GR

ORIGINAL SUBMISSION

000001.001

THOMAS O. HENTELEFF
RICHARD S. MOREY
KINSEY S. REAGAN
PETER R. MATHERS
ANTHONY L. YOUNG
ANNE V. MAHER
BONNIE A. BEAVERS
DANIEL R. DWYER
GLENN E. DAVIS
STACY L. EHRLICH
JENNIFER A. DAVIDSON
STACEY L. VALERIO
JONATHAN M. WEINRIEB
CARRIE C. HORN

OF COUNSEL:
WILLIAM J. HARDY

LAW OFFICES
KLEINFELD, KAPLAN AND BECKER, LLP
1140 NINETEENTH STREET, N.W.
WASHINGTON, D.C. 20036-6606
TELEPHONE (202) 223-5120
FACSIMILE (202) 223-5619
www.kkblaw.com

WEST COAST OFFICE:
ONE MARKET STREET
STEUART TOWER, SUITE 1450
SAN FRANCISCO, CA 94105-1313
TELEPHONE (415) 538-0014
FACSIMILE (415) 538-0016

VINCENT A. KLEINFELD
1907-1993

ALAN H. KAPLAN
1930-2001

August 18, 2005

RECEIVED
AUG 19 2005
OFFICE OF
FOOD ADDITIVE SAFETY

Via Hand Delivery

Antonia Mattia, Ph.D.
Division of Biotechnology and GRAS Notice Review
Office of Food Additive Safety (HFS-255)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740

**RE: GRAS notification for plant phytosterols for use in egg products including egg whites, and egg substitutes**

Dear Dr. Mattia:

Enclosed please find for filing a GRAS notice for the use of plant phytosterols in egg products including egg whites, and egg substitutes, submitted on behalf of Michael Foods, Inc.

On August 2, 2005, we met with representatives of your office and the FSIS Labeling and Consumer Protection Staff to discuss this GRAS notification. At that time, we explained that phytosterols are suitable for use in products such as egg whites and egg substitutes because those products contain low levels of fat, saturated fat, cholesterol and sodium. As a result, these products are appropriate for use with phytosterols, which are intended to reduce blood total and LDL cholesterol levels (as explained in FDA's approved health claim for phytosterols, 21 CFR 101.83).

In addition to egg whites and egg substitutes, other egg products may also contain sufficiently low levels of fat, saturated fat, cholesterol and sodium to be appropriate for use with phytosterols – such as, for example, a combination of egg whites and whole eggs. In order to ensure that the GRAS notice covers all of the egg products in which phytosterols are suitable for use, we have described the foods in which the substance is intended to be used as "egg products including egg whites, and egg substitutes."

Thank you for y

Sincerely,

Daniel R. Dwyer\

THOMAS O. HENTELEFF
RICHARD S. MOREY
KINSEY S. REAGAN
PETER R. MATHERS
ANTHONY L. YOUNG
ANNE V. MAHER
BONNIE A. BEAVERS
DANIEL R. DWYER
GLENN E. DAVIS
STACY L. EHRLICH
JENNIFER A. DAVIDSON
STACEY L. VALERIO
JONATHAN M. WEINRIEB
CARRIE C. HORN

OF COUNSEL:
WILLIAM J. HARDY

LAW OFFICES
KLEINFELD, KAPLAN AND BECKER, LLP
1140 NINETEENTH STREET, N.W.
WASHINGTON, D.C. 20036-6606
TELEPHONE (202) 223-5120
FACSIMILE (202) 223-5619
www.kkblaw.com

WEST COAST OFFICE:
ONE MARKET STREET
STEUART TOWER, SUITE 1450
SAN FRANCISCO, CA 94105-1313
TELEPHONE (415) 538-0014
FACSIMILE (415) 538-0016

VINCENT A. KLEINFELD
1907-1993
ALAN H. KAPLAN
1930-2001

August 18, 2005

RECEIVED
AUG 19 2005
OFFICE OF
FOOD ADDITIVE SAFETY

## I. GRAS Exemption Claim

### A. Claim of Exemption From The Requirement for Premarket Approval Requirements Pursuant to Proposed 21 CFR § 170.36(c)(1)

Plant phytosterols have been determined to be generally recognized as safe, and therefore, exempt from the requirement of premarket approval, under the conditions of intended use as described below. The basis for this finding is described in the following sections and the enclosed expert panel report.

### B. Name and Address of Notifier

Hershell R. Ball, Jr., Ph.D.
Vice President
Michael Foods, Inc.
120 Tower Street South
Gaylord, MN 55334
952.258.4000

All correspondence should be sent to Michael Foods, Inc.

### C. Common or Usual Name of the Notified Substance

Plant phytosterols

### D. Conditions of Use

The intended use of the plant phytosterols will be for a new use as an ingredient in egg products including egg whites, and egg substitutes at levels up to 1000 mg/50grams (20 mg phytosterol/g egg product). The recommended serving will be 2 fl. oz. or 56.7 grams, delivering 1134 mg phytosterol/serving.

## E. Basis for the GRAS Determination

Pursuant to 21 CFR § 170.30, plant phytosterols have been determined to be GRAS by scientific procedures. A comprehensive search of the scientific literature, along with several GRAS notices to FDA on phytosterols and phytosterol esters, were also utilized for this review.

## F. Availability of Information

The data and information that serve as a basis for this GRAS are available for the Food and Drug Administration's review and copying during reasonable business hours at the offices of:

Edward A. Steele
AAC Consulting Group
7361 Calhoun Place, Suite 500
Rockville, MD 20855-2765
Telephone: 301-838-3120
Facsimile: 301-838-3182

# II. Detailed Information About the Identity of the Substance

## A. Identity

The phytosterols that are the subject of this GRAS notice are derived from plant sources and further purified in product manufacture. The composition and specifications of the phytosterol product is given in Table 1 below. The range of sterol composition in the phytosterol product is:

Total sterols->95%
β-sisterol-40-58%
Campesterol-20-30%
Stigmasterol-11-22%
Brassicasterol-0-6%
Campestanol-0-2%
Sistostanol-0-5%

## B. Method of Manufacture

The initial starting material is termed a distillate, typically with 10-17% phytosterol content. It is extracted primarily from soybeans, but may contain smaller amounts of phytosterols from

various oilseeds such as canola, corn, cottonseed and sunflower oil. The product may also contain phytosterols derived from tall oil (the starting material being tall oil pitch), but the total addition will be less than 5% of the total volume of vegetable oil derived product on a w/w basis. All of these oilseeds and tall oil have been noted in prior GRAS notifications as oil sources for phytosterol production and are considered suitable for food-grade oils and by-products.

The distillate is refined by a primary distillation followed by a molecular film distillation, thus separating the mono- and di-glycerides, methyl esters and tocopherols from the crude sterols. Transesterification converts any remaining glycerides to free sterols. The resulting crude sterols are purified by recrystallization and filtration. Impurities are further removed by filtration and methanol/heptane wash. Heating and vacuum removal reduces residual solvent to <10 ppm methanol/heptane. The end product typically contains 94-97% sterols. The main components are beta-sitosterol, campesterol and stigmasterol. The general process used for phytosterol production is similar for all the major producers by distillation of the crude plant oil distillate, recrystallization, transesterification of waxy sterols and triglycerides, further filtration and solvent purification and removal of impurities/residual solvent.

## C. Specifications for Plant Phytosterols

**Table 1. Specifications and Physical Properties of Notified Phytosterol**

| **Property** | **Phytosterols** |
|---|---|
| **Specifications** | |
| Total sterol content % | 85 min. |
| β-sisterol % | 40 min |
| Campesterol % | 20 min |
| Stigmasterol % | 11 min |
| Brassicasterol % | 0.3 min |
| Residue on ignition (%) | 0.5 max. |
| Lead by AA | <0.2 ppm |
| Heavy metals | 10 ppm max. |
| Solvent (methanol/heptane) | 10 ppm max |
| | |
| **Microbiological analysis** | |
| Total plate count | 3000/g |
| Coliforms | 10/g |
| Yeast and Mold | 100/g |
| *E. coli* | Negative/g |
| *Salmonella* | Negative/25 g |
| | |
| **Physical properties** | |
| Bulk density | ~0.6 g/ml |
| Appearance | White to yellow amber solid |
| Solubility | Partly soluble in fats, oils and ethanol<br>Insoluble in water |
| Melting point (°C) | 135-145 |

## III. Self-Limiting Levels of Use

The level of intake of the egg products including egg whites, and egg substitutes with phytosterols is considered to be similar to consumption of shell eggs by the consumer. The most useful estimate of egg intake is survey data for eaters only. A reasonable assumption is that health conscious consumers of egg and egg substitute products will consume eggs directly in scrambled egg or omelet preparations much as the consumer does with shell eggs. Krebs-Smith *et al.* (1997) of USDA conducted a two-day survey on eaters only and found that mean and 90$^{th}$ percentile egg consumption was 27 and 87 g/day respectively. For the population as a whole, with a mean and 90$^{th}$ percentile of 27 and 87 grams, daily intake from use of egg products and egg substitutes containing 20 mg phytosterol/g egg products/substitutes is estimated as 540 and 1740 mg/person/day respectively.

