition of a lipase derived from *Candida rugosa* (GRN 000081, FDA has no questions Feb 7, 2002), or a chemical process catalyzed by sodium methoxide. The enzymatic transesterification reaction is followed by separation of the catalyst and the crude mixture, that has the same fatty acid composition as the parent oil, may undergo winterization that results in the top fraction having a more unsaturated fatty acid composition. This fraction undergoes further processing. The chemical transesterification reaction is followed by neutralization of the sodium methylate *via* the addition of water and an aqueous citric acid solution. The transesterified oils undergo processing similar to that used for traditional vegetable oils, which include bleaching (in the chemical process), steam deodorization or molecular distillation.

A schematic representation of the manufacturing processes employed in the production of vegetable oil-based **Cardia**Beat™ products is presented in Figure 2 (enzymatic process) and in Figure 3 (chemical process).

A regular grade **Cardia**Beat™ product (have lower concentration of phytosterol esters) is produced by either the enzymatic or the chemical processes to include about 25% phytosterol esters. A high-grade material (have higher content of phytosterol esters) is produced, using a chemical process, with higher amounts of phytosterols to produce a product that includes about 60% phytosterol esters.

It should be noted that the Expert Panel Review (See Appendix I) also contains a description of an enzymatic manufacturing process employing solid fats: however Enzymotec Ltd, no longer produces solid fat products by the enzymatic method that included the use of hexane.

Figure 2 Enzymatic Manufacturing Process for the Production of Vegetable Oil-Based CardiaBeat™

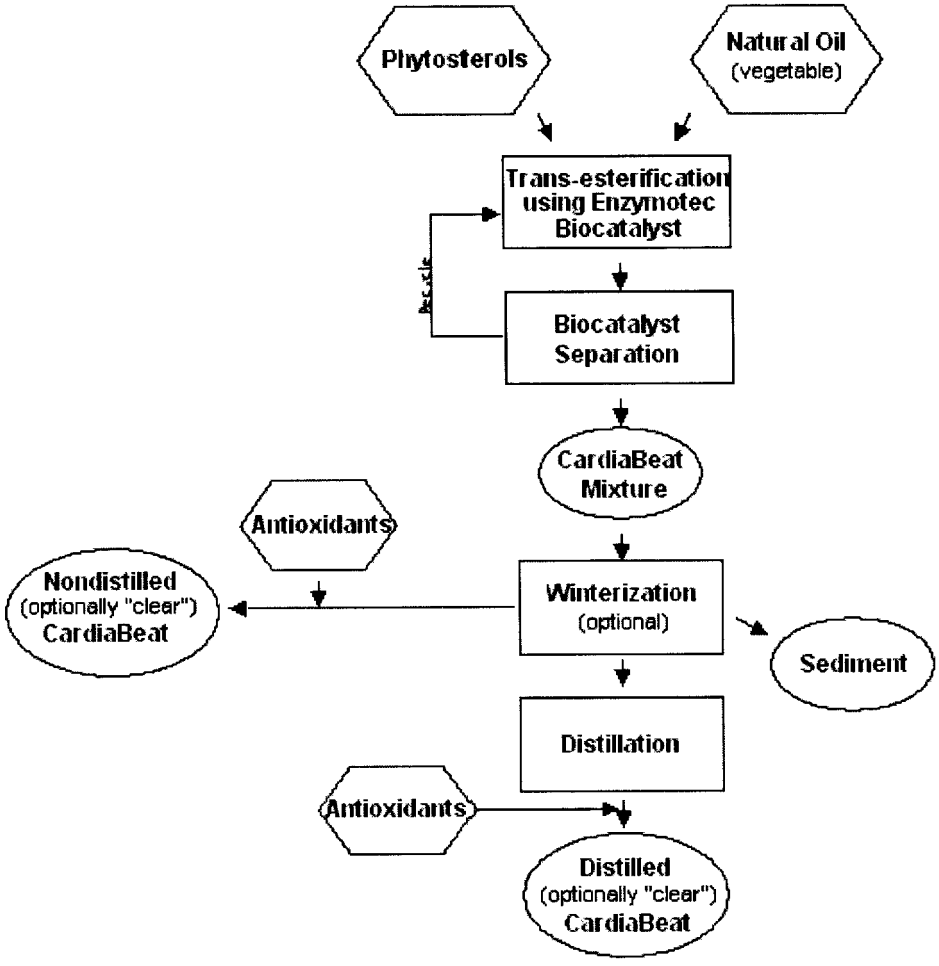
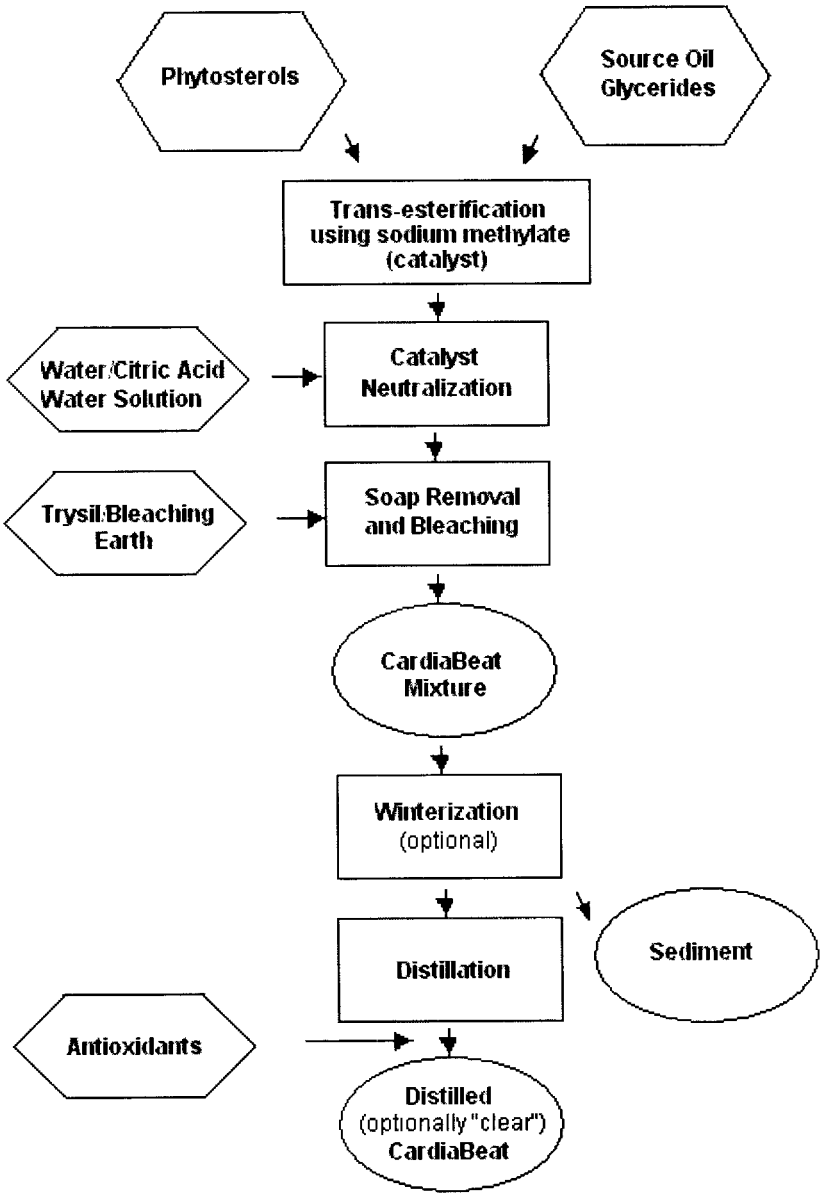


Figure 3 Chemical Manufacturing Process for the Production of Vegetable Oil-Based CardiaBeat™



C. Specifications for Food Grade Material

The chemical specifications for regular grade distilled and nondistilled vegetable oil-based **Cardia**Beat™ are presented in Table 4, and the specifications for high grade vegetable oil-based **Cardia**Beat™ are presented in Table 5. For details concerning the analytical methods employed in the analysis of **Cardia**Beat™ products see Appendix I (**EXPERT PANEL CONSENSUS STATEMENT regarding THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF *CARDIABEAT™* (VEGETABLE OILS/FATS/FISH OILS CONTAINING TRANSESTERIFIED PLANT PHYTOSTEROL ESTERS**).

Table 4 Chemical Specifications for Regular Grade Distilled and Nondistilled Vegetable Oil-Based CardiaBeat™

Specification parameter	Specification value	Method of analysis
Identification		
Solubility	Freely to very soluble in hexane	FCC (2003)
Infrared spectroscopy	Identical to standard	USP 197 (2004)
Purity		
Triglyceride	Between 35 and 65%	AOCS Cd 11d-96 (1999)
Diglyceride	Between 10 and 20%	AOCS Cd 11d-96 (1999)
Ratio 1,3/1,2 Diglycerides	Greater than 2.0	AOCS Cd11d-96 (1999)
Monoglyceride	Less than 5%	AOCS Cd 11d-96 (1999)
Total phytosterols	Greater than 14%	Enzymotec internal method
Phytosterol esters	Greater than 20%	Calculated based on difference between total and free phytosterols
Free phytosterols	Less than 2.5%	Enzymotec internal method
Free fatty acids	Less than 10%	AOCS Ca 5a-40 (1999)
Peroxide value	Less than 10 meq/kg	AOCS Cd 8-53 (1999)
Ash	Less than 1 mg/kg	USP 281 (2004)
Water	Less than 1%	Enzymotec internal method based on Karl Fischer method
Lead	Less than 0.1 mg/kg	EPA 200.7

Table 5 Chemical Specifications for High Grade Vegetable Oil-Based CardiaBeat™

Specification parameter	Specification value	Method of analysis
Identification		
Solubility	Very soluble in hexane	FCC (2003)
Infrared spectroscopy	Identical to standard	USP 197 (2004)
Purity		
Triglyceride	Between 7 and 40%	AOCS Cd 11d-96 (1999)
Diglyceride	Between 5 and 18%	AOCS Cd 11d-96 (1999)
Ratio 1,3/1,2 Diglycerides	Greater than 2.0	AOCS Cd 11d-96 (1999)
Monoglyceride	Less than 5%	AOCS Cd 11d-96 (1999)

Table 5 Chemical Specifications for High Grade Vegetable Oil-Based CardiaBeat™		
Specification parameter	**Specification value**	**Method of analysis**
Total phytosterols	Greater than 36%	Enzymotec internal method
Phytosterol esters	Greater than 50%	Calculated based on difference between total and free phytosterols
Free phytosterols	Less than 10%	Enzymotec internal method
Free fatty acids	Less than 0.5%	AOCS Cd-5a-40 (1999)
Peroxide value	Less than 5 meq/kg	AOCS Cd 8-53 (1999)
Ash	Less than 1 mg/kg	USP 281 (2004)
Water	Less than 1.0%	Enzymotec internal method based on Karl Fischer method
Lead	Less than 0.1 mg/kg	EPA 200.7

The microbial specifications for regular and high grade distilled and nondistilled vegetable oil-based **Cardia**Beat™ are presented in Table 6. For details concerning the analytical methods employed in the analysis of vegetable oil-based **Cardia**Beat™ products see Appendix I (**EXPERT PANEL CONSENSUS STATEMENT regarding THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF *CARDIABEAT™* (VEGETABLE OILS/FATS/FISH OILS CONTAINING TRANSESTERIFIED PLANT PHYTOSTEROL ESTERS**).

Table 6 Microbial Specifications for Regular and High Grade Vegetable Oil-Based CardiaBeat™		
Specification Parameter	**Specification Value**	**Analytical Method**
Total aerobic count	<1000 CFU/g	Israel Standard SI 885 Part 3 (1999)
Mold	<100 CFU/g	Israel Standard SI 885 Part 8 (1999)
Yeast	<100 CFU/g	Israel Standard SI 885 Part 8 (1999)
Coliforms	ND/g	USP 61 (2000)
Salmonella	ND/g	Israel Standard SI 885 Part 7 (1999)
Staphlyococcus aureus	ND/g	USP 61 (2000)

D. Additional Chemical Characterization

D.1 Potential Epichlorohydrin and 1,3-Dichloro-2-propanol Residues

The ion exchange resin which is used to bind/immobilize the lipase enzyme is manufactured using epichlorohydrin which potentially could give rise to epichlorohydrin and 1,3-dichloro-2-propanol residues in vegetable oil-based **Cardia**Beat™. The potential residues from two Lots of the preconditioned resin using thermal desorption/purge and trap GC/MS indicated residues of epichlorohydrin in the solid resin of 1.2 to 2.4 ppm. The ratio of vegetable oil-based **Cardia**Beat™ to the ion exchange resin is approximately 100:1 (w/w) and thus the level of epichlorohydrin would be 1/100th this level as summarized in the following Table 7. Assuming

that all the epichlorohydrin reacted with water to form 1,3-dichloro-2-propanol and adjusting for the differences in molecular weight, the potential worst case levels of 1,3-dichloro-2-propanol in vegetable oil-based **Cardia**Beat™ can also be determined.

Table 7 Potential Worst Case Residual Levels of Epichlorohydrin and 1,3-Dichloro-2-propanol in Vegetable Oil-Based CardiaBeat™ Processed with Different Resin Batches

Chemical Residue	Residue Levels (ppm)	
	Lot 1	Lot 2
Epichlorohydrin	<0.012	<0.024
1,3-Dichloro-2-propanol	<0.0084	<0.017

These levels would be considered to be worst case residue levels since vegetable oil-based **Cardia**Beat™ undergoes a distillation process designed to remove free fatty acids with boiling points of approximately 330°C at 760 mmHg. Under these conditions, components such as epichlorohydrin and 1,3-dichloro-2-propanol that have much lower boiling points (116°C and 174°C, respectively) are expected to be removed. In addition, the supplied resins undergo a washing procedure prior to use.

Based on an estimated 90th percentile intake of vegetable oil-based **Cardia**Beat™ of 33.6 g/day, the exposures to epichlorohydrin and 1,3-dichloro-2-propanol from consumption of vegetable oil-based **Cardia**Beat™ can be determined and compared to permitted levels (Table 8). As calculated the potential residue levels are below what would be considered to have adverse health effects.

Table 8 Comparison of Potential Exposures to Epichlorohydrin and 1,3–Dichloro-2-propanol from Consumption of Vegetable Oil-Based CardiaBeat™ to Permitted Consumption Levels

Chemical Residue	Potential Daily Residue Exposure (µg/day)		Permitted Exposure[1] (µg/day)
	Lot 1	Lot 2	
Epichlorohydrin	0.40	0.81	22.11
1,3-Dichloro-2-propanol	0.28	0.57	1.75

[1] Permitted exposure to give risk of 1 in 1 x10^6 (FDA, 2002)

D.2 Methanol Residues

The production of vegetable oil-based **Cardia**Beat™ involves the addition of sodium methoxylate, which may result in the presence of methanol residues in the final product. Several lots of vegetable oil-based **Cardia**Beat™ products manufactured by the chemical method were analyzed for residual methanol solvent using a GC/MS head space method. It should be noted that since the completion of the Expert Panel Report (Appendix I), Enzymotec

Ltd. has completed additional residual methanol analysis, and that the detection limit in the new analysis is 1 ppm, as opposed to 30 ppm in the previous analysis. The results of the more recent residual methanol analysis are presented in Table 8.

Table 8 Residual Methanol Solvent Content of Manufactured Lots of Various CardiaBeat™ Products

Solvent	High Grade, Canola Oil-Based CardiaBeat™		Regular Grade, Canola Oil-Based CardiaBeat™	
	C2000144-5	C2000-25-281104	C4500-PL2005-NWD	C4500-MLC-2
Methanol (ppm)	<1.0	<1.0	<1.0	<1.0

The U.S. Environmental Protection Agency (U.S. EPA) has chosen to use a reference dose of 0.5 mg/kg body weight/day for methanol to determine drinking water standards (Marcus, 1993). However, these values are below the current exposure to methanol in food products (10 to 40 mg/day from fruit juice consumption) and may be very conservative. The U.S. Food and Drug Agency (FDA) has stated in a number of reviews that 7.1 to 8.4 mg/kg body weight/day is considered to be a safe level (FDA, 1993, 1996) based on human data and a safety factor of 10.

Based on the consumption estimates of **Cardia**Beat™ of 33.6 g/day (see section 5.2) and levels of residual methanol of <30 µg/g or <1µg/g, this represents a trivial proportion of current methanol exposure from food and is well below the acceptable exposure. Additionally, due to the distillation process and the volatile nature of methanol, it is expected that the majority of the methanol present will have evaporated and that methanol residues present in the final **Cardia**Beat™ products will be negligible.

D.3 Vitamin E and Vitamin K Content

Vegetable oils are recognized as source of fat-soluble vitamins, and in particular vitamin E and K. The vitamin E content of vegetable oils is generally divided between different tocopherol isomers that are known to vary greatly in their biological activities. The vitamin E content of processed soybean oil per 100 g portion, as represented by α-tocopherol, has been reported to range between 8.10 and 15.09 mg, depending on processing methods (USDA, 2005). Palm oil and cocoa butter tend to be low in α-tocopherol containing between 1.80 and 3.81 mg/100 g oil, while canola and olive oil tend to have higher α-tocopherol content, ranging between 14.35 and 21.80 mg/100g oil. The production of **Cardia**Beat™ is expected to remove most tocopherols naturally present in the source oils employed; however tocopherols are re-introduced into **Cardia**Beat™ as antioxidants. The amount of tocopherols introduced as antioxidants are expected to represent the final tocopherol content of **Cardia**Beat™, and are presented in Table 7 along with the tocopherol content of the source oil before processing.

Table 10 Tocopherol Content of Source Oils and the Amount of Tocopherols Added to Vegetable Oil-Based CardiaBeat™ Products						
	Cocoa Butter Source Oil	Cocoa Butter-Based CardiaBeat™		Soybean Source Oil	Soybean Oil-based CardiaBeat™	
		Enzymatic Process	Chemical Process		Enzymatic Process	Chemical Process
Tocopherols (mg/g)						
Alpha (α)	<0.01	0.33	0.20	0.06	0.22	0.27
Delta (δ)	<0.01	0.43	0.29	0.12	0.39	0.41
Gamma (γ)	0.16	1.47	0.81	0.35	1.06	1.16
Total	0.16	2.23	1.30	0.53	1.67	1.84

Based on the intake estimates for **Cardia**Beat™, the 90 percentile all user intake of tocopherols resulting from the consumption of vegetable oil-based **Cardia**Beat™ is estimated to range between 43.68 and 74.93 mg tocopherols/day.

The normal levels of vitamin K in some vegetable oils such as soybean and canola oil are considered to be high compared to other vegetable oils such as sunflower and corn oils. However, 60% of vitamin K is usually obtained from green leafy vegetables. The levels of vitamin K_1 in canola based **Cardia**Beat™ ranges from 51 to 107 µg/100g of oil. Although the canola source oil was not analysed for vitamin K_1 levels, low eurcic acid canola oil normally contains 122 µg/100g (USDA, 2005) suggesting a small decrease in vitamin K_1 levels in some forms of **Cardia**Beat™.

The proposed use level of **Cardia**Beat™ products is relatively small, 1.1 to 2.6 g/serving and will only replace a small proportion of the current levels of regular vegetable oils in processed foods and thus will have no impact on the consumption of vitamin E or K from oil sources in the diet.

III. Self-Limiting Levels of Use

No technological self-limiting levels of use were identified for vegetable oil-based **Cardia**Beat™. As this product acts as an oil in proposed food uses, the use of vegetable oil-based **Cardia**Beat™ would be limited along the same lines as other vegetable oils. Generally speaking, vegetable oils tend to represent a small portion of finished product and when used alone, for example as a salad dressing, the serving sizes consumed are limited to several tablespoons.

IV. Basis for GRAS Determination

Pursuant to 21 CFR §170.30, vegetable oil-based **Cardia**Beat™ intended for use in foods by Enzymotec Ltd, as defined in Appendix I, has been determined to be GRAS based on scientific

procedures. This determination is based on the opinion of experts (the Expert Panel) who are qualified by their scientific training and experience to evaluate the safety of vegetable oil-based **Cardia**Beat™ as a component of food. Further support for the safety of vegetable oil-based **Cardia**Beat™ is well established based on the safety of phytosterols.

Enzymotec Ltd has conducted an animal and a clinical trial in which the efficacy of **Cardia**Beat™ was examined. In the animal study, 8-week-old apoE^{0} mice were administered gavage doses of a placebo, canola oil, or regular grade canola oil-based **Cardia**Beat™ in addition to their normal chow, with 5 rats employed in each dosing group, for a period of 10 weeks. The administered doses of treatment oils were equivalent to approximately 13.9 mg/day or 463 mg/kg/day. As the administered formulations of regular grade canola oil-based **Cardia**Beat™ contained 25% phytosterols from soy, this represents an exposure of approximately 2.5 mg phytosterols/day, equivalent to 83 mg/kg body weight/day. At the conclusion of the treatment period, the plasma lipid profile of the mice and parameters pertaining to lipid peroxidation were examined. During the feeding period no significant signs of induced toxicity were inspected in the daily evaluation of the animals. Neither major adverse events nor minor adverse effects were detected in mice from all treatment groups. The consumption of canola oil-based regular grade **Cardia**Beat™ was observed to induce a decrease in serum cholesterol levels. Additionally, the mice receiving canola oil-based regular grade **Cardia**Beat™ were reported to exhibit a significant reduction in serum oxidation as compared to both the canola oil fed and control mice.

The clinical trial conducted by Enzymotec Ltd. was of crossover design and was completed by 21 volunteers who consumed olive-oil based diets supplemented with olive oil, a commercially available phytosterol esters product or regular grade olive oil-based **Cardia**Beat™ for periods of 4 weeks. Consumption of the diets was preceded by, and separated by, a 4-week washout period in which the volunteers consumed their typical diets. Baseline control diets provided either up to 200 mg/day phytosterols in the olive-oil based diet or 21.4 g of commercial product of a low-fat sunflower oil-phytosterol ester margarine containing 1.7 g/day phytosterols. The regular grade olive-oil based **Cardia**Beat™ supplemented diet provided 1.7 g of phytosterols/day in a total of 9.2 g/day product. At the end of each dietary phase, blood lipid levels were examined.

No adverse effects or indications of side effects were reported to result from the consumption of diets supplemented with commercial phytosterols, or regular grade olive oil-based **Cardia**Beat™. The consumption of **Cardia**Beat™ was reported to have a beneficial and distinct hypocholesterolemic effect on both total and LDL-cholesterol levels, as compared to both olive oil and commercially available phytosterol esters control diets.

In addition to the animal and clinical trials conducted by Enzymotec Ltd., the safety of **Cardia**Beat™ is supported by the safety of its individual components, phytosterols and diglycerides. Subchronic and chronic toxicity studies conducted in several laboratory animal

species, have indicated that there are no adverse effects associated with dietary consumption of phytosterols. Developmental and reproductive toxicity studies indicated that dietary phytosterols have no adverse effect on reproductive performance, and no significant positive results were reported in genotoxicity or mutagenicity assays. NOAELs of 3 g of phytosterol esters and 4.8 g of phytosterols/kg body weight were reported in 13-week toxicity studies conducted in rats, while NOAELs of 1.5 to 5.6 g phytosterols/kg body weight/day were reported in reproductive toxicity studies also conducted in rats. In clinical trials, the administration of up to 9.0 g phytosterols/day was reported to have no adverse effect on lipid soluble vitamin or carotenoid levels. The majority of the clinical trials reviewed indicated that consumption of phytosterols had a beneficial effect on LDL cholesterol levels and cardiovascular disease risk factors, with these effects being observed at phytosterol doses as low as 1.5 g phytosterols/day. Additionally, a review of clinical trials conducted in individuals heterozygous for phytosterolemia indicated that the consumption of 2.2 g phytosterols/day was reported to produce no adverse effects in these individuals.

DAG is present in the diet as a minor constituent of most edible oils and plant derived fats, and it is produced endogenously as a product of triacylglycerol (TAG) digestion. Naturally occurring DAG is primarily present in the 1,2-DAG (or 2,3-DAG) isoform of DAG, whereas in most edible oils 1,3-DAG has been identified as the predominant isoform. The daily dietary intake of diacylglycerol has been estimated to range between 1 and 10 g/person/day. The safety of diglycerides is also supported by a chronic toxicity study conducted male and female Sprague-Dawley rats that indicated no adverse effects were associated with the consumption of up to 2,650 mg/kg body weight/day of a DAG rich oil in the diet. Genotoxicity assays conducted both *in vitro* and *in vivo* have demonstrated that DAG oil and DAG oil subjected to intense heating conditions does not possess genotoxic potential. In clinical trials, the consumption of up to 45 g/day of a DAG rich oil with a composition similar to the DAG composition of **Cardia**Beat™ (7:3 1,3-DAG to 1,2-DAG ratio) for up to 24 weeks has been reported to be well tolerated and produce no adverse effects.

The scientific evidence presented in Appendix I (**EXPERT PANEL CONSENSUS STATEMENT regarding THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF *CARDIABEAT™* (VEGETABLE OILS/FATS/FISH OILS CONTAINING TRANSESTERIFIED PLANT PHYTOSTEROL ESTERS**) does not indicate that **Cardia**Beat™, or any of its constituents, would produce adverse effects on human health when consumed at the intended conditions of food use described herein. The data and information summarized in this report demonstrate that **Cardia**Beat™, meeting appropriated food grade specifications and manufactured in accordance with current good manufacturing practice, would be GRAS based on scientific procedures under the conditions of intended use in foods.

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Appendix 1

EXPERT PANEL CONSENSUS STATEMENT REGARDING THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF *CARDIABEAT™* (VEGETABLE OILS/FATS/FISH OILS CONTAINING TRANSESTERIFIED PLANT PHYTOSTEROL ESTERS)

1.0 INTRODUCTION

At the request of Enzymotec, Ltd., an Expert Panel (the "Panel") of independent scientists, qualified by their relevant national and international experience and scientific training to evaluate the safety of food ingredients, was convened on July 12th, 2005 to conduct a critical and comprehensive evaluation of the available pertinent data and information, and determine whether, under the conditions of intended use to replace normal vegetable oils/fats/fish oil combinations as a food ingredient, a number of plant phytosterol ester containing vegetable oil/fats/fish oil combinations referred to as **Cardia**Beat™ would be Generally Recognized as Safe (GRAS), based on scientific procedures. The Panel consisted of the below-signed qualified scientific experts: Dr. Joseph F. Borzelleca (Medical College of Virginia), Dr. W. Gary Flamm (Flamm Associates), and Dr. Ernst J. Schaefer (Tufts University). *Curricula vitae* evidencing the Panel members' qualifications for evaluating the safety of food ingredients are provided in Attachment 1.

The Panel, independently and collectively, critically examined a comprehensive package of scientific information and data compiled from the literature and other published sources through May 2005 by CANTOX. In addition, the Panel evaluated other information deemed appropriate or necessary, including data and information provided by Enzymotec, Ltd. The data evaluated by the Panel included information pertaining to the method of manufacture and product specifications, supporting analytical data, intended use-levels in specified food products, consumption estimates for all intended uses, and a comprehensive assessment of the available scientific literature pertaining to the safety of **Cardia**Beat™.

Following independent, critical evaluation of such data and information, the Panel unanimously concluded that under the conditions of intended use in traditional foods described herein, **Cardia**Beat™, meeting appropriate food-grade specifications, is GRAS based on scientific procedures. A summary of the basis for the Panel's conclusion, excluding confidential data and information, is provided below.

000023

2.0 MANUFACTURING AND COMPOSITION

The manufacturing process for the formation of a family of **Cardia**Beat™ products involves a transesterification reaction between the triglycerides in the source oil and the added soy phytosterol fraction using an enzymatic process, involving the addition of an immobilized GRAS

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lipase isolated from *Candida rugosa* (GRN 000081, Feb 7, 2002), or a chemical process, involving the addition of sodium methoxide. The source oils employed in the transesterification reaction can include any common commercial oil or fat meeting food grade standards such as soybean oil, canola oil, olive oil, sesame oil, cocoa butter, or palm oil. The saturated vegetable oils (*e.g.*, cocoa butter and palm oil) are manufactured by the enzymatic process in the presence of an organic solvent (hexane). Following reaction and separation of the catalyst, the crude mixture that has the same fatty acid composition as the parent oil may undergo winterization that results in the top fraction having a more unsaturated fatty acid composition. This fraction undergoes further processing. Each of the transesterified oils undergoes processing similar to that used for traditional vegetable oils including bleaching (chemical process) and steam deodorization or molecular distillation. Finally, permitted antioxidants are added to **Cardia**Beat™ products for stability. **Cardia**Beat™ products can be regular grade (have lower concentrations of phytosterol esters) materials or high-grade material (have higher content of phytosterol esters) produced, using a chemical process, with higher amounts of phytosterols.

Fish oil derived **Cardia**Beat™ is produced from an enriched DHA/EPA fatty acid ethyl ester fraction prepared from fish oil. The resulting phytosterol ester preparation may undergo blending with fish oil.

All processing chemicals used in the manufacture of **Cardia**Beat™ are appropriate for food-use. In order to ensure a consistent product, Enzymotec, Ltd. has established numerous chemical and microbiological specification parameters for the final regular and high grade **Cardia**Beat™ preparations, which are presented in Tables 2.1 to 2.5 in Attachment 2. Analyses of representative lots of **Cardia**Beat™ demonstrated compliance with final product chemical and microbiological specifications. Additionally, analysis results of the long-term stability (12 weeks) of **Cardia**Beat™, as well as the stability of **Cardia**Beat™ at high temperatures (180 to 185°C), indicate conformity to product specifications under appropriate long-term storage and deep frying conditions.

3.0 INTENDED USE AND ESTIMATED EXPOSURE

CardiaBeat™ does not occur naturally; however, it's components, phytosterol esters, individual fatty acids, and DAG (diacylglycerol), are readily encountered in the food supply. Phytosterols are produced endogenously in plants and therefore are encountered in all plants and plants based food products (Piironene and Lampi, 2004). EPA and DHA are omega-3 fatty acids that occur naturally in cold-water fish, and this represents the primary source of EPA and DHA in the diet (Kris-Etherton *et al.*, 2000; Arab, 2003). DAG is present in the diet as a minor constituent of most edible oils and plant derived fats, and it is produced endogenously as a product of triacylglycerol (TAG) digestion (Taguchi *et al.*, 2001; Yasunaga *et al.*, 2004). The estimated daily dietary intake of phytosterols from the typical western diet has been estimated to range

between 160 and 360 mg/day (Connor, 1968; Salen *et al.*, 1970; Cerqueira *et al.*, 1979). The daily dietary intake of EPA and DHA in the U.S. has been estimated to range between 100 and 200 mg/day (Kris-Etherton *et al.*, 2000). The adequate daily dietary intake of EPA and DHA fatty acids has been reported to be 650 mg/day for adults, and pregnant and nursing women are recommended to consume a minimum of 300 mg DHA/day (Simopoulos *et al.*, 1999; Kris-Etherton *et al.*, 2000). The daily dietary intake of DAG has been estimated to range between 1 and 10 g/person/day (FDA, 2000d).