## IV. Summary of the Basis for the Notifier's Determination that Plant Phytosterols Are GRAS

An independent panel of recognized experts, qualified by their scientific training and relevant national and international experience to evaluate the safety of food and food ingredients, was requested by Michael Foods, Inc. to determine the Generally Recognized As Safe (GRAS) status of plant phytosterols for use as ingredients in egg products including egg whites, and egg substitutes. A comprehensive search of the scientific literature, along with several GRAS notices to FDA on phytosterols and phytosterol esters, was also utilized for this review.

The generally accepted ADI for phytosterols based on animal studies at the highest doses tested is about 130 mg/kg/day. This equates to a daily intake of 9.1 g/day for a 70 kg adult. Moreover, even this ADI is likely to be conservative. The data reviewed in the GRAS determination assessment document support a conclusion that dietary phytosterols have a very low order of toxicity and are safe at significant levels of consumption. Clinical studies have shown safe consumption at levels including 18 g/day and 25-50 g/day. The estimated daily intake (EDI) for phytosterols from consumption of egg products including egg whites, and egg substitutes would be significantly below these levels, at 540 and 1740 mg/day at the mean and 90$^{th}$ percentile levels, respectively.

Following independent, critical evaluation, the Expert Panel conferred and unanimously agreed to the decision described in the Expert Panel Statement. The Expert Panel members have concluded that plant derived phytosterols, meeting the specifications cited above, are safe for addition to egg products including egg whites, and egg substitutes at levels up to 1000 mg phytosterol per 50 grams egg product (1134 mg/recommended serving). It is also their opinion that other qualified and competent scientists, reviewing the same publicly available toxicological and safety information, would reach the same conclusion. Therefore, they have concluded that these ingredients, when used as described, are GRAS based on scientific procedures.

Sincerely,

Daniel R. Dwyer

Counsel to Michael Foods, Inc.

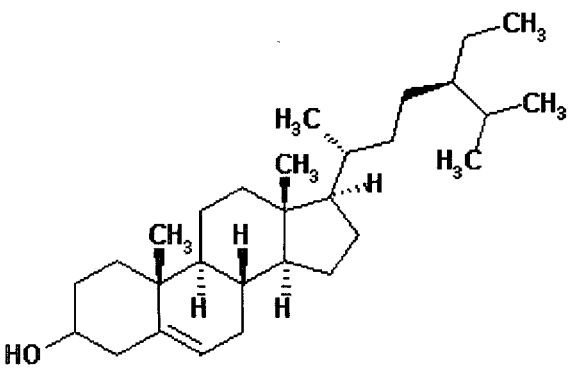
## EXPERT PANEL STATEMENT

## DETERMINATION OF THE GRAS STATUS OF PLANT PHYTOSTEROLS FOR USE AS INGREDIENTS IN EGG PRODUCTS INCLUDING EGG WHITES, AND EGG SUBSTITUTES

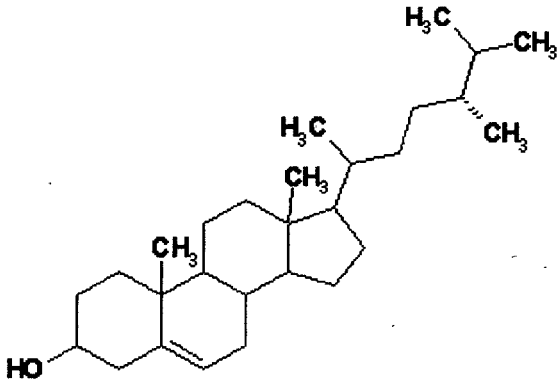
The undersigned, an independent panel of recognized experts, qualified by scientific training and relevant national and international experience to evaluate the safety of food and food ingredients, was requested by Michael Foods, Inc. to determine the Generally Recognized as Safe (GRAS) status of plant phytosterols for use as ingredients in egg products including egg whites, and egg substitutes. A comprehensive search of the scientific literature through July 2005, along with several GRAS notices to FDA on phytosterols and phytosterol esters, were also utilized for this review. The Expert Panel independently evaluated this information and other materials deemed necessary or appropriate. Following independent, critical evaluation, the Expert Panel conferred and unanimously agreed to the decision described herein.

### INTRODUCTION

Plant sterols, also called phytosterols, are present in all plants and in foods containing plant-based raw materials. Vegetable oils are in general rich in free phytosterols and their fatty acid esters. In normal diets, vegetable oils and products based on them are generally also acknowledged to be the richest sources of phytosterols. They are of keen interest due to their cholesterol-reducing properties. In a recent review by Kritchevsky (1997), the principal plant sterols were noted as follows: β-sitosterol which comprises 45-95 % of the total sterols present in plant oils, campesterol which may total 30 % of the total sterols in seed oils and stigmasterol which may account for as much as 25 % of the total sterols of seed oils. Brassicasterol is also present in appreciable amounts in plant oils. The structures of these compounds are represented by the following chemical formulae:



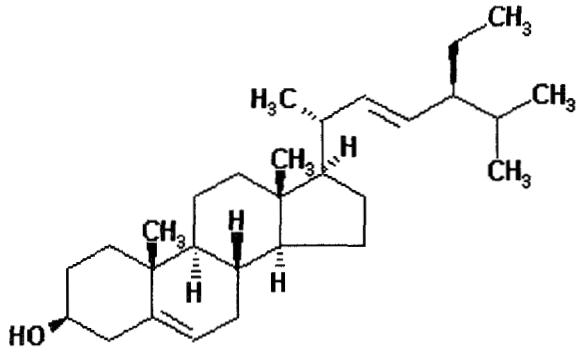

**Sitosterol**
**RN: 5779-62-4**



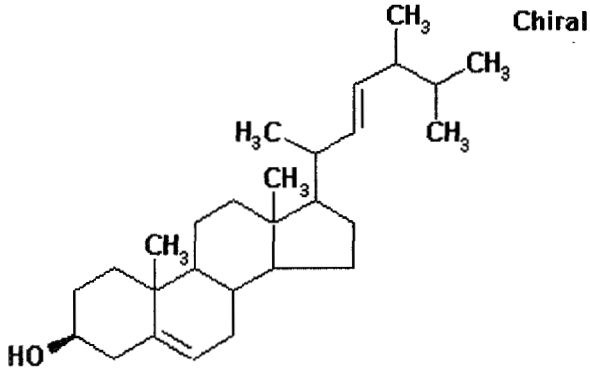

**Campesterol**
**RN: 474-62-4**



000009




**Stigmasterol**
**RN: 83-48-7**




**Brassicasterol**
**RN: 474-67-9**

The phytosterols that are the subject of this safety and equivalency assessment are comprised primarily of the sterols mentioned above. The product will be supplied as a purified phytosterol complex in the form of a white to yellow amber granular solid, fine powder or prills.

The families of phytosterols and phytosterol esters have been extensively tested in a comprehensive series of safety evaluations and have been demonstrated to be safe for use as ingredients in food and dietary supplements. Further, their efficacy has been demonstrated in multiple clinical trials evaluating their ability to lower serum cholesterol and reduce LDL-cholesterol.

## REGULATORY STATUS OF PHYTOSTEROLS

As described below, the phytosterols and phytosterol esters have also been the topic of several GRAS Notices submitted to the Food and Drug Administration (FDA) for use as ingredients in a variety of foods that are used as a part of a low fat diet to reduce serum cholesterol. FDA has responded to each of these submissions with recognition of their request and a statement that they had no questions regarding the conclusions that the phytosterols and phytosterol esters are GRAS for the intended applications. FDA has also indicated that they will use enforcement discretion as commercially available food products containing these ingredients enter the marketplace. FDA has also issued an interim final rule in September 2000 in response to filings by both Unilever Bestfoods and McNeil Nutritionals that permit a specific health claim related to lowering serum cholesterol and reducing the risk of coronary heart disease (CHD).

The intended applications of the phytosterols product identified above will be for use as an ingredient in egg products including egg whites, and egg substitutes. The phytosterol product, as demonstrated below, is essentially equivalent to previously notified phytosterols in GRAS notices. Phytosterols and phytosterol esters are already incorporated in products that are currently available in the marketplace. FDA has previously recognized their use in a final rule published in the Federal Register (65 FR 54685, 2000) on September 8, 2000 in response to petition filed by Lipton (plant sterols petitioner) and a petition filed by McNeil Consumer Health Care (plant stanol esters petitioner) for a health claim associating plant sterol/stanol esters and reduced risk of coronary heart disease. Based on the totality of publicly available evidence, the agency concluded that plant sterol/stanol esters may reduce the risk of coronary heart disease. The applications in these petitions were for spreads and dressings for salads containing at least 1.6 grams of plant sterol esters per reference amount customarily consumed to bear a health claim about reduced risk of CHD. McNeil's petition requested a similar application primarily as a spread at a level of 1.7 grams of plant stanol esters per serving of spread per reference amount customarily consumed to bear a health claim about reduced risk of CHD.

There were several other GRAS notice submissions to the agency that followed in subsequent years. In each case, FDA responded that they had no questions on the proposed use and did not object to the GRAS notice. These include the following:

**1. GRAS Notice No. GRN 000039: Responses dated April 24 and November 2, 2000 from FDA to a submission from Novartis Consumer Health, Inc. (Novartis) on tall oil phytosterols informing FDA of their view that tall oil phytosterols are GRAS, through scientific procedures, for use as an ingredient in vegetable oil spread to reduce the absorption of cholesterol from the gastrointestinal tract at levels up to 12 % free phytosterols.**

The Novartis GRAS panel concluded that given the similarities between the composition, level of use, and intended use of tall oil phytosterols compared to that of the related

substances, vegetable oil sterol esters and plant stanol esters, that the intended use of tall oil phytosterols does not raise safety questions and that any potential adverse nutritional effects (i.e. uptake on fat soluble vitamins) has been addressed and adequately resolved in the course of the determinations that vegetable oil sterol esters and plant stanol esters are GRAS. The Novartis panel concluded that tall oil phytosterols are GRAS, through scientific procedures, when used in vegetable oil-based spreads to help maintain a healthy cholesterol level, provided that the phytosterols as an ingredient meet the specifications cited in the report, are used in accordance with GMP's in an amount not to exceed 12 % phytosterol plus phytostanol in the finished product. FDA's response to Novartis stated that based on the information provided by Novartis, as well as other information available to FDA, the agency has no questions at this time regarding Novartis' conclusion that tall oil phytosterols are GRAS under the intended conditions of use.

**2. GRAS Notice No. GRN 000048: Response on November 27, 2000 from FDA to a submission from Cargill Incorporated informing FDA of their view that vegetable oil phytosterol esters are GRAS, through scientific procedures for use as an ingredient in vegetable oil spreads, dressings for salads, bars, and yogurt.**

Cargill's GRAS panel concluded that the composition of vegetable oil phytosterol esters is equivalent to that of another marketed ingredient (i.e. vegetable oil sterol esters, also known as plant sterol esters), which was the subject of the Lipton submission to FDA referenced above. FDA's previous evaluation included both plant stanol esters and tall oil phytosterols as ingredients in spreads. This consumption would be approximately 3 grams of plant sterols or plant stanols per person per day (which is equivalent to approximately 5 grams of plant sterol esters or plant stanol esters per person per day). At that time, the cumulative dietary exposure from their use in food products, such as dressings for salads, bars and yogurt, were not considered. FDA acknowledged receipt and consideration of information regarding the use of plant sterol esters and plant stanol esters in a broader array of food categories than spreads and concluded that the petitioners had met the burden to demonstrate to FDA's satisfaction that the use of plant sterol esters and plant stanol esters in this broader array of food categories was safe and lawful. FDA's response to Cargill stated that based on the information provided by Cargill, as well as other information available to FDA, the agency has no questions at this time regarding Cargill's conclusion that vegetable oil phytosterol esters are GRAS under the intended conditions of use.

**3. GRAS Notice No. GRN 000053: Response from FDA dated December 20, 2000 to a submission from Procter and Gamble informing FDA of P&G's view that phytosterol esters are GRAS, as determined through scientific procedures, for use as an ingredient in vegetable oil, at a level up to 13.3 per cent by weight, for home use applications such as baking, frying, and salad dressings.**

P&G's GRAS panel concluded that the composition of the ingredient that P&G would add to vegetable oil is equivalent to the ingredient "vegetable sterol esters" that is

marketed by Lipton in its Take Control® brand of vegetable spread. The panel also considered additional phytosterol intake from consumption of P&G's vegetable oil product compared to the consumption of phytosterol esters contained in currently available spreads and assessed stability during typical home cooking conditions. FDA's response to P&G stated that, based on the information provided by P&G, as well as other information available to FDA, the agency has no questions at this time regarding P&G's conclusion that phytosterol esters are GRAS under the intended conditions of use.

4. **GRAS Notice No. GRN 000061: Response from FDA dated April 18, 2001 to a submission from Archer Daniels Midland Company informing FDA of ADM's view that plant sterols are GRAS, as determined through scientific procedures, for use as an ingredient in vegetable oil spreads, dressings for salad, health drinks, health bars and yogurt-type products at a level of 1 gram per serving, and as a raw material in the manufacture of plant sterol esters for use as an ingredient in the same foods at a level of 1.65 grams (i.e., 1 gram sterol equivalent) per serving.**

ADM considers that the ingredients plant sterols and plant sterol esters are similar to the ingredient vegetable oil sterol esters, which is marketed by Lipton and which FDA previously evaluated for use in vegetable oil based spreads as discussed above. In ADM's view, Lipton's submission of January 11, 1999, presents a thorough evaluation of the available literature. This literature was evaluated by a panel of individuals (Lipton's GRAS panel) whom Lipton considered qualified by scientific training and experience to evaluate the safety of substances added to food. ADM notes that several relevant studies that were not publicly available at the time of Lipton's submission have now been published. Because the publicly available information draws on studies that included both sterol esters and free sterols, ADM concludes that this literature supports the safety of both of its ingredients.

ADM notes that Lipton's GRAS panel set an acceptable daily intake (ADI) for vegetable oil sterol esters of 130 milligrams/kilogram/day (as the free phytosterol), equivalent to 9.1 grams per day for a 70 kg person. ADM discusses the rationale that Lipton's GRAS panel used to set this ADI.

ADM notes that its own estimated daily intake (i.e., 10.6 grams per day at the 90th percentile level) exceeds the ADI set by Lipton's GRAS panel by 16 percent. ADM discusses the rationale for its own conclusion that dietary exposure to plant sterols and plant sterol esters from ADM's proposed uses remains within a safe range. To a large extent, ADM relies on consumers' previous exposure to a drug product (i.e., Cytellin) marketed by Eli Lilly as a treatment for hypercholesterolemia during the 1950's to 1980's. The primary component of Cytellin (β-sitosterol) was the same as the primary component of plant sterols. More than 1800 people participated in clinical studies conducted with Cytellin to assess effects on blood cholesterol levels, with no reported adverse effects. Individuals who used the marketed drug typically consumed dosages ranging from 9 to 30 grams per day. More recently, plant sterols as the esters have been the subject of

additional studies and there has been general consumption in the United States. For all of these reasons, ADM considers that the ADI set by Lipton's GRAS panel is a conservative number.

Based on the 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 the ingredients plant sterols and plant sterol esters are GRAS under the intended conditions of use.

**5. GRAS Notice No. 000112: Response from FDA dated February 4, 2003 to a submission from Teriaka Ltd. informing FDA of their view that phytosterols are GRAS, through scientific procedures, for use as an ingredient in margarine and vegetable-based spreads (margarine-like); yogurt and yogurt-like products; milk-based juice beverages; ice cream and non-standardized ice cream products; cream cheese and cream cheese-like products; snack bars (health bars); salad dressing, mayonnaise, French dressing, and dressings for salads; and white breads, white rolls and buns, and comparable non-standardized white bread products.**

Teriaka describes the products it manufactures containing phytosterols as vegetable fat mixtures with phytosterols, vegetable oil mixtures with phytosterols, or anhydrous milk fat mixtures with phytosterols, depending on the fat used to produce the sterol-enriched ingredient. The phytosterols that Teriaka uses are either from vegetable oils (soybean, cottonseed, corn, sunflower seed, canola, or peanut) or tall oil extracted from pine trees (*Pinus pinaster* and *Pinus sylvestris*). Teriaka notes that the vegetable oil phytosterols that it processes into a fatty mixture are the subject of a previous GRAS notice, i.e., GRN 000061. Teriaka notes that the tall oil phytosterols that it processes into a fatty mixture predominantly contain β-sitosterol, β-sitostanol, campesterol, and campestanol and compares the composition of these tall oil phytosterols with that of the tall oil phytosterols that are the subject of a previous GRAS notice, i.e., GRN 000039. Teriaka estimates that per capita intake of phytosterols from use in fatty mixtures would be 1.6 grams per person per day (g/p/d) at the mean and 3.6 g/p/d at the 90th percentile.

In its notice, Teriaka discusses published studies addressing the safety of acute, sub-chronic and chronic intakes of phytosterols and the potential for genotoxic, reproductive, or developmental effects of phytosterols. Teriaka also discusses published studies addressing the metabolism of plant sterols in animals, the effect of phytosterols on sex hormones in animals and humans, the effect of phytosterols on fat-soluble vitamins and carotenoids, and the safety of the cumulative intake of plant sterols. Teriaka notes that most of these studies were discussed in previous GRAS notices and Food Master Files submitted to FDA by other manufacturers (i.e., GRN 000039, GRN 000048, GRN 000053, GRN 000061, FMF 000625, and FMF 000626). Teriaka concludes that its ingredients containing either vegetable or tall oil phytosterols are safe for their intended use.

Based on the information provided by Teriaka, as well as other information available to FDA, the agency has no questions at this time regarding Teriaka's conclusion that phytosterols added as a component of a fatty mixture are GRAS under the conditions of their intended use.

**5. FDA letter dated February 14, 2003 to Cargill regarding enforcement discretion with respect to expanded use of an interim health claim rule about plant sterol/stanol esters and reduced risk of coronary heart disease.**

Cargill cited new scientific evidence and comments submitted to FDA in the plant sterol/stanol esters health claim rulemaking, and requested that FDA exercise enforcement discretion until such time as a final rule is issued that extends the authorized health claim to all the forms and sources of phytosterols, and product forms, that may effectively reduce blood cholesterol levels. Cargill further stated that the scientific literature now supports expanding the health claim to free forms of plant sterols and stanols; and to a wider range of products, including low-fat products. Cargill further stated that current science shows that the lowest effective daily intake of free phytosterols is 800 mg/day with the minimum addition of 400 mg free phytosterols per serving, and that the phytosterol substance should consist of at least 80 percent sitosterol, campesterol, stigmasterol, sitostanol, and campestanol (combined weight).