The FDA has determined that vegetable or plant phytosterol esters are GRAS as food ingredients in vegetable oils, spreads, salad dressings, bars and yogurt, as well as a number of other food uses proposed in several GRAS notifications to which the FDA has not objected too. Additionally, the FDA has published an Interim Final Rule (IFR), which authorized the use, on food labels and in food labelling, of health claims relating to the relationship between phytosterol esters and a reduced risk of Coronary Heart Disease (CHD) (FDA, 2000c). The IFR authorizes the use of a health claim relating plant sterol esters and reduced risk of CHD on labelling of spreads and dressings for salad containing at least 0.65 g of phytosterol esters per serving, and providing a minimum of 1.3 g/day. Additionally, the FDA has established a maximum daily intake of 3.0 g of DHA and EPA in its final rule concerning the use of menhaden oil (FDA, 2005). A GRAS Notice submitted concerning the use of a DAG oil providing a 90th-percentile intake of approximately 300 to 500 mg/kg body weight/day was met with a no questions from the FDA (FDA, 2003b).

Regular grade **Cardia**Beat™ preparations are proposed for use as food ingredients at levels of 2.6 g/serving, while high grade preparations are proposed for use at levels of 1.1g/serving which are estimated to provide an average of 0.65 g of phytosterol esters per serving. The individual proposed food-uses and use-levels for **Cardia**Beat™ and the corresponding use-levels for phytosterol esters are summarized in Table A3-1 in Attachment 3, and include 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. The food-uses of regular and high grade fish oil **Cardia**Beat™ have been restricted due to concerns relating to the level of EPA + DHA provided by the fish oil **Cardia**Beat™ products, and these are presented in Table A3-2 in Attachment 3. Additionally, phytosterol esters are currently permitted for addition to foods in the United States and the intake of phytosterol esters resulting from the previously approved GRAS uses was added to the intake resulting from the proposed food uses for **Cardia**Beat™. The previously approved GRAS food uses for phytosterol esters are summarized in Table A3-3 in Attachment 3, and include baked goods, beverages and beverage bases, fats and oils, grain products and pastas, and milk products.

The consumption of regular and high grades of **Cardia**Beat™ from all proposed food-uses, and the resulting intake of phytosterol esters from all proposed and approved food-uses of

phytosterol esters, was estimated using the USDA CSFII 1994-1996 and the 1998 Supplemental Children's Survey (USDA CSFII 1998) (USDA, 2000). In total, approximately 95.5% of the total U.S. population was identified as potential consumers of **Cardia**Beat™ and phytosterol esters from the proposed food-uses (19,684 actual users identified). On an all-user basis, the mean intake of regular grade non-fish oil based **Cardia**Beat™ by the total U.S. population from all proposed food-uses was estimated to be 20.3 g/person/day, equivalent to 0.39 g/kg body weight/day. The heavy consumer (90th percentile) all-user intakes of **Cardia**Beat™ from all proposed food-uses were estimated to be 33.6 g/person/day, 0.76 g/kg body weight/day. On an all-user basis, the mean intake of high grade non-fish oil based **Cardia**Beat™ by the total U.S. population from all proposed food-uses was estimated to be 8.4 g/person/day, equivalent to 0.16 g/kg body weight/day. The 90th percentile all-user intakes of high grade non-fish oil based **Cardia**Beat™ from all proposed food-uses was estimated to be 14.0 g/person/day, 0.32 g/kg body weight/day. Based on the composition of regular and high grade **Cardia**Beat™, the 90th percentile all-user consumption of DAG is expected to range between 4.27 and 5.75 g/person/day, equivalent to 0.061 to 0.082 mg/kg body weight/day.

Consumption of all of the proposed food-uses of **Cardia**Beat™ would provide all-user mean and 90th percentile phytosterol ester intakes of 5.06 and 8.40 g/person/day (0.10 to 0.19 mg/kg body weight/day). When the approved food uses were added to the proposed food uses of **Cardia**Beat™, the all-user mean and 90th percentile intake of phytosterol esters was determined to be 6.63 and 12.14 g/person/day (0.13 and 0.25 g/kg body weight/day), respectively. The all-user mean and 90th percentile intakes of phytosterol esters estimated from all currently proposed food-uses of **Cardia**Beat™ and the approved food uses of phytosterol esters are similar to the levels reported to be well tolerated in clinical trials. Based on the estimated intake of **Cardia**Beat™ and phytosterol esters, the resulting 90th percentile all-user intakes of phytosterols, EPA + DHA, and DAG were calculated. Based on the specifications for **Cardia**Beat™, the 90th percentile all-user intake of phytosterols, resulting from both the proposed food uses of **Cardia**Beat™ and the approved GRAS uses of phytosterol esters, is expected to be approximately 8.25 g phytosterol/person/day, equivalent to 0.17 g/kg body weight/day.

In total, approximately 92.5 and 94.3% of the total U.S. population was identified as potential consumers of regular and high grade fish oil based **Cardia**Beat™, respectively (19,060 and 19,438 actual users identified, respectively). On an all-user basis, the mean intake of regular grade fish oil **Cardia**Beat™ by the total U.S. population from all proposed food-uses was estimated to be 4.9 g/person/day (0.09 g/kg body weight/day). The 90th percentile all-user intakes of regular grade fish oil **Cardia**Beat™ from all proposed food-uses was observed to be 9.9 g/person/day (0.18 g/kg body weight/day). On an all-user basis, the mean intake of high grade fish oil **Cardia**Beat™ by the total U.S. population from all proposed food-uses were estimated to be 3.3 g/person/day (0.06 g/kg body weight/day). To calculate the intake of EPA and DHA resulting from the proposed food-uses of regular and high grade fish oil **Cardia**Beat™,

results of the chemical analysis of both forms of fish oil **Cardia**Beat™ were employed. Approximately 44.5% of regular grade fish oil **Cardia**Beat™ was determined to be DHA and EPA (72.5% of the oil is fatty acids and DHA + EPA comprise 61.4% of these by weight) and 38.7% of the high grade fish oil **Cardia**Beat™ is comprised of EPA +DHA. Based on these calculations, the 90th percentile all-user intakes of EPA and DHA were calculated to range between 2.27 and 4.41 g/person/day, equivalent to between 32.4 and 63.9 mg/kg body weight/day.

The type of intake methodology used to estimate intakes of **Cardia**Beat™ and phytosterol esters, and the resulting calculated intakes of phytosterols, EPA + DHA, and DAG, is generally considered to be 'worst case' as a result of several conservative assumptions made in the consumption estimates. For example, it is often assumed that all food products within a food category contain the ingredient at the maximum specified level of use. In addition, it is well established that the length of a dietary survey affects the estimated consumption of individual users. Short-term surveys, such as the typical 2- or 3-day dietary surveys, overestimate consumption of food products that are consumed relatively infrequently.

4.0 PRECLINICAL AND CLINICAL DATA PERTAINING TO *CARDIA*BEAT™

Enzymotec Ltd has conducted an animal and a clinical trial in which the efficacy of **Cardia**Beat™ was examined. In an animal trial conducted by Enzymotec Ltd., 8-week-old apoE^{0} mice were administered a regular chow along with gavage doses of a placebo, canola oil, or regular grade canola oil or fish oil **Cardia**Beat™, with 5 rats employed in each dosing group, for a period of 10 weeks. The administered doses of canola oil and **Cardia**Beat™ were equivalent to approximately 13.9 mg/day or 463 mg/kg body weight/day, providing 2.5 mg phytosterols/day, 83 mg/kg body weight/day, while the regular grade fish oil **Cardia**Beat™ also provided 7.5 mg DHA + EPA/day, 250 mg DHA + EPA/kg body weight (FDA, 1993; personal communication from Enzymotec). At the conclusion of the treatment period, the plasma lipid profile of the mice and parameters pertaining to lipid peroxidation were examined. The consumption of both canola oil and fish oil based regular grade **Cardia**Beat™ was observed to induce a decrease in serum cholesterol levels. The mice receiving both formulations of regular grade **Cardia**Beat™ were reported to exhibit a reduction in serum oxidation as compared to both the canola oil fed and control mice.

The clinical trial conducted by Enzymotec Ltd. was of crossover design and was completed by 21 volunteers who consumed olive oil-based diets (control), supplemented with olive oil, DHA and EPA, commercially available phytosterols, regular grade olive oil **Cardia**Beat™, and regular grade fish oil **Cardia**Beat™ for periods of 4 weeks separated by 4-week washout periods. Baseline control diets provided either up to 200 mg/day phytosterols in the olive oil-based diet or 21.4 g of commercial product of a low-fat sunflower oil-phytosterol ester margarine containing

1.7 g/day phytosterols. The total consumption of **Cardia**Beat™ was approximately 9.2 g/day, providing 1.7 g of phytosterols/day and approximately 5.1 g EPA + DHA/day. The consumption of **Cardia**Beat™ was reported to have a beneficial effect on total, HDL and LDL-cholesterol levels, as well postprandial triglyceride concentrations as compared to the consumption of DHA and EPA. These effects were accompanied by a significant decrease in body weight, BMI, and percentage body fat in individuals consuming **Cardia**Beat™. No significant effects on apolipoprotein B levels were reported in individuals consuming **Cardia**Beat™, however a significant increase in the ratio of apoA/apoB was reported. This as considered to be beneficial as it represents an increase in the ratio of antiatherogenic lipoproteins to proatherogenic lipoproteins.

5.0 DATA PERTAINING TO THE SAFETY OF PHYTOSTEROLS

Phytosterols are sterols present in plants that are structurally related to cholesterol, but differ in their side chain configuration. There are a wide variety of phytosterol structures; however, β-sitosterol, campesterol, and stigmasterol are the most predominant phytosterols identified in nature. Phytosterol esters are comprised of a phytosterol moiety joined *via* an ester bond to a fatty acid. Studies have been performed in both human and animals to elucidate the metabolism of phytosterols, which have indicated that the phytosterol esters is cleaved into the component phytosterol and fatty acid, and therefore these components are discussed independently. Animal toxicity studies have been conducted to examine the toxicological effects of phytosterol consumption and these, as well as other relevant animal studies, are presented in support of the safety of phytosterol consumption. Clinical trials examining the safety of phytosterol consumption were identified and the results of these are supported by trials focussing on the effects on phytosterol consumption of circulating lipid soluble vitamin and carotenoid levels and circulating cholesterol levels. Furthermore, clinical trials examining the safety of phytosterol consumption in individuals heterozygous for phytosteroleamia were identified.

5.1 Absorption, Distribution, Metabolism, and Elimination

Following consumption, the ester bond of phytosterol esters is hydrolyzed, separating the molecule into its component phytosterol and fatty acid (Mattson *et al.*, 1977). Free phytosterols are unable to cross the intestinal wall alone and so they are incorporated into micelles, which also contain fatty acids, phospholipids, and bile salts, and the micelles are taken up by enterocytes (Trautwein *et al.*, 2003). Within the enterocytes phytosterols are incorporated into chylomicrons which are excreted into lymphatic ducts located in the intestinal wall, which in turn deposit the chylomicrons into systemic circulation for distribution and metabolism (Boberg and Skrede, 1988; Ling and Jones, 1995; Cormack, 2001; Trautwein *et al.*, 2003). Approximately 5% of an ingested dose of phytosterols is absorbed into the systemic circulation (Sylven and

Borgstrom, 1969; Salen *et al.,* 1989; Miettinen *et al.,* 1990; Pegel *et al.,* 1997; Sanders *et al.*, 2000; Katan *et al.*, 2003; Trautwein *et al.*, 2003).

Once in systemic circulation, phytosterols are distributed to the liver where they are incorporated in lipoproteins, which are then secreted back into systemic circulation and distributed to various tissues (Tilvis and Miettinen, 1986; Leikin and Brenner, 1989). In rats and rabbits, phytosterols have been reported to favourably distribute to the abdominal organs, the lung, the liver, the intestinal tract, the kidneys, the spleen, the adrenal glands, and the bone marrow of male and female rats and rabbits, as well as and the ovaries of female rats and rabbits (Bhattacharyya and Lopez, 1979; Sanders *et al.*, 2000). The strongest retention of phytosterols 96 hours after dosing was reported to occur in the lung where 50% of an administered dose of sitosterol was 96 hours after administration (Sanders *et al.*, 2000).

In the liver, phytosterols are converted into bile acids for excretion and although the exact mechanism behind this conversion is unknown it is believed to involve hydroxylation of phytosterols at C_{21} followed by peroxisomal or mitochondrial β-oxidation (Lund *et al.,* 1991). This process results in the creation of C_{21} bile acids, which can be secreted into the gastrointestinal tract for excretion in the feces (Boberg *et al.*, 1990a,b). Analysis of the feces of hypercholesterolemic subjects who consumed 1,005 mg/day of free sitosterol for 9 weeks indicated that over 95% of the sitosterol in the feces was unesterified (Miettinen and Vanhanen, 1994). Sanders *et al.* (2000) reported that the predominant route of phytosterol excretion in the rats was the feces, in which 75 to 96% of an administered dose was recovered within 24 hours of dosing. In humans, the elimination of phytosterols also has been reported to occur primarily in the bile with minor proportions occurring through the skin (Bhattacharyya *et al.,* 1983; Boberg and Skrede, 1988; Weststrate *et al.*, 1999; Ayesh *et al.*, 1999; Sudhop *et al.*, 2002). Bhattacharyya *et al.* (1983), reported that phytosterols were absorbed from the diet and distributed to skin surface lipids from the plasma, and then lost during the sloughing of the epidermis.

5.2 Toxicological Studies

5.2.1 Acute Studies

No effects were observed following the administration f 3.2 g sitosterol/kg body weight; therefore, the oral LD_{50} value is greater than 3.2 g/kg body weight (Gupta *et al.*, 1980).

5.2.2 Subchronic and Chronic Studies

Repeated dose toxicity studies involving phytosterols and phytosterol esters have been conducted in rats, rabbits, and dogs (Shipley *et al.*, 1958; Hepburn *et al.*, 1999; Kim *et al.*, 2002). In all of the identified subchronic toxicity studies, phytosterol esters formulations with a composition similar to that of **Cardia**Beat™ were administered. The studies ranged in length from 90 days to 22 months in length. Phytosterol esters doses administered ranged between

160 to 9,000 mg/kg body weight/day and these were either administered in the diet or by gavage. The results of the toxicity studies examined indicated that there are no adverse effects associated with phytosterol consumption in any of the previously mentioned animal species. Although several authors noted significant differences in some of the parameters examined, no authors determined these differences to be of any toxicological or biological significance. NOAELs of 3,000 mg phytosterol ester/kg body weight/day for male and female Sprague-Dawley rats in a 13-week toxicity study, and 6,600 mg phytosterol ester/kg body weight/day, equivalent in this instance to approximately 4,100 mg phytosterol/kg body weight/day mg/kg, for male and female Wistar rats in a 90-day toxicity study were identified.

Additionally, several studies were identified in which safety-related endpoints were examined although the studies themselves were not conducted to assess the safety of phytosterols or phytosterol esters (Baker *et al.*, 1999; Ntanios *et al.*, 2002; Kritchevsky *et al.*, 2003; Wilund *et al.*, 2004). Phytosterols were reported to induce no significant changes in uterine weights when administered to 21 to 22 day old female Wistar rats, while increases in uterine weights were observed in the rats administered both weak and strong phytoestrogens as positive controls (Baker *et al.*, 1999). When diets containing phytosterols were administered to mice with varying degrees of sitosteroleamia, it was concluded by the authors that plant phytosterols are not more atherogenic in normal, hypercholesterolemic, or G5G8-/- mice (Wilund *et al.*, 2004). Additionally, no safety concerns or aortic foam cell formation was associated with the consumption of up to 3,408 mg/kg body weight/day of phytosterol esters by golden Syrian hamsters for 12 weeks (Ntanios *et al.*, 2003). In rabbits, no signs of toxicity were observed following the consumption of diets enriched with 500 mg of a phytosterol rich spread for 60 days (Kritchevsky *et al.*, 2003).

In a several animal studies conducted by Ratnayake *et al.* (2000a, b, 2003), stroke-prone spontaneously hypertensive (SHRSP) rats consuming diets containing canola oil and various vegetable oils were reported to have a shortened lifespan and were reported to be subject to a greater risk of hemorrhagic stroke. The conclusions of Ratnayake *et al.* (2000a,b, 2003) were contradicted by the studies of Tatematsu *et al.*, 2004 who again fed different vegetable oil, oil fractions enriched in phytosterols and vegetable oils with added phytosterols isolated from soybean oil to groups (12 male rats) of male SHRSP rats for up to 180 days. Whereas canola oil again significantly shortened the life span of the rats, free fatty acid fractions (containing the phytosterols) did not shorten the life span of the rats indicating that a different component of the canola oil was responsible for this shortening of life span.

5.2.3 Developmental and Reproductive Toxicity Studies

A 2-generational reproductive study was carried out in Wistar rats in which male and female rats were administered diets containing 0 (control), 1.6, 3.2, or 8.1% phytosterol esters, for a 10-week pre-mating period, and subsequently to the male and female F_1 and F_2 offspring (Waalkens-Berendsen *et al.*, 1999). Phytosterol esters were reported to have no effect on any of the fertility or reproductive parameters examined, to produce no adverse developmental or

reproductive effects, and to possess no estrogenic activity. The authors concluded that the NOAEL was the highest dosing level of 8.1% phytosterol esters in the diet, which is equivalent to 2.5 to 9.1 g of phytosterol esters/kg body weight/day, equivalent to between 1.54 and 5.62 g phytosterol/kg body weight/day, depending on the phase of the study.

5.2.4 Genotoxicity Studies

Phytosterols derived from common edible vegetable oils, and phytosterol esters derived from the same oils both produced negative results in Ames, chromosomal aberration, and cell mutation assays conducted in various strains of *S. typhimurium* and *E. coli*, human peripheral blood lymphocytes, and L5178Y mouse lymphoma cells, respectively, in both the absence and presence of S9 microsomal fractions (Wolfreys and Hepburn, 2002). In a micronucleus assay, examination of the bone marrow of rats administered 1,000, 2,000, and 4,000 mg phytosterol esters/kg body weight/day indicated that exposure to phytosterol esters was not toxic to the bone marrow (Wolfreys and Hepburn, 2002). Cytoplasmic graining was observed in rat hepatocytes collected 2 to 4 hours following exposure to 800 or 2,000 mg/kg body weight of phytosterol esters; however, no cytoplasmic graining was reported in the hepatocytes collected 12 to 14 hours after exposure and no further indications of an increase in cellular proliferation were identified leading to the conclusion that the graining was likely to be spurious and unrelated to exposure to phytosterol esters.

5.2.5 Other Animal Studies

Baker *et al.* (1999) examined the estrogenic potential of phytosterol esters both *in vitro* and *in vivo*. The authors reported that phytosterols did not compete with H^3-E_2 for binding to the oestrogen receptor at concentrations of up to 10-4 mol/L, while β-estradiol did displace H^3-E_2 from the oestrogen receptors. Additionally, phytosterols did not stimulate transcriptional activity, while a strong, dose-dependent stimulation of transcriptional activity was observed in the yeast incubated with β-estradiol.

5.2.6 Clinical Studies

In clinical trials conducted to examine the safety of phytosterol consumption, no adverse effects were reported to result from the consumption of up to 9.0 g phytosterols/day for periods of 8 to 52-weeks in length (Davidson *et al.,* 2001; Hendriks *et al.*, 2003; Katan *et al.*, 2003; St-Onge and Jones, 2003). The results of clinical trials conducted to examined various effects of phytosterol consumption, though not specifically addressing the safety of phytosterol consumption, indicate that there are no adverse effects associated with the consumption of up to 8.6 g phytosterols/day for periods as long as 52 weeks.

No clinical trials were identified in which a significant decrease in the circulating levels of lipid soluble vitamins was observed following the consumption of 0.83 to 3.6 g of phytosterols for periods of 3.5 to 52 weeks (Hendriks *et al.*, 1999, 2003; Hallikainen *et al.*, 2000; Christiansen *et*

al., 2001; Davidson *et al.*, 2001; Judd *et al.*, 2002; Ntanios *et al.*, 2002; Seki *et al.*, 2003). Several clinical trials were identified in which the consumption of phytosterols was reported to induce a significant decrease in plasma carotenoid levels; however, it also was reported that individuals consuming one or more servings of β-carotene rich vegetables or β-carotene enriched foods were reported to exhibit a significant increase in plasma α- and β-carotene levels and no significant change in any other plasma carotenoid concentrations while consuming up 3.2 g phytosterols/day (Hendriks *et al.*, 1999, 2003; Judd *et al.*, 2002; Noakes *et al.*, 2002; Ntanios *et al.*, 2002; Raeini-Sarjaz *et al.*, 2002; Quilez *et al.*, 2003). Additionally, no significant changes in plasma α- and β-carotene concentrations were reported following the administration of up to 9.0 g phytosterols/day for periods as long as 6 months in length (Hallikainen *et al.*, 2000; Christiansen *et al.*, 2001; Davidson *et al.*, 2001; Seki *et al.*, 2003).

All of the clinical trials reviewed, with the exception of one conducted by Seki *et al.* (2003), indicated that consumption of spreads, oils, milk, yoghurt, orange juice, ground beef, and bakery products containing phytosterol esters resulted in a decrease in total and LDL cholesterol levels (Hendriks *et al.*, 1999, 2003; Hallikainen *et al.*, 2000; Amundsen *et al.*, 2001; Christiansen *et al.*, 2001; Nigon *et al.*, 2001; Stalenhoef *et al.*, 2001; Judd *et al.*, 2002; Matvienko *et al.*, 2002; Noakes *et al.*, 2002; Ntanios *et al.*, 2002; Simons, 2002; Kwiterovich *et al.*, 2003; Cleghorn *et al.*, 2003; Lee *et al.*, 2003; Quílez *et al.*, 2003; Clifton *et al.*, 2004; Devaraj *et al.*, 2004). The trial conducted by Seki *et al.* employed the lowest phytosterol doses of all the clinical trials reviewed, 450 mg/day, and reported no significant change in LDL cholesterol levels and a significant decrease in VLDL cholesterol levels. Additionally, clinical trials have indicated that individuals heterozygous for phytosterolaemia do not accumulate phytosterols and therefore do not experience the problems of phytosterolaemic individuals (Stalenhoef *et al.*, 2001; Kwiterovich *et al.*, 2003).

5.3 Summary

Phytosterol esters are hydrolyzed into their component phytosterols and fatty acids in the gastrointestinal tract. The resulting phytosterols are poorly absorbed, and those that are absorbed tend to be secreted into the bile and excreted in the feces. Subchronic and chronic toxicity studies conducted in several laboratory animal species, have indicated that there are no adverse effects associated with dietary consumption of phytosterols. Additionally, no adverse effects were reported in developmental and reproductive toxicity studies, and no significant positive results were reported in genotoxicity or mutagenicity assays. NOAELs of 3 g of phytosterol esters and 4.8 g of phytosterols/kg body weight were reported in 13-week toxicity studies conducted in rats, while NOAELs of 1.5 to 5.6 g phytosterols/kg body weight/day were reported in reproductive toxicity studies also conducted in rats. In clinical trials, the administration of up to 9.0 g phytosterol/day was reported to have no adverse effect on lipid soluble vitamin or carotenoid levels and produces no serious adverse effect in normal individuals or those heterozygous for phytosterolemia.

Based on the food uses for which phytosterol esters have been approved, and the proposed food uses for **Cardia**Beat™, the 90th percentile all-user intakes of phytosterol esters was determined to be 12.14 g/person/day or 0.25 g/kg body weight/day, which is estimated to be equivalent to 7.28 g phytosterols/day or 0.15 g/kg body weight/day. On a body weight basis, this intake is approximately 14 to 27-fold lower than dietary NOAELs reported in animal toxicity studies conducted in male and female rats. Additionally, NOAELs at least 8 times higher than the estimated 90th percentile all-user intake of phytosterols were reported in developmental and reproductive toxicity studies conducted in rats. In clinical trials, the administration of phytosterol doses similar to the 90th percentile all-user intake of phytosterols have been reported to be well tolerated and produce no adverse effects. Overall, the intake of phytosterol esters, and resultant phytosterols, resulting from the use of **Cardia**Beat™ is expected to pose no safety concerns.

6.0 DATA PERTAINING TO THE SAFETY OF EPA AND DHA

DHA and EPA are long-chain polyunsaturated fatty acids, also referred to as n-3 or omega-3 fatty acids, that are present at high levels in fish oils (PDRNS, 2001). Studies have been performed in both human and animals to elucidate the metabolism of EPA and DHA. Animal toxicity studies have been conducted to examine the toxicological effects of EPA and DHA consumption and these, as well as other relevant animal studies, are presented in support of the safety of DHA and EPA consumption. Clinical trials examining the safety of EPA and DHA consumption were identified and the results of these are supported by trials focussing on the effects of EPA and DHA consumption on bleeding time, glycemic control, and LDL cholesterol levels.

6.1 Absorption, Distribution, Metabolism, and Elimination

In the gastrointestinal tract, EPA and DHA triglycerides are hydrolyzed into monoglycerides and free fatty acids (PDRNS, 2001). The free fatty acids and monoglycerides then diffuse across the intestinal cell wall into enterocytes, where they are reassembled into triacylglycerols, which are then packaged into chylomicrons with phospholipids, cholesterol, and apolipoproteins (Nelson and Cox, 2000; PDRNS, 2001). Enterocytes excrete chylomicrons into lymphatic ducts located in the intestinal wall, which transport the chylomicrons into systemic circulation for distribution and metabolism (Cormack, 2001). EPA and DHA free fatty acids were reportedly completely absorbed when consumed with low and high fat meals, while 20 and 69% of ethyl esters and triglycerides, respectively, were absorbed when consumed with a low fat meal, and 60 and 90% were absorbed with a high fat meal, respectively (Lawson and Hughes, 1988b).

From general circulation, EPA and DHA are distributed to various tissues including the brain, the eye, the liver, the kidney, red blood cells, and adipose tissue, where they are incorporated into membrane phospholipids (Vidgren *et al.*, 1997; Fenton *et al.*, 2001; Yasui *et al.*, 2001;

Berson *et al.*, 2004). DHA is preferentially taken up by the brain and is transported across the placenta into foetal circulation during development (PDRNS, 2001). Postprandial uptake of EPA has been reported to be more rapid than postprandial uptake of DHA (Hodge *et al.,* 1993; Gibney and Daly, 1994); however, the uptake DHA has been reported to be more complete than that of EPA (London *et al.,* 1991; Gibney and Hunter, 1993; Hodge *et al.,* 1993; Gibney and Daly, 1994). An extensive study of the incorporation of fish oil fatty acids was conducted by Katan *et al.* (1997), who reported that EPA and DHA were incorporated into cholesterol esters to varying degrees within 3 days of fish oil administration, with half-maximal concentrations reached after 4.8 and 10.3 days for EPA and DHA, respectively. Additionally, the time required for the incorporation of EPA and DHA into membranes varied between tissues, with the slowest times observed in adipose tissues and red blood cells.

Generally, the metabolism of fatty acids occurs in the mitochondria *via* β-oxidation, which involves a progressive, cyclical, shortening of the fatty acid carbon chain to produce acetyl CoA, (Linscheer and Vergroesen, 1994; Krummel, 1996). DHA does not undergo mitochondrial β-oxidation to the same extent as shorter chain fatty acids, leading to some preservation of DHA in tissue membrane phospholipids where it plays an important role in maintaining membrane fluidity (Hsia *et al.*, 1989). Approximately 10% of DHA is retro-converted to EPA by a process involving β-oxidation and reductase and isomerase enzymes (Brossard *et al.*, 1996; PDRNS, 2001). In red blood cells, EPA and DHA are employed in the synthesis of eicosanoids, which produce compounds that regulated smooth muscle contraction, inflammation, and platelet function (Nelson and Cox, 2000). With a chain length the same as arachidonic acid, but with one more double bond, EPA may substitute for arachidonic acid in the pathways of eicosanoid synthesis. This substitution results in the formation of similar structures with very different activities, which combine to result in an overall anti-inflammatory effect (Terano *et al.,* 1984; James *et al.,* 2000).

Studies of the decay of the EPA and DHA have demonstrated that EPA levels decline more rapidly than DHA levels. In a study conducted by Brown *et al.* (1991) to examine the elimination of EPA and DHA, volunteers were fed 3 different fish oil diets for 6 weeks each with 6 weeks washout between each. Twelve weeks after fish oil supplementation, only 16% of EPA was retained in erythrocytes, as compared to 44% of DHA. After 18 weeks, DHA levels were still higher than baseline levels (Brown *et al.,* 1991). Similarly, Von Schacky *et al.* (1999) found that DHA levels had not returned to normal 20 weeks after cessation. It has been suggested that the reason for the longer staying power of DHA is a result of the conversion of EPA to DHA, but there is little human evidence of this, and it is not universally supported (Hodge *et al.,* 1993).

6.2 Toxicological Studies

6.2.1 Acute Studies

The administration of a single dose of 2,000 mg/kg body weight of an oil containing approximately 45% DHA was reported to produce no serious adverse effects on clinical parameters or macroscopic necropsy observations (Kroes *et al.*, 2003).

6.2.2 Subchronic and Chronic Studies

Several studies were identified in which the effects of DHA and EPA consumption was examined in rats and pigs and although these studies were not conducted specifically to assessment to safety of EPA and DHA consumption, they measured safety-related endpoints and therefore, support the safety and tolerability of EPA and DHA. (Arterburn *et al.*, 2000; Hung *et al.*, 2000; Minami *et al.*, 2002; Meritt *et al.*, 2003; Poulsen and Kruger, 2004). In general, all of the data reviewed indicated that doses of up to 1.3 g/kg body weight/day of EPA or DHA are well tolerated and produce no serious adverse effect when administered for periods of up to 25 weeks in length. One 9-week study was identified in which administration of 1.0 g EPA/kg body weight/day to ovariectomised rats was reported to negatively influence bone density; however no further animal or clinical trials were identified to substantiate this result (Kruger *et al.,* 1998; Albertazzi and Coupland, 2002; Poulsen and Kruger, 2004).

6.2.3 Developmental and Reproductive Toxicity Studies

An assessment of the reproductive safety of a DHA oil was identified in which male and female Sprague-Dawley-derived (Cr1:CD(SD)BR) rats were administered diets containing 0 (control), 0.6, 6.0 or 30% of the oil, equivalent to approximately 33 to 1,512 mg DHA/kg body weight/day, prior to mating, during the mating period, and after the mating period (Hammond *et al.*, 2001a). DHA was reported to have no effects on the reproductive performance of the male and female rats, or on the physical development of the pups. In a follow up analysis of the developmental effects of the DHA rich oil, male and female Sprague-Dawley rats were provided with diets providing approximately 39 to 1,800 mg DHA/kg body weight/day and male and female New Zealand white rabbits were administered gavage doses of 180, 600 or 1,800 mg/kg body weight of the oil throughout Days 6 through 19 of gestation (Hammond *et al.*, 2001b). No compound-related gross pathological changes or developmental toxicity was observed in both maternal and foetal rats and rabbits at any of the dose levels. The authors reported NOELs of 1,823 mg DHA/kg body weight/day for pregnant rats and foetuses, and NOELs of 600 and 1,800 mg DHA rich oil/kg body weight/day for pregnant rabbits and foetuses, respectively.

6.2.4 Genotoxicity Studies

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Negative results were reported for Ames assays in which several strains of S. typhimurium were exposed to doses of a DHA rich oil ranging from 0.5 to 5.0 mg/plate, with and without metabolic activation (Kroes *et al.*, 2003). Additionally, the application of 1.25, 2.5 or 5.0 mg of the oil, with

and without metabolic activation, to Chinese hamster fibroblast cells was reported to induce no chromosomal aberrations (Kroes *et al.*, 2003).

5.2.5 Clinical Studies

The FDA has established that the daily intake of EPA + DHA fatty acids should be limited to 3 g/day due to the potential adverse effects of greater consumption on bleeding times, fasting glucose control in individuals with NIDDM, and LDL cholesterol levels. The majority of the clinical trials reviewed addressed these three issues and examined the effect of EPA + DHA doses greater than the 3 g/day limit established by the FDA.

More than 40 clinical trials examining the effect of DHA and EPA consumption on bleeding time and parameters related to blood clotting and fibrinolysis were reviewed. The trials consisted of EPA and DHA dosing regimes ranging between 3.3 g/day for 3 weeks and 10 g/day for 6 weeks, with only one of the reviewed trials observing a significant increase in bleeding times to a level above the established normal range of bleeding times. The clinical trial that reported the significant increase in bleeding times was a 6-week open challenge study of questionable design, with 8 patients with chronic glomerular disease and no control group, (Lenzi *et al.*, 1996). Clinical trials examining parameters of blood clotting revealed that the incorporation of DHA and EPA into phospholipids membranes significantly altered clotting mediators and responses; however the physiological response to these alterations was generally determined to have little biological significance (Nilsen *et al.*, 1993; Scheurlen *et al.*, 1993; Leaf *et al.*, 1994; Prisco *et al.*, 1994; Turini *et al.*, 1994; Luostarinen *et al.*, 1995; Prisco *et al.*, 1995; Mori *et al.*, 1997; Hansen *et al.*, 2000). In a 1-year study conducted by Eritsland *et al.* (1995a, 1996), no increase in plasminogen activator inhibitor (PAI) activity was observed in individuals concurrently administered aspirin or warfarin with doses of 3.4 g DHA + EPA/day, despite similar bleeding times to controls on aspirin or warfarin only. Freese and Mutanen (1997a) reported no effects on PAI following 4 weeks of a mean dose of 5.2 g DHA + EPA/day, whereas Nilsen *et al.* (1993) reported a significant increase in PAI activity following >2 months of 5.1 g DHA + EPA/day; however a similar significant increase in PAI activity also was observed in the placebo group calling into question the validity of these results.

Approximately 20 clinical trials were identified in which the effect on DHA and EPA on variables related to glycemic control were examined. Of these trials, the vast majority reported that doses of >3 g DHA + EPA/day did not alter blood glucose or glycosylated haemoglobin concentrations. Furthermore, the majority of the trials also indicated that DHA and EPA also had no effect on plasma insulin levels (McVeigh *et al.*, 1994; Fasching *et al.*, 1996; McGrath *et al.*, 1996; Sheehan *et al.*, 1997; Sirtori *et al.*, 1997, 1998; Luo *et al.*, 1998). In particular, the results of a 12-week study conducted in hyperlipidemic NIDDM patients indicated that 10.1 g DHA + EPA/day had no significant effect on fasting glucose and glycosylated haemoglobin levels (Morgan *et al.*, 1995). Similar results were reported by Connor *et al.* (1993) who administered 6 g DHA + EPA/day to NIDDM patients for six months, and Luo *et al.* (1998) who administered 6 g

fish oil (1.2 g DHA + EPA)/day and measured glycemic control parameters using a euglycemic-hyperinsulinemic clamp. An increase in fasting glucose levels was reported in two clinical trials, both of which involved patients with abnormal lipid metabolism who were administered 3.6 to 3.84 g DHA + EPA/day for 6 to 8 weeks (Mori *et al.*, 2000; Silva *et al.*, 1996). Mori *et al.* (2000) reported that glucose levels increased significantly with 3.84 g EPA/day, but not with 3.84 g DHA/day, and that fasting insulin levels were significantly increased following both treatments. Silva *et al.* (1996) reported a significant increase in blood glucose levels of receiving 3.6 g DHA + EPA/day for a period of 2 months.

More than 40 clinical trials were identified in which the effect of DHA and EPA supplementation on plasma lipids and triacylglycerols, and specifically LDL cholesterol levels were examined. While some trials did report an increase in LDL cholesterols levels associated with DHA and EPA consumption, the majority clearly indicated that DHA + EPA supplementation did not significantly alter or significantly decrease LDL cholesterol levels. Alternatively, Adler and Holub (1997) reported that dietary supplementation with garlic powder, concurrent with daily fish oil supplementation, negated the minor significant increase in LDL observed with fish oil alone. Nordøy *et al.* (1998) observed that the increase in LDL cholesterol following supplemental treatment of hyperlipidemic individuals with fish oil ω-3 fatty acids does not occur in individuals consuming a cholesterol-inhibiting drug such as simvastatin. In hypercholesterolemic patients undergoing chronic treatment with simvastatin, Balestrieri *et al.* (1996) reported daily treatment with 5.1 g DHA + EPA/day had no effect on LDL cholesterol levels. Additionally, it should be noted that in all of the studies in which an increase in LDL cholesterol was observed, the subjects were those with established health conditions such as NIDDM, hypertriglyceridemia, IDDM, hypercholesterolemia, or hypertension (Connor *et al.*, 1993; Harris *et al.*, 1993, 1997; Gray *et al.*, 1996; Otto *et al.*, 1996; Rossing *et al.*, 1996; Swahn *et al.*, 1998; Mori *et al.*, 2000). Furthermore, the clinical trials in which LDL cholesterol levels were unchanged contained both healthy patients and patients with pre-existing conditions, including CHD, and these patients were not affected by DHA and EPA doses as high as 10.1 g/day.

Clinical trials were identified in which several additional parameters relating to the liver and kidney function, lipid peroxidation, visual acuity, and measures of the immune system were examined and these indicated that DHA and EPA had no adverse effects on any of these parameters. No adverse effects on liver or kidney function were reported following the administration of up to 6 g of DHA and EPA/day for periods as long as a year (Clark *et al.*, 1993; Henderson *et al.*, 1994; Eritsland *et al.*, 1995b; Fasching *et al.*, 1996; Gray *et al.*, 1996; Lenzi *et al.*, 1996; Rossing *et al.*, 1996; Silva *et al.*, 1996; Badalamenti *et al.*, 1997; Harris *et al.*, 1997; Nordøy *et al.*, 1998; Grundt *et al.*, 2003). Additionally, no adverse health effects were reported to result from disruption of immune system mediators caused by consumption of EPA and DHA for periods of up to 6 months (Gogos *et al.*, 1998; Almallah *et al.*, 2000; Kelley *et al.*, 1999). The maximum tolerated dose of fish oil in terminally ill patients was reported to be 21 g/day, providing approximately 13.2 g DHA + EPA/day for a 70 kg individual, at which point Grade 3

(non-life threatening and lead to at least a 50% reduction in the administered fish oil dose) gastrointestinal adverse effects were reported (Burns *et al.*, 1999).

6.3 Summary

EPA and DHA are well absorbed from the gastrointestinal tract, distributed to cellular membranes in a variety of tissues in the body including the brain, the eye, the liver, the kidney, red blood cells, and adipose tissue, and metabolized in those tissues where they are often employed in the synthesis of eicosanoids. DHA has been reported to possess low toxicity in acute, subchronic, and chronic animal toxicity studies. Additionally, no reproductive or developmental toxicity has been reported for DHA, which also has been reported to produce negative results *in vitro* mutagenicity and genotoxicity studies. In subchronic and reproductive toxicity studies, NOAELs of 500 to 1,823 mg/kg body weight/day were reported following the dietary administration of DHA to rats. A NOAEL of 600 mg/kg body weight/day was reported for DHA in a reproductive toxicity study conducted in rabbits. In clinical trials, no serious adverse events were reported following dietary consumption of EPA and DHA has been examined for periods of up to 4 years in length and individuals have been administered daily doses of up to 10.1 g EPA + DHA.

The amount of DHA and EPA provided from the proposed food-uses of **Cardia**Beat™ is expected to range between 2.28 and 4.41 g/person/day. Subchronic and chronic animals trials have indicated that DHA and EPA possess low oral toxicity. Clinical trials conducted with DHA and EPA doses that are double the highest estimated 90th percentile intake provided by the proposed food uses of fish oil **Cardia**Beat™ have indicated that DHA and EPA consumption produced no adverse effects on bleeding times, LDL cholesterol levels, and fasting glucose levels in individuals with NIDDM. Furthermore, the current consumption estimates likely overestimate the consumption of all population groups considering that all manufacturers are unlikely to use the maximum regulatory limit in all permitted food types and the use of short-term food consumption databases to estimate long-term consumption. Additionally, the DHA + EPA intake provided are 90th percentile intakes whereas the limit established by the FDA is a mean intake. Therefore, the amount of DHA and EPA provided by the estimated 90th percentile all-user intake of **Cardia**Beat™ is not expected pose any safety concerns.

7.0 DATA PERTAINING TO THE SAFETY OF DIACYLGLYCEROL

Diglycerides are contained in **Cardia**Beat™ as 12 to 20% by weight of the finished product. DAG are produced endogenously during the digestion of TAG, and are also encountered in the diet through the consumption of vegetable and animal fats and oils (Flickinger and Matsuo, 2003; Yasunaga *et al.*, 2004). Edible oils generally contain 2 to 10 % DAG, of which the majority is present as 1,3-DAG (Flickinger and Matsuo, 2003; Kondo *et al.*, 2003). Studies have been performed in both human and animals to elucidate the metabolism of DAG, as well as the

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relationship between TAG and DAG metabolism. Animal toxicity studies have been conducted to examine the safety of DAG consumption and these, as well as animal studies examining the digestibility of DAG, are presented in support of the safety of DAG consumption. Clinical trials examining the safety and efficacy of DAG consumption were identified and presented to provide support for the safety of long-term consumption of DAG by humans.

7.1 Absorption, Distribution, Metabolism, and Elimination

Following ingestion, diglycerides and triglycerides are both digested by pancreatic lipases to produce 2-monoglycerides and 1-monoglycerides, respectively (Flickinger and Matsuo, 2003; Kondo *et al.*, 2003). The breakdown products of tri and diglycerides are then absorbed across the intestinal wall into enterocytes, where triglycerides are reassembled by the sequential addition of free fatty acid to monoglycerides by monoacylglyceride (MAG) acetyltransferase (MGAT) and DAG acetyltransferase (DGAT) (Flickinger and Matsuo, 2003; Kondo *et al.*, 2003). Triglycerides are then packaged into chylomicrons and secreted into the lymph, and eventually reach generally circulation (Nelson and Cox, 2000). The digestion products of diglycerides are absorbed from the gastrointestinal tract with equal efficiency as the triglyceride digestion products, approximately 96% of an ingested dose; however, consumption of diglycerides results in lower circulating levels of triglycerides than does triglyceride consumption in both humans and animals (Taguchi *et al.*, 2000, 2001; Tada *et al.*, 2001).

The metabolism of diglycerides is similar to that of triglycerides, with the primary difference being that diglyceride consumption results in the formation of fewer triglycerides (Taguchi *et al.*, 2000, 2001; Tada *et al.*, 2001). Once in circulation, triglycerides are metabolized as a source of energy, producing approximately 38 kJ of metabolizable energy/g (Taguchi *et al.*, 2001). Both DAG and TAG oils have high digestibility values, with approximately 96% of an ingested dose absorbed from the gastrointestinal tract and the remaining 4% is excreted in the faeces (Taguchi *et al.*, 2001). The clearance of chylomicrons from the blood stream is slow, allowing triglycerides to remain in circulation for approximately 10 hours facilitating the deposition of TAG into the arterial wall in the formation of plaques (Taguchi *et al.*, 2000). Chylomicrons containing triglycerides are cleared from circulation either by enzymatic hydrolysis or by receptor-mediated uptake into the tissues, where triglycerides are completely consumed in the production of metabolic energy (Taguchi *et al.*, 2001).

7.2 Toxicological Studies

7.2.1 Subchronic and Chronic Studies

A chronic toxicity study was identified in which the effects of the dietary administration of up to 2,650 mg DAG oil/kg body weight/day for 105 weeks were examined in male and female Sprague-Dawley rats (Soni *et al.*, 2001). The authors concluded that the consumption of diets containing up to 5.3% DAG oil, providing 2,650 mg DAG oil/kg body weight/day, for 2 years did not produce any adverse effects in male and female Sprague-Dawley rats. Additionally, in a 4

week digestibility study, the consumption of diets providing approximately 36 mg DAG oil/ kg body weight/day was reported to produce no adverse effects on the food consumption or the body weight gain of male Sprague-Dawley rats (Taguchi *et al.*, 2001).

7.2.2 Genotoxicity Studies

An oil containing more than 80% DAG, with a DAG composition similar to that of **Cardia**Beat™, was employed in Ames assays conducted in *S. typhimurium* strains TA98, TA100, TA1535, TA1537 and *E. coli* WP2*uvr*A (Kasamatsu *et al.*, 2005). No mutagenicity was observed following the incubation of the bacteria with either the natural DAG oil or a DAG oil that had been heated to simulate deep drying conditions, at doses ranging from 5 to 5,000 µg/plate, both with and without metabolic activation. The same heated and unheated oils were employed in a chromosomal aberration assay conducted in Chinese hamster lung cells, and neither DAG oil was observed to induce chromosomal aberrations following either short-term or continuous incubation periods, with and without metabolic activation (Kasamatsu *et al.*, 2005). In an *in vivo* micronucleus assay examining the bone marrow cells of ICR(Crj:CD-1) mice orally administered 500, 1,000, or 2,000 mg oil/kg body weight of the same heated and unheated DAG oils, no significant increase in the incidence of micronucleated polychromatic erythrocytes was observed in any of the DAG oil groups (Kasamatsu *et al.*, 2005). A significant difference in the polychromatic erythrocyte/normochromatic erythrocyte ratio was observed in the mid and high-dose groups receiving unheated and heated DAG oil, respectively; however, this was attributed to intraspecific variability and determined by the authors to be of no toxicological significance.

7.2.3 Clinical Studies

The safety of high-dose diacylglycerol oil consumption in men and women was examined in a 12-week clinical trial conducted by Yasunaga *et al.* (2004) in which study participants were instructed to consume an equivalent of at least 500 mg/kg body weight/day DAG or TAG oil/day. There were no significant differences observed in the number or severity of complaints reported by individuals in the TAG and DAG oil groups, and no serious adverse effects or biologically or toxicologically significant changes in any of the measured parameters were observed in any of the study participants. The authors concluded that the consumption of up to 500 mg DAG oil/kg body weight for 12 weeks was well tolerated and produced no adverse effects.

Several clinical trials were identified in which the effects of the consumption of oils containing high amount of 1,3-DAG was examined (Nagao *et al.*, 2000; Taguchi *et al.*, 2000; Tada *et al.*, 2001, 2005; Maki *et al.*, 2002). The trials ranged from 1 day to 24 weeks in length and employed healthy male, diabetic male, or overweight and obese male and female participants. The DAG oil was administered in various forms, baked goods, spreads, bars, and emulsions, resulting in daily DAG oil intakes of 10 to 45 g/person/day. No significant differences were observed between the adverse events reported by the DAG and TAG oil groups in any of the clinical trials reviewed.

7.3 Summary

A chronic toxicity study conducted male and female Sprague-Dawley rats indicated that no adverse effects are associated with the consumption of up to 2,650 mg/kg body weight/day of a DAG rich oil in the diet. Genotoxicity assays conducted both *in vitro* and *in vivo* have demonstrated that DAG oil and DAG oil subjected to intense heating conditions does not possess genotoxic potential. In clinical trials, the consumption of up to 45 g/day of a DAG rich oil with a composition similar to the DAG composition of **Cardia**Beat™ (7:3 1,3-DAG to 1,2-DAG ratio) for up to 24 weeks has been reported to be well tolerated and produce no adverse effects.

8.0 OVERALL SUMMARY

CardiaBeat™ are a family of oils that result from transesterification of different vegetable oils/fish oil/fats with vegetable oil phytosterols to form a mixture containing phytosterol esters and containing a significant amount of diglycerides derived from the original triglyceride fraction of the original oil or fat. The daily dietary intake of phytosterols from the typical western diet has been estimated to range between 160 and 360 mg/day. The FDA has determined that the intake of EPA and DHA from the diet should not exceed 3 g/day and the adequate daily dietary intake of EPA and DHA fatty acids has been reported to be 650 mg/day for adults and 300 mg DHA/day for and pregnant and nursing women. The daily dietary intake of mono- and diglycerides resulting from their use as food additives has been reported to be 3.61 g/person/ day, equivalent to 51.6 mg/kg body weight/day for a 70 kg individual.

The mean and 90th percentile all-user intakes of phytosterol esters resulting from all proposed food uses or regular and high grade **Cardia**Beat™ were determined to be 5.06 and 8.40 g/person/day (0.10 and 0.19 g/kg body weight/day), respectively, and with the addition of foods that are GRAS for phytosterol use to the intake assessment, mean and 90th percentile all-user intakes of phytosterol esters were calculated to be 6.63 g/person/day (0.13 g/kg body weight/day) and 12.14 g/person/day (0.25 g/kg body weight/day), respectively. Based on compositional data available for phytosterol esters, this intake is expected to correspond to an all-user 90th percentile phytosterol intake of approximately 8.25 g phytosterol/ person/day (0.17 g/kg body weight/day). Under the conditions of intended use of regular and high grade non-fish oil based **Cardia**Beat™, the all-user mean and 90th percentile daily intakes are estimated to be 4.9 and 9.9 g/person/day (0.09 and 0.18 g/kg body weight/day), respectively, for regular grade fish oil **Cardia**Beat™ and 3.3 and 5.9 g/person/day (0.06 and 0.11 g/kg body weight/day), respectively, for high grade fish oil **Cardia**Beat™. Based on the composition of fish oil based **Cardia**Beat™ products the 90th percentile all-user intake of EPA and DHA was estimated to range between 2.28 and 4.41 g/person/day (0.043 and 0.079 g/kg body weight/day), and based on the composition of non-fish oil based **Cardia**Beat™ products the intake of DAG was estimated to range between 4.27 and 5.75 g/person/ day (0.10 and 0.13 g/kg body weight/day).

As detailed herein, the safety of **Cardia**Beat™ is supported by the safety of its component phytosterols, EPA and DHA fatty acids, and diglycerides. The safety of these components is supported by their natural presence in the diet and published toxicological and clinical data conducted pertaining to phytosterols and phytosterol esters, EPA and DHA, and DAG rich oils.

Phytosterols are lipid-soluble and available scientific evidence indicates that they follow the same digestion and intestinal absorption pathways as dietary fat. The safety of phytosterols is supported by various experimental animal studies, which indicate that dietary phytosterols do not produce adverse effects on mortality, body weight gain, organ weights, food consumption, or clinical observations. Furthermore, no significant positive results were identified in the available experimental data regarding the genetic toxicity of phytosterols. Reproductive toxicity studies indicated that that dietary phytosterols have no adverse effect on reproductive performance. On a body weight basis, the 90th percentile all-user intake of phytosterols is at least 12-fold lower than the lowest reported NOAEL in animal toxicity studies. Prospective clinical trials and intervention studies assessing intakes of phytosterols ranging from 0.04 to 9 g/person/day indicate that these levels of intake, which are similar to the estimated 90th percentile all-user intakes from the intended food uses of **Cardia**Beat™, are generally well tolerated and do not produce adverse effects.

EPA and DHA are lipid-soluble fatty acids and available scientific evidence indicates that it follows the same digestion and intestinal absorption pathways as dietary fat. The safety of EPA and DHA is supported by various experimental animal studies, which indicate that DHA does not produce adverse effects on mortality, body weight gain, organ weights, food consumption, or clinical observations. Furthermore, no positive results were identified in the available experimental data regarding the genetic and reproductive toxicity of DHA. On a body weight basis, the 90th percentile all-user intake of EPA + DHA is at least 6-fold lower than the lowest reported NOEL in animal toxicity studies. Prospective clinical trials and intervention studies assessing intakes of EPA and DHA ranging from 1.5 to 10.1 g/person/day indicate that these levels of intake, which are 2 to 5 times larger than the estimated 90th percentile all-user intakes from the intended food uses of regular and high grade fish oil **Cardia**Beat™, are generally well tolerated and do not produce adverse effects. In particular, these studies demonstrated that doses of EPA and DHA greater than 3.0 g/day have no adverse effect on bleeding time, control of fasting glucose levels in NIDDM individuals, and LDL cholesterol levels.

DAG is a dietary fat and available scientific evidence indicates that it follows the same digestion and intestinal absorption pathways other dietary fats. The safety of DAG oil consumption is supported by experimental animal studies, which indicate that dietary phytosterols, do not produce adverse effects on mortality, body weight gain, organ weights, food consumption, or clinical observations. Furthermore, no positive results were identified in the available experimental data regarding the genetic toxicity of DAG oils. On a body weight basis, the 90th percentile all-user intake of EPA and DHA is at 33 times lower than doses reported to produce

no adverse effects in rats. Prospective clinical trials and intervention studies assessing intakes of phytosterols ranging from 500 mg to 45 g/person/day indicate that these levels of intake, which are up to 9 times higher than the estimated 90th percentile all-user intake resulting from the proposed food uses of **Cardia**Beat™, are generally well tolerated and do not produce adverse effects.

The scientific evidence presented above does not indicate that CardiaBeat™, or any of its constituents, would produce adverse effects on human health when consumed at the intended conditions of food use described herein. The data and information summarized in this report demonstrate that CardiaBeat™, meeting appropriate food grade specifications and manufactured in accordance with current good manufacturing practice, would be GRAS based on scientific procedures under the conditions of intended use in foods.

9.0 CONCLUSION

We, the Expert Panel, have, independently and collectively, critically evaluated the data and information summarized above and concluded that **Cardia**Beat™, a family of vegetable oils/fats/fish oils containing transesterified plant phytosterol esters, meeting appropriate food-grade specifications, is Generally Recognized as Safe (GRAS) based on scientific procedures under the conditions of intended use in foods specified herein.

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APPENDIX A

PRODUCT SPECIFICATIONS FOR *CARDIABEAT™*

A1.1 Chemical Specifications for Regular Grade Distilled and Nondistilled CardiaBeat™

Specification parameter	Specification value	Method of analysis
Identification		
Solubility	Freely to very soluble in hexane	FCC (2003)
Infrared spectroscopy	Identical to standard	USP 197 (2004)
Purity		
Triglyceride	Between 35 and 65%	AOCS Cd 11d-96 (1999)
Diglyceride	Between 10 and 20%	AOCS Cd 11d-96 (1999)
Ratio 1,3/1,2 Diglycerides	>2.0	AOCS Cd11d-96 (1999)
Monoglyceride	<5%	AOCS Cd 11d-96 (1999)
Total phytosterols	>14%	Enzymotec internal method
Phytosterol esters	>20%	Calculated based on difference between total and free phytosterols
Free phytosterols	<2.5%	Enzymotec internal method
Free fatty acids	<10%	AOCS Ca 5a-40 (1999)
Peroxide value	<10 meq/kg	AOCS Cd 8-53 (1999)
Ash	<1 mg/kg	USP 281 (2004)
Water	<1%	Enzymotec internal method based on Karl Fischer method
Lead	<0.1 mg/kg	EPA 200.7

A1.2 Chemical Specifications for Regular Grade Fish Oil CardiaBeat™

Specification parameter	Specification value	Method of analysis
Identification		
Solubility	Very soluble in hexane	FCC (2003)
Infrared spectroscopy	Identical to standard	USP 197 (2004)
Purity		
Triglyceride	Between 7 and 40%	AOCS Cd 11d-96 (1999)
Diglyceride	Between 5 and 18%	AOCS Cd 11d-96 (1999)
Ratio 1,3/1,2 Diglycerides	>2.5	AOCS Cd11d-96 (1999)
Monoglyceride	Less than 5%	AOCS Cd 11d-96 (1999)
DHA + EPA	Greater than 25%	AOCS Ce 1-62 (1999)
Total phytosterols	Greater than 36%	Enzymotec internal method
Phytosterol esters	Greater than 50%	Calculated based on difference between total and free phytosterols
Free phytosterols	Less than 10%	Enzymotec internal method
Free fatty acids	Less than 2.0%	AOCS Cd 5a-40 (1999)
Ethyl esters	Less than 10%	Enzymotec internal method
Peroxide value	Less than 5 meq/kg	AOCS Cd 8-53 (1999)
Ash	Less than 1 mg/kg	USP 281 (2004)
Water	<1.0%	Enzymotec internal method based on Karl Fischer method
Lead	<0.1 mg/kg	EPA 200.7

A1.3 Chemical Specifications for High Grade Vegetable Oil CardiaBeat™

Specification parameter	Specification value	Method of analysis
Identification		
Solubility	Very soluble in hexane	FCC (2003)
Infrared spectroscopy	Identical to standard	USP 197 (2004)
Purity		
Triglyceride	Between 7 and 40%	AOCS Cd 11d-96 (1999)
Diglyceride	Between 5 and 18%	AOCS Cd 11d-96 (1999)
Ratio 1,3/1,2 Diglycerides	Greater than 2.0	AOCS Cd 11d-96 (1999)
Monoglyceride	Less than 5%	AOCS Cd 11d-96 (1999)
Total phytosterols	Greater than 36%	Enzymotec internal method
Phytosterol esters	Greater than 50%	Calculated based on difference between total and free phytosterols
Free phytosterols	Less than 10%	Enzymotec internal method
Free fatty acids	Less than 0.5%	AOCS Cd-5a-40 (1999)
Peroxide value	Less than 5 meq/kg	AOCS Cd 8-53 (1999)
Ash	Less than 1 mg/kg	USP 281 (2004)
Water	Less than 1.0%	Enzymotec internal method based on Karl Fischer method
Lead	Less than 0.1 mg/kg	EPA 200.7

A1.4 Chemical Specifications for High Grade Fish Oil CardiaBeat™

Specification parameter	Specification value	Method of analysis
Identification		
Solubility	Very soluble in hexane	FCC (2003)
Infrared spectroscopy	Identical to standard	USP 197 (2004)
Purity		
Triglyceride	Between 7 and 40%	AOCS Cd 11d-96 (1999)
Diglyceride	Between 5 and 18%	AOCS Cd 11d-96 (1999)
Monoglyceride	Less than 5%	AOCS Cd 11d-96 (1999)
DHA + EPA	Greater than 25%	AOCS Ce 1-62 (1999)
Total phytosterols	Greater than 36%	Enzymotec internal method
Phytosterol esters	Greater than 50%	Calculated based on difference between total and free phytosterols
Free phytosterols	Less than 10%	Enzymotec internal method
Free fatty acids	Less than 2.0%	AOCS Cd 5a-40 (1999)
Ethyl esters	Less than 10%	Enzymotec internal method
Peroxide value	Less than 5 meq/kg	AOCS Cd 8-53 (1999)
Ash	Less than 1 mg/kg	USP 281 (2004)
Water	<1.0%	Enzymotec internal method based on Karl Fischer method
Lead	<0.1 mg/kg	EPA 200.7

A1.5 Microbial Specifications for High and Regular Grade CardiaBeat™

Specification Parameter	Specification Value	Analytical Method
Total aerobic count	<1000 CFU/g	Israel Standard SI 885 Part 3 (1999)
Mold	<100 CFU/g	Israel Standard SI 885 Part 8 (1999)
Yeast	<100 CFU/g	Israel Standard SI 885 Part 8 (1999)
Coliforms	ND/g	USP 61 (2000)
Salmonella	ND/g	Israel Standard SI 885 Part 7 (1999)
Staphlyococcus aureus	ND/g	USP 61 (2000)

APPENDIX B

SUMMARY OF THE INDIVIDUAL PROPOSED FOOD-USES AND USE-LEVELS FOR *CARDIABEAT™*, AND THE CORRESPONDING USE-LEVELS FOR PHYTOSTEROL ESTERS IN THE UNITED STATES

Table B1-1 Summary of the Individual Proposed Food-Uses and Use-Levels for Regular and High Grade Non-Fish Oil Based CardiaBeat™ and the Corresponding use-Levels of Phytosterol Esters in the United States

Food Category	Proposed Food-Use	Regular Grade CardiaBeat™ Use-Level (g/RACC)[a]	Regular Grade CardiaBeat™ Use-Level (%)	High Grade CardiaBeat™ Use-Level (g/RACC)[a]	High Grade CardiaBeat™ Use-Level (%)	Phytosterol ester Use-Level (g/RACC)[a]	Phytosterol ester Use-Level (%)
Baked Goods and Baking Mixes	Cakes	2.6	2.08 - 4.7	1.1	0.88 – 2.0	0.65	0.52 - 1.18
	Cookies	2.6	6.5 - 8.67	1.1	2.75 - 3.67	0.65	1.62 - 2.17
	Grain-Based Crackers	2.6	8.67 - 17.33	1.1	3.67 - 7.33	0.65	2.17 - 4.33
	French Toast, Pancakes, and Waffles	2.6	2.36 - 3.06	1.1	1 - 1.29	0.65	0.59 - 0.76
	Pastries	2.6	2.08 - 4.7	1.1	0.88 - 2.0	0.65	0.52 - 1.2
	Pies	2.6	2.08	1.1	0.88	0.65	0.52
	Quick Breads	2.6	4.7	1.1	1.99	0.65	1.2
	Yeast Breads and Rolls	2.6	5.2	1.1	2.2	0.65	1.3
Fats and Oils	Butter	2.6	17.33	1.1	7.33	0.65	4.33
	Fat-Based Sauces	2.6	17.33	1.1	7.33	0.65	4.33
	Margarine, and Margarine-Like Spreads	2.6	17.33	1.1	7.33	0.65	4.33
	Mayonnaise and Mayonnaise-Type Dressings	2.6	17.33	1.1	7.33	0.65	4.33
	Oils (including vegetable shortening)	2.6	17.33	1.1	7.33	0.65	4.33
	Salad Dressings (regular and low calorie)	2.6	8.67	1.1	3.67	0.65	2.17
Frozen Dairy Desserts and Mixes	Ice Cream and Frozen Milk Desserts	2.6	2.17	1.1	0.92	0.65	0.54
	Frozen Yogurt	2.6	2.17	1.1	0.92	0.65	0.54
Gelatins, Puddings, and Fillings	Puddings, Custards and Other Milk Desserts	2.6	2.17	1.1	0.92	0.65	0.54
Grain Products and Pastas	Frozen Grain-Based Meals	2.6	1.08	1.1	0.46	0.65	0.27
	Grain Mixtures	2.6	1.86	1.1	0.79	0.65	0.46

Table B1-1 Summary of the Individual Proposed Food-Uses and Use-Levels for Regular and High Grade Non-Fish Oil Based CardiaBeat™ and the Corresponding use-Levels of Phytosterol Esters in the United States

Food Category	Proposed Food-Use	Regular Grade CardiaBeat™ Use-Level (g/RACC)[a]	Regular Grade CardiaBeat™ Use-Level (%)	High Grade CardiaBeat™ Use-Level (g/RACC)[a]	High Grade CardiaBeat™ Use-Level (%)	Phytosterol ester Use-Level (g/RACC)[a]	Phytosterol ester Use-Level (%)
	Pastas	2.6	1.86	1.1	0.79	0.65	0.46
	Grain-based Patties	2.6	1.86	1.1	0.79	0.65	0.46
	Rice and Other Cereal Grains	2.6	1.86	1.1	0.79	0.65	0.46
Gravies and Sauces	White Sauces and Milk Gravies	2.6	2.08	1.1	0.88	0.65	0.52
Hard Candy	Hard Candy	2.6	17.33	1.1	7.33	0.65	4.33
Milk	Milk	2.6	1.08	1.1	0.46	0.65	0.27
Milk Products	Cheese (Natural and Cream)	2.6	2.36 - 52	1.1	1 - 22	0.65	0.59 - 13
	Cheese (Processed and Spreads)	2.6	8.67	1.1	3.67	0.65	2.17
	Cheese (Imitation)	2.6	8.67	1.1	3.67	0.65	2.17
	Cheese Mixtures	2.6	8.67	1.1	3.67	0.65	2.17
	Creams and Cream Substitutes	2.6	17.33	1.1	7.33	0.65	4.33
	Evaporated, Condensed, and Dry Milks	2.6	8.67	1.1	3.67	0.65	2.17
	Flavored Milk and Milk Drinks	2.6	1.08	1.1	0.46	0.65	0.27
	Milk-Based Meal Replacements	2.6	1.08	1.1	0.46	0.65	0.27
	Sour Cream	2.6	8.67	1.1	3.67	0.65	2.17
	Milk, Fluid, Imitation	2.6	1.08	1.1	0.46	0.65	0.27
	Yogurt	2.6	1.15	1.1	0.49	0.65	0.29
Soft Candy	Candies and Chocolate	2.6	6.5	1.1	2.75	0.65	1.62
Soups and Soup Mixes	Grain Based Soups	2.6	1.06	1.1	0.45	0.65	0.26
	Cheese Soups	2.6	1.06	1.1	0.45	0.65	0.26
Snack Foods	Grain-Based Salty Snacks	2.6	8.67	1.1	3.67	0.65	2.17

[a] RACC – Reference Amounts Customarily Consumed Per Eating Occasion (21 CFR §101.12). When a range of values is reported for a proposed food-use, particular foods within that food-use may differ with respect to their RACC.