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

## IDENTITY, COMPOSITION AND SPECIFICATIONS

The chemical/physical properties and specifications of the phytosterol product of this GRAS notification are shown in Table 2.

| Table 2. Specifications and Physical Properties of Notified Phytosterol | |
|---|---|
| **Property** | **Phytosterols** |
| **Specifications** | |
| Total sterol content % | 85 min. |
| β-sisterol % | 40 min |
| Campesterol % | 20 min |
| Stigmasterol % | 11 min |
| Brassicasterol % | 0.3 min |
| Residue on ignition (%) | 0.5 max. |
| Lead by AA | <0.2 ppm |
| Heavy metals | 10 ppm max. |
| Solvent (methanol/heptane) | 10 ppm max |
| **Microbiological analysis** | |
| Total plate count | 3000/g |
| Coliforms | 10/g |
| Yeast and Mold | 100/g |
| *E. coli* | Negative/g |
| *Salmonella* | Negative/25 g |
| **Physical properties** | |
| Bulk density | ~0.6 g/ml |
| Appearance | White to yellow amber solid |
| Solubility | Partly soluble in fats, oils and ethanol<br>Insoluble in water |
| Melting point (°C) | 135-145 |

The chemical/physical properties and specifications of the prior GRAS notified phytosterol products are shown in Table 3.

| Table 3. Specifications of Prior Notified Phytosterol Products | | | | |
|---|---|---|---|---|
| **Property\ Specification** | **GRN 39 Tall oil sterols** | **GRN 53 Vegetable Sterol** | **GRN 61 Vegetable Sterol** | **GRN 112 Tall oil and vegetable sterols** |
| Sterol content | >95% | >95% | >90% | >99% |
| Solvents | <0.5% | | | <5% |
| Water | <5% | | | <5% |
| Residue on ignition | <0.1% | | <0.1 | <0.5% |
| Lead | <0.25 ppm | <0.1ppm | | <0.25 ppm |
| Heavy metals | <10 ppm | | <10 ppm | <10 ppm |

As the comparison of properties and specifications in tables 2 and 3 demonstrate, the specifications of the phytosterols are essentially identical for the phytosterol product that is the subject of this notification and phytosterols that have been previously accepted by FDA under GRAS notification. The lead specification is <0.2 ppm for sterol products.

Sterol content is generally greater than 90% for the sterol products and over 85% for the sterol product being notified .

The composition of the phytosterol and phytosterol ester products for this GRAS notification are shown in Table 4 in comparison to the composition of the phytosterol products for prior GRAS notified sterols.

**Table 4. Comparison of Composition of Prior Notified and Subject Notified Phytosterol Products**

| Constituent | GRN 39 Tall oil sterols | GRN 53 Vegetable sterol | GRN 61 Vegetable sterol | GRN 112 Tall oil and vegetable sterols | Notified Phytosterol |
|---|---|---|---|---|---|
| Total as sterols | >95 . | >95 | >90 | >99 | >95% |
| β-sisterol | 38-60 | 40-45 | 40-58 | 75-80 | 40-58% |
| Campesterol | 9-18 | 20-25 | 20-28 | 6-9 | 20-30% |
| Stigmasterol | | 20-25 | 14-23 | <2 | 11-22% |
| Brassicasterol | | 0.5 | 0-6 | <2 | 0-6% |
| Campestanol | 2-14 | | 0-2 | 0-2 | 0-2 |
| Sistostanol | 14-34 | | 0.4 | 10-14 | 0-5% |

As the composition data in table 4 shows, there is substantial equivalence in the composition of the sterol constituents in the phytosterols of the subject notification and prior GRAS notified substances. The vegetable sterols being notified are almost exactly the same as those in GRN 61 from vegetable sources.

Two lots of the phytosterol product have been analyzed for pesticides by FDA Method 304 for fatty foods. No pesticide residues were found for organophosphates at the limit of detection (LOD) of 0.05 ppm, organonitrogens at the LOD of 0.5 ppm and organochlorinated compounds at the LOD of 0.2 ppm.

## MANUFACTURING PROCESS

Information on the manufacture and refining of the phytosterol product is summarized as follows. The initial starting material is termed a deodorizer distillate, typically with 10-17% phytosterol content. It is extracted primarily from soybeans, but may contain smaller amounts of phytosterols from various oilseeds such as canola, corn, cottonseed and sunflower oil. The product may also contain phytosterols derived from tall oil (the starting material being tall oil pitch), but the total addition will be less than 5% of the total volume of vegetable oil derived product on a w/w basis. All of these oilseeds and tall oil have been noted in prior GRAS notifications as oil sources for phytosterol production and are considered suitable for food-grade oils and by-products.

The distillate is refined by a primary distillation followed by a molecular film distillation, thus separating the mono- and di-glycerides, methyl esters and tocopherols from the crude sterols. Transesterification converts any remaining glycerides to free sterols. The resulting crude sterols are purified by recrystallization and filtration. Impurities are further removed by filtration and methanol/heptane wash. Heating and vacuum removal reduces residual solvent to <10 ppm methanol/heptane. The end product typically

contains 94-97% sterols. The main components are beta-sitosterol, campesterol and stigmasterol. The general process used for phytosterol production is similar for all the major producers by distillation of the crude plant oil distillate, recrystallization, transesterification of waxy sterols and triglycerides, further filtration and solvent purification and removal of impurities/residual solvent.

**USE AND EXPOSURE**

The phytosterols esters that are the subject of this notice are comprised primarily of the plant sterols mentioned above in the specifications. The intended application of these phytosterol products will be for a new use as an ingredient in egg products including egg whites, and egg substitutes at levels up to 1000 mg/50 grams (20 mg phytosterol/g egg product). The recommended serving will be 2 fl. oz. or 56.7 grams, delivering 1134 mg phytosterol/serving. The egg products including egg whites, and egg substitutes will be sold to the consumer and end users as liquid products under refrigerated or frozen conditions.

The proposed level of addition to egg products including egg whites, and egg substitutes (1000 mg/50 grams or 1134 mg/serving) is nearly the same amount per food serving that has been proposed and accepted in prior GRAS notifications for phytosterols. The levels of addition to foods typically result in phytosterol consumption of approximately 1 gram per serving. The food types accepted for this level of addition of phytosterols include margarines and table spreads, yogurt, yogurt-like products, milk based juice beverages, ice cream/non-standardized ice cream, cream cheese/cream cheese analogs, snack (health) bars, salad dressing, mayonnaise, white bread rolls and buns, non-standardized white bread products and health drinks. Other food types have been approved for phytosterol esters, typically up to 1.6 grams/serving.

According to AC Nielsen Survey of grocery store sales, annual volume of refrigerated egg substitutes is approximately 66 million lbs. and refrigerated egg whites, 9 million lbs. Frozen egg white and egg substitutes sold are approximately 4 million lbs. Using USDA intake of 261 eggs/person/year in 2001, with a census of 283 million, total egg consumption was grossly 7887 million lbs or about 28 lbs/person/year by a population disappearance measure. This may be high given the wastage of 10-20% likely to occur in the use of eggs. The current market of refrigerated and frozen egg white and egg substitutes are 79 million lbs. Thus, the refrigerated and frozen egg white and egg substitute market is roughly 1% of the total shell egg market for the total US population.

A more useful estimate of egg intake is survey data for eaters only. A reasonable assumption is that health conscious consumers of egg and egg substitute products will consume eggs directly in scrambled egg or omelet preparations much as the consumer does with shell eggs. Krebs-Smith *et al.* (1997) of USDA conducted a two-day survey on eaters only and found that mean and 90$^{th}$ percentile egg consumption was 27 and 87 g/day respectively. Highest consumption was in males 20-39 with a mean of 37 grams

and 90th percentile of 107 grams; females in this age group ate a mean of 24 grams and 90th percentile of 79 grams.

Research by Michael Foods, Inc. of over 1000 respondents suggests that the primary consumers of egg and egg substitute products are persons over 50 years of age. Mean and 90th percentile intakes of eaters only of age 40-59 were 34 and 94 grams in males and 22 and 74 grams in females respectively. For individuals over 60, corresponding values were 32 and 88 grams in males and 22 and 65 grams in females. For the population as a whole, with a mean and 90th percentile of 27 and 87 grams, daily intake from use of egg products and egg substitutes containing 20 mg phytosterol/g egg products/substitutes is estimated as 540 and 1740 mg/person/day respectively.

As noted before in the discussion of prior notifications on phytosterols, FDA has accepted the safety of cumulative intake from various food additions in a single GRAS notification as high as 10.6 grams/person/day. A consumer eating the recommended serving of egg products including egg whites, and egg substitutes would have an intake of 1134 mg of phytosterols, an amount commonly agreed to be necessary to confer health benefit against the risk of CHD. Consumption at the 90th percentile in the highest general consumption group (20-39 year old males) would be 2.1 grams, well below the accepted intake of 10.6 grams that was the subject of a prior GRAS notification.

## SAFETY STUDIES

### Phytosterols and Phytosterol Esters

There are many published safety studies available on plant phytosterols and phytosterol esters demonstrating their low order of toxicity. The key studies are summarized below. It is important to note that man does not synthesize these compounds and exposure is strictly from vegetable sources in the diet. Further, plant sterols are an essential component of the membranes of all eukaryotic organisms. This review includes the safety information on phytosterol esters as well as phytosterols due to the fact that phytosterol esters are readily converted *in vivo* to phytosterols and the data and findings from key studies are considered applicable to phytosterol safety assessment.