Table B1-2 Summary of the Individual Proposed Food-Uses and Use-Levels for Regular and High Grade Fish Oil Based CardiaBeat™ and the Corresponding use-Levels of Phytosterol Esters in the United States

Food Category	Proposed Food-Use	Regular Grade CardiaBeat™ Use-Level (g/RACC)[a]	Regular Grade CardiaBeat™ Use-Level (%)	High Grade CardiaBeat™ Use-Level (g/RACC)[a]	High Grade CardiaBeat™ Use-Level (%)	Phytosterol ester Use-Level (g/RACC)[a]	Phytosterol ester Use-Level (%)
Baked Goods and Baking Mixes	Grain-Based Crackers	2.6	8.67 - 17.33	1.1	3.67 - 7.33	0.65	2.17 - 4.33
	Pastries	2.6	2.08 - 4.7	1.1	0.88 - 2.0	0.65	0.52 - 1.2
	Quick Breads	2.6	4.7	1.1	1.99	0.65	1.2
	Yeast Breads and Rolls	-	-	1.1	2.2	0.65	1.3
Fats and Oils	Fat-Based Sauces	2.6	17.33	1.1	7.33	0.65	4.33
	Margarine, and Margarine-Like Spreads	2.6	17.33	1.1	7.33	0.65	4.33
	Mayonnaise and Mayonnaise-Type Dressings	2.6	17.33	1.1	7.33	0.65	4.33
	Salad Dressings (regular and low calorie)	2.6	8.67	1.1	3.67	0.65	2.17
Frozen Dairy Desserts and Mixes	Frozen Yogurt	2.6	2.17	1.1	0.92	0.65	0.54
Grain Products and Pastas	Frozen Grain-Based Meals	-	-	1.1	0.46	0.65	0.27
	Grain Mixtures	-	-	1.1	0.79	0.65	0.46
Gravies and Sauces	White Sauces and Milk Gravies	2.6	2.08	1.1	0.88	0.65	0.52
Milk Products	Flavored Milk and Milk Drinks	2.6	1.08	1.1	0.46	0.65	0.27
	Milk-Based Meal Replacements	2.6	1.08	1.1	0.46	0.65	0.27
	Milk, Fluid, Imitation	2.6	1.08	1.1	0.46	0.65	0.27
	Yogurt	2.6	1.15	1.1	0.49	0.65	0.29

[a] RACC – Reference Amounts Customarily Consumed Per Eating Occasion (21 CFR §101.12). When a range of values is reported for a proposed food-use, particular foods within that food-use may differ with respect to their RACC.

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Table B1-3 Summary of the Food-Uses and Use Levels for Phytosterols that are Generally Recognized as Safe (GRAS) in the United States

Food Category	Food-Use	Use Level (%)
Baked Goods	White Breads, Rolls, Buns, and Comparable Non-Standardized White Bread Products (GRN 000112)	1.3*
Beverages and Beverage Bases	Health Drinks (GRN 000061)	0.41
Fats and Oils	Salad Dressing (GRN 000061, *000112)	3.33
	Mayonnaise (GRN 000112)	4.33*
	Vegetable Oil (for home use for baking, frying, salad dressings)	13.3
	Vegetable Oil Spread (GRN 000039, 000061, 000112)	12.0
Grain Products and Pastas	Health Bars (GRN 000061, 000112)	2.5
Milk Products	Cream Cheese and Cream Cheese-Like Products (GRN 000112)	2.17*
	Ice Cream and Non-standardized Ice Cream Products (GRN 000112)	0.54*
	Milk-based juice beverages (GRN 000112)	0.27*
	Yogurt and Yogurt-type Products (GRN 000061, 000112)	0.44 (regular) 0.83 (frozen)

* Use-levels not specified in GRAS notices. A use-level of 0.65/RACC, corresponding to the requirements for health claims regarding phytosterol esters and risk of CHD (21 CFR § 101.83), was assumed (CFR, 2004).

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APPENDIX C

SUMMARY TABLES OF THE REVIEWED NON-CLINICAL STUDIES

Table C1-1 Non-Clinical Studies Conducted With Phytosterols, EPA + DHA, and DAG			
Number, Sex, Strain, Species, and Age	**Route of Administration, Dosage, and Duration of Intervention**	**Observations Relevant to Safety**	**Reference**
Acute Studies conducted with phytosterols			
Mice (sex, strain, and age not specified)	Intraperitoneal, single administration of 0 to 3.2 g/kg body weight of β-sitosterol	Intraperitoneal LD_{50} value is > 3.2 g/kg body weight	Gupta *et al.*, 1980
Subchronic, developmental and reproductive studies conducted with phytosterols			
50 animals (10 rats/group), male and female rats (strain and age not specified)	Dietary, 2,500 mg/kg body weight/day (a), 8 to 22 months	No detectable changes in growth, blood cell counts, blood urea nitrogen, serum protein levels, or histological appearance of any organ or tissue	Shipley *et al.*, 1958
63 animals (10 to 16 rats/group), male and female Sprague-Dawley rats aged 4 weeks	Gavage, 0, 1,000, 3,000, or 9,000 mg/kg body weight/day, 13 weeks	No toxicologically significant differences were observed between the treatment and control groups. Based on cardiomyopathy observed in male rats in the high dose group, NOAEL was reported to be 3,000 mg/kg body weight/day.	Kim *et al.*, 2002
200 animals (20 rats/sex/group), 28 day old male and female Wistar rats	Dietary (0, 0.16, 1.6, 3.2 and 8.1% of diet), 0, 160, 1,600, 3,200, and 8,100 mg/kg body weight/day (a), 90 days	No toxicologically or biologically significant differences were observed between the treatment and control groups. NOAEL was reported to be highest dose level (8.1% of diet)	Hepburn *et al.*, 1999
100 animals (10 rats/group), 21-22 day old female Wistar rats	Gavage, 0, 5, 50, or 500 mg phytosterols, 0, 5, 20, 40, 80 mg weak positive control, or 0.4 mg strong positive control/kg body weight/day, 3 days	No significant difference in uterine weight was observed between rats administered phytosterols and negative controls. No estrogenic effects observed.	Baker *et al.*, 1999
120 animals (20 hamsters/group), male and female Syrian Golden FB hamsters aged 11 weeks	Dietary (0, 0.24, 0.48, 0.96, 1.92, or 2.84% of diet), 0, 288, 572, 1,152, 2,304, or 3,408 mg/kg body weight/day (a), 12 weeks	No safety concerns associated with consumption of all diets containing phytosterol esters. Significant decrease in plasma total, and LDL cholesterol as well as aortic foam cell formation.	Ntanios *et al.*, 2003
6 New Zealand White rabbits (age not specified)	Dietary, 2,000 mg/kg body weight/day, for 348 to 842 days	No gross or microscopic abnormalities observed in the blood vessel, heart, thyroid, spleen, liver, or intestinal tract of any of the rabbits.	Shipley *et al.*, 1958
6 New Zealand White rabbits (age not specified)	Dietary, 2,000 mg/kg body weight/day, for70 or 212 days	No gross or microscopic abnormalities observed in the blood vessel, heart, thyroid, spleen, liver, or intestinal tract of any of the rabbits. No accumulation of phytosterols observed in the vasculature or the tissues	Shipley *et al.*, 1958

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24 male New Zealand White rabbits (age not specified)	Dietary, 266 to 295 mg/kg body weight/day, 60 days	No adverse effects or signs of toxicity were reported.	Kritchevsky *et al.*, 2003
13 dogs (strain, sex, and age not specified)	Dietary, 500 or 1,000 mg/kg body weight/day, 8 to 22 months	No significant differences in the body weight, serum composition, formed blood elements, or gross or macroscopic tissue examinations of the treated dogs	Shipley *et al.*, 1958.
80 animals (10 rats/group), female G5G8+/+, or -/-, *Ldlr*+/+ or -/- mice aged 8 weeks	Dietary, typical western diet (not specific phytosterol dose administered), 7 months	No relationship between plasma phytosterol concentrations and atherosclerosis was observed	Wilund *et al.*, 2004
196 male Stroke Prone Spontaneously Hypertensive (SHRSP) rats aged 35 days	Dietary, casein based diets containing 27, 27, 36, 97, 114, 201, or 204 g phytosterols/100 g diet (providing approximately 21, 21, 28, 74, 90, 158, or 161 mg phytosterols/kg body weight/day), duration of lifespan.	A significant decrease in lifespan was observed in the rats administered diets containing oils rich in phytosterols. The observed decrease in survival time correlated with lower RBC membrane deformability indexes, lower RBC cholesterol concentrations, and higher RBC phytosterol concentrations.	Ratnayake *et al.*, 2000a
84 male Stroke Prone Spontaneously Hypertensive (SHRSP) rats aged 26 to 29 days	Dietary, casein based diets containing 0.02 (control) or 1.4g phytosterols/100 g diet (providing approximately 0.01 or 1.1 mg phytosterols/kg body weight/day), duration of lifespan.	While a significant increase in deaths due to stroke were correlated to increased phytosterol consumption; however the authors acknowledged that similar results had been observed in SHRSP rats fed diets rich in other vegetable oils low in phytosterols.	Ratnayake *et al.*, 2003
84 male Stroke Prone Spontaneously Hypertensive (SHRSP) rats 4 weeks of age	Dietary, conventional diets of which 0.4% comprised a phytosterol mixture, 180 days	No significant correlation was observed between the SHRSP rat survival time and the amount of phytosterols consumed in the diet or isolated in the tissues.	Tatematsu *et al.*, 2004
117 male and 116 female Crl:(WI)WU BR Wistar rats of 4 to 5 weeks of age	Dietary, casein based diets comprised of 0 (control), 1.6, 3.2, or 8.1 % phytosterols (providing between 0 and 9.1 g phytosterols/kg body weight/day), 32 weeks.	Consumption of diets providing up to 9.1 g phytosterols/kg body weight did significantly alter any of the examined reproductive or developmental parameters through 3 generations of rats. NOAEL was determined to be diets containing 8.1% phytosterols (providing 2.5 to 9.1 g phytosterols/kg body weight/day)	Waalkens-Berendsen *et al.*, 1999
Acute Studies conducted with EPA and DHA			
5 male ICR mice and 10 Crj/CD(SD)IGS] Sprague-Dawley male and female rats	Single gavage dose of 2,000 mg/kg body weight, 14 day observation period	No adverse effects were observed in the mice while water diarrhea was the only adverse effect reported in 2 out of the 10 rats.	Kroes *et al.*, 2003
Subchronic, developmental and reproductive studies conducted with EPA and DHA			

24 male type 2 diabetic Otsuka Long-Evans Tokushima Fatty (OLETF) rats of 5 weeks of age	Dietary, diets providing 0 or 1.0 g EPA/kg body weight/day, 25 weeks	No significant differences were observed in the body weight gain, food consumption, or glucose tolerance of the treatment and control animals. Consumption of the diet containing EPA significantly lowered plasma triacylglycerol levels and abdominal fat accumulation, as well as improve insulin resistance. Additionally, no adverse effects or signs of toxicity were reported in OLETF rats consuming a diet providing 1.0 g EPA/kg body weight/day.	Minami *et al.*, 2002
60 female Sprague-Dawley rats, 45 of which were ovariectomized, 15 of which subject to sham operations	Dietary, calcium adequate diets providing 0, 0.1, or 1.0 g EPA/kg body weight/day, 9 weeks	No significant difference was noted between the bone breaking strength and serum type-1 collagen concentrations of the treatment and control rats. Ovariectomized rats consuming 1.0 g EPA/kg body weight/day were reported to have a significantly lower femur bone density than the control group, the sham control group, and the low dose EPA group.	Poulsen and Kruger, 2004
Female sham operated and ovariectomized Balb/c mice aged 8 weeks	Dietary, diets comprised of 0 or 5% fish oil, providing approximately 652 and 1,070 mg/kg body weight of EPA and DHA, respectively, 16 weeks	A significant reduction in bone loss was observed in the EPA treated group, which the authors attributed to a decrease in the activation of osteroclast progenitors resulting in a decrease in osteoclast activity.	Sun *et al.*, 2003
30 male Sprague-Dawley rats of 4 weeks of age	Dietary, diets containing 2% DHA or EPA esters, providing approximately 1,000 mg/kg body weight/day, 3 weeks	No adverse effects were reported to result from the consumption of either DHA or EPA. Consumption of EPA and DHA were reported to have beneficial effects on lipid metabolism and leukotriene synthesis.	Hung *et al.*, 2000
160 male and female CD rats aged 25 days	Gavage, 0 (control), 0.5, or 1.25 g/kg body weight/day of an oil containing 51.7% DHA, 90 days	One death was observed during the experimental period; however, it was determined by the authors not to be treatment related. No significant differences were observed between the treatment and control groups in any of the parameters examined. The authors determined the NOAEL to be the highest dose level examined, providing 0.5 to 0.625 g DHA/kg body weight/day.	Arterburn *et al.*, 2000

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34 male C57BL/6L mice of 3 to 4 months of age	Dietary, standard chow and high fat diets containing 0 (control), 2.32, 4.15, 5.00, 6.00, 6.84, or 11.44% EPA + DHA (estimated to provided 0, 3.48, 6.22, 7.5, 9.0, 10.26, and 17.16 g EPA +DHA/kg body weight/day), 6 weeks	The authors reported no adverse effects in mice consuming either the control or EPA and DHA enriched diets. Significant decreases in body weight gain, the adiposity of epidydimal and subcutaneous fat depots, serum leptin levels, leptin gene expression, serum triglyceride levels and serum insulin levels were observed in EPA + DHA treated mice. No significant changes were observed in the blood glucose levels, food consumption, and serum non-essential fatty acid levels.	Ruzickova *et al.*, 2004
Male and Female newborn piglets	Dietary, formula containing 0.34 or 1.6 mg DHA/g formula (providing approximately 387or 653 mg DHA/kg body weight/day for male piglets and 412 or 691 mg DHA/kg body weight/day for female piglets)	The consumption of DHA was reported to have no significant effects on the body weights, clinical signs, food consumption, clinical chemistry, hematology, organ weight, or gross or histopathology of any the piglets receiving either dose of DHA.	Meritt *et al.*, 2003
240 male and female Sprague-Dawley-derived (Cr1:CD(SD)BR) rats	Males: Dietary, administered diets containing 0 (control), 0.6, 6.0 or 30% of *Schizotrichium sp.* oil, providing approximately 33 to 1,512 mg DHA/kg body weight/day, 10 weeks prior to, during, and for 3 weeks following the mating period. Females: Dietary, administered diets containing 0 (control), 0.6, 6.0 or 30% of *Schizotrichium sp.* oil, providing approximately 40 to 1,680 mg DHA/kg body weight/day, 2 weeks prior to the mating period and throughout the mating, gestation and lactation periods.	No compound-related adverse effects were observed in either the male or female rats. Three male rats died during the course of the experimental period; however, these deaths were reported to be unrelated to the consumption of the *Schizotrichium sp.* oil. Consumption of diets supplemented with DHA was reported to have no effect on the reproductive performance of the male and female rats, or on the physical development of the pups.	Hammond *et al.*, 2001a
125 female Sprague-Dawley-derived (Cr1:CD(SD)BR) rats and 110 artificially inseminated female New Zealand white rabbits	Rats: Dietary, administered diets containing 0 (control), 0.6, 6.0 or 30% of *Schizotrichium sp.* oil, providing approximately 39 to 1,800 mg DHA/kg body weight/day, during Days 6 though 15 of gestation. Rabbits: Gavage, 0 (control) 180, 600 or 1,800 mg/kg body weight of the *Schizotrichium sp.* oil, during Days 6 through 19 of gestation.	A decrease in food consumption and body weight gains as well as a slight increase in abortions was observed in rabbits in the high-dose group. No compound-related gross pathological changes were recorded in either rats or rabbits. Additionally, no developmental toxicity was observed in either rats or rabbits at any of the dose levels. The authors reported NOELs of 1,823 mg DHA/kg body weight/day for pregnant rats and fetuses, and NOELs of 600 and 1,800 mg microalgae oil/kg body weight/day for pregnant rabbits and fetuses, respectively.	Hammond *et al.*, 2001b
Subchronic, developmental and reproductive studies conducted with DAG			

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360 animals (60 rats/sex/group), male and female Srague-Dawley rats aged 4 weeks	Dietary (0, 2.65, or 5.3% of diet), 0, 1,325, or 2,650 mg/kg body weight/day (a), 105 weeks	No significant effects on food consumption, body weight, mortality, or histolofical parameters. No toxicologically significant effects of hematological, urinalysis, or blood chemistry parameters.	Soni *et al.*, 2001
16 male Sprague-Dawley rats aged 5-weeks	Dietary (0.2%), 36 mg/kg body weight/day, 4 weeks	No significant changes in food consumption or body weight gain.	Taguchi *et al.*, 2001

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APPENDIX D

SUMMARY TABLES OF THE REVIEWED CLINICAL STUDIES

Table D1-1 Clinical Studies Conducted with Phytosterols, EPA + DHA, and DAG				
Sample Size and Subject Demographics	**Study Design**	**Dosage**	**Observations Relevant to Safety**	**Reference**
Clinical studies conducted with Phytosterols				
Healthy males and females of 35 to 64 years of age	52-week randomized, double-blind, placebo controlled	1.6 g/day	A significant reduction is plasma carotenoid concentrations were observed. No significant differences in lipid soluble vitamin concentrations. Significant decrease in total and LDL cholesterol levels. No significant difference in HDL cholesterol, triglyceride, or lipoprotein levels.	Hendriks *et al.*, 2003
Healthy normocholesterolemic and mildly hypercholesterolemic males and females of 35 to 64 years of age	52-week randomized, double-blind, placebo controlled	0.83, 1.61, or 3.24 g/day	No significant change in Vitamin K or D levels. Significant decrease plasma lipid adjusted α- and β-carotene concentrations. Significant decrease in total and LDL cholesterol levels. Significant decrease in the ratio of LDL/HDL cholesterol. No significant change in triglyceride concentrations was observed.	Hendriks *et al.*, 1999
Individuals heterozygous for phytosterolemia	28- and 16-week treatment protocols	2.2 g/day	No significant change in any of the variables measured. Significant decrease in total and LDL cholesterol levels. No significant change in HDL cholesterol or triglyceride levels.	Kwiterovich *et al.*, 2003
Hypercholesterolemic males and females of 25 to 64 years of age	6-month randomized, double-blind, placebo controlled	0, 1.5, or 3.0 g/day	No significant changes in plasma retinol, α-tocopherol, or α-and β-carotene levels. Significant decrease in total and LDL cholesterol levels. No significant change in HDL cholesterol or triglyceride levels.	Christiansen *et al.*, 2001
Hypercholesterolemic males and females of 20 to 75 years of age	12-week, 3-way, double-blind, randomized, crossover	2.0 to 2.3 g/day	Reported a significant increase in plasma α- and β-carotene and no significant change in any other plasma carotenoid concentrations. Significant decrease in total and LDL cholesterol in both 2- and 3-way analysis. No change in triacylglyerol or HDL cholesterol levels.	Noakes *et al.*, 2002
Healthy males with slightly elevated total cholesterol	12-week, randomized, double-blind, controlled	0.04 or 0.45 g/day	No significant differences in blood α-tocopherol, retinol, or β-carotene concentrations. Significant decrease in apoC total-, and VLDL cholesterol levels. No significant changed in LDL cholesterol levels.	Seki *et al.*, 2003

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Male and female type 2 diabetes patients of 52 to 68 years of age	12 week randomized, double-blind, placebo-controlled	1.6 g/day	A downward trend in total and LDL cholesterol levels and an upward trend in HDL cholesterol levels were observed.	Lee *et al.*, 2003
Males and females of 18 to 70 years of age with moderately elevated plasma total cholesterol	11 week, randomized, double-blind crossover design	0 or 2.1 g/day	Significant decrease in total and LDL cholesterol levels. No significant change in HDL cholesterol or triglyceride levels.	Cleghorn *et al.*, 2003
Healthy male and female adults of 18 to 65 years in age	8-week, randomized, double-blind, controlled	0, 3.0, 6.0, or 9.0 g/day	No significant differences in lipid-soluble vitamin levels or corrected plasma carotenoid levels.	Davidson *et al.*, 2001
Normocholesterolemic males and females	8-week, randomized, double-blind, placebo controlled, repeated measures	3.2 g/day	Significant increase in α-tocopherol and β-carotene levels, significant decrease in lycopene and α-carotene levels, and no change in γ-tocopherol levels. Significant decrease in total and LDL cholesterol in both 2- and 3-way analysis. No change in triacylglyerol, lipoprotein or HDL cholesterol levels.	Quílez *et al.*, 2003
Hyperlidemic males and females of 22 to 76 years of age	2 month randomized, double-blind, placebo controlled Latin square	1.6 g/day (0.8 g β-sitosterol, 0.4 g campesterol, and 0.32 g stigmasterol/day)	Significant decrease in total and LDL cholesterol levels. No significant change in HDL cholesterol, lipoprotein, or triglyceride levels.	Nigon *et al.*, 2001
Male and female adults of 20 to 73 years of age with mild hypercholesterolemia	8 week randomized, double-blind, placebo-controlled trial	0 or 2.0 g/day	Significant decrease in apoB, total-, and LDL cholesterol levels. No significant changed in HDL cholesterol or triglyceride levels.	Devaraj *et al.*, 2004
Children with family history of hypercholesterolemia	8 week, double-blind, crossover	1.60 ± 0.13 g/day	Significant decrease in apoB, total-, and LDL cholesterol levels. No significant changed in HDL cholesterol or triacylglycerol levels.	Amundsen *et al.*, 2001
Individuals heterozygous for phytosterolemia	8 week open feeding study with 2 week run-in and 2 week washout	~3 g/day	Significant decrease in total plasma cholesterol levels due to changes in LDL cholesterol levels. No change in plasma triglyceride levels.	Stalenhoef *et al.*, 2001
Mean and women of ≥18 years of age with primary hypercholesterolemia	4 week, randomized, double-blind study with 4 parallel treatments	0, 0 + 400 μg cerivastatin, ~2 g, or ~2 g + 400 μg cerivastatin	Significant decrease in total and LDL cholesterol levels. No significant change in HDL, or triglyceride levels.	Simons, 2002
Hypercholesterolemic males and females of 30 to 65 years of age	4-week, Latin square, double-blind, with 3 test periods	~0.1 or 2.0 g/day	No significant changes in Vitamin D, retinol, α-carotene or lycopene concentrations or cholesterol ratios. Significant decrease in total and LDL cholesterol levels. No significant change in HDL, VLDL, lipoprotein, or triglyceride levels.	Hallikainen *et al.*, 2000

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Hypercholesterolemic male college students	4 week randomized, triple-blind	2.7 g/day (4.0 g β-sitosterol, 2.2 g campesterol, and 1.7 g stigmasterol/day)	Significant decrease in total and LDL cholesterol concentrations, and in the total:LDL cholesterol ratio. Increase in HDL cholesterol levels.	Matvienko *et al.*, 2002
Males and females of 25 to 65 years of age with normal to slightly elevated cholesterol levels	3-week, crossover, parallel design	0 or 3.6 g/day	No significant changes in lipid soluble vitamin levels. Significant decrease in plasma carotenoid levels, although they remained within normal ranges. Significant decrease in total and LDL cholesterol levels, and a similar decrease in apolipoprotein levels. No significant change in HDL cholesterol or triglyceride levels.	Judd *et al.*, 2002
Normal and hypercholesterolemic Japanese men and women of 24 to 67 years of age	3-week, double-blind, crossover, with a 1-week washout	1.8 g/day	Significant decrease in β-carotene levels. No significant differences in vitamin A or E levels were observed. Significant decrease in total and LDL cholesterol levels and no significant change in triglycerides or HDL-and VLDL cholesterol levels.	Ntanios *et al.*, 2002
Hypercholesterolemic males between the ages of 37 and 61	3-week, randomized, double-blind, placebo-controlled crossover design with a 5-week washout	1.92 g/70 kg body weight/day	No significant effects on serum carotenoid or lipid-soluble vitamin concentrations.	Raeini-Sarjaz *et al.*, 2002
Males and females, aged 20 to 75 years, with a moderately elevated plasma cholesterol level	3 week, single-blind, controlled, randomized, incomplete crossover with 4 test periods	1.6 g/day (0.8 g sitosterol, 0.3 g stigmasterol, and 0.3 g campesterol/day)	Significant decrease in total and LDL cholesterol levels in all food groups. No significant change in triglyceride concentrations were observed. Significant increase in HDL cholesterol in bread group	Clifton *et al.*, 2004
Clinical studies conducted with EPA + DHA				
Retinitis Pigmentosa patients of 18 to 55 years of age	4 years, randomized, controlled, double-masked trial	1.2g DHA/day and 15,00 IU Vitamin A/day or placebo and 15,00 IU Vitamin A/day	No significant difference between treatment group and placebo.	Berson *et al.*, 2004
Male X-linked retinitis pigmentosa patients with a mean age of 16 years	4-year, placebo controlled	400 mg DHA/day or 400 mg placebo oil/day	Increase in plasma DHA levels, no negative effects on any of the parameters examined, and no difference in adverse events reported between groups. Administration of DHA was reported to have no adverse effects	Wheaton *et al.*, 2003
Patients of 55 to 69 years of age, with CHD and normal plasma lipid levels	28 months, randomized, double-blinded, placebo-controlled	12 g fish oil/day, providing 1.92 g DHA and 2.88 g EPA/day control: 12 g olive oil	LDL cholesterol was significantly increased within the fish oil group but not significantly different from that of the control group. Significant decrease in TG levels was observed. No effects of fish oil on other parameters.	Sacks *et al.*, 1995

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Patients with lupus nephritis randomized to receive 1 of 2 initial treatments, 22 to 66 years of age	2 year placebo-controlled, double-blind crossover of 2 treatments separated by a 10-week washout	1.7g DHA + 2.7g EPA/day in 1st year, 15 placebo capsules/day in 2nd year Placebo in 1st year, 1.7 g DHA + 2.7 g EPA/day in 2nd year	Fish oil increased bleeding time; however, the statistical significance was compromised by a significant treatment order effect. Fish oil had no effect on HDL or LDL cholesterol. Significant decrease in serum TG and VLDL cholesterol levels following treatment with fish oil. Fish oil had no effect on GFR, serum creatinine levels, or urinary protein or IgG excretion. Serum complement (C3 and C4) and antibodies to double-stranded DNA antibodies were not affected by treatment.	Clark *et al.*, 1993
Patients aged 48 to 67 years with coronary atherosclerosis	24 months, randomized, double-blinded, placebo-controlled	6 g fish oil capsules/day (1.29 g DHA and 2.12 g EPA./day) for 3 months, followed by 3 capsules/day (0.64 g DHA and 1.06 g EPA/day) for 21 months placebo oil capsules containing no marine ω-3 fatty acids and reflective of fatty acid composition of typical European diet	No effects reported on total or HDL cholesterol following fish oil compared with placebo. Compared with placebo, TG levels were significantly lower in the fish oil group after 18, months of treatment. Compared with the placebo group LDL cholesterol was significantly greater in the fish oil group after 24 months of treatment, but not when compared to baseline levels. Reported no significant side effects of fish oil compared to control.	von Schacky *et al.*, 1999
Male and female hypercalciuria patients of 25 to 71 years of age	18-month clinical trial	1,800 mg EPA/day	A significant reduction in urinary calcium was observed.	Yasui *et al.*, 2001
610 patients with coronary artery disease and undergoing CAB surgery with mean ages of 59.9±8.8 years.	1-year post-operation (until 5-7 days before angiographic evaluation) patients randomized to receive either 300 mg aspirin or warfarin, and simultaneously randomized to fish oil treatment	300 mg aspirin + 1.28 g DHA + 2.04 g EPA/day 300 mg aspirin/day warfarin + 1.28 g DHA + 2.04 g EPA/day warfarin to attain normalized anticoagulant ratio	Supplementation with fish oil had no significant effect on any measured haemostatic parameter, glucose homeostasis, total, LDL, or HDL cholesterol levels, or apo A-I and B-100 concentrations compared to control. At 9 months, total serum TG was significantly lower in the fish oil group. Reported that fish oil supplements generally well tolerated with few adverse effects, mainly gastrointestinal complaints. No patients withdrew from study due to fish oil treatment. At 9 months, mean ALAT activity was significantly higher in the fish oil group although the authors determined the change "presumably of no clinical consequence". No significant group differences observed for ASAT and GGT activities or serum thiobarituric acid-reactive substances.	Eritsland *et al.*, 1995a, 1996

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Patients of 28 to 87 years of age with acute myocardial infarction	1 year, prospective, randomized, double-blind, placebo-controlled study	3.4 to 3.5 g EPA + DHA/day placebo: 4 g corn oil/day	Plasma TBA-MDA was significantly greater following treatment with EPA + DHA compared to placebo.	Grundt *et al.*, 2003
Normotensive IDDM patients with diabetic nephropathy of 24 to 44 years of age	Randomized, double-blind, 1year	21 mL cod liver oil/day, providing 2.6 g DHA and 2.0 g EPA/day 21 mL olive oil	Reported "no serious side effects", although, 3 withdrawals from fish oil group due to nausea. Glycemic control remained constant throughout study. Mean TG and VLDL cholesterol levels were significantly lower in the fish oil group at 6 months, but not 12 months. Serum total and LDL cholesterol were significantly greater after 6 and 12 months of fish oil treatment. Neither treatment impaired increase in albuminuria or reduction in renal function in patients with nephropathy.	Rossing *et al.*, 1996
Symptomatic menopausal Japanese women of 46 to 62 years of age	48 week, double blind, placebo controlled	1,737 mg EPA ethyl ester, 3.36 mg Vitamin E and 2 mg estriol per day	Significant decrease in total, HDL, and LDL cholesterol, TG, and apo A-I, A-II, B, E, and B/A-I levels.	Kurabayashi *et al.*, 2000
Patients of 56 to 60 years of age, with generalized solid tumours	until death; mean 213-481 days	well-nourished; 18 g fish oil + 0.2 g vitamin E/day (2.07 g DHA + 3.06 g EPA/day) malnourished; 18 g fish oil + 0.2 g vitamin E/day (2.07 g DHA + 3.06 g EPA/day) well-nourished; placebo (sugar) malnourished; placebo (sugar) healthy control group	Significant increase in T-helper cells and TNF synthesis and significant decrease in T suppressor cells in malnourished individuals consuming fish oil. No effect of fish oil on numbers or percentage of T cell populations or on *in vitro* IL-1 and IL-6 production by PBMNC. Patient survival significantly increased with supplemental fish oil. Reported no serious toxicity, some mild abdominal discomfort and transient diarrhea.	Gogos *et al.*, 1998
Patients with active distal proctocolitis	6 months	15 mL fish oil extract/day (2.4 g DHA + 3.2 g EPA/day) placebo = 15 mL sunflower oil (2.6 g oleic acid + 7.9 g linoleic acid)	Following 3 and 6 months of fish oil treatment, a significant decrease in the number of cells expressing HLA-DR and, after 6 months, CD3 was observed. A significant decrease in the percentage of IgM containing cells was also observed after 3 and 6 months. No adverse effects were reported, apart from minor nausea at the very first few doses.	Almallah *et al.*, 2000

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Patients of 46 to 72 years of age with NIDDM and hypertriglycidemia	consecutive 6-month dietary intervention; randomized, double-blind, placebo-controlled cross-over	15 g fish oil/day, providing 1.9 g DHA and 4.1 g EPA/day placebo: 15 g olive oil/day	No significant changes in fasting plasma glucose, glycosylated haemoglobin, C-peptide concentrations, or in urinary glucose levels. Following 6 months of fish oil intake, diabetic control did not deteriorate. Significant increase in LDL cholesterol and significant decrease in VLDL cholesterol, and total and VLDL-TG levels in fish oil group. No changes in plasma total and HDL cholesterol.	Connor *et al.*, 1993
Hypertryglyceridemic patients undergoing CABG with mean ages of 61.0±8.1years (fish oil) and 61.7±8.9 years (control)	6 months post operation, patients randomized to receive either 300 mg aspirin or warfarin, and simultaneously randomized to fish oil treatment	4 g fish oil ω-3 fatty acid concentrate/day, providing 3.4 g DHA + EPA/day control: no K-85	Reported fish oil well tolerated. No effect of fish oil treatment on fibrinolytic parameters measured, apart from a slight yet significant reduction in PAI-1 antigen concentration. Significant increase in median fasting plasma glucose of fish oil group; however, glucose levels of both groups decreased compared to baseline. Median fasting serum insulin significantly decreased in the fish oil group. Oral glucose tolerance test demonstrated no significant differences in any measured glucose control parameters. LDL-and total cholesterol concentrations lower than baseline following both fish oil and control treatments. Treatment with fish oil significantly reduced TG levels compared to control.	Eritsland *et al.*, 1994a,b
Healthy adults of 25 to 46 years of age	180 days; randomized, double-blind, placebo-controlled	2.5 g n-3 ethyl esters PUFA/day, providing 1.1 g DHA + 1.4 g EPA/day 5.1 g n-3 ethyl esters PUFA /day, providing 2.4 g DHA + 2.7 g EPA/day 7.7 g n-3 ethyl esters PUFA /day, providing 3.6 g DHA + 4.4 g EPA/day placebo capsules	Significant time-dependent increases in DHA and EPA concentrations, and a significant decrease in arachidonic acid concentration, in RBC membranes at all doses. Reported a significant increase in α-tocopherol content of RBCs after 30 days of treatment at mid and high doses. γ-Tocopherol was significantly reduced at all doses following 180 days.	Palozza *et al.*, 1996
Healthy males with a mean age of 32±4 years	4 months, randomized, double-blind, placebo-controlled	1.4g DHA and 2.04g EPA/day or 4 g olive oil/day (control)	Reported no significant effect of DHA + EPA on all measured parameters of coagulation or fibrinolysis. Significant inhibition of collagen-induced platelet aggregation and TxB2 production in DHA + EPA dose group.	Prisco *et al.*, 1994; 1995

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Healthy volunteers, 23 to 39 years old	18 weeks	0.96 g DHA + 1.29 g EPA/day for 18 weeks 1.92 g DHA and 2.58 g EPA/day for 6 weeks, followed by 0.96 g DHA + 1.29 g EPA/day for 12 weeks	No significant effect on platelet aggregation, TxB_2 production, or urinary thromboxane metabolites after 6 weeks. After 18 weeks, a significant decrease in collagen-induced aggregation and 11-dehydro-TxB_2 urinary metabolites	Tremoli *et al.*, 1995
Healthy males, 20 to 40 years of age	120 days (basal diet for 30 days followed by 90 days with supplement; total fat content and α-tocopherol similar between diets)	basal diet with safflower oil replaced by oil providing 6 g DHA/day basal diet	Circulating polymorphonuclear leukocytes and WBC significantly lower after 50 and 83 days of DHA treatment. No effect on number of B cells, total T cells, helper T cells, suppressor T cells, cytotoxic T cells, or NK in circulation, delayed type hypersensitivity response, *in vitro* lymphocyte proliferation, or serum concentrations of IgG, C3 and ILR2.	Kelley *et al.*, 1998
Healthy males, 20 to 40 years of age	120 days (basal diet for 30 days followed by 90 days with supplement)	basal diet with safflower oil replaced with 6 g DHA/day basal diet	NK cell activity significantly reduced on day 83 of treatment compared with pre-treatment activity. Significant reductions in the *in vitro* secretion of TNF α and iIL 1β by PBMNC following 83 days of supplementation. Significant reduction in the *in vitro* secretion of prostaglandin E2 and leukotriene B4 by PBMNC on day 83 of supplementation compared to pre-treatment values.	Kelley *et al.*, 1999
Healthy males with a mean age of 33 years	120 days	stabilization diet for 30 days, followed by 6 g DHA/day for 90 days control: stabilization diet for 120 days; <0.05 g DHA/day	No effect on bleeding time, platelet aggregation or soluble clotting factors.	Nelson *et al.*, 1997
Patients with ventricular tachyarrythmia of 48 to 73 years of age	16 weeks	8 capsules/day, providing 4.3 g DHA + EPA/day control, 8 capsules of corn oil rich in n-6 PUFA	Significant decrease in plasma TG concentration following supplemental DHA and EPA. No significant changes in total, HDL or LDL cholesterol levels. Supplemental DHA and EPA had no effect on the number of ventricular extrasystoles/48 h, following 16 weeks of treatment.	Christensen *et al.*, 1995
Schizophrenia or schizoaffective disorder patients between the ages of 18 and 65	16 week, double blind	3 g EPA/day or 3 g mineral oil (placebo)/day	No significant improvement in schizophrenic symptoms, two adverse events – diarrhea and upper respiratory infection	Fenton *et al.*, 2001

Patients with severe hypertriglyceridemia of 35 to 57 years of age	16 weeks, randomized, double-blind, prospective parallel group	4 g fish oil concentrate/day, providing 1.5 g DHA and 1.9 g EPA/day placebo: 4 g corn oil	Treatment with fish oil had no effect on blood glucose level compared to baseline. Reported no adverse effects related to treatment. Treatment with fish oil significantly increased HDL and LDL cholesterol, and significantly reduced total cholesterol, VLDL and TG compared to baseline. Treatment with fish oil had no effect on baseline measures of liver and kidney function.	Harris *et al.*, 1997
Patients of 43 to 63 years of age with untreated hypertension	16 week, randomized, double-blind, placebo-controlled	0.6 g DHA + 3.4 g EPA ethyl esters/day control: 4 g corn oil/day, containing 56% linoleic and 26% oleic acids	Reported no serious side effects observed. Treatment with DHA + EPA had no effect on platelet count, PAI-1, tPA, or FVIIC. Fibrinogen level significantly increased over baseline in both groups. Treatment with DHA + EPA had no effect on proinsulin level compared to baseline or the control group.	Toft *et al.*, 1997
Patients aged 30 to 71 years with primary type IIb or type IV hyperlipidemia	14 weeks, randomized, double-blind, placebo-controlled	4 g ω-3 fatty acid ethyl ether concentrate/day, providing 3.4 g DHA and EPA/day placebo: 4 g corn oil	No adverse effects due to treatment. K-85 fish oil concentrate had no effect on fasting blood glucose level compared with baseline. Fish oil concentrate had no significant effect on LDL cholesterol. Serum TG and VLDL cholesterol significantly reduced from baseline following fish oil treatment.	Mackness *et al.*, 1994
Patients with unresectable adenocarcinoma of the pancreas	median of 3 months	dose escalation at weekly intervals from 2 g fish oil/day, by 2 g, to a maximum of 16 g/day (0.12 g DHA + 0.18 g EPA/g)	Median MTD was reported to be 3.6 g DHA + EPA/day. Reported "no serious toxicity", although transient diarrhea occurred in a " number of patients". Steatorrhea, occurring in 25% of patients, was managed with supplemental pancreatic enzyme.	Wigmore *et al.*, 1996
healthy males with total cholesterol >5.2 mmol/L of 33 to 59 years of age	12 weeks, randomized, placebo-controlled	900 mg garlic powder pills + 12 g oil placebo/day 12 g fish oil/day, providing 1.44 g DHA and 2.16 g EPA/day 900 mg garlic + 12 g fish oil/day, providing 1.44 g DHA and 2.16 g EPA/day placebo: 900 mg garlic placebo + 12 g oil placebo (evening primrose oil)/day	In fish oil group LDL cholesterol increased significantly compared to baseline and other treatments. LDL cholesterol was significantly reduced compared to baseline following treatment with fish oil + garlic. Both fish oil and fish oil + garlic treatments significantly reduced serum TG. No subjects withdrew from study due to treatment and no serious side effects reported.	Adler and Holub, 1997

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Patients with combined hyperlipidemia aged 36 to 44 years	12 weeks, randomized, double-blind, placebo-controlled	4 g concentrated n-3 fatty acid ethyl esters/day, providing 3.4 g DHA and EPA/day	No change in bleeding time, fibrinogen, Hcy, TFPI, or FVIIC levels. Reported platelet count was significantly reduced in DHA + EPA group. PAI was reported to be significantly increased following DHA + EPA treatment.	Grundt *et al.*, 1999
		placebo: 4 g corn oil/day		
Healthy, non-smoking males "having body mass index, blood pressure, and plasma cholesterol toward the higher end of the normal range" with a mean age of 45.7±0.6 years	12 weeks; 120 subjects randomized to 7 dietary groups consuming either a diet composed of 40% of energy from fat or 30% of energy from fat	40% Diet with 1.5-2.4 g DHA and 1.32 g EPA/day	Significant decrease in collagen and PAI-1 induced platelet aggregation, and TxB_2 levels.	Mori *et al.*, 1997
		40% Diet with 0.8 g DHA and 1.32 g EPA/day		
		40% Diet with 2.3-3.2 g DHA and 2.64 g EPA/day		
		40% Diet with 1.6 g DHA and 2.64 g EPA/day		
		30% Diet with: 1.5-2.4 g DHA and 1.32 g EPA/day		
Patients with NIDDM and hyperlipidemia of 46 to 61 years of age	12 weeks, randomized, double-blind, placebo-controlled	9 g fish oil/day (2.5 g DHA and 2.6 g EPA/day)	Reported no significant effect of either fish oil treatment on fasting glucose and glycosylated haemoglobin concentrations compared to baseline measures. No significant differences in these values between treatments following the treatment period. Reported no significant effects on lipid parameters within each fish oil or control treatment. Pooled dose levels indicated total and VLDL-TG were significantly reduced following 12 weeks of fish oil treatment compared to baseline and corn oil.	Morgan *et al.*, 1995
		placebo: 9 g corn oil		
		18 g fish oil/day, (4.9 g DHA and 5.2 g EPA/day		
		placebo: 18 g corn oil/day		
Outpatients of 50 to 62 years of age with history of myocardial infarction >3 months prior to study	12 weeks	4 g ω-3 ethyl esters fatty acids concentrate from fish oil/day, providing 3.0 g DHA and EPA/day	Antithrombin III was significantly increased from baseline in fish oil group, however, value was not significantly different from that of placebo. Significant decrease in total triglycerides, VLDL-TG, and VLDL cholesterol in fish oil group. Significant increase in mean LDL cholesterol and LDL apo B following treatment with fish oil.	Swahn *et al.*, 1998
		placebo 4 g corn oil/day		
patients with CHD and elevated serum lipids	67 ± 30 days randomized, double-blind, placebo-controlled	6 g n-3 fatty acid ethyl esters/day, providing 5.1 g DHA and EPA/day	Fibrinogen, PAI-1 and TAT were significantly increased with fish oil treatment; however, similar significant increases were also observed with placebo.	Nilsen *et al.*, 1993
	85 ± 23 days	placebo: 6 g corn oil/day		

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Otherwise healthy IDDM patients of 30.4±3.7 years of age	2 months	10 g marine oil concentrate/day, providing 1.0 g DHA and 3.6 g EPA/day	Reported glucose and fructosamine levels were unchanged from baseline following treatment with DHA + EPA fatty acid concentrate.	Bagdade *et al.*, 1996
Patients 47 to 58 years of age with hypertriglyceridemia or mixed hyperlipidemia	2 months, randomized, double-blind	12 g fish oil/day, providing 1.44 g DHA and 2.16 g EPA/day 12 g soya oil/day	No major side effects reported with fish oil treatment. Significant increase in blood glucose of fish oil group as compared to baseline. Glucose significantly increased by 19.9% in NIDDM patients and by 7.2% in the non-diabetic patients. Fish oil had no significant effect on any measured cholesterol related parameters, including LDL cholesterol, compared to baseline or soy oil. Treatment with fish oil had no effect on baseline measures of liver and kidney function. No major side effects reported with fish oil treatment.	Silva *et al.*, 1996
Patients undergoing elective percutaneous intraluminal coronary angioplasty	56 days; 12-14 before through 6 months after angioplasty; randomized, double-blind, placebo-controlled	8.1 g fish oil ethyl esters of ω-3 fatty acids/day, providing 2.8 g DHA and 4.1 g EPA/day placebo: 8.1 g fatty acid ethyl esters of corn oil	Adverse events were similar and unremarkable between fish oil and placebo groups. All bleeding times were within normal ranges. DHA and EPA displaced arachadonic acid and linoleic acids in plasma and red blood cell phospholipids. Fish oil treatment had no effect on LDL cholesterol. TG levels significantly decreased with fish oil treatment.	Leaf *et al.*, 1994
Males of 52.8 to 60.4 years of age with hypertension not adequately controlled with antihypertensive drugs	8 weeks	18 g fish oil/day, providing 1.28 g DHA and 2.16 g EPA/day 18 g placebo: corn oil	Reported fish oil was well tolerated with no study withdrawals due to treatment. Blood glucose levels were unchanged from baseline following 12 weeks of fish oil treatment. Significant increase in baseline LDL cholesterol was reported in the fish oil group. In the fish oil group, TG were significantly reduced after 4 weeks. Blood chemistry and liver function were unchanged from baseline following 12 weeks of fish oil treatment.	Gray *et al.*, 1996
Male outpatients with mild to moderate essential hypertension with a mean age of 44.8 ±6.4 years	8 week, randomized, double-blind, crossover trial	2.7 g EPA/day	A decrease in systolic blood pressure and intracellular sodium content, and an increase in erythrocyte membrane EPA content	Miyajima *et al.*, 2001

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Patients with primary FDL or FHTG of 43 to 46 years of age	8 weeks	3 g fish oil (0.6 g DHA + 0.9 g EPA)/day for 2 weeks, followed by 6 g fish oil (1.2 g DHA + 1.8 g EPA)/day for 6 weeks	One patient withdrew due to gastrointestinal side effects. No significant effects on hemorrhealogical parameters, except for a decrease in the mean red blood aggregation of FHTG patients. No significant change in mean LDL cholesterol in FHTG patients. Significant decrease in total and VLDL TG, and VLDL cholesterol following fish oil treatment. In FDL patients there was a significant decrease in total and VLDL cholesterol, VLDL TG, and apo B following fish oil treatment.	Otto *et al.*, 1996
Healthy males of 36 to 65 years of age	7 weeks	4 g DHA capsule/day (3.6 g DHA ethyl ester/day) 4 g EPA capsule/day (3.8 g EPA ethyl ester/day) placebo = 4 g corn oil/day	No significant difference in LDL cholesterol levels. Significant decrease in TG following both DHA and EPA treatments. Total cholesterol and apo A-I significantly lower in EPA group than in placebo and DHA groups. HDL cholesterol significantly increased in DHA group than in placebo or EPA groups. Mild, transient side effects, notably belching were reported.	Grimsgaard *et al.*, 1997
Healthy, non-smoking men of 36 to 56 years of age	7 weeks, double blind, placebo controlled	3.8 g EPA, 3.6 g DHA, or 4 g corn oil/day	No significant changes in PAI-1 activity, and no relationship between TG and phospholipid n-3 fatty acid levels and PAI-1 activity after EPA or DHA consumption.	Hansen *et al.*, 2000
Healthy, non-obese, normotensive, normolipemic males of 21 to 47 years of age	7 weeks, randomized, double-blind, placebo-controlled	4 g purified n-3 fatty acids ethyl ether /day, providing 1.2 g DHA + 2.2 g EPA/day 12 g n-3 triglycerides/day, providing 1.4 g DHA + 2.2 g DHA/day placebo 4 g corn oil	Reported no adverse effects with treatment. Following 7 weeks, both n-3 fatty acids treatments significantly reduced collagen-induced platelet aggregation as compared to the placebo. No significant effects of treatment on any other measured variable. Following 7 weeks, no treatment effects on total cholesterol or TG concentrations. Reported no adverse effects with treatment.	Hansen *et al.*, 1993a
Male and female dislipidemic volunteers aged 40 to 69 years	7 weeks, double blind, parallel design	3 g DHA/day, 3 g EPA/day or a placebo	Significant decrease in plasma total and VLDL TG, an increase in SAC, and a trend of lower pulse pressure and total vascular resistance	Nestel *et al.*, 2002
Patients with stable angina pectoris and accepted for CAB surgery of 48 to 69 years of age	6 weeks, randomized, double-blind, placebo-controlled	9 g n-3 fatty acid ethyl esters/day, providing 2.84 g DHA and 4.72 g EPA/day placebo: 9 g corn oil/day	No effect on measured parameters	Almdahl *et al.*, 1993

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Healthy males of 28 to 31 years of age	6 weeks	20 g seal oil/day (1.7 g DHA + 1.3 g EPA /day) 20 vegetable oil capsules (evening primrose oil)/day	Significant decrease in fibrinogen concentration and increase in protein C concentration following seal oil supplementation. No significant differences reported for all other thrombogenic parameters measured. Seal oil had no significant effect on glucose levels following 6 weeks of treatment. Seal oil had no significant effect on the levels of selected cardiovascular risk factors, including LDL cholesterol when compared to control.	Conquer *et al.*, 1999
Patients with CF, 12.2 ± 5.4 years old, and patients without CF, 13.4 ± 6.3 years old	6 weeks, randomized, double-blind, placebo-controlled	CF + 8 g fish oil/day (2.2 g DHA and 3.2 g EPA/day) non-CF + 2.2 g DHA and 3.2 g EPA/day CF + 1.6 mg EPA/day non-CF + 1.6 mg EPA/day	No increase on bleeding incidence or platelet aggregation. No changes in any other haemostatic parameter examined. Noted adverse effects of diarrhea and eructation in some CF patients treated with fish oil. Serum glucose levels unchanged from baseline following treatment with fish oil in both CF and non-CF patients. No significant differences in any other measured parameters.	Henderson *et al.*, 1994
Patients of 29 to 59 years of age with familial hypercholesterolemia (type IIa) randomized to one of three treatments	double-blind, placebo-controlled, crossover; 3 treatment periods of 6 weeks separated by 6- to 8-week washout periods	1.8 g DHA and 3.3 g EPA/day (treatment 1) for 1st period, 40 mg HMG-CoA reductase inhibitor/day (treatment 2) for 2nd period, and combination of both (treatment 3) for 3rd period Treatment 2, treatment 3, and then treatment 1 Treatment 3, treatment 1, and then treatment 2	No adverse effects reported with treatment. Six weeks of fish oil treatment had no effect on resting bleeding time. Exercise induced changes in red blood cell count, hematocrit, white blood cell count, and platelet count were similar between treatments. Significant decrease in exercise-induced shortening of bleeding times observed, however bleeding times were still shorter than resting bleeding times. Six weeks of fish oil treatment significantly reduced serum TG levels, but had no effect on total, HDL, and LDL cholesterol.	Hansen *et al.*, 1993b

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8 patients with chronic glomerular disease of 19 to 70 years of age	6 weeks; open study, 4 patients of group A also in group B, 1 patient (NIDDM) duplicated in group B	9 g fish oil triglycerides/day, (2.97 g DHA + EPA/day)	Significant increase in bleeding time, and decrease in plasma TxB_2 and plasma TG. No other significant effects were reported. No effect of either fish oil treatment on total, HLD or LDL cholesterol levels or on Lp A. TG were significantly reduced following both fish oil treatments. No effect of either fish oil treatment on creatinine clearance, and serum albumin and creatinine. Reported a significant reduction of proteinuria following 7.69 g DHA +EPA, but not 2.97 g DHA + EPA	Lenzi *et al.*, 1996
		9 g ethyl esters of ω-3 fatty acids/day (7.65 g DHA + EPA/day)		
Mildly hypertriacylglycerolemic males	6 weeks, double blind, placebo controlled crossover with a 12-week washout period	1.7 g EPA + 1.3 g DHA/day	Increased platelet EPA and DHA, decrease in CHD risk factors, and an increase in LDL, and LDL oxidizability	Leigh-Firbank *et al.*, 2002
Hypertensive patients concurrently treated with diuretics or beta-blockers with a mean age of 61±3 years	6 weeks, randomized, double-blind, placebo-controlled crossover of consecutive 6-week treatment periods	4 g fish oil/day, providing 1.5 g DHA and 1.9 g EPA/day	Fish oil had no effect on total, HDL, or LDL cholesterol levels. Following treatment with fish oil, plasma TG was significantly reduced as compared to placebo.	Lungershausen *et al.*, 1994
		placebo: corn oil		
NIDDM patients of 46 to 61 years of age	6 weeks, randomized, double-blind, placebo-controlled crossover with 6-week washout period	10 g fish oil/day, providing 1.2 g DHA and 1.8 g EPA/day	No significant effect of fish oil on blood glucose level, or glycosylated haemoglobin and LDL, compared to baseline or placebo. No significant effect of fish oil on any measured cholesterol related parameters, including LDL cholesterol, compared to baseline or placebo. Plasma thiobarituric acid-reactive substances were significantly greater following treatment with fish oil compared to baseline and placebo	McGrath *et al.*, 1996
		placebo: 10 g olive oil		
NIDDM patients of 45 to 64 years of age	6 weeks, randomized, double-blind, placebo-controlled crossover with a 6-week washout between treatment periods	10 g fish oil/day, providing 1.2 g DHA and 1.8 g EPA/day	Fasting glucose levels were unchanged from baseline following treatment with fish oil.	McVeigh *et al.*, 1994
		placebo: 10 g olive oil		

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Mildly hypercholesterolemic males of 20 to 65 years of age	6 weeks	3.68 g DHA ethyl ester/day	One subject "withdrew because of gastrointestinal symptoms". Both DHA and EPA significantly increased fasting insulin compared to olive oil. No effect of treatment on total serum cholesterol. DHA significantly increased serum LDL cholesterol and LDL particle size. A significant decrease in oxidative stress and no changes in markers of inflammation were reported	Mori *et al.*, 2000
		3.84 g EPA ethyl ester/day		
		4 g olive oil capsule/day		
Healthy males, 25 to 38 years of age	6 weeks	Group I = 3.35 g DHA + 4.5 g EPA supplement/day; total 7.85 g/day	No significant effect on bleeding time or platelet aggregation. Significant reduction in platelet aggregation induced by the endoperoxide analogue U 46619 and a significant increase in sensitivity to iloprost, an anti-aggregatory prostacyclin analogue.	Scheurlen *et al.*, 1993
		Group II = 6.5 g DHA + 3.5 g EPA supplement/day; total 10 g/day		
Healthy males of 25 to 28 years of age	6 weeks	13.8 g sardine oil/day, providing 1.2 g DHA and 3.3 g EPA/day	Significant decrease in LTB_4, and 5-HEPE levels. Similar effects not observed following treatment with vegetable oil.	Turini *et al.*, 1994
		control: 13.8 g vegetable oil/day		
Male and female hypertensive type 2 diabetic patients of 40 to 75 years of age	6 week, double-blind, placebo controlled, parallel design	4 g of EPA, DHA or olive oil (placebo)/day	No significant changes in platelet aggregation, fibrinolytic function or vascular function. DHA significantly decreased collagen aggregation and TXB_2 levels	Woodman *et al.*, 2002
Postmenopausal women aged 50 to 75 years	5 weeks, 3 treatment periods separated by 7-week washout periods	2.0 g EPA/day and 1.4 g DHA/day	No evidence of increased lipid peroxidation	Higdon *et al.*, 2000

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Patients aged 38.9 to 56 years, with combined hyperlipidemia	5 week randomized, double-blind, placebo-controlled treatment period following 5-10 weeks of treatment with Simvastatin	20 mg Simvastatin/day + 1.56 g DHA and 1.8 g EPA/day 20 mg Simvastatin/day + 4 g corn oil/day	Reported no registered side effects due to treatment. Glucose and insulin levels were not affected by treatment with fish oil compared to control. Significant decreases in total cholesterol, TG, and apo E, in addition to the significant reductions reported with Simvastatin alone. LDL cholesterol and other measured variables were not significantly different following treatment with fish oil compared to corn oil. Liver and serum enzymes were not affected by treatment with fish oil compared to control. No significant effects of fish oil on plasma lipid peroxides; however, α-tocopherol was increased following control and not fish oil treatments.	Nordøy *et al.*, 1998
cirrhotic patients with ascites	1 month	12 g fish oil concentrate/day to healthy patients (2.76 g DHA and 3.24 g EPA/day) 12 g fish oil concentrate/day to patients with renal failure 12 g fish oil concentrate/day to healthy controls	No increase in bleeding time of either patient group following 1 month of supplemental fish oil. Authors pooled groups and reported a significant increase in bleeding time after treatment. GFR and urine volume were significantly increased, without changes in sodium excretion or free water clearance, in both control and normal renal function fish oil groups. No changes in urinary excretion of prostaglandin E2 and 6-keto-PGF1α were reported for any group.	Badalamenti *et al.*, 1997
Hypercholesterolemic patients of 39 to 49 years of age	28 days	20 g fish oil/day, providing 2.32 g DHA and 3.56 g EPA/day	No significant changes in any of the parameters examined.	Chin and Dart, 1994
Patients with familial hypercholesterolemia on long term treatment with simvastatin with a mean age of 45.2±15.0 years	4 week; randomized, double-blind, placebo-controlled crossover of 2, 4-week treatment periods separated by a 4-week washout	6 g fish oil ethyl ester/day, providing 2.55 g DHA and 2.55 g EPA/day control: 6 g olive oil	Treatment with fish oil had no effect on any measured parameter, in patients concurrently administered simvastatin. Reported no complaints of side effects related to treatment.	Balestrieri *et al.*, 1996
Patients undergoing elective CABG surgery	4 weeks prior to CABG; in addition, all patients received 325 mg aspirin/day until 7-10 days before CABG	3.2 g evening primrose oil/day; 3.2 g fish Oil/day; providing 1.26 g DHA and 1.9 g EPA/day	No effects on platelet adhesivity and aggregation (ADP- and collagen-induced), and TxA2 and 12-HETE synthesis were-reported for any treatment. Total dose of 3.16 g DHA + EPA/day.	Brister and Buchanan, 1998