### Absorption, Distribution, Metabolism and Excretion

A major metabolic effect of dietary plant sterols is the inhibition of absorption and subsequent compensatory stimulation of the synthesis of cholesterol. The ultimate effect is a lowering of serum cholesterol due to enhanced elimination of cholesterol in stools. Sanders *et al.* (2000) conducted a series of studies to determine the relative extent of intestinal absorption and tissue distribution of the β-sitosterol, β-sitostanol, campesterol, campestanol and stigmasterol in the rat. $^{14}C$ –labeled samples of cholesterol, β-sitosterol, or β-sitostanol or $^{3}H$-labeled samples of β-sitostanol, campesterol, campestanol or stigmasterol were orally administered to male and female CD strain rats weighing between 140 and 170 grams (4-5 weeks old) in acclimatized metabolism cages for 24 hours and urine and feces were collected. For the rats dosed with $^{3}H$-labeled samples, glass traps were fitted to monitor expired air. A standard pelleted diet, virtually

phytosterol free, was restricted to a few pellets for the 24 hours prior to dosing and then provided *ad libitum* from 2 hours after dosing. Urine and feces were collected up to 96 hours after the dose. Animals were sacrificed and either prepared for whole body autoradiography or tissue and carcass remains were assayed for $^{14}C$ or $^{3}H$. Elimination from the body was mainly in the feces and was initially very rapid, but traces of material were still being excreted at 4 days after dosing. Recovery results are shown in Table 7. While total absorption of the phytosterols could not be quantified without biliary excretion data, it was clear that cholesterol was absorbed to the greatest extent (27% of the dose in females at 24 hours). Campesterol (13%) was absorbed more than β-sitosterol and stigmasterol (both 4%), which were absorbed more than β-sitostanol and campestanol (1-2 %). The absorption of phytosterols was slightly greater in females than males (27 vs. 24 %). For each test material, the overall pattern of tissue distribution of radioactivity was similar, with the adrenal glands, ovaries and intestinal epithelia showing the highest levels and the longest retention of radioactivity. The importance of this observation was unclear and will be addressed in the 90-day study below. The findings relative to absorption and excretion of the β-sitosterol and β-sitostanol were in agreement with earlier work by Ikeda and Sugano (1978).

**Table 7: Recovery of $^{14}C$ and $^{3}H$ from Rats Gavaged with Labeled Sterols or Stanols**

| Substance | No. of Rats | Gender | Feces (%) | Total (%) |
|---|---|---|---|---|
| $^{14}C$-β sitosterol | 4 | M | 96.54±7.85 | 100.00±7.67 |
| $^{14}C$-βsitosterol | 4 | F | 85.23±3.84 | 97.85±6.20 |
| $^{14}C$-β sitostanol | 4 | M | 87.93±4.00 | 95.96±6.20 |
| $^{14}C$-β sitostanol | 4 | F | 88.09±3.61 | 99.47±2.98 |
| $^{3}H$-β sitostanol | 3 | F | 75.05±15.86 | 95.03±2.24 |
| $^{3}H$-Campesterol | 3 | F | 76.45±2.88 | 94.17±3.14 |
| $^{3}H$-Campestanol | 3 | F | 90.39±1.05 | 97.97±2.46 |
| $^{3}H$-Stigmasterol | 3 | F | 84.65±2.64 | 95.03±0.33 |
| $^{14}C$-Cholesterol | 3 | M | 51.32±17.02 | 81.23±12.8 |
| $^{14}C$-Cholesterol | 3 | F | 58.70±5.59 | 91.55±1.15 |

Heinemann *et al.* (1993) compared the absorption of cholesterol and several phytosterols in 10 human subjects. Mean hourly absorption (%) was cholesterol, 31.2±9.1; campestanol, 12.5±4.8; campesterol, 9.6±13.8; stigmasterol, 4.8±6.5; and sitosterol 4.2±4.2. Ostlund *et al.* (2002) examined absorption of phytosterols in humans using intravenous di-deuterated phytosterols and phytostanols followed two weeks later by orally administered penta-deuterated phytosterols. The oral–to-intravenous tracer ratio provided a measure of absorption. Absorption of sitosterol and campesterol was 0.512±0.038% and 1.89±0.27%, respectively. Absorption of the corresponding stanols was considerably lower being 0.0441±0.004% for sitostanol and 0.155±0.017% for campestanol. Conversion of sitosterol to acidic metabolites extracted from feces has been reported in rats by Boberg *et al.* (1990) and vervet monkeys by Kritchevsky *et al.* 1981).

## Safety Evaluations/Toxicity Studies

### Genotoxicity Studies

As part of the comprehensive program of safety assessment of phytosterols and phytosterol esters, Wolfreys and Hepburn (2002) assessed their mutagenic potential in a bacterial mutation assay and an *in vitro* chromosome aberration assay. In addition, an *in vitro* mammalian cell gene mutation assay and two *in vivo* mutagenicity studies, namely rat bone marrow micronucleus and liver unscheduled DNA synthesis (UDS) assays, were conducted on phytosterol esters only. Phytosterols and phytosterol esters did not show any evidence of genotoxicity in any of these assays. A breakdown product of cholesterol is 4-cholesten-3-one and there was a concern that the amount of 4-cholesten-3-one in the gut may increase following supplementation of foods with phytosterol esters. An assessment of the mutagenic activity of 4-cholesten-3-one and its major fecal breakdown product 5 beta-cholestan-3-one was completed in two *in vitro* assays, a bacterial mutation assay and an *in vitro* chromosome aberration assay. Neither of these compounds showed any evidence of mutagenic or clastogenic activity.

Since oxidation of phytosterols may occur at low levels under extreme heating conditions, Lea *et al.* (2004) assessed the genotoxicity and subchronic toxicity of a phytosterol oxide concentrate containing approximately 30% phytosterol oxides. This level of phytosterol oxides far exceeds that seen in commercial batches of phytosterol esters or products containing phytosterol esters after frying or baking. The phytosterol oxide profile was representative of that produced in frying and baking experiments with a phytosterol ester enriched spread. The phytosterol oxide concentrate was also fed at levels of 0, 0.2%, 0.6% and 1.2% of the diet of rats for 90 days. The results show that phytosterol oxides did not possess any genotoxic potential in any of the 3 *in vitro* genotoxicity assays (bacterial mutation, chromosome aberration and micronucleus) nor any notable sign of systemic toxicity following subchronic administration in the diet at levels up to 1.6% phytosterol oxide concentrate for 90 consecutive days in the rat.

### Estrogenicity

#### *In vivo* and *In vitro* assays

Baker *et al.* (1999) used competitive binding with the immature rat uterine estrogen receptor (ER) to measure the ability of phytosterols to bind to ERs and also the transcriptional activation of estrogen responsive genes in an estrogen-inducible yeast screen. Phytosterols did not display any activity in these *in vitro* assays. Uterotrophic assays were also conducted to investigate the potential for phytosterols to elicit an estrogenic response when administered orally to groups of 10 immature female rats at doses of 0, 5, 50 or 500 mg/kg/day for 3 consecutive days. The phytosterols were a well-characterized mixture of β-sitosterol (47.9%), campesterol (28.8%) and stigmasterol (23.3%) and a mixture of phytosterol esters of the same sterols listed. No estrogenic activity was exhibited at any of the dose levels in the immature female rats using uterine weights as the endpoint.

**Subchronic Toxicity Studies**

Hepburn *et al.* (1999) fed a characterized mixture of various plant phytosterols as part of an extensive safety evaluation program in a subchronic rat toxicity study for 90 days. The major phytosterols present in the diet were β-sitosterol (48.7%), campesterol (25.8%), and stigmasterol (21.6%). Phytosterols were sourced from a variety of vegetable oil distillates (mainly soybean). Groups of Alpk:$AP_fSD$ (Wistar derived) rats (20 males and 20 females /group) were fed diets containing phytosterol esters at levels of 0, 0.16, 1.6, 3.2 and 8.1% (w/w). These doses are equivalent to 0.1, 1.0, 2.0, or 5.0% of the respective sterol mixture. Throughout the study, clinical observations, body weights, and food and water consumption were measured. At the end of the study, a full post-mortem examination was conducted, cardiac blood samples were drawn for clinical parameters, select organs were weighed, and a full tissue list was taken for subsequent histopathological examination. There were no treatment-related changes that were considered to be of toxicological significance. Further, there were no specific treatment-related changes in the adrenal gland, ovary or intestine even though earlier data showed localization in these organs. The mean consumption of phytosterol esters over the course of the study was calculated to be 6.3 grams/kg body weight/day (3.9 grams sterol/kg/day) and 6.8 grams phytosterol esters /kg body weight /day (4.2 grams sterol/kg/day) for males and females, respectively. Therefore, the phytosterol concentration of 8.1% was considered to be the no-observed-adverse-effect level (NOAEL) following daily oral administration for 90 days. This was equivalent to a dose of 6.6 grams/kg body weight/day phytosterol ester or 4.1 grams phytosterol/kg body weight /day.

Previously, Malini and Vanithakumari (1990) reported a study in which male and female Wistar rats (10/sex/treatment) were dosed subcutaneously with 0, 2.5, 5 or 10 mg beta-sitosterol/kg body weight/day for 60 days and found no difference in a number of blood parameters but it was not possible to make an adequate toxicological assessment since only the liver and kidneys were histologically examined.