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		3.2 g primrose oil + fish oil/day, (1 g DHA and 1.52 g EPA/day) placebo: gelatin capsules		
Healthy Finnish adults or 22 to 44 years of age	4 weeks supplement with 12-week follow-up; supplements provided as 1.19 mg/kJ (1 g/200 kcal) calculated energy expenditure	Fish muscle oil concentrate (mean 2.3 g DHA/day; range 1.8-3.4 g DHA/day) (mean 2.9 g EPA/day; range 2.2-4.2 g EPA/day) linseed oil (mean 5.9 g linolenic acid/day; range 4.2-8.4 g linolenic acid/day)	Reported that supplements were well tolerated and the sole adverse effect reported was abnormally long bleeding times. Significant reduction in ADP-induced platelet aggregation in fish oil-supplemented subjects 12 weeks after supplementation.. Total cholesterol and TG significantly reduced in fish oil group following treatment compared with linseed oil.	Freese and Mutanen, 1997a
Healthy volunteers of 22 to 42 years of age	4-week supplementation with 12-week follow-up	Fish oil + 2.0-3.5 g sunflower oil/day; (mean 12.2 g fatty acids/day; n-3:n-6 = 4.0; 2.45 g DHA + 3.04 g EPA/day) linseed oil (mean 11.9 g fatty acids/day; n-3:n-6 = 3.6; 6.21 g α-linolenic acid/day)	Fish oil was reportedly well tolerated with no gastrointestinal side effects. Postprandial and fasting values of FVIIC activity, PAI-1, and ADP-induced aggregation were significantly increased, and collagen-induced aggregation significantly decreased. Fasting value of glucose was significantly increased following fish oil supplementation from fasting samples taken before supplementation. Significant decrease in fasting TG and cholesterol following fish oil supplementation.	Freese and Mutanen, 1997b
Mildly hypertriglyceridemic patients of 34 to 68 years of age	4 weeks; randomized, double-blind, placebo-controlled crossover with a 1-week washout between treatments	1 g fish oil ethyl esters/10 kg body weight/day, (~2.0 g DHA and ~3.5 g EPA/day) placebo: olive oil ethyl esters	Reported no side effects with treatment. Fish oil had no effect on bleeding time. Oxidation of apoprotein B-containing lipoprotein was significantly increased following treatment with fish oil. Significant increase in LDL cholesterol, LDL-apo B, apo B and HDL_2 cholesterol following treatment with fish oil. TG levels were significantly decreased in fish oil group.	Harris *et al.*, 1993
males of 39 to 60 years of age with serum triglyceride concentrations of 1.5-4 mmol/L (normal to mildly high)	4 week treatment period in randomized, double-blind crossover with 6 week washout period between treatments	30 mL fish oil (ESKIMO-3)/day containing 1.5 IU vitamin E/g; providing 3.2 g DHA and 5.4 g EPA/day 30 mL ESKIMO-3/day containing 4.5 IU vitamin E/g; providing 3.2 g DHA and 5.4 g EPA/day	Significant increase in PAI-1 activity, and decrease in TxB_2 and LTB_4 levels. No change in plasma prostacyclin or fibrinogen. Significant increase in fasting plasma glucose concentration following fish oil treatment with 1.5 IU vitamin E/day and in insulin/glucose ratio following treatment with 4.5 IU vitamin E/day. Insulin concentrations were not affected following either treatment.	Luostarinen *et al.*, 1995; Engström *et al.*, 1996

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Healthy volunteers of 23 to 40 years of age	4 weeks, randomized, double-blind, crossover of 2 treatment periods of 4 weeks, each followed by a washout of 4 weeks	1.44 g DHA and 2.16 g EPA/day or 12 g olive oil (control)	No significant effect on platelet aggregation, fibrinogen, or any measures of coagulation. Significant decrease in prothrombin time reported in both fish oil and olive oil groups.	Misso and Thompson, 1995
Hypertensive males with hyperlipidemia of 33 to 64 years of age	4 weeks, randomized, double-blind, placebo-controlled crossover of 2, 4-week, treatment periods with a 4 week washout	Fish oil providing 2.8 g DHA + 1.8 g EPA/day	No effect on bleeding time, β-thromboglobulin, or platelet count.	Mundal *et al.*, 1993
		placebo: olive oil		
Male and female normal volunteers	4 weeks, placebo controlled, parallel design	4 g of EPA, DHA, or safflower oil (placebo)/day	EPA and not DHA decrease platelet activation.	Park and Harris, 2002
Healthy, non-smoking, normolipemic males of 27 to 58 years of age	3 weeks per diet with 6-week washout between high and low saturated fat diets	high saturated fat with 9 fish oil capsules/day, providing 1.2 g DHA and 2.1 g EPA./day.	Reported fish oil was well tolerated without adverse effects. Significant effect of fish oil not demonstrated for any haemostatic parameter measured.	Nordøy *et al.*, 1994
		high saturated fat with 9 olive oil capsules/day		
Healthy, non-obese males of 18 to 34 years of age	crossover of 3 weeks of nonrandomized saturated fat diet followed by 3 weeks of randomized n-3 or n-6 diet, with 8-week washout between diets	Saturated fat diet - "trace" amounts of DHA + EPA	Significant increase in fasting FVIIC activity and von Willebrand factor levels. Significant decrease in β-thromboglobulin and platelet counts. Fasting values for apo B and total and LDL cholesterol were significantly lower following the n-3 and n-6 diets compared to the saturated fat diet. Fasting values for total TG and apo AII were significantly lower, and HDL_2 cholesterol significantly higher, in the n-3 diet compared to the n-6 and saturated diets.	Sanders *et al.*, 1997
		n-3 diet – 2 g DHA + 3 g EPA/day		
		n-6 diet – 5 g linoleic acid		
Healthy normolipidemic males of 22 to 26 years of age	3 weeks of fish oil dietary supplementation subsequent to 3 weeks of soybean oil dietary supplementation	"low" cholesterol diet 20 g fish oil/day, providing 1.6 g DHA and 7.2 g EPA/day	LDL cholesterol was unchanged from baseline following fish oil treatment in both groups. Fish oil diet resulted in significantly lower total and VLDL cholesterol, and total and VLDL-TG, compared with baseline and soybean oil treatments at both levels of dietary cholesterol.	Tsai and Lu, 1997
		"high" cholesterol diet 20 g fish oil/day, providing 1.6 g DHA and 7.2 g EPA/day		

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Patients aged 34 to 76 years with diagnosis of cancer not amendable to curative treatment	2 weeks per dose	dose-escalation in groups of 2 starting at 0.1 g fish oil/kg bw/day (0.249 g DHA + 0.378 g EPA/g fish oil)	No life-threatening toxicity (grade 4 or 5) observed up to maximum treatment of 0.5 g fish oil/kg bw/day. Dose-limiting toxicities (grade 3) were all gastrointestinal. MTD estimated to be 0.3 g fish oil/kg bw/day. Thus, for 70 kg individual MTD is equivalent to 13.2 g DHA + EPA/day.	Burns *et al.*, 1999
hyperlipidemic, NIDDM patients of 55 to 67 years of age	2 weeks, randomized, open, crossover of 2 treatment periods of 2 weeks separated by an 8-week washout	22 mL fish oil/day, providing 1.784 g DHA and 2.890 g EPA/day	Treatment with fish oil had no effect on any measured parameter of glycemic control, including control of blood glucose levels. Treatment with fish oil had no effect on LDL cholesterol levels compared to baseline. Following treatment with fish oil, there were significant reductions in total and VLDL cholesterol, total and VLDL-TG, and apo B. Reported no adverse effects on parameters of liver and kidney function.	Fasching *et al.*, 1996
		900 mg Gemfibrozil/day		
Healthy males of 18 to 55 years of age	14 days	1.2 g DHA + 2.2 g EPA/day	Reported "none of the volunteers suffered from adverse effects or discomfort that could be ascribed to the treatment."	Krokan *et al.*, 1993
		2.4 g DHA + 4.4 g EPA/day		
		4.2 g DHA + 7.7 g EPA/day		
		1.4 g DHA + 2.2 g EPA/day		
		2.9 g DHA + 4.4 g EPA/day		
Hypertensive, mildly obese and dyslipidemic patients of 57 to 61 years of age	13- days, non-randomized crossover of 3 treatment periods containing 4, 20-h fasting periods; each treatment period separated by a 3-week washout	Period I: 1.8g DHA and 2.7g EPA/day, administered after fasting and followed by refeeding	No effects of fish oil treatment on fibrinogen and platelet count. Significant reduction in platelet aggregation and adhesion, and α_2-anti-plasmin following period I but not periods II or III. No effects of fish oil treatment on cholesterol related parameters measured, including LDL cholesterol.	Yosefy *et al.*, 1996
		Period II: fasting followed by refeeding without DHA or EPA		
		Period III: 1.8g DHA and 2.7g EPA/day, without fasting and refeeding		
healthy volunteers aged 18 to 22 years	1 week	30 g fish oil/day, providing 3.6 g DHA and 5.4 g EPA/day	No effect of fish oil treatment on fibrinogen or C-reactive protein levels.	de Maat *et al.*, 1994

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patients undergoing elective total esophagectomy with a mean age of 55 (treatment) and 60 years (control)	7 days	IV administration of 28.45 mg DHA/day and 28.16 mg EPA/day Control IV administration of 20% soybean fat emulsion	Reported no adverse effects observed with treatment. Bleeding time unchanged with fish oil treatment.	Roulet *et al.*, 1997
Women 24 to 28 weeks pregnant	Randomized, double-blind, controlled clinical trials lasting until the birth of the baby	Eggs supplemented with DHA, ~133 mg DHA/egg Control: Normal eggs ~ 33 gm DHA/egg	DHA intake increased gestation by an average of 6 days	Smuts *et al.*, 2003
Clinical trials conducted with DAG				
Generally healthy male and female adults aged 19 to 71 years	24-weeks, randomized, double blind, controlled, parallel trial	Food products infused with DAG oil providing 16 to 45 g DAG oil/day	No significant difference observed in the adverse events reported by the DAG and TAG oil treatment groups. The mean body weight loss, mean fat loss, and decrease in abdominal fat were all greater in the individuals consuming the products containing DAG oil as opposed to those containing TAG oil. No significant differences were observed in the total, LDL, HDL, or non-HDL cholesterol and triacylglycerol levels of the two test oil groups.	Maki *et al.*, 2002
Generally healthy male volunteers of 27 to 49 years of age	16-weeks, double blind, controlled trial	10 g test oil/day	Significantly larger decreases in body weight, body mass index, waist circumference, and abdominal, visceral, and subcutaneous fat areas were reported for the DAG oil treatment group as compared to the TAG oil treatment group. There were no significant differences in the changes in the hip circumference, the waist to hip circumference ratio, hepatic fat levels, or body fat levels observed in the DAG and TAG oil groups. No significant differences were observed in the serum lipid levels within or between either test group.	Nagao *et al.*, 2000
Male and female volunteers of good overall health, 23 to 50 years of age	12-weeks, double blind, controlled, parallel trial	500 mg test oil/kg body weight/day	No serious adverse effects were reported. No biologically or toxicologically significant changes in blood pressure, lipid profile, or blood chemistry observed. Significant decrease in several body weight and size measurements of individuals consuming DAG oil products.	Yasunaga *et al.*, 2004

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Male and female diabetic patients aged 35 to 73 years	12-weeks, randomized, single blind, controlled parallel trial	Food products infused with DAG oil providing an average of 10.4 g/day	No adverse effects resulted from the consumption of either DAG or TAG oils. A significant decrease in serum triglyceride and HbA_{1c} levels was observed in the individuals consuming the DAG oil while no significant differences were observed in the blood glucose levels, body weight, or body mass index of the DAG oil group.	Yamamoto *et al.*, 2001
Healthy normocholesterolemic or moderately hypercholesterolemic men aged 29 to 50 years	2-week, randomized crossover design	10 g DAG or TAG oil and 500 mg phytosterols/day	No side effects were reported by any of the study participants and both DAG oil and TAG oil containing mayonnaises were reported to be well tolerated by all study participants. No significant differences were observed in the dietary intake or serum TG levels of either dosing group. In the individuals consuming the DAG oil and phytosterols, a significant decrease in total and LDL cholesterol levels was observed, and these were significantly greater than those observed in the individuals consuming the TAG oil and phytosterols.	Meguro *et al.*, 2001
Male volunteers aged 31 to 40 years	Single exposure	Creamed test meals containing 30 g/m^2 of either DAG or TAG oil	Serum triacylglycerol and cholesterol concentrations were significantly lower at 2, 3 and 8 h after loading of DAG than those after loading of TAG. The area under the curve of serum TAG was significantly lower after DAG loading than after TAG loading.	Tada *et al.*, 2001
Normolipidemic male volunteers aged 25 to 42 years	Single exposure, crossover design with 7 day washout period	Fat emulsion providing 10, 20, or 44 g of DAG or TAG oil	TAG levels were significantly lower 4 and 6 hours after DAG emulsion consumption as compared to TAG emulsion consumption. TG, cholesterol, and phospholipid concentrations at 4 hours after ingestion of DG emulsion were significantly lower than those after TG emulsion ingestion. No significant differences were observed for VLDL, LDL and HDL lipids between the test emulsions.	Taguchi *et al.*, 2000
Male and female type 2 diabestes mellitus patients aged 46 to 70 years	Single exposure	Creamed test meals containing 30 g/m^2 of either DAG or TAG oil	DAG loading significantly suppressed increases in postprandial serum TAG and lipids in RLP as compared with TAG loading. No significant differences in serum levels of insulin, free fatty acids, and ketone bodies during fat loading were observed between DAG and TAG test oils.	Tada *et al.*, 2005

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Abbreviations: apo = Apolipoprotein; ASAT = Aspartate Amino Transferase; ALAT = alanine amino transferase; CAB = Coronary Artery Bypass; CABG = Coronary artery bypass grafting; CF = Cystic Fibrosis; CHD = Coronary Heart Disease; FDL = Familial dysbetalipoproteinemia; FHTG = Familial hypertriglyceridemia; FVIIC = Coagulation Factor VII; GFR = Glomerular filtration rate; GGT = γ-glutamyl transferase; Hcy = Homocystein; HDL = High Density Lipoprotein; IDDM = Insulin Dependant Diabetes Mellitus; IL = Interleukin; LDL = Low Density Lipoportein; Lp = Lipoprotein; MTD = Maximum Tolerated Dose; NIDDM = Non-Insulin Dependant Diabetes Mellitus; NK = Natural Killer Cells; PAI = Plaminogen Activator Inhibitor; PBMNC = Peripheral Blood Mononuclear Cells; RBC = Red Blood Cells; SAC = systemic arterial compliance; TBA-MDA = Thiobarbituric acid-Malondialdehyde; TAT = Thrombin-antithrombin III; TG = triglyceride; TNF = Tumour Necrosis Factor; t-PA= tissue plasminogen activator; TPF = Tissue factor pathway inhibitor; TxB_2 = Thomboxane B_2; TxA_2 = Thomboxane A_2; VLDL = Very Low Density Lipoprotein; WBC = White Blood Cells

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CFSAN/Office of Food Additive Safety
December 20, 2005

Agency Response Letter
GRAS Notice No. GRN 000177

William C. Franke, Ph.D.
President
Heart Blend Foods LLC
14 Silvers Lane
Cranbury, NJ 08512

Re: GRAS Notice No. GRN 000177

Dear Dr. Franke:

The Food and Drug Administration (FDA) is responding to the notice, dated July 15, 2005, that Heart Blend Foods LLC (Heart Blend) submitted in accordance with the agency's proposed regulation, proposed 21 CFR 170.36 (62 FR 18938; April 17, 1997; Substances Generally Recognized as Safe (GRAS); the GRAS proposal). FDA received the notice on July 18, 2005, filed it on July 20, 2005, and designated it as GRAS Notice No. GRN 000177.

The subject of the notice is plant sterol esters. The notice informs FDA of the view of Heart Blend that plant sterol esters are GRAS, through scientific procedures, for use as an ingredient in ground roasted coffee at 1.0 gram (g) per 8 fluid (fl) ounce (oz) serving.

Heart Blend obtains the plant sterol esters from Archer Daniels Midland, who submitted GRAS notices for plant sterol esters in November 2000 (GRN 000061) and, more recently, in August 2005 (GRN 000176). Heart Blend's notice describes its intended conditions of use for plant sterol esters and the estimated daily intake that would result from that use. GRN 000177 incorporates by reference the information contained in GRNs 000048, 000053, 000061, and 000112; and in Food Master File (FMF) 000625.

As described in GRN 000061, the main sterol components of the ingredient plant sterol esters are beta-sitosterol, campesterol, and stigmasterol. The sterols are derived from oil seeds such as corn, palm, soy, rape, and sunflower. In the manufacturing process, the sterols are esterified with vegetable oil fatty acids. The fatty acids are preferentially derived from soy, sunflower, safflower, and canola. Corn, peanut, cottonseed, and palm may also be used as sources. Food grade specifications exist for plant sterol esters.

Heart Blend intends plant sterol esters for use as an ingredient in ground roasted coffee that, when brewed according to package directions, will yield 1.0 g plant sterol esters per 8 fl oz

serving. Brand name, brand positioning, price, product information, and directions for preparation are intended to inform consumers about the presence of plant sterol esters in the roasted ground coffee. The requirement to use a wire mesh "permanent" filter will limit consumption predominantly to home brewed coffee because wire mesh filters are not commonly used in commercial food service operations. A typical paper filter will trap the plant sterol esters in the filter, resulting in little or no plant sterol esters in the filtered coffee beverage.

Heart Blend provides an estimate of the consumption of plant sterol esters in brewed coffee. Assuming that 1.0 g plant sterol esters is equivalent to 0.6 g plant sterols, Heart Blend estimates that its use of plant sterol esters would result in the consumption of 1.4 g/day (d) at the mean and 2.5 g/d at the 90th percentile.

The notifier notes that a panel of individuals who evaluated the data and information that are the basis for a determination that vegetable sterol esters are GRAS considered that an acceptable daily intake (ADI) of 130 milligrams per kilogram body weight per day, as the free sterol, was appropriate for vegetable sterol esters.[1] Heart Blend considers that plant sterol esters are similar to vegetable sterol esters and that for a 70 kilogram person, an ADI of 9.1 g/d is appropriate. Based on a review of literature from January 2000 to June 2005, Heart Blend concludes that the original safety assessment of these esters remains valid.

Based on the information provided by Heart Blend, as well as other information available to FDA, the agency has no questions at this time regarding Heart Blend's conclusion that plant sterol esters are GRAS under the intended conditions of use. The agency has not, however, made its own determination regarding the GRAS status of the subject use of plant sterol esters. As always, it is the continuing responsibility of Heart Blend to ensure that food ingredients that the firm markets are safe, and are otherwise in compliance with all applicable legal and regulatory requirements.

In accordance with proposed 21 CFR 170.36(f), a copy of the text of this letter, as well as a copy of the information in your notice that conforms to the information in proposed 21 CFR 170.36(c)(1), is available for public review and copying on the homepage of the Office of Food Additive Safety (on the Internet at http://www.cfsan.fda.gov/~lrd/foodadd.html).

Sincerely,

Laura M. Tarantino, Ph.D.
Director
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition

[1]Heart Blend references Lipton's GRAS panel, from FMF 000625, for the ADI for vegetable oil sterol esters.

FDA/Center for Food Safety & Applied Nutrition
Hypertext updated by pmg/rxm January 30, 2006

Appendix 11

21 CFR Part 172

[Docket No. 90F-0446]

Food Additives Permitted for Direct Addition to Food for Human Consumption: Dimethyl Dicarbonate

AGENCY: Food and Drug Administration, HHS.

ACTION: Final rule.

SUMMARY: The Food and Drug Administration (FDA) is amending the food additive regulations to provide for the safe use of dimethyl dicarbonate as a yeast inhibitor in dealcoholized and low alcohol wines. This action is in response to a petition filed by Miles, Inc. (formerly Mobay Corp.).

DATES: Effective January 26, 1993; written objections and requests for a hearing by February 25, 1993.

ADDRESSES: Written objections may be sent to the Dockets Management Branch (HFA-305), Food and Drug Administration, rm. 1-23, 12420 Parklawn Dr., Rockville, MD 20857.

FOR FURTHER INFORMATION CONTACT: Rosalie M. Angeles, Center for Food Safety and Applied Nutrition (HFS-207), Food and Drug Administration, 200 C St. SW., Washington, DC 20204, 202-254-9515.

SUPPLEMENTARY INFORMATION: In a notice published in the **Federal Register** of November 20, 1990 (55 FR 48292), FDA announced that a food additive petition (FAP 0A4213) had been filed by Mobay Corp., 1575 I St. NW., Washington, DC 20005, proposing that § 172.133 *Dimethyl dicarbonate* (21 CFR 172.133) be amended to provide for the safe use of dimethyl dicarbonate as a yeast inhibitor in dealcoholized and low alcohol wine. The petitioner currently operates under the name of Miles, Inc.

FDA has evaluated data in the petition and other relevant material and concludes that the proposed use of dimethyl dicarbonate in dealcoholized wine and low alcohol wine is safe. Dealcoholized wine and low alcohol wine will generally be consumed as substitutes for, rather than in addition to, wine. Thus, these uses will not increase consumer exposure to dimethyl dicarbonate or its decomposition products compared to that already deemed safe at the time § 172.133 was promulgated (53 FR 41325, October 21, 1988).

Dimethyl dicarbonate is unstable in aqueous solution and breaks down almost immediately after addition to beverages. In wine and other aqueous liquids, the principal breakdown products are methanol and carbon dioxide. Methyl ethyl carbonate, as well as carbomethoxy amino- and hydroxy-adducts of amines, sugars, and fruit acids, are also formed in minor amounts. Dimethyl carbonate is present as an impurity in dimethyl dicarbonate. Dimethyl dicarbonate also may react with traces of ammonia or ammonium ions in wines to form trace quantities of methyl carbamate, a compound that has been shown to cause cancer in laboratory animals (Ref. 1). In dealcoholized wine and low alcohol wine, the level of methyl carbamate formation is expected to be similar to that formed in standard wine because the critical parameters governing methyl carbamate formation, Ph and ammonium ion concentration, are not expected to be altered by the dealcoholization process (reverse osmosis) employed in the manufacture of dealcoholized wine and low alcohol wine (Ref. 2).

I. Determination of Safety

Under section 409(c)(3)(A) of the Federal Food, Drug, and Cosmetic Act (the act) (21 U.S.C 348(c)(3)(A)), the so-called "general safety clause" of the statute, a food additive cannot be approved for a particular use unless a fair evaluation of the data available to FDA establishes that the additive is safe for that use. Under section 409(c)(5)(A) of the act (21 U.S.C. 348(c)(5)(A)), among the relevant factors to be considered in determining whether a proposed use of a food additive is safe is the probable consumption of the additive and of any substance formed in or on food because of the use of the additive. The concept of safety embodied in the Food Additives Amendment of 1958 is explained in the legislative history of the provision: "Safety requires proof of a reasonable certainty that no harm will result from the proposed use of an additive. It does not--and cannot--require proof beyond any possible doubt that no harm will result under any conceivable circumstance." (H. Rept. 2284, 85th Cong., 2d sess. 4 (1958)). This definition of safety has been incorporated into FDA's food additive regulations (21 CFR 170.3(i)). The anticancer or Delaney clause of the Food Additives Amendment (section 409(c)(3)(A) of the act) provides further that no food additive shall be deemed to be safe if it is found to induce cancer when ingested by man or animal.

In the past, FDA has refused to approve the use of an additive that contained or was suspected of containing even minor amounts of a carcinogenic chemical, even though the additive as a whole had not been shown to cause cancer. The agency now believes, however, that developments in scientific technology and experience with risk assessment procedures make it possible for FDA to establish the safety of additives that contain carcinogenic chemicals but that have not themselves been shown to cause cancer.

In the preamble to the final rule permanently listing D&C Green No. 6 published in the **Federal Register** of April 2, 1982 (47 FR 14138), FDA explained the basis for approving the use of a color additive that had not been shown to cause cancer, even though it contained a carcinogenic impurity. Since that decision, FDA has approved the use of other color additives and food additives on the same basis.

An additive that has not been shown to cause cancer but that contains a carcinogenic impurity, or whose use will lead to the formation of trace amounts of a carcinogenic substance in or on food, may be properly evaluated under the general safety clause of the statute using risk assessment procedures to determine whether there is a reasonable certainty that no harm will result from the proposed use of the additive.

The agency's position is supported by *Scott* v. *FDA*, 728 F. 2d. 322 (6th Cir. 1984). That case involved a challenge to FDA's decision to approve the use of D&C Green No. 5, which contains a carcinogenic chemical but has itself not been shown to cause cancer. Relying heavily on the reasoning in the agency's decision to list this color additive, the U.S. Court of Appeals for the Sixth Circuit rejected the challenge to FDA's action and affirmed the listing regulation.

II. Safety of the Petitioned Use

In evaluating the safety of the food additive, dimethyl dicarbonate, FDA reviewed the byproducts formed during hydrolysis and the reaction of the food additive with other constituents found in wines. The results of that evaluation were discussed in the preamble to the final rule establishing § 172.133 and are included in the discussion below.

FDA finds that the petitioned use level of 100 to 200 parts per million (ppm) of dimethyl dicarbonate will result in virtually no exposure of consumers to the additive itself. Dimethyl dicarbonate is unstable in aqueous solution and breaks down almost immediately after addition to the food (beverages) to form primarily carbon dioxide and methanol. The instability of dimethyl dicarbonate is confirmed by data submitted by the petitioner showing that dimethyl dicarbonate cannot be detected by analysis of food to which it has been added (Ref. 2).

To establish that dimethyl dicarbonate is safe for use as an inhibitor of yeast in wine, dealcoholized wine, and low alcohol wine, the petitioner submitted data from acute, subchronic, and chronic toxicity studies. In the subchronic and chronic toxicity studies, rats received either water, orange juice, or wine treated with 4,000 ppm of dimethyl dicarbonate (20 times the proposed use level in wine or wine substitutes) as the drinking fluid while the controls received water, orange juice, or wine. These studies showed no adverse effects from water, orange juice, or wine treated with dimethyl dicarbonate.

In another chronic toxicity study, dogs received either water or orange juice treated with 4,000 ppm of dimethyl dicarbonate as the drinking fluid. This study also revealed no adverse effects from the water or orange juice treated with dimethyl dicarbonate.

The petitioner also submitted a two-generation reproduction study in which rats received drinking fluids that were treated with dimethyl dicarbonate (4,000 ppm). This study revealed no treatment-related adverse effects. These chronic and other multigeneration (lifetime) studies of dimethyl dicarbonate also did not produce any evidence that dimethyl dicarbonate is a carcinogen.

III. Safety of Substances That May Be Present in Wine or Wine Substitutes Due to the Use of the Additive

Because dimethyl dicarbonate may contain impurities and decomposes into other chemical species when added to aqueous solutions, such as wine, dealcoholized wine, and low alcohol wine, FDA has also evaluated the safety of the chemicals found in wine, dealcoholized wine, and low alcohol wine as a result of the use of dimethyl dicarbonate.

A. Minor Impurities and Reaction Products

The minor reaction products formed in wine, dealcoholized wine, and low alcohol wine from the use of dimethyl dicarbonate include methylethyl carbonate and carbomethoxy amino- and hydroxy-adducts of amines, sugars, and naturally occurring fruit acids such as lactic acid, citric acid, and ascorbic acid (vitamin C). Dimethyl carbonate, an impurity in dimethyl dicarbonate, is also present in minor amounts in wine, dealcoholized wine, and low alcohol wine, as a result of the use of the additive.

The petitioner presented data to show that the addition of 100 to 200 ppm of dimethyl dicarbonate to wine, dealcoholized wine, or low alcohol wine is effective in inhibiting the growth of most species of yeast found in such products. According to the U.S. Department of Agriculture Food Consumption Survey, 1977 1978, the 90th percentile consumption level for "drinkers only" of these products is 232 grams per person per day (g/person/day). Based upon a level of addition of dimethyl dicarbonate of 100–200 ppm, on consumption of 232 g of wine or wine substitutes, and on data submitted by the petitioner, the agency estimates that the maximum daily consumption of the minor reaction products resulting from the addition of dimethyl dicarbonate to wine or wine substitutes is from 2 to 5 milligrams per person per day (mg/person/day). Because these reaction products were formed in the dimethyl dicarbonate-treated fluids (water and wine) used in the subchronic and chronic rat and dog studies submitted by the petitioner, the safety of the reaction products is evidenced by the findings of no treatment-related adverse effects in these studies.

The safety of methylethyl carbonate was further evaluated in a subchronic toxicity study in rats in which the substance was added to the drinking water at levels of 0, 1,000, 3,000, and 10,000 ppm for 3 months. The average daily consumption of methylethyl carbonate ranged from approximately 0.1 mg/kilogram (kg) to 1 g/kg body weight/day. No adverse effects in rats from drinking the water treated with methylethyl carbonate were seen in this study.

A teratogenicity study was conducted with pregnant female rats of the Long-Evans FB30 strain. The animals were fed diets containing methylethyl carbonate at levels of 0, 100, 1,000, and 10,000 ppm. No signs of toxicity were noted. However, there was a dose-related reduction in fluid intake and a slight decrease in body weight gain in pregnant females receiving methylethyl carbonate throughout the gestational period. The reduced fluid intake appears to be attributable to the bad taste and smell of the water containing the methylethyl carbonate. All test and control females were sacrificed at day 20, Cesarean sections were performed, and the fetuses were examined. No embryotoxic or teratogenic effects were found in this examination.

To establish the safety of dimethyl carbonate, the petitioner submitted a subchronic study in rats in which dimethyl carbonate was incorporated into the drinking water at levels of 0, 1,000, 3,000 and 10,000 ppm. An increase in body weight gain was observed in male rats at all treatment levels. No adverse effects were found in this study at any level.

B. Carbon Dioxide

Carbon dioxide, one of the principal hydrolysis products of dimethyl dicarbonate, is a natural product of animal metabolism. Carbon dioxide is present in solution as the carbonate and bicarbonate anions, however, and is routinely used to carbonate beverages (Ref. 3). The levels of carbon dioxide present in wine or wine substitutes as a result of the use of dimethyl dicarbonate are well below the levels found in carbonated beverages. Thus, the agency has no evidence that carbon dioxide would be harmful under the intended conditions of use.

C. Methanol

Methanol is the principal reaction product of concern resulting from the addition of dimethyl dicarbonate to wine. Theoretically, complete hydrolysis of dimethyl dicarbonate would yield 2 moles of methanol and 2 moles of carbon dioxide from each mole of dimethyl dicarbonate added to wine or wine substitute. On a weight basis, this yield corresponds to approximately 48 mg of methanol for each 100 mg of the additive added to a liter (L) of wine or wine substitute. To estimate a worst-case exposure of consumers to methanol from the proposed use of the additive, the agency assumed complete hydrolysis of dimethyl dicarbonate to methanol and carbon dioxide. Based on the addition of 100 to 200 mg dimethyl dicarbonate to 1 L of wine or wine

substitute and on a beverage intake of 232 g/person/day (90th percentile consumption level), the agency estimates that the daily intake of methanol from this use of dimethyl dicarbonate would range from 11 to 22 mg/day (0.18 to 0.36 mg/kg body weight for a 60-kg person) (Ref. 4).