Recently, Kim *et al.* (2002) conducted a 13-week repeated gavage dose toxicity study in male and female Sprague-Dawley rats. Three treatment groups of plant sterol esters consisted of doses of 1000, 3,000 and 9000 mg/kg/day along with a negative control group. The composition of the plant sterol esters (sterols had been isolated from soybean and then esterified) consisted of about 49.4% sitosterol, 27.9% campesterol, 18.5% stigmasterol, and others. Each group consisted of 10 (low and middle dose groups) or 16 (negative control and highest dose groups) rats of each sex. The only findings observed were a suppression of body weight gains in both sexes and cardiomyopathy in males at a dose level of 9000 mg/kg. The target organ appeared to be the heart for males, but not for females. The NOAEL of plant sterol esters administered by gavage was considered to be 3000 mg/kg/day for both sexes. It should be noted that the gavage route is generally not suitable for assessing dietary ingredients because of its stress on the animals and its concentrated exposure versus being consumed as a dietary component.

**Reproductive Toxicity Study**

As part of an extensive safety assessment program on phytosterol esters, Waalkens-Berendson *et al.* (1999) conducted a two-generation reproduction study in Wistar rats in which the possible effect of phytosterol esters on male and female reproductive performance and on the growth and development of the offspring were studied. Four groups of 28 rats/sex/group were fed test diets containing 0, 1.6, 3.2 or 8.1% (w/w) phytosterol esters for a period of 10 weeks prior to the mating period and over two successive generations and a wide range of reproductive and developmental parameters including sexual maturation and estrous cycle length were determined. The phytosterol esters were sourced from a variety of common vegetable oil distillates (mainly soybean). The major phytosterols present in the diet β-sitosterol (48.7%), campesterol (25.8%), and stigmasterol (21.6%). Macroscopic and microscopic examinations were conducted including a histological examination of selected organs from $F_1$- and $F_2$-weanlings and from $F_0$- and $F_1$- parental animals. Daily clinical observations did not reveal any unusual findings. In both generations, no effects were observed on pup mortality (calculated on litter basis), precoital time, mating index, male and female fertility index, female fecundity index, gestation index, duration of gestation, number of females with stillborn pups, post-implantation loss and pup development. Furthermore, phytosterol esters had no effect on sexual maturation parameters (preputial separation and vaginal opening) and estrous cycle length. There were no dose-related effects on selected organs following histological examination. Therefore, a nominal phytosterol ester concentration of 8.1% (equivalent to a dose of 2.5-9.1 grams phytosterol ester /kg body weight/day or 1.54-5.62 grams phytosterol/kg body weight/day (dependent on the period of the study) was considered to be the no-observed-adverse-effect level (NOAEL) following daily oral administration of phytosterol esters for two successive generations.

Awad *et al.* (1998) reported a 33% reduction in serum testosterone in SD rats fed a diet containing 2% phytosterols and 0.2% cholic acid for 22 days. The phytosterol mixture contained 56% β-sitosterol, 28% campesterol, 10% stigmasterol, and 6% dihydrobrassicasterol. A 55% reduction in aromatase activity was the only effect observed in the testes. The authors suggest that phytosterol feeding may reduce the risk of prostate cancer by the lowering the activities of the enzymes of testosterone metabolism.

**Chronic Toxicity/Carcinogenicity Studies**

There are several long –term studies on phytosterols reported in the literature. Shipley *et al.* (1958) found no evidence of toxicity in a series of studies in rats, rabbits or dogs when phytosterols were fed at high levels for periods up to 2 years. In the rat study, groups of 10 rats were fed 5% phytosterols for 8, 18 and 22 months. Unfortunately, due to deficiencies in the design compared with current toxicological testing standards, it is not possible to adequately assess the potential toxicity of repeat dosing from these studies. More recently, two chronic toxicity/carcinogenicity studies on a related material, gamma-oryzanol, which is a mixture of ferulic acid esters of phytosterols (including beta-sitosterol) from rice bran oil were completed and reported by Tamagawa *et al.* (1992a) in

rats, and Tamagawa *et al.* (1992b) in mice at doses up to 2000 mg/kg body weight. No treatment related changes were observed in either rats or mice and there were no significant increase in tumors above background levels.

**Studies on Minor Constituents - Stigmastanol and Campestanol**

These two minor components common in phytosterols have been extensively studied and reported as a part of the research to assess the safety of stanols and stanol esters. Findings in toxicity experiments with phytostanol esters reflect those obtained with phytosterol esters. Turnbull *et al.* (1999a) found in *in vitro* gene mutation assays using bacterial or mammalian cells no genotoxicity. Turnbull *et al.* (1999c) also reported that phytostanol esters had no adverse effects when fed to Wistar rats at levels of 0.5 g/day for 13 weeks. *In vitro* and *in vivo* tests by Turnbull *et al.* (1999b) or estrogenic activity of phytostanol esters proved negative. Tests in rats for developmental toxicity by Slesinski *et al.* (1999) found no evidence of toxicity. Whittaker *et al.* (1999) also reported no effects in a two-generation reproductive study of plant stanol esters in rats.

**Safety Evaluation in Humans**

Weststrate *et al.* (1999) conducted a study in healthy, normolipidemic human volunteers (12 male, 12 female, mean age 36 years) to assess the impact of a margarine enriched with phytosterol esters on fecal concentrations of bile acids and sterols. Volunteers consumed 40 grams of control margarine for 21 consecutive days if male, and 28 days if female. After randomly allocating half of the volunteers to the control margarine for another 21 or 28 days respectively, the remaining subjects consumed 40 grams of a margarine containing 8.6 grams vegetable oil phytosterol (46% (w/w) β-sitosterol, 26% campesterol, 20% stigmasterol) for the same time periods. The phytosterol-enriched spread significantly enhanced fecal neutral sterol concentrations from about 40 mg/g to 190 mg/g dry weight basis. Fecal neutral sterol metabolites increased from about 30 mg/g to about 50 mg/g. The major parent sterols excreted were cholesterol, sitosterol, campesterol and stigmasterol. Sitosterol, campesterol and stigmasterol comprised 28%, 15% and 12% of the total fecal neutral sterols reflecting the composition of the sterol-enriched margarine. The major sterol metabolites were formed by predominantly oxidation at the 3- position and metabolites saturated at the 5, 6 position in a Beta configuration. Fecal secondary bile acid concentration was reduced by vegetable oil phytosterols from 7.6 mg/g dry feces to 6.0 mg/g suggesting more rapid transit time. There was also a slightly significantly increase in fecal concentration of 4-cholesten-3-one but this was still in line with literature values for subjects on high or low fat diets. No sterol oxides were detected in the feces. Thus, there is little likelihood that vegetable oil sterols will increase colon cancer risk.

In another study by Ayesh *et al.* (1999) in normolipidemic volunteers, the effect of a phytosterol ester-enriched margarine (conducted in an identical manner to that of Weststrate *et al.*, 1999) was studied on fecal short-chain fatty acids (SCFAs) and fecal bacterial enzyme activities, viable fecal microflora count, female sex hormones and cholesterol. When compared to the control group values, the test group showed a

significant reduction in serum total and LDL-cholesterol concentrations of 18 and 23% respectively, in fecal lactic acid concentration and in serum progesterone levels. There were no other significant treatment related effects. Within this study, apart from a few exceptions, the bacterial profile seen in the fecal samples remained essentially unchanged in either the control or test group. The few statistically significant changes noted from day 1, a decrease in the bacterial amounts in total microbes, staphylococci and lactobacilli, were observed in both the control and treatment groups and were considered to be biologically unimportant.

A study by Davidson *et al.* (2001) evaluated the safety and tolerability of esterified phytosterols administered in reduced-fat spread and salad dressing to 84 free-living men and women at levels of 0, 3.0 g/day, 6.0 g/day or 9.0 g/day for an eight-week treatment period. Side effects did not differ among the groups during the study and there were no study related serious adverse effects. No changes were observed in clinical chemistry in response to phytosterol intake. Due to their mechanism of action, phytosterol-enriched products could conceivably affect fat-soluble vitamins and carotenoids. In this study, blood concentrations of all fat-soluble vitamins remained within normal reference ranges, and there were no differences in serum vitamin responses. Further, in the current study, there were significant reductions in α-and β-carotene in the 9.0g/day group vs. control subjects. This finding is similar to those reported in previous studies by Weststrate and Meijer (1998) and Maki *et al.* (2001) and is expected given the high lipophilicity of these carotenoids. In addition, though decreased after intervention, carotenoid levels remained within normal ranges throughout the study. All groups in this study receiving phytosterols had significant increases in serum campesterol, but β-sitosterol did not differ from the control. Total, low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterol responses did not differ significantly. The total HDL-cholesterol response in the 9.0 g/day group was significantly different from the control group response (-9.6% vs. 2.6%). Also, a median increase of 7.8% in serum triglycerides was observed in the control group, which differed significantly from the response in the 3.0 g/day group (-13.3%). The authors conclude that phytosterol esters are well tolerated and show no evidence of adverse effects at a daily intake of up to 9.0 g/day of phytosterols for eight weeks.