The agency considers the daily intake of methanol from the addition of dimethyl dicarbonate to wine or wine substitutes, even when added to the amount of methanol naturally present in other foods such as fresh fruits and vegetables and grain alcohol, to be safe. The no observed adverse effect level (NOAEL) in humans for methanol is 71 to 84 mg/kg body weight (Ref. 5). Because the NOAEL is derived from studies in humans, an acceptable daily intake (ADI) of 7.1 to 8.4 mg/kg body weight (426 to 500 mg/person for a 60-kg adult) is derived from the NOAEL by using a 10-fold safety factor (Ref. 5). The levels of methanol that occur naturally in fruit juices average 140 mg/L (140 ppm) and an additional 50 to 100 mg/L (50 to 100 ppm) may result from the use of dimethyl dicarbonate in wine (Ref. 4). Based upon consumption data from the U.S. Department of Agriculture Food Consumption Survey, 1977–1978, the total methanol exposure from these sources would be up to 50 to 60 mg/person/day (or one-tenth of ADI). There is, therefore, a large margin of safety between the methanol intake from the subject uses and the amount which can be safely ingested.

D. Methyl Carbamate

1. *Carcinogenicity.* Reaction of dimethyl dicarbonate with naturally occurring ammonia or ammonium ions in wine or wine substitutes may result in the formation of trace amounts of methyl carbamate, which has been shown to be carcinogenic in rats (Ref. 1). FDA has evaluated the safety of this reaction byproduct using risk assessment procedures to estimate the upper-bound limit of risk presented by the presence of this chemical as an impurity in wine treated with dimethyl dicarbonate. Based on this evaluation, the agency has concluded that under the proposed conditions of use, dimethyl dicarbonate is safe.

2. *Basis for evaluation.* The risk assessment procedures that FDA used in this evaluation are similar to the methods that it has used to examine the risk associated with the presence of minor carcinogenic impurities in various food and color additives (see e.g., 49 FR 13018, April 2, 1984). This evaluation of the risk from the use of dimethyl dicarbonate has two aspects: (1) Assessment of the probable exposure to methyl carbamate produced in food from the use of dimethyl dicarbonate; and (2) extrapolation of the risk observed in the animal bioassay to the conditions of probable exposure to humans.

Based on an estimate of the level of methyl carbamate that may be produced from the addition of dimethyl dicarbonate to wine or wine substitutes as a yeast inhibitor, as well as the estimated average daily intake of wine over a lifetime, FDA estimated the worst-case exposure to methyl carbamate to be 2.4 micrograms per person per day (µg/person/day) (Refs. 4, 6, and 7).

The agency used data in a carcinogenesis bioassay report on methyl carbamate conducted by the National Toxicology Program (NTP) (Ref. 6) to estimate the upper-bound level of lifetime human risk from exposure to this chemical stemming from the proposed use of dimethyl dicarbonate. The bioassay report consisted of results from studies of methyl carbamate in both rats and mice. The bioassay in B6C3F1 mice was reported by NTP to be negative. The bioassay of methyl carbamate in F344/N rats consisted of a 2-year chronic study and a parallel study with sacrifices at 6, 12, and 18 months. The 2-year study employed a high dosage level of 200 mg/kg body weight. The parallel study employed one dosage level of 400 mg/kg body weight. In the 2-year chronic study, an increase in hepatocellular neoplasms was found at the high dose in female F344/N rats. In the parallel study, hepatocellular neoplasms were found at 6 months in both sexes, and the sacrifices at the later times revealed a classic progression from benign to highly malignant neoplasms dependent upon the length of time of exposure. The NTP concluded that "there was clear evidence of carcinogenic activity for male and female F344/N rats given methyl carbamate as indicated by incidences of hepatocellular neoplastic nodules and hepatocellular carcinoma" (Ref. 1).

3. *Results of evaluation.* Using the NTP bioassay report, the Center for Food Safety and Applied Nutrition's Quantitative Risk Assessment Committee (QRAC) estimated the human cancer risk from the potential exposure to methyl carbamate stemming from the proposed use of dimethyl dicarbonate as a yeast inhibitor in wine (Ref. 7).

The QRAC used a quantitative risk assessment procedure (linear proportional model) to extrapolate from the dose used in the animal experiment through zero to cover the very low doses expected to be encountered under the proposed conditions of use of the additive. This procedure is not likely to underestimate the actual risk from the very low doses and may, in fact, exaggerate it because the extrapolation models used are designed to estimate the maximum risk consistent with the data. For this reason, the estimate can be used with confidence to determine to a reasonable certainty whether any harm will result from the proposed conditions and a maximum 200 ppm level of use of the food additive.

Based on a worst-case exposure to methyl carbamate (2.4 µg/person/day), FDA estimated, using the linear proportional model, that the upper-bound limit of individual lifetime risk from potential exposure to methyl carbamate is 2.4×10^{-8} or less than 1 in 42 million. Because of numerous conservatisms in the exposure estimate, lifetime averaged individual daily exposure to methyl carbamate is expected to be substantially less than the estimated daily intake, and, therefore, the calculated upper-bound risk would be less than 1 in 42 million. Thus, the agency concludes that there is a reasonable certainty of no harm from the exposure to methyl carbamate that may result from the use of up to 200 ppm of dimethyl dicarbonate in wine, dealcoholized wine, or low alcohol wine.

4. *Need for specifications.* The agency also has considered whether a specification is necessary to control the amount of methyl carbamate that may be formed in wine or wine substitutes treated with the additive. The agency finds that the amount of methyl carbamate formed in wine or wine substitutes may be controlled by limiting the amount of dimethyl dicarbonate that may be added to the wine or wine substitute to 200 ppm or less rather than setting a specification for the level of methyl carbamate impurity in the wine product. The petitioner submitted data to show that the maximum level of methyl carbamate impurity formed in commercial wine is less than 10 parts per billion for each 100 ppm of dimethyl dicarbonate added to wine. A 200 ppm level of dimethyl dicarbonate is sufficient to control the growth of all significant genera and species of yeast in wine and in wine substitutes that have been adequately pasteurized or ultra-filtered according to current good manufacturing practices to reduce the microbial count to 500 per milliliter or less.

E. Ethyl Carbamate

The agency is aware that ethyl carbamate, an animal carcinogen occurs

as a "natural" contaminant in wine. The agency is in the process of obtaining as much information as possible about the levels of such ethyl carbamate contamination. In addition, in cooperation with the wine industry, a program has been instituted to find and control the formation of ethyl carbamate so as to reduce its concentration to the lowest levels possible (Ref. 8).

The petitioner submitted studies in which gas chromatography/mass spectroscopy was used to measure the formation of ethyl carbamate (urethane) in dimethyl dicarbonate treated-wine and model wine solutions, in the presence of high concentrations of ammonium ions. These studies, conducted over a 12-month period, did not show formation of ethyl carbamate in excess of endogenous levels found in wine. These studies also did not show evidence of formation of ethyl carbamate by transesterification of methyl carbamate. Thus, there is no evidence that the use of dimethyl dicarbonate affects the level of ethyl carbamate in wine.

IV. Conclusion on Safety

FDA has evaluated all of the data in the petition pertaining to the use of dimethyl dicarbonate in dealcoholized wine and low alcohol wine and has determined that the additive is safe for the proposed use.

To ensure the safe use of the additive, FDA, under 21 U.S.C. 348(c)(1)(A) and in accordance with section 403 of the act (21 U.S.C. 343), finds that it is necessary to require that the label of the package containing the additive include, in addition to other information required by the act: (1) The name of the additive, "dimethyl dicarbonate," and (2) directions to provide that not more than 200 ppm of dimethyl dicarbonate will be added to the dealcoholized wine or low alcohol wine.

In accordance with § 171.1(h) (21 CFR 171.1(h)), the petition and the documents that FDA considered and relied upon in reaching its decision to approve the petition are available for inspection at the Center for Food Safety and Applied Nutrition (address above) by appointment with the information contact person listed above. As provided in 21 CFR 171.1(h), the agency will delete from the documents any materials that are not available for public disclosure before making the documents available for inspection.

V. Environmental Impact

The agency has carefully considered the potential environmental effects of this action. FDA has concluded that the action will not have a significant impact on the human environment, and that an environmental impact statement is not required. The agency's finding of no significant impact and the evidence supporting that finding, contained in an environmental assessment, may be seen in the Dockets Management Branch (address above) between 9 a.m. and 4 p.m., Monday through Friday.

VI. 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. NTP Technical Report on the Toxicology and Carcinogenesis Studies of Methyl Carbamate in F344/N Rats and B6C3F1 Mice, NTP, U.S. Department of Health and Human Services, Report No. 328, 1986.

2. Memorandum from the Food and Color Additives Review Section to the Direct Additives Branch, "Dimethyl Dicarbonate (DMDC) in Dealcoholized and Low-alcohol Wines," dated October 4, 1990.

3. Mones, Martha, "Carbonated Beverages," in "Encyclopedia of Chemical Technology," 4:710–725, 1978.

4. Memorandum from the Regulatory Food Chemistry Branch to the GRAS Review Branch, "Dimethyl Dicarbonate in Wine. Submission of September 5, 1986; Exposure Estimate for Methyl Carbamate and Methanol in Wine," dated January 14, 1987.

5. Memorandum from the Standards and Monitoring Branch to the Division of Regulatory Guidance, "Methanol in Brandy," dated December 18, 1989.

6. Memorandum from QRAC to the Office of Toxicological Sciences, "Methyl Carbamate in Wine," dated October 28, 1986.

7. Memorandum from QRAC to the Office of Toxicological Sciences, "Methyl Carbamate in Wine," dated November 20, 1987.

8. "Ethyl Carbamate Voluntary Program," Final Agreement Between the Wine Institute, the Association of American Vintners, and FDA, January 7, 1988.

VII. Objections

Any person who will be adversely affected by this regulation may at any time on or before February 25, 1993, file with the Dockets Management Branch (address above) written objections thereto. Each objection shall be separately numbered, and each numbered objection shall specify with particularity the provisions of the regulation to which objection is made and the grounds for the objection. Each numbered objection on which a hearing is requested shall specifically so state. Failure to request a hearing for any particular objection shall constitute a waiver of the right to a hearing on that objection. Each numbered objection for which a hearing is requested shall include a detailed description and analysis of the specific factual information intended to be presented in support of the objection in the event that a hearing is held. Failure to include such a description and analysis for any particular objection shall constitute a waiver of the right to a hearing on the objection. Three copies of all documents shall be submitted and shall be identified with the docket number found in brackets in the heading of this document. Any objections received in response to the regulation may be seen in the Dockets Management Branch between 9 a.m. and 4 p.m., Monday through Friday.

List of Subjects in 21 CFR Part 172

Food additives, 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 172 is amended as follows:

PART 172—FOOD ADDITIVES PERMITTED FOR DIRECT ADDITION TO FOOD FOR HUMAN CONSUMPTION

1. The authority citation for 21 CFR part 172 continues to read as follows:

Authority: Secs. 201, 401, 402, 409, 701, 706 of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 321, 341, 342, 348, 371, 376).

2. Section 172.133 is amended by revising the introductory text and paragraphs (b) and (c)(2) to read as follows:

§ 172.133 Dimethyl dicarbonate.

Dimethyl dicarbonate (CAS Reg. No. 4525–33–1) may be safely used in wine, dealcoholized wine, and low alcohol wine, in accordance with the following prescribed conditions:

* * * * *

(b) The additive is used or intended for use as an inhibitor of yeast in wine, dealcoholized wine, and low alcohol wine under normal circumstances of bottling where the viable yeast count has been reduced to 500 per milliliter or less by current good manufacturing practices such as flash pasteurization or filtration. The additive may be added to wine, dealcoholized wine, or low alcohol wine in an amount not to exceed 200 parts per million (ppm).

(c) * * *

(2) Directions to provide that not more than 200 ppm of dimethyl dicarbonate will be added to the wine, dealcoholized wine, or low alcohol wine.

* * * * *

Dated: January 15, 1993.

Michael R. Taylor,

Deputy Commissioner for Policy.

[FR Doc. 93-1795 Filed 1-25-93; 8:45 am]

BILLING CODE 4160-01-F

DEPARTMENT OF HEALTH AND HUMAN SERVICES

Food and Drug Administration

21 CFR Part 172

[Docket No. 94F-0189]

Food Additives Permitted for Direct Addition to Food for Human Consumption; Dimethyl Dicarbonate

AGENCY: Food and Drug Administration, HHS.

ACTION: Final rule.

SUMMARY: The Food and Drug Administration (FDA) is amending the food additive regulations to provide for the safe use of dimethyl dicarbonate (DMDC) as a yeast inhibitor in sports drinks and fruit or juice sparklers. This action is in response to a petition filed by Miles, Inc. (now Bayer Corp.).

DATES: Effective May 29, 1996; written objections and requests for a hearing by June 28, 1996.

ADDRESSES: Written objections may be sent to the Dockets Management Branch (HFA-305), Food and Drug Administration, 12420 Parklawn Dr., rm. 1-23, Rockville, MD 20857.

FOR FURTHER INFORMATION CONTACT: Martha D. Peiperl, Center for Food Safety and Applied Nutrition (HFS-217), Food and Drug Administration, 200 C St. SW., Washington, DC 20204, 202-418-3077.

SUPPLEMENTARY INFORMATION:

I. Background

In a notice published in the Federal Register of June 28, 1994 (59 FR 33299), FDA announced that a food additive petition (FAP 4A4420) had been filed by Miles, Inc., Mobay Rd., Pittsburgh, PA 15205-9741 (now Bayer Corp., 100 Bayer Rd., Pittsburgh, PA 15205-9741), proposing that the food additive regulations in § 172.133 *Dimethyl dicarbonate* (21 CFR 172.133) be amended to provide for the safe use of DMDC as a yeast inhibitor in sports drinks and fruit or juice sparklers. The petition defines sports drinks as carbonated or noncarbonated, nonjuice-containing (less than or equal to 1 percent juice), flavored or unflavored beverages containing added electrolytes (5-20 milliequivalents (meq)/liter sodium ion (Na+) and 3-7 meq/liter potassium ion (K+)). Fruit or juice sparklers are defined as carbonated, dilute beverages containing juice, fruit flavor, or both, with juice content not to exceed 50 percent.

DMDC is currently approved in § 172.133 for use as a yeast inhibitor in wine, dealcoholized wine, and low alcohol wine (53 FR 41325, October 21, 1988; and 58 FR 6088, January 26, 1993) and in ready-to-drink tea beverages (59 FR 5317, February 4, 1994) (hereinafter referred to as the October 1988 final rule, the January 1993 final rule, and the February 1994 final rule, respectively).

As discussed below, FDA has evaluated data in the petition and other relevant material and concludes that DMDC is efficacious in preventing the growth of yeasts and molds in sports drinks and fruit or juice sparklers and that the proposed use of DMDC is safe.

II. Determination of Safety

Under the so-called "general safety clause" in section 409(c)(3)(A) of the Federal Food, Drug, and Cosmetic Act (the act) (21 U.S.C. 348(c)(3)(A)), a food additive cannot be approved for a particular use unless a fair evaluation of the data available to FDA establishes that the additive is safe for that use. FDA's food additive regulations (21 CFR 170.3(i)) define safe as "a reasonable certainty in the minds of competent scientists that the substance is not harmful under the intended conditions of use."

The food additive anticancer or Delaney clause in section 409(c)(3)(A) of the act (21 U.S.C. 348(c)(3)(A)) further provides that no food additive shall be deemed to be safe if it is found to induce cancer when ingested by man or animal. Importantly, however, the Delaney clause applies to the additive itself and not to the impurities in the additive. That is, where an additive itself has not been shown to cause cancer, but contains a carcinogenic impurity, the additive is properly evaluated under the general safety clause using risk assessment procedures to determine whether there is a reasonable certainty that no harm will result from the proposed use of the additive, *Scott* v. *FDA*, 728 F.2d 322 (6th Cir. 1984).

III. Safety of DMDC in Sports Drinks and Fruit or Juice Sparklers

DMDC is currently permitted as a yeast inhibitor in wine and wine substitutes (dealcoholized wine and low-alcohol wine) and in ready-to-drink tea beverages under § 172.133. In the October 1988, January 1993, and February 1994 final rules, the agency concluded that, because DMDC decomposes almost immediately after addition to aqueous beverages, there will be virtually no exposure to the additive from the consumption of the above-listed beverages.

Data submitted in the petition to support the proposed use of the additive at levels up to 250 parts per million (ppm) in sports drinks and fruit or juice sparklers are consistent with these findings. Specifically, data from a study of sparkling juice drink formulated with 250 ppm DMDC showed no detectable amount of the additive (limit of detection (LOD) = 40 parts per billion (ppb)) after 4 hours (Ref. 1). A study of water with 250 ppm DMDC added yielded the same result (Ref. 1). Based on these data and data incorporated from the petition that resulted in the October 1988 final rule (FAP 2A3636), the agency concludes that there will be virtually no consumer exposure to DMDC, per se, from the use of the additive in sports drinks and fruit or juice sparklers. Therefore, FDA concludes that DMDC itself presents no hazard to the consumer.

IV. Safety of Substances That May be Present in Sports Drinks and Fruit or Juice Sparklers Due to the Use of the Additive

DMDC is unstable in aqueous solution and breaks down almost immediately after addition to beverages. In aqueous liquids, the principal breakdown products are methanol and carbon dioxide. Dimethyl carbonate (DMC) may be present as an impurity in DMDC. Section 172.133 sets a specification of 0.2 percent DMC in DMDC. DMDC also may react with traces of ammonium ions in beverages to produce methyl carbamate (MC), a known carcinogen.

In previous evaluations of DMDC, the agency, in accordance with § 171.1 (21 CFR 171.1), reviewed the safety not only of DMDC but also of its decomposition products in aqueous beverages. The results of the agency's analysis of the additive's use in wine and wine substitutes were discussed extensively in the October 1988 and January 1993 final rules, and its use in ready-to-drink tea beverages was discussed in the February 1994 final rule. The agency applied the same type of analysis as in past reviews to its review of the petitioned use of DMDC. Aspects of the safety evaluation that were not previously addressed in final rules for other uses of DMDC are discussed below.

A. Methanol

As stated in previous final rules on DMDC, the tolerable (safe) level of exposure to methanol is 7.1 to 8.4 milligrams per kilogram body weight per day (mg/kg body weight/day), or approximately 426 to 504 mg/person/day for a 60 kg adult. FDA estimates that the cumulative methanol exposure for a consumer at the 90th percentile from its presence naturally in untreated fruit juice and wine and from all uses of

DMDC, including its currently regulated uses and the proposed use in sports drinks and fruit or juice sparklers, is 59 mg/person/day (Ref. 2). This estimate is based on a maximum level of methanol that can be derived from DMDC of 48.7 ppm methanol per 100 ppm DMDC used. This level is less than one-seventh of the tolerable safe level. The agency, therefore, concludes that there is an adequate margin of safety between total methanol consumption from all sources, including the petitioned use of DMDC, and the amount of methanol that can be safely ingested.

B. Methyl Carbamate

The reaction of ammonium ions in beverages with DMDC produces MC, a known carcinogen. The petitioner provided data showing that MC was detected at a level of 3.7 ppb in a fruit sparkler formulated with 250 ppm DMDC. MC was not detected in DMDC-treated sports drinks, using an analytical method with an LOD of 0.5 ppb. Using the residual level of 3.7 ppb and the LOD of 0.5 ppb for MC in fruit sparklers and sports drinks, respectively, the agency estimates the exposure to MC for all ages from the petitioned use of DMDC to be 1.5 microgram/person/day at the 90th percentile (Ref. 1). Using established procedures for quantitative risk assessment, the agency estimates that the 90th percentile upper-bound lifetime risk from potential exposure to MC from the petitioned use of DMDC is 1.5×10^{-8}, or less than 1 in 67 million, and the 90th percentile upper-bound lifetime risk from exposure to MC from all approved and petitioned uses of DMDC is 1.8×10^{-8}, or less than 1 in 56 million (Refs. 1 and 3).

Therefore, the agency concludes that there is a reasonable certainty of no harm from the exposure to MC that may result from the use of up to 250 ppm of DMDC in sports drinks and fruit or juice sparklers.

V. Conclusion on Safety

FDA has evaluated all of the data in the petition pertaining to the use of DMDC in sports drinks and fruit or juice sparklers, as well as other data in its files, and concludes that the additive is safe for its proposed use.

To ensure the safe use of the additive in sports drinks and fruit or juice sparklers, FDA, under 21 U.S.C. 348(c)(1)(A), finds that it is necessary to require directions on the food additive label limiting the level of use of the additive in these beverages to 250 ppm.

In accordance with § 171.1(h), the petition and the documents that FDA considered and relied upon in reaching its decision to approve the petition are available for inspection at the Center for Food Safety and Applied Nutrition by appointment with the information contact person listed above. As provided in § 171.1(h), the agency will delete from the documents any materials that are not available for public disclosure before making the documents available for inspection.

VI. Environmental Impact

The agency has carefully considered the potential environmental effects of this action. FDA has concluded that the action will not have a significant impact on the human environment, and that an environmental impact statement is not required. The agency's finding of no significant impact and the evidence supporting that finding, contained in an environmental assessment, may be seen in the Dockets Management Branch (address above) between 9 a.m. and 4 p.m., Monday through Friday.

The agency received one comment on the environmental assessment in response to the filing notice published in the Federal Register of June 28, 1994 (59 FR 33299). The comment states that approval of the subject additive could have two environmental benefits due to switching from hot-fill bottling of sports drinks and sparklers to cold-fill. The comment claims that this switch could greatly reduce water usage in the bottling process and could reduce cooling water flow into municipal wastewater treatment plants. However, the comment did not provide quantitative data on the magnitude of the claimed environmental benefits of the approval of this petition. FDA has concluded that the comment does not affect the agency's determination that the approval of this petition will have no significant impact on the environment. This comment can be seen at the Dockets Management Branch, along with the petitioner's environmental assessment and the agency's finding of no significant impact.

VII. Objections

Any person who will be adversely affected by this regulation may at any time on or before June 28, 1996, file with the Dockets Management Branch (address above) written objections thereto. Each objection shall be separately numbered, and each numbered objection shall specify with particularity the provisions of the regulation to which objection is made and the grounds for the objection. Each numbered objection on which a hearing is requested shall specifically so state. Failure to request a hearing for any particular objection shall constitute a waiver of the right to a hearing on that objection. Each numbered objection for which a hearing is requested shall include a detailed description and analysis of the specific factual information intended to be presented in support of the objection in the event that a hearing is held. Failure to include such a description and analysis for any particular objection shall constitute a waiver of the right to a hearing on the objection. Three copies of all documents shall be submitted and shall be identified with the docket number found in brackets in the heading of this document. Any objections received in response to the regulation may be seen in the Dockets Management Branch between 9 a.m. and 4 p.m., Monday through Friday.

VIII. 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. Memorandum from the Chemistry Review Branch to the Direct Additives Branch, "FAP 4A4420–Dimethyl Dicarbonate as a Yeast Inhibitor in Sports Drinks and in Fruit or Juice Sparkling Beverages," dated July 8, 1994.

2. Memorandum from the Chemistry Review Branch to the Direct Additives Branch, "FAP 4A4420–DMDC as a Yeast Inhibitor in Sports Drinks and Sparkling Fruit or Juice Beverages. Background Methanol Exposure," dated May 8, 1996.

3. Memorandum from the Direct Additives Branch to the Quantitative Risk Assessment Committee, "Estimation of the Upper-Bound Lifetime Risk from Methyl Carbamate (MC) Formed by the Reaction of Ammonium Ions with Dimethyl Dicarbonate (DMDC) During the Use of DMDC as Requested in FAP 4A4420 (Miles Inc.)," dated May 23, 1995.

List of Subjects in 21 CFR Part 172

Food additives, 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 172 is amended as follows:

PART 172—FOOD ADDITIVES PERMITTED FOR DIRECT ADDITION TO FOOD FOR HUMAN CONSUMPTION

1. The authority citation for 21 CFR part 172 continues to read as follows:

Authority: Secs. 201, 401, 402, 409, 701, 721 of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 321, 341, 342, 348, 371, 379e).

2. Section 172.133 is amended by adding new paragraphs (b)(3) and (b)(4)

and by revising paragraph (c)(2) to read as follows:

§ 172.133 Dimethyl dicarbonate.

* * * * *

(b) * * *

(3) Inhibitor of yeast in carbonated or noncarbonated, nonjuice-containing (less than or equal to 1 percent juice), flavored or unflavored beverages containing added electrolytes (5–20 milliequivalents (meq)/liter sodium ion (Na+) and 3–7 meq/liter potassium ion (K+)). The additive may be added to the beverage in an amount not to exceed 250 ppm.

(4) Inhibitor of yeast in carbonated, dilute beverages containing juice, fruit flavor, or both, with juice content not to exceed 50 percent. The additive may be added to the beverage in an amount not to exceed 250 ppm.

(c) * * *

(2) Directions to provide that not more than 200 ppm of dimethyl dicarbonate will be added to the wine, dealcoholized wine, or low alcohol wine and not more than 250 ppm of dimethyl dicarbonate will be added to the ready-to-drink tea or to the beverages described in parts (b)(3) and (b)(4) of this section.

Dated: May 17, 1996.

William K. Hubbard,

Associate Commissioner for Policy Coordination.

[FR Doc. 96–13303 Filed 5–28–96; 8:45 am]

BILLING CODE 4160–01–F

DEPARTMENT OF THE TREASURY

Internal Revenue Service

26 CFR Parts 301 and 602

[TD 8671]

RIN 1545–AS83

Taxpayer Identifying Numbers (TINs)

AGENCY: Internal Revenue Service (IRS), Treasury.

ACTION: Final regulations.

SUMMARY: This document contains final regulations relating to requirements for furnishing a taxpayer identifying number on returns, statements, or other documents. These regulations set forth procedures for requesting a taxpayer identifying number for certain alien individuals for whom a social security number is not available. These numbers are called "IRS individual taxpayer identification numbers." These regulations also require foreign persons to furnish a taxpayer identifying number on their tax returns.

DATES: These regulations are effective May 29, 1996.

For dates of applicability of these regulations, see § 301.6109–1(h).

FOR FURTHER INFORMATION CONTACT: Lilo A. Hester, (202) 874–1490 (not a toll-free number).

SUPPLEMENTARY INFORMATION:

Paperwork Reduction Act

The collection of information contained in these final regulations has been reviewed and approved by the Office of Management and Budget in accordance with the Paperwork Reduction Act (44 U.S.C. 3507) under control number 1545–1461.

An agency may not conduct or sponsor, and a person is not required to respond to, a collection of information unless the collection of information displays a valid control number.

The estimated annual burden for the collection of information contained in § 301.6109–1(d) is reflected in the burden of Form W–7.

Comments concerning the accuracy of this burden estimate and suggestions for reducing this burden should be sent to the Internal Revenue Service, Attn: IRS Reports Clearance Officer, PC:FP, Washington, DC 20224, and to the Office of Management and Budget, Attn: Desk Officer for the Department of the Treasury, Office of Information and Regulatory Affairs, Washington, DC 20503.

Books or records relating to this collection of information must be retained as long as their contents may become material in the administration of any internal revenue law. Generally, tax returns and tax return information are confidential, as required by 26 U.S.C. 6103.

Background

On June 8, 1995, the IRS published in the Federal Register (60 FR 30211) the withdrawal of the notice of proposed rulemaking published in the Federal Register on September 27, 1990 at 55 FR 39427, a notice of proposed rulemaking, and a notice of public hearing relating to taxpayer identifying numbers as contained in the Income Tax Regulations (26 CFR part 301) under section 6109 of the Internal Revenue Code (Code).

Written comments responding to the notice of proposed rulemaking were received, and a public hearing was held on September 28, 1995. After consideration of all the comments, the proposed regulations under 6109 of the Code are adopted as revised by this Treasury decision. The comments and revisions are discussed below.

Explanation of Provisions and Revisions

A. Principal Changes

Section 6109 of the Code generally provides that, when required by regulations, a person must furnish a taxpayer identifying number (TIN) for securing proper identification of that person on any return, statement, or other document made under the Code. The notice of proposed rulemaking contains two principal changes to the existing regulations. The first change is the introduction of a new IRS-issued TIN, called an IRS individual taxpayer identification number (ITIN), for use by alien individuals, whether resident or nonresident, who currently do not have, and are not eligible to obtain, social security numbers. The Social Security Administration generally limits its assignment of social security numbers to individuals who are U.S. citizens and alien individuals legally admitted to the United States for permanent residence or under other immigration categories which authorize U.S. employment. Therefore, this change is designed to help taxpayers (who need a TIN but cannot qualify for a social security number) maintain compliance with TIN requirements under the Code and regulations.

The second change is to modify the existing rule set forth in § 301.6109–1(g) that currently excludes from the general requirement of providing a TIN, foreign persons that do not have either (1) income effectively connected with the conduct of a U.S. trade or business or (2) a U.S. office or place of business or a U.S. fiscal or paying agent. Under these regulations, the exclusion is modified to require that any foreign person who makes a return of tax (i.e., income, gift, and estate tax returns, amended returns, or refund claims, but excluding information returns) furnish its TIN on that return. This change is intended to address the IRS' and Treasury's concern that, without TINs, taxpayers cannot be identified efficiently and tax returns cannot be processed effectively.

B. Comments

Regarding the assignment of ITINs under § 301.6109–1(d)(3)(iii) of the proposed regulations, commentators suggested that the IRS develop a process whereby either (1) the Social Security Administration (SSA) issues the ITIN when the individual is not eligible for a social security number, or (2) the Immigration and Naturalization Service (INS) (within the Department of Justice) and the U.S. consulate offices (within the Department of State) issue the ITIN

Federal Register

Friday,
September 8, 2000

3R

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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46. Letter from Alan M. Rulis, FDA, to Vivian A. Chester, and Edward B. Nelson, McNeil Consumer Healthcare, May 17, 1999.

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.

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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

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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; washout 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

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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

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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

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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

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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)

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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

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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

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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PART 217—APPLICATION FOR ANNUITY OR LUMP SUM

1. The authority citation for part 217 continues to read as follows:

Authority: 45 U.S.C. 231d and 45 U.S.C. 231f.

2. Section 217.9, paragraph (b)(1), is amended by adding directly after the words "paragraph (b)(2)", the words "and paragraph (b)(3)", and by adding a new paragraph (b)(3) to read as follows:

§ 217.9 Effective period of application.

* * * * *

(b) * * *

(3) *Application for spouse annuity filed simultaneously with employee disability annuity application.* When the qualifying employee's annuity application effective period is determined by the preceding paragraph (b)(2) of this section, a spouse who meets all eligibility requirements may file an annuity application on the same date as the employee claimant. The spouse application will be treated as though it were filed on the later of the actual filing date or the employee's annuity beginning date.