While the question has been raised about the effect of dietary phytosterols on circulating carotenoids, no data exist on optimal plasma levels of these compounds and on the effects of relatively small changes in their concentration. Further, there is little consistency in the published data. Hallikainen *et al.* (2000a) studied the effects of stanol and sterol esters in hypercholesterolemic subjects and found in one study with stanol for weeks that serum β-carotene and alpha-tocopherol levels were reduced significantly. When the data was corrected for total cholesterol, β-carotene levels normalized and α-tocopherol levels rose. In a second study by this group (2000b), in which sterol esters were fed for 4 weeks at doses from 0, 0.8, 1.6, 2.4 and 3.2 g/day, and the data was calculated as millimoles of carotenoid or tocopherol per mole of cholesterol, there was no difference.

Nestel *et al.* (2001) conducted a randomized single-blind study with sterol esters and non-esterified plant stanols in breakfast cereals, bread and spreads for 12 weeks in 37 subjects consuming 2.4 g/day. They found no reduction in plasma carotenoids or tocopherols. Judd *et al.* (2002) fed a group of 26 men and 27 women diets in which plant sterol esters were fed at 3.5 g/day in salad dressing and reported a significant reduction in plasma and LDL-cholesterol and triglycerides along with a reduction in plasma total carotenoids. Ntanios *et al.* (2002) reported that a plant sterol –enriched margarine containing 12 g/100g free sterol for 3 weeks in a typical Japanese diet lowered plasma cholesterol and lipoproteins but did no affect levels of vitamins A and E. They did observe a 21% reduction in β carotene. When Amundsen *et al.* (2002) included a plant sterol –enriched spread in the diet of children with familial hypercholesterolemia for 8 weeks; plasma total and LDL-cholesterol were reduced significantly. Plasma tocopherol levels were unaffected, but lycopene and β-carotene levels were reduced. Noakes *et al.* (2002) reported that the addition of carotenoids to a diet of subjects consuming plant sterols or stanols is effective in maintaining plasma carotenoid concentrations.

Eli Lilly conducted clinical trials and marketed Cytellin, a drug for hypercholesteremia through 1985, which was voluntarily withdrawn due to the advent of the statins and its poor palatability. The drug contained mixed phytosterols, but was mainly composed of β-sistosterol. As noted in the review by Pollak and Kritchevsky (1981), subjects received doses as high as 24 g/day for three years without any apparent adverse effects. In a trial with 13 patients with coronary atherosclerosis, doses as high as 52 g/day were given with no reported adverse clinical effects.

**Sitosterolemia**

Sitosterolemia, also known as phytosterolemia, is an extremely rare recessive lipid storage disease that results in atherosclerosis and xanthomatosis. Bhattacharyya and Connor (1974) were the first to describe this lipid storage disease in two sisters. Salen *et al.* (1989) demonstrated increased sitosterol absorption, decreased removal, and expanded body pools compensating for reduced cholesterol synthesis in sitosterolemia and xanthomatosis. In a published workshop on the efficacy and safety of plant stanols and sterols in the management of blood cholesterol levels, Katan *et al.* (2003) noted that the homozygous state occurs in about 1 in 5 million people, with a wide margin of uncertainty. Patients are prone to premature atherosclerosis and CHD. Sitosterol absorption in homozygous individuals is typically 15% to 25% as opposed to 5% or less in normal persons and excretion is reduced. Typical ranges for plasma concentrations are 14 to 65 mg/dL for sitosterol and 7 to 21 mg/dL for campesterol. Berge *et al.* (2000) identified seven different mutations in two adjacent, oppositely oriented genes that encode new members of the adenosine triphosphate (ATP)-binding cassette (ABC) transporter family (six mutations in ABCG8 and one in ABCG5) in nine patients with sitosterolemia. The two genes are expressed at highest levels in liver and intestine. The authors suggest from their data that ABCG5 and ABCG8 normally cooperate to limit intestinal absorption and to promote biliary excretion of sterols, and that mutated forms of these transporters predispose to sterol accumulation and atherosclerosis.

The genetics of sitosterolemia have been reviewed by Lee *et al.* (2001). They also proposed two molecular mechanisms to explain the highly selective absorption and retention of cholesterol by the body and the regulation of sterol excretion. Glueck *et al.* (1991 and 1992) found that in hypercholesterolemic subjects who had premature coronary disease and their first-degree relatives that their also exhibited elevated levels of cholesterol and phytosterols. The authors implied that phytosterolemia may have contributed to CHD. In seven subjects in the 1992 Glueck study, serum cholesterol levels were 7.09 mmol/L whereas the concentration of campesterol, stigmasterol, and sitosterol combined was only 43.86 micromol/L. They implied that even a slight increase in phytosterols may increase deposition in the aorta. They further showed that when these subjects were placed on a low fat/low cholesterol/low phytosterol diet, all of these levels dropped substantially. Salen *et al.* (1985) had earlier demonstrated in a sitosterolemic subject that cholesterol accounted for over 80% of plasma, tissue and atheroma sterol. Sudhop *et al.* (2002) reported on subjects with and without a family history of CHD. There were no differences in levels of serum lipids, lipoproteins, or cholesterol precursors. Average serum cholesterol levels of the two groups were identical: 242$\pm$46 mg/dl in 27 controls and 242$\pm$31 mg/dl in 26 test subjects. The serum campesterol level in the control group was 0.38 mg/dl compared to 0.50 mg/dl in the group with a family history of heart disease, while sitosterol levels were 0.31 mg/dl and 0.40 mg/dl, respectively. Levels of phytosterol in the sera of the test group were small, representing 0.21% (campesterol) or 0.17% (sitosterol). In the control group, the percentage of serum sterol represented by campesterol is 0.16% and by sitosterol is 0.13%. Clearly, these differences are small and it is difficult to speculate that the resulting atherosclerotic effect is due to these differences.

In a study reported by Becker *et al.* (1993), sitosterol and sitostanol were compared in the treatment of severe familial hypercholesterolemia in childhood. Serum and fecal excretion patterns were measured following a 3-month administration of sitosterol pastilles (2 gm three times per day), followed by sitostanol for 7 months at a dose of 0.5 gm three times a day. All children completed the study and no adverse effects were observed. Both sterols were highly effective in reducing serum lipoprotein levels of LDL-cholesterol. Sitostanol was even more effective at a dose four-fold lower than sitosterol. Finally, Vanstone *et al.* (2002) demonstrated in hypercholesterolemic individuals that unsterified sterols and stanols at a dietary dosage of 1.8 g/day were equally effective in lowering plasma LDL-cholesterol levels by suppressing cholesterol absorption.

## SAFETY ASSESSMENT

There have been many studies on the beneficial cholesterol-lowering effects of phytosterols and phytosterol esters and a representative number of these peer-reviewed published studies have been presented in this document. It is clear from a review of the

toxicological studies reported above that phytosterols and phytosterol esters do not produce adverse effects even at high doses (except in individuals with phytosterolemia, an inherited and rare lipid disorder). In studies with Cytellin, doses as high as 24 g/day were tolerated with no adverse effects in humans for years.

As noted in the ADM GRAS notification (GRN 61) and the Lipton GRAS notification (FMF 625), the generally accepted ADI for phytosterols based on animal studies at the highest doses tested is about 130 mg/kg/day. This equates to a daily intake of 9.1 g/day for a 70 kg adult. The ADM GRAS notice concluded that a higher ADI was appropriate, of 10.6 g/day, and FDA did not object to this conclusion. Moreover, even this ADI is likely to be conservative. The data reviewed in this document support a conclusion that dietary phytosterols have a very low order of toxicity and are safe at significant levels of consumption. For example, as discussed in the Teriaka notice (p. 100), clinical studies have shown safe consumption at levels including 18 g/day and 25-50 g/day.

The estimated daily intake (EDI) for phytosterols from consumption of egg products including egg whites, and egg substitutes would be significantly below these levels, at 540 and 1740 mg/day at the mean and 90$^{th}$ percentile levels, respectively. We estimate that the highest level of consumption could occur at the 90$^{th}$ percentile among 20-39 year old males, at 2.1 g/day.

Because phytosterols in egg products including egg whites, and egg substitutes have not previously been the subject of a GRAS notification, we considered the cumulative effect of consuming phytosterols in egg products including egg whites, and egg substitutes in addition to the phytosterol intake that may occur from other foods that have been the subject of GRAS notifications. The most recent GRAS notification was Teriaka's, which covered almost all of the food categories that have been the subject of prior GRAS notifications. Teriaka estimated that the addition of phytosterols to fat-based spreads, dressing for salads, snack bars (health), milk-based juice beverages, yogurt-like products, cream cheese-like products, ice cream-like products, and white bread products at 0.75 g per serving (except for yogurt, which would have 1.5 g per serving) would result in phytosterol intake of 1.6 g/p/d (mean) and 3.6 g/p/d (90th percentile). (See Teriaka GRAS notice, pp. 58, 101.)

Teriaka's notice did not cover phytosterols in vegetable oil, which was discussed in GRN 000053. That notice reported a 90$^{th}$ percentile EDI of up to 2.1 g/day. The simple addition of this figure to the consumption estimates discussed above would result in an EDI of 5.7 g/day (at the 90$^{th}$ percentile).

If the EDI for egg products including egg whites, and egg substitutes were simply added to the above EDI for all other food categories, the result (7.4 g/day at the 90$^{th}$ percentile) would still be below the most conservative ADI (9.1 g/day). This is a highly conservative estimate for two reasons.