* * * * *

3. Section 217.30 is amended by removing paragraph (b), redesignating paragraph (c) as paragraph (b), and by adding a new paragraph (c) to read as follows:

§ 217.30 Reasons for denial of application.

* * * * *

(c) The applicant files an application more than three months before the date on which the eligible person's benefit can begin except if the application is for an employee disability annuity or for a spouse annuity filed simultaneously with the employee's disability annuity application.

Dated: June 18, 2002.

By Authority of the Board.

For the Board.

Beatrice Ezerski,

Secretary to the Board.

[FR Doc. 02–15911 Filed 6–24–02; 8:45 am]

BILLING CODE 7905–01–P

DEPARTMENT OF HEALTH AND HUMAN SERVICES

Food and Drug Administration

21 CFR Part 173

[Docket No. 89F–0452]

Secondary Direct Food Additives Permitted for Direct Addition to Food for Human Consumption; Materials Used as Fixing Agents in the Immobilization of Enzyme Preparations

AGENCY: Food and Drug Administration, HHS.

ACTION: Final rule.

SUMMARY: The Food and Drug Administration (FDA) is amending the food additive regulations to provide for the safe use of dimethylamine-epichlorohydrin and acrylamide-acrylic acid resins, individually or together, as fixing agents for the immobilization of glucose isomerase enzyme preparations. This action is in response to a petition filed by Enzyme Bio-Systems Ltd.

DATES: This rule is effective June 25, 2002. Submit written objections and requests for a hearing by July 25, 2002.

ADDRESSES: Submit written objections and requests for a hearing to the Dockets Management Branch (HFA–305), Food and Drug Administration, 5630 Fishers Lane, rm. 1061, Rockville, MD 20852. Submit electronic objections to *http://www.fda.gov/dockets/ecomments.*

FOR FURTHER INFORMATION CONTACT: Rosalie M. Angeles, Center for Food Safety and Applied Nutrition (HFS–206), Food and Drug Administration, 5100 Paint Branch Pkwy., College Park, MD 20740, 202–418–3107.

SUPPLEMENTARY INFORMATION:

I. Background

In a notice published in the **Federal Register** of November 17, 1989 (54 FR 47828), FDA announced that a food additive petition (FAP 9A4175) had been filed by Enzyme Bio-Systems Ltd., International Plaza, Route 9W, Englewood Cliffs, NJ 07632. The petition proposed to amend the food additive regulations to provide for the safe use of dimethylamine-epichlorohydrin copolymer (DEC) and acrylamide-acrylic acid resin (AAR) as fixing agents for immobilizing glucose isomerase enzyme.

DEC and AAR will be used, individually or together, to immobilize glucose isomerase enzymes for the purpose of converting glucose to a mixture of glucose and fructose for the production of high fructose corn syrup (HFCS). The glucose isomerase immobilized with the petitioned polymers may be used as a substitute for one or more of the immobilized glucose isomerases currently in use.

In its evaluation of the safety of the petitioned substances, FDA has reviewed the safety of the additives and the chemical impurities that may be present in them resulting from the manufacturing processes. Although the petitioned polymers have not been shown to cause cancer, they may contain minute amounts of carcinogenic impurities resulting from their manufacture. DEC may contain traces of unreacted epichlorohydrin and its degradation product, 1,3-dichloro-2-propanol. AAR may contain minute amounts of the unreacted monomer, acrylamide. These chemical impurities have been shown to cause cancer in test animals. Residual amounts of reactants and their impurities commonly are found as contaminants of chemical products, including food additives.

II. Determination of Safety

Under the general safety standard of the Federal Food, Drug, and Cosmetic Act (the act) (21 U.S.C. 348(c)(3)(A)), a food additive cannot be approved for a particular use unless a fair evaluation of the data available to FDA establishes that the additive is safe for that use. FDA's food additive regulations (21 CFR 170.3(i)) define safe as a "reasonable certainty in the minds of competent scientists that the substance is not harmful under the intended conditions of use."

The food additives anticancer, or Delaney, clause of the act (21 U.S.C. 348(c)(3)(A)) provides that no food additive shall be deemed safe if it is found to induce cancer when ingested by man or animal. Importantly, however, the Delaney clause applies to the additive itself and not to impurities in the additive. That is, where an additive itself has not been shown to cause cancer, but contains a carcinogenic impurity, the additive is evaluated properly under the general safety standard using risk assessment procedures to determine whether there is reasonable certainty that no harm will result from the intended use of the additive (*Scott* v. *FDA*, 728 F.2d 322 (6th Cir. 1984)).

III. Safety of the Petitioned Use of the Additives

FDA has estimated that the petitioned use of the additives, DEC and AAR, will result in a daily intake of 210 micrograms per person per day (μg/p/d) and 83 μg/p/d, respectively (Ref. 1).

FDA has evaluated the safety of DEC and AAR under the general safety

standard and concludes that the estimated dietary exposure to the additives resulting from the petitioned uses is safe. In reaching this conclusion, FDA reviewed all available toxicological data and used risk assessment procedures to estimate the upper-bound limit of lifetime human risk presented by the carcinogenic impurities that may be present in the petitioned additives. The chemical impurities considered are acrylamide in AAR and epichlorohydrin and 1,3-dichloro-2-propanol in DEC.

The risk evaluation of the carcinogenic impurities has two aspects: (1) Assessment of exposure to the impurities from the petitioned use of the additives; and (2) extrapolation of the risk observed in the animal bioassays to the conditions of exposure to humans.

A. Acrylamide

FDA has estimated the upper-bound exposure to acrylamide from the petitioned use of AAR to be 2 nanograms per person per day (ng/p/d), corresponding to a dietary concentration of 0.67 part-per-trillion (pptr) in the daily diet (3 kg) (Ref. 2). This estimate is conservative, as it does not account for the removal of impurities, including acrylamide, from the crude HFCS during the purification process.

1. Acrylamide as a Neurotoxin

Acrylamide is a recognized neurotoxin. To derive the safe exposure level to acrylamide as a neurotoxin, the agency used a study by J. D. Burek et al. (Ref. 3). FDA, using an uncertainty factor of 1,000 (equivalent to a safety factor), determined the acceptable daily intake of acrylamide with respect to neurotoxicity to be 12 μg/p/d based on the neurotoxicity evaluation and absence of a neurotoxic effect (Refs. 4 and 5). Therefore, based on the agency's estimate that the exposure to acrylamide will not exceed 2 ng/p/d, FDA concludes that the exposure to acrylamide from the petitioned use of AAR does not pose a neurotoxic risk.

2. Acrylamide as a Carcinogen

To estimate the upper-bound limit of lifetime human risk from exposure to acrylamide as a carcinogen resulting from the petitioned use of AAR, the agency used published data from a long-term rat bioassay on acrylamide, conducted by Johnson et al., in addition to unpublished data from this bioassay in the agency's files (Refs. 6 and 7). The authors of this bioassay reported that acrylamide administered to rats via drinking water is associated with statistically significant increased incidences of thyroid follicular adenomas and testicular mesotheliomas in male rats, and of mammary tumors (adenomas or adenocarcinomas, fibromas or fibroadenomas, adenocarcinomas alone), central nervous system tumors (brain astrocytomas, brain or spinal cord glial tumors), and uterine tumors (adenocarcinomas) in female rats.

Based on the agency's estimate that exposure to acrylamide will not exceed 2 ng/p/d, FDA estimates that the upper-bound limit of lifetime human risk from exposure to acrylamide from the petitioned use of the subject additive is 2.2×10^{-8} or 22 in 1 billion (Ref. 8). Considering that this estimated upper-bound risk is based on very conservative assumptions, the agency believes that the probable lifetime human risk would be significantly less than the estimated upper-bound limit of lifetime human risk. Therefore, the agency concludes that there is reasonable certainty that no harm from exposure to acrylamide would result from the petitioned use of AAR.

B. Epichlorohydrin

FDA has estimated the exposure to epichlorohydrin from the petitioned use of DEC to be 2.1 ng/p/d or 0.7 pptr of the daily diet (Refs. 1 and 9). This estimate is conservative, as it does not account for the removal of residual impurities, including epichlorohydrin, during the processing of the crude HFCS.

The agency used data from a carcinogenesis bioassay conducted by Konishi et al. (Ref. 10), on rats fed epichlorohydrin via their drinking water, to estimate the upper-bound limit of lifetime human risk from exposure to this chemical resulting from the petitioned use of DEC. The authors reported that the test material caused significantly increased incidence of stomach papillomas and carcinomas in rats.

Based on the agency's estimate that exposure to epichlorohydrin will not exceed 2.1 ng/p/d, FDA estimates that the upper-bound limit of lifetime human risk from exposure to epichlorohydrin resulting from the petitioned use of the subject additive is 9.5×10^{-11} or 95 in 1 trillion (Ref. 8). Considering that this upper-bound estimated risk is based on very conservative assumptions, the agency believes that the probable lifetime human risk would be significantly less than the estimated upper-bound limit of lifetime human risk. Therefore, FDA concludes that there is reasonable certainty that no harm from exposure to epichlorohydrin would result from the petitioned use of DEC.

C. 1,3-Dichloro-2-propanol (DCP)

DCP is the product of epichlorohydrin degradation in water. The current regulation for the use of DEC resin establishes a residual limit for DCP at 1,000 ppm in the DEC resin (21 CFR 173.60 (b)(3)). The agency has estimated that exposure to DCP from the petitioned use for DEC will not exceed 210 ng/p/d (Refs. 1 and 9). This estimate is conservative, as it does not account for the removal of residual impurities, including DCP, during the processing of the crude HFCS.

The agency used data from a 1986 drinking water bioassay in rats (Ref. 11) to estimate the worst case upper-bound lifetime cancer risk from exposure to DCP from the petitioned use of DEC. This risk was calculated as 1.2×10^{-7} or 12 in 100 million (Refs. 12 and 13). Considering that this upper-bound estimated risk is based on very conservative assumptions, the agency believes that the probable lifetime human risk would be significantly less than the upper-bound limit of lifetime human risk. Therefore, FDA concludes that there is reasonable certainty that no harm from exposure to DCP would result from the petitioned use of DEC.

D. Need for Specifications

The agency also has considered whether specifications are necessary to control the amount of acrylamide present as an impurity in AAR and epichlorohydrin and DCP in DEC. The agency finds that specifications are not necessary for the following reasons:

1. The agency would not expect these impurities to become components of food at other than extremely low levels because of the low levels at which acrylamide, epichlorohydrin, and DCP may be expected to remain as impurities following production and purification of the additives and HFCS, and

2. The upper-bound limits of lifetime human risk from exposure to acrylamide, epichlorohydrin, and DCP are very low, 2.2×10^{-8}, 9.5×10^{-11}, and 1.2×10^{-7} respectively.

IV. Conclusions

FDA has evaluated data in the petition and other relevant material. Based on this information, the agency concludes that: (1) The proposed use of the additives as fixing agents in the immobilization of glucose isomerase enzyme preparations is safe, (2) that the additives will achieve their intended technical effect, and therefore, (3) the regulations in § 173.357 (21 CFR 173.357) should be amended as set forth below.

In accordance with § 171.1(h) (21 CFR 171.1(h)), the petition and the

documents that FDA considered and relied upon in reaching its decision to approve the petition are available for inspection at the Center for Food Safety and Applied Nutrition by appointment with the information contact person. As provided in § 171.1(h), the agency will delete from the documents any materials that are not available for public disclosure before making the documents available for inspection.

V. Environmental Impact

The agency has determined under 21 CFR 25.32(j) that this action is of a type that individually or cumulatively does not have a significant effect on the human environment. Therefore, neither an environmental assessment nor an environmental impact statement is required.

VI. Paperwork Reduction Act of 1995

This final rule contains no collection of information. Therefore, clearance by the Office of Management and Budget under the Paperwork Reduction Act of 1995 is not required.

VII. Objections

Any person who will be adversely affected by this regulation may at any time file with the Dockets Management Branch (see **ADDRESSES**) written objections by July 25, 2002. Each objection shall be separately numbered, and each numbered objection shall specify with particularity the provisions of the regulation to which objection is made and the grounds for the objection. Each numbered objection on which a hearing is requested shall specifically so state. Failure to request a hearing for any particular objection shall constitute a waiver of the right to a hearing on that objection. Each numbered objection for which a hearing is requested shall include a detailed description and analysis of the specific factual information intended to be presented in support of the objection in the event that a hearing is held. Failure to include such a description and analysis for any particular objection shall constitute a waiver of the right to a hearing on the objection. Three copies of all documents are be submitted and are to be identified with the docket number found in brackets in the heading of this document. Any objections received in response to the regulation may be seen in the Dockets Management Branch between 9 a.m. and 4 p.m., Monday through Friday.

VIII. References

The following references have been placed on display in the Dockets Management Branch (see **ADDRESSES**) and may be seen by interested persons between 9 a.m. and 4 p.m., Monday through Friday.

1. Memorandum dated November 22, 1989, from the Food and Color Additives Review Section to the Direct Additives Branch, "FAP 9A4175: Enzyme Bio-Systems Ltd. Dimethylamine-Epichlorohydrin Resin (DEC) and Acrylic Acid-Acrylamide Resin (AAR) as Fixing Agents for Glucose Isomerase Immobilized Enzyme Preparations. Submission of 9–25–89."
2. Memorandum dated August 17, 1998, from the Division of Product Policy, Scientific Support Branch, Chemistry and Environmental Review Team (CERT) to the Division of Petition Control, "FAP 9A4175 (MATS# 438)—Enzyme Bio-Systems Ltd. Exposure to Acrylamide Monomer from the Use of Acrylic Acid-Acrylamide Resin (AAR) as a Fixing Agent for Glucose Isomerase Immobilized Enzyme Preparations. Division of Petition Control (DPC, HFS–215) Verbal Request dated 8–4–98."
3. Burek, J. D., R. R. Albee, J. E. Beyer, et al., "Subchronic Toxicity of Acrylamide Administered to Rats In the Drinking Water Followed by Up to 144 Days of Recovery," *Journal of Environmental Pathology and Toxicology*, 4:157–182, 1980.
4. Memorandum dated September 9, 1997, from the Division of Health Effects Evaluation to the Division of Product Policy, "Acrylamide, New Information and Re-evaluation of the Neurotoxicity Potential and Tentative ADI of Acrylamide as a Migrant."
5. Memorandum dated January 24, 2000, from the Division of Health Effects Evaluation to the Division of Product Policy, "Final Safety Evaluation of Acrylamide-Acrylic Acid Resin (AAR) and Dimethylamine-epichlorohydrin Resin (DEC) as Fixing Agents for Immobilized Glucose Isomerase Used in Foods. Memo of Div. of Product Manufacture and Use, Chemistry and Environmental Review Team (CERT) 4/28/99, Received 5/5/99. QRAC Concurrence of Estimation of the Upper Bound Lifetime Risk from Residual Epichlorohydrin and Acrylamide (S. Henry Memo Dated Dec. 20, 1999)."
6. Johnson, K. A., S. J. Gorzinski, K. M. Bodner, R. A. Campbell, C. H. Wolf, M. A. Friedman, and R.W. Mast, "Chronic Toxicity and Oncogenicity Study on Acrylamide Incorporated in the Drinking Water of Fischer 344 Rats," *Toxicology and Applied Pharmacology*, 85:154–168, 1986.
7. Memorandum of Conference, FDA, CFSAN, Washington, DC Cancer Assessment Committee Meeting on Acrylamide, February 13 and June 6, 1985, and May 31, 1996.
8. Memorandum dated May 7, 1999, from the Regulatory Policy Branch to the Quantitative Risk Assessment Committee, "Estimation of the Upper-Bound Lifetime Risk from Residual Epichlorohydrin and Acrylamide Monomers in Dimethylamine-Epichlorohydrin and Acrylic Acid-Acrylamide Resins, Respectively, for Use as Fixing Agents in Immobilizing Glucose Isomerase Enzyme Preparation: Use Requested in Food Additive Petition No. 9A4175 from Enzymes Bio-Systems Ltd."
9. Memorandum dated August 7, 1997, from the Division of Product Policy to Division of Petition Control, "FAPs 9A4175, 3B3677, 6B3940, 3B3696, 9B4131, 9B4132 and 9B4133. DPC Request to Identify and Address Unresolved Issues in the Pending Acrylamide Petitions."
10. Konishi, Y. et al., "Forestomach Tumors Induced by Orally Administered Epichlorohydrin in Male Wistar Rats," *Gann*, 71:922–923, 1980.
11. Research and Consulting Co., AG, Project 017820, Report Parts 1–4, February 24, 1986: 104-Week Chronic Toxicity and Carcinogenicity Study with 1,3-Dichloropropan-2-ol in the Rats; Food Master File 000543, Vol. 11.
12. Memorandum dated August 24, 1998, from the Executive Secretary, Cancer Assessment Committee, to the Chairman, Cancer Assessment Committee, "FAP 9A4175: Worst-Case Cancer Risk Assessment for 1,3-dichloropropanol (DCP)."
13. Memorandum dated March 25, 1999, from Division of Health Effects Evaluation to the Executive Secretary, Cancer Assessment Committee, "Expedited Risk Assessment for 1,3-dichloropropanol Memo of August 24, 1998. Accepting Risk Estimate for Regulation of FAP 9A4175."

List of Subjects in 21 CFR Part 173

Food additives.

Therefore, under the Federal Food, Drug, and Cosmetic Act and under authority delegated to the Commissioner of Food and Drugs, 21 CFR part 173 is amended as follows:

PART 173—SECONDARY DIRECT FOOD ADDITIVES PERMITTED IN FOOD FOR HUMAN CONSUMPTION

1. The authority citation for 21 CFR part 173 continues to read as follows:

Authority: 21 U.S.C. 321, 342, 348.

2. Section 173.357 is amended in the table in paragraph (a)(2) by alphabetically adding entries for "Acrylamide-acrylic acid resin" and "Dimethylamine-epichlorohydrin resin" to read as follows:

§ 173.357 Materials used as fixing agents in the immobilization of enzyme preparations.

* * * * *

(a) * * *

(2) * * *

Substances	Limitations
Acrylamide-acrylic acid resin: Complying with § 173.5(a)(1) and (b) of this chapter.	May be used as a fixing material in the immobilization of glucose isomerase enzyme preparations for use in the manufacture of high fructose corn syrup, in accordance with § 184.1372 of this chapter.

Substances	Limitations
* * * * *	
Dimethylamine-epichlorohydrin resin: Complying with § 173.60(a) and (b) of this chapter.	May be used as a fixing material in the immobilization of glucose isomerase enzyme preparations for use in the manufacture of high fructose corn syrup, in accordance with § 184.1372 of this chapter.
* * * * *	

* * * * *

Dated: June 17, 2002.

Margaret M. Dotzel,

Associate Commissioner for Policy.

[FR Doc. 02–15901 Filed 6–24–02; 8:45 am]

BILLING CODE 4160–01–S

DEPARTMENT OF HEALTH AND HUMAN SERVICES

Food and Drug Administration

21 CFR Part 510

New Animal Drugs; Change of Sponsor's Name and Address

AGENCY: Food and Drug Administration, HHS.

ACTION: Final rule.

SUMMARY: The Food and Drug Administration (FDA) is amending the animal drug regulations to reflect a change of sponsor's name and address for Akey, Inc.

DATES: This rule is effective June 25, 2002.

FOR FURTHER INFORMATION CONTACT: Lonnie W. Luther, Center for Veterinary Medicine (HFV–101), Food and Drug Administration, 7500 Standish Pl., Rockville, MD 20855, 301–827–0209, e-mail: lluther@cvm.fda.gov.

SUPPLEMENTARY INFORMATION: Akey, Inc., P.O. Box 607, Lewisburg, OH 45338, has informed FDA of a change of name and address to North American Nutrition Companies, Inc., C.S. 5002, 6531 St., Rt. 503, Lewisburg, OH 45338. Accordingly, the agency is amending the regulations in 21 CFR 510.600(c) to reflect the change.

This rule does not meet the definition of "rule" in 5 U.S.C. 804(3)(A) because it is a rule of "particular applicability." Therefore, it is not subject to the congressional review requirements in 5 U.S.C. 801–808.

List of Subjects in 21 CFR Part 510

Administrative practice and procedure, Animal drugs, Labeling, Reporting and recordkeeping requirements.

Therefore, under the Federal Food, Drug and Cosmetic Act and under authority delegated to the Commissioner of Food and Drugs and redelegated to the Center for Veterinary Medicine, 21 CFR part 510 is amended as follows:

PART 510—NEW ANIMAL DRUGS

1. The authority citation for 21 CFR part 510 continues to read as follows:

Authority: 21 U.S.C. 321, 331, 351, 352, 353, 360b, 371, 379e.

2. Section 510.600 is amended in the table in paragraph (c)(1) by removing the entry for "Akey, Inc." and by alphabetically adding a new entry for "North American Nutrition Companies, Inc.", and in the table in paragraph (c)(2) by revising the entry for "017790" to read as follows:

§ 510.600 Names, addresses, and drug labeler codes of sponsors of approved applications.

* * * * *

(c) * * *

(1) * * *

Firm name and address	Drug labeler code
* * * * * * *	
North American Nutrition Companies, Inc., C.S. 5002, 6531 St., Rt. 503, Lewisburg, OH 45338	017790
* * * * * * *	

(2) * * *

Drug labeler code	Firm name and address
* * * * * * *	
017790	North American Nutrition Companies, Inc., C.S. 5002, 6531 St., Rt. 503, Lewisburg, OH 45338
* * * * * * *	

Dated: May 24, 2002.

Andrew J. Beaulieu,

Acting Director, Office of New Animal Drug Evaluation, Center for Veterinary Medicine.

[FR Doc. 02–15900 Filed 6–24–02; 8:45 am]

BILLING CODE 4160–01–S

DEPARTMENT OF DEFENSE

Office of the Secretary

32 CFR Part 199

RIN 0720–AA28

TRICARE; Revisions to Coverage Criteria for Transplants, Cardiac and Pulmonary Rehabilitation and Ambulance Services

AGENCY: Office of the Secretary, DoD.

ACTION: Final rule.

SUMMARY: This final rule implements a number of regulatory revisions relating to TRICARE coverage for transplants and related services, cardiac and pulmonary rehabilitation and ambulance services. The revisions are clarification of TRICARE coverage and time limitations on preauthorizations for solid organ and stem cell transplantation for beneficiaries whose conditions are considered appropriate

U.S. Food and Drug Administration
Department of Health and Human Services
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CFSAN/Office of Food Additive Safety
January 13, 2006

Agency Response Letter
GRAS Notice No. GRN 000176

Dr. Luis A. Mejia
Archer Daniels Midland Company
1001 N. Brush College Road
Decatur, IL 62521

Re: GRAS Notice No. GRN 000176

Dear Dr. Mejia:

The Food and Drug Administration (FDA) is responding to the notice, dated July 7, 2005, that you submitted on behalf of Archer Daniels Midland Company (ADM) in accordance with the agency's proposed regulations, proposed 21 CFR 170.36 (62 FR 18938; April 17, 1997); Substances Generally Recognized as Safe (GRAS)). FDA received the notice on July 12, 2005, filed it on July 14, 2005, and designated it as GRN 000176. In a letter dated September 9, 2005, you provided additional clarifying information.

The subjects of the notice are plant sterols and plant sterol esters from vegetable oils or sterols/stanols from tall oil (hereinafter referred to as phytosterols in this letter). The notice informs FDA of the view of ADM that phytosterols are GRAS, through scientific procedures, for use as an ingredient in margarines and vegetable oil spreads, dressings for salads, beverages, snack bars, dairy analogs (including soy milk, ice cream and cream substitutes), cheese and cream, baked foods, ready-to-eat breakfast cereals, mayonnaise, pasta and noodles, sauces, salty snacks, processed soups, puddings, yogurt, confections, vegetarian meat analogs at a level up to 0.4 gram (g) sterol equivalents per serving; in fruit/vegetable juices at a level up to 1 g sterol equivalents per serving. In edible vegetable oils, including diacylglycerol oil, the use level could be up to 4 g/100g sterol equivalents per serving.

As part of its notice, ADM includes the report of a panel of individuals (ADM's GRAS panel) who evaluated the data and information that are the basis for ADM's GRAS determination. ADM considers the members of its GRAS panel to be qualified by scientific training and experience to evaluate the safety of substances added to food. ADM's GRAS panel evaluated estimates of dietary exposure, the method of manufacture, specifications for the ingredient, and published and unpublished studies on phytosterols, derived from either edible vegetable oils or from tall oil. ADM's GRAS panel concluded that phytosterols meeting food-grade specifications are GRAS by scientific procedures for their intended use.

Phytosterols from vegetable oils were the subject of GRN 000061. GRN 000176 incorporates GRN000061 by reference. GRN 000061 includes the identity and composition of, as well as, the method of manufacture for phytosterols from vegetable oil. The main sterol components of these phytosterols are beta-sitosterol, campesterol and stigmasterol. The sterols are derived from oil seeds such as corn, palm, soy, rape, and sunflower. The fatty acids are preferentially derived from soy, sunflower, safflower, and canola. Corn, peanut, cottonseed and palm may also be used as sources. GRN 000061 also includes food grade specifications for phytosterols from vegetable oil.

GRN 000176 discusses the identity and composition of, as well as, the method of manufacture for phytosterols from tall oil. The tall oil, derived from pine trees, (*Pinus pinaster* and/or *P. syvestris*) was the subject of GRN 000112. The main components of these phytosterols are beta-sitosterol, beta-sitostanol, campesterol, and campestanol. ADM provides food grade specifications for the phytosterols from tall oil.

In GRN 000176, ADM intends to use 0.4 g of sterol per serving in most food categories. ADM bases this level on the interim health claim rule (65 FR 54685; September 8, 2000), and the agency's enforcement discretion letter (Ref 1). ADM estimates that the sterol intake for individuals, including the already existing and newly proposed food categories, is 3.9 g/day at the 90th percentile intake.

In its notice, ADM states its intention to use phytosterols in several food categories for which a standard of identity exists. We note that an ingredient that is lawfully added to food products may be used in a standardized food only if it is permitted by the applicable standard of identity.

Based on information provided by ADM, as well as other information available to FDA, the agency has no questions at this time regarding ADM's conclusion that phytosterols are GRAS under the intended conditions of use. The agency has not, however, made its own determination regarding the GRAS status of the subject use of phytosterols. As always, it is the continuing responsibility of ADM to ensure that food ingredients that the firm markets are safe, and are otherwise in compliance with all applicable legal and regulatory requirements.

In accordance with proposed 21 CFR 170.36(f), a copy of the text of this letter, as well as a copy of the information in your notice that conforms to the information in proposed 21 CFR 170.36 (c) (1), is available for public review and copying on the homepage of the Office of Food Additive Safety (on the Internet at http://www.cfsan.fda.gov/~lrd/foodadd.html).

Sincerely,

Laura M. Tarantino, Ph.D.
Director
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition

References

000255

1. Letter dated February 14, 2003, from Christine L. Taylor of FDA to Fred L. Shinnick.

U.S. Food and Drug Administration
Department of Health and Human Services
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CFSAN/Office of Food Additive Safety
March 16, 2006

Agency Response Letter
GRAS Notice No. GRN 000181

Dr. Hershell R. Ball
Vice President
Michael Foods Inc.
120 Tower Street South
Gaylord, MN 55334

Re: GRAS Notice No. GRN 000181

Dear Dr. Ball:

The Food and Drug Administration (FDA) is responding to the notice, dated August 18, 2005, that you submitted on behalf of Michael Foods, Inc. (hereinafter referred to as Michael Foods) in accordance with the agency's proposed regulations, proposed 21 CFR 170.36 (62 FR 18938; April 17, 1997); Substances Generally Recognized as Safe (GRAS)). FDA received the notice on August 19, 2005, filed it on August 23, 2005, and designated it as GRN 000181. In letters dated August 18, 2005, September 1, 2005, and January 30, 2006, you provided additional clarifying information.

The subject of the notice is plant phytosterols. The notice informs FDA of the view of Michael Foods that phytosterols are GRAS, through scientific procedures, for use as an ingredient in egg products, including egg whites and egg substitutes, at levels up to 20 milligrams (mg) plant sterol per gram (g) of egg product, providing 1100 mg phytosterol per serving.

In a letter dated January 30, 2006, Michael Foods amended its notice to limit use to egg substitutes and other similar or related products that are not "egg products" within the meaning of 9 CFR 590.5. This amendment limits the GRAS notice to cover only products that contain eggs in a relatively small proportion or historically have not been considered by consumers as products of the egg food industry.

As part of its GRAS notice Michael Foods includes the report from a panel of individuals (Michael Foods' GRAS panel) who evaluated the data and information that are the basis for its GRAS determination. Michael Foods considers the members of its GRAS panel to be qualified by scientific training and experience to evaluate the safety of substances added to food. Michael Foods' GRAS panel evaluated previously submitted GRAS notices GRN 000039, 000048, 000053, 000061, 000112, Food Master Files 000625 and 000626, estimates of dietary exposure, methods of manufacture, specifications for the ingredient, and published and unpublished

studies on phytosterols and phytostanols derived from vegetable oils or tall oil.

GRN 000181 includes the identity and composition of, as well as, the method of manufacture for phytosterols from vegetable oil. The main sterol components of these phytosterols are beta-sitosterol, campesterol, brassicasterol, and stigmasterol. Food grade specifications for phytosterols from vegetable oil are provided in the notice.

The product may also contain phytosterols and phytostanols derived from tall oil. However, the total addition of phytosterols and phytostanols from tall oil is expected to be less than 5% of the total volume of vegetable oil derived product on a weight/weight basis. The main sterol and stanol components of tall oil derived from tall oil pitch are beta-sitosterol, campesterol, stigmasterol, brassicasterol, campestanol, and sitostanol.

Michael Foods provides a cumulative estimate of intake for phytosterols in the diet from the intended use described in its notice in addition to the phytosterol intake that may occur from other foods that have been the subject of previous submissions. Michael Foods notes that its intended use in egg products will not add significantly to the overall intake of phytosterols in food.

Based on information provided by Michael Foods, and other information available to FDA, the agency has no questions at this time regarding Michael Foods' conclusion that phytosterols are GRAS under the intended conditions of use in egg products that are regulated by FDA and, thus, are not egg products within the meaning of 9 CFR 590.5. The agency has not, however, made its own determination regarding the GRAS status of the subject use of phytosterols. As always, it is the continuing responsibility of Michael Foods to ensure that food ingredients that the firm markets are safe, and are otherwise in compliance with all applicable legal and regulatory requirements.

In accordance with proposed 21 CRF 170.36(f), a copy of the text of this letter, as well as a copy of the information in your notice that conforms to the information in proposed 21 CFR 170.36(c)(1), is available for public review and copying on the homepage of the Office of Food Additive Safety (on the Internet at http://www.cfsan.fda.gov/~lrd/foodadd.html).

Sincerely,

Laura M. Tarantino, Ph.D.
Director
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition

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SUBMISSION END