- First, the simple addition of separate intake estimates is likely to overstate intake, as compared with a consumption analysis that takes into account that not all foods are consumed by all consumers on the same day.

- Second, it is likely that phytosterols from egg products including egg whites, and egg substitutes would be substituted for other sources of phytosterols in the diet. Like other foods containing this ingredient, egg products including egg whites, and egg substitutes will be prominently labeled with the FDA-authorized health claim, and so would likely be used interchangeably with other sources of phytosterols in the diet. Thus, it is unlikely that the addition of phytosterols to egg products including egg whites, and egg substitutes would result in any significant increase in consumption above previously estimated intake levels.

Because the available information provides a sound, highly conservative basis for concluding that the EDI for all food categories that contain phytosterols (including egg products including egg whites, and egg substitutes) will be below the most conservative ADI, the panel did not consider it necessary to conduct further consumption analyses.

The panel considered the questions raised by FDA at 66 FR 50824, 50826 (Oct. 5, 2001), including questions about consumption of phytosterols by pregnant and breast-feeding women and children. It concluded that the available data indicate that phytosterols are safe for use by the population generally.

In summary, the panel concluded that the proposed addition of phytosterols to egg products including egg whites, and egg substitutes is reasonably expected to be safe for all population groups consuming these products.

## CONCLUSION

Based on a critical evaluation of the publicly available data and information summarized above, the Expert Panel members, whose signatures appear below, have individually and collectively concluded that plant derived phytosterols, meeting the specifications cited above, are safe for addition to egg products including egg whites, and egg substitutes at levels up to 1000 mg phytosterol per 50 grams egg product (1134 mg/recommended serving).

It is also our opinion that other qualified and competent scientists, reviewing the same publicly available toxicological and safety information, would reach the same conclusion. Therefore, we have also concluded that these ingredients, when used as described, are GRAS based on scientific procedures.

| | |
|---|---|
| James W. Barnett, Jr., Ph.D., DABT | Date |
| W. Gary Flamm, Ph.D., FACT, FATS | Date |
| Stanley M. Tarka, Jr., Ph.D. | Date |

## REFERENCES


Amundsen AL *et al.* (2002). Plant Sterol Ester-Enriched Spread Lowers Plasma Total and LDL-Cholesterol in Children with Familial Hypercholesterolemia. *Am J Clin Nutr.76:338-344.*

Awad AB *et al.* (1998). Phytosterol Feeding Induces Alteration in Testosterone Metabolism in Rat tissues. *J. Nutr Biochem 9: 712-717.*

Ayesh R *et al.* (1999). Safety Evaluation of Phytosterol Esters. Part 5. Faecal Short-chain Fatty Acid and Microflora Content, Faecal Bacterial Enzyme Activity and Serum Females Sex Hormones in Healthy Normolipidaemic Volunteers Consuming a Control Diet with or without a Phytosterol Ester–Enriched Margarine. *Food Chem Toxicol 37:1127-1138.*

Baker VA *et al.* (1999). Safety Evaluation of Phytosterol Esters. Part 1. Assessment of Oestrogenicity Using a Combination of *In vivo* and *In vitro* Assays. *Food Chem Toxicol 37: 13-22.*

Becker M *et al.* (1993). Treatment of Severe Familial Hypercholesterolemia in Childhood with Sitosterol and Sitostanol. *J Pediatr. 122: 292-296.*

Berge KE *et al.* (2000). Accumulation of Dietary Cholesterol in Sitosterolemia Caused by Mutations in Adjacent ABC Transporters. *Science. 290:1771-1775.*

Bhattacharyya AK and Connor WE. (1974). β-Sitosterolemia and Xanthomatosis. A Newly Described Lipid Storage Disease in Two Sisters. *J Clin Investigation. 53: 1033-1043.*

Boberg KM *et al.* (1990). Formation of $C_{21}$ Bile Acids from Plant Sterols in the Rat. *J Biol Chem 265: 7967-7975.*

Davidson *et al.* (2001). Safety and Tolerability of Esterified Phytosterols Administered in Reduced-Fat spread and Salad Dressing to Healthy Adult Men and Women. *J Am Coll Nutr. 20:307-319.*

FDA Letter (2003) to Cargill Health and Food Technologies (February 14, 2003) Regarding Enforcement Discretion with Respect to Expanded Use of an Interim Health Claim Rule about Plant Sterol/Stanol Esters and Reduced Risk of Coronary Heart Disease: *FDA/CFSAN/Office of Nutritional Products, Labeling, and Dietary Supplements.*

*Federal Register 65 FR pp 54685-54739*, (September 8, 2000)-Food Labeling: Health Claims; Plant Sterol/Stanol Esters and Coronary Heart Disease; Interim Final Rule.

*Food Chemicals Codex, Fifth Edition (2004)*, pp. 444-445. Monograph for Soybean Oil (unhydrogenated), Institute of Medicine, National Academy of Sciences, Washington, DC.

Glueck CJ *et al.* (1991). Relationships of Serum Plant Sterols (Phytosterols) and Cholesterol in 595 Hypercholesterolemic Subjects, and Familial Aggregation of Phytosterols, Cholesterol, and Premature Coronary Heart Disease in Hyperphytosterolemic Probands and their First-Degree Relatives. *Metabolism. 40: 842-848.*

Glueck CJ *et al.* (1992). Serum and Dietary Phytosterols, Cholesterol, and Coronary Heart Disease in Hyperphytosterolemic Probands. *Clin Biochem. 25: 331-334.*

GRAS Notice No. GRN 000048 (November 27, 2000): *FDA/CFSAN/OPA*: Agency Response Letter to Cargill, Inc.

GRAS Notice No. GRN 000053 (December 20, 2000): *FDA/CFSAN/OPA*: Agency Response Letter to The Procter & Gamble Company.

GRAS Notice No. GRN 000061 (April 18, 2001): *FDA/CFSAN/OPA*: Agency Response Letter to Archer Daniels Midland Company.

GRAS Notice No. GRN 000112 (February 4, 2003): *FDA/CFSAN/OPA*: Agency Response Letter to Phoenix Regulatory Associates on behalf of Teriaka Ltd.

GRAS Notice GRN 000039 (April 24 and November 2, 2000): *FDA/CFSAN/OPA*: Agency Response Letters to Novartis Consumer Health.

Hallikainen MA *et al.* (2000a). Comparison of the Effects of Plant Sterol Ester and Plant Stanol Ester-Enriched Margarines in Lowering Serum Cholesterol Concentrations in Hypercholesterolaemic Subjects on a Low-Fat Diet. *Eur J Clin Nutr. 54: 715-725.*

Hallikainen MA *et al.* (2000b). Plant Stanol Esters Affect Serum Cholesterol Concentrations of Hypercholesterolemic Men and Women in a Dose-Dependent Manner. *J Nutr. 130: 767-776.*

Heinemann T *et al.* (1993). Comparison of Intestinal Absorption of Cholesterol with Different Plant Sterols in Man. Eur J Clin Investigation 23: 827-831.

Hepburn PA *et al.* (1999). Safety Evaluation of Phytosterol Esters. Part 2. Subchronic 90-Day Oral Toxicity Study on Phytosterol Esters- A Novel Functional Food. *Food Chem Toxicol 37:521-532.*

Ikeda I and Sugano M. (1978). Comparison of Absorption and Metabolism of β-Sitosterol and β-Sitostanol in Rats. *Atherosclerosis 30: 227-237.*

Judd JT *et al.* (2002). Plant Sterol Esters Lower Plasma Lipids and Most Carotenoids in Mildly Hypercholesterolemic Adults. *Lipids.37: 33-42.*

Katan MB *et al.* (2003). Efficacy and Safety of Plant Stanols and Sterols in the Management of Blood Cholesterol Levels. *Mayo Clin Proc. 78: 965-978.*

Kim J.-C *et al.* (2002). Subchronic Toxicity of Plant Sterol Esters Administered by Gavage to Sprague-Dawley Rats. *Food Chem Toxicol 40:1569-1580.*

Krebs-Smith SM, Guenther PM, Cook A, Thompson FE, Cucinelli J and Udler J. 1997. "Foods Commonly Eaten in the US--- Quantities Consumed Per Eating Occasion and in a Day", NFS-Report No. 91-3, United States Department of Agriculture. National Technical Information Service, US Department of Commerce, PB98-111719.

Kritchevsky D *et al.* (1981). Identification of Acidic Steroids in Feces of Monkeys Fed β-Sitosterol. *Lipids 16: 77-78.*

Kritchevsky, D. (1997), Phytosterols: Chapter 24 pp235-243: in *Dietary Fiber in Health and Disease*. Edited by Kritchevsky and Bonfield, Plenum Press, New York.

Lea LJ *et al.* (2004). Safety Evaluation of Phytosterol Esters. Part 8. Lack of Genotoxicity and Subchronic Toxicity with Phytosterol Oxides. *Food Chem Toxicol. 42:771-783.*

Lee H *et al.* (2001). Genetic Basis of Sitosterolemia. *Curr Opin Lipidol. 12: 141-149.*

Maki KC *et al.* (2001). Lipid Responses to a Plant Sterol-Enriched Reduced-Fat Spreads Incorporated into a National Cholesterol Education Program Step1 Diet. *Am J Clin Nutr. 74:33-43.*

Malini T and Vanithakumari G. (1990). Rat Toxicity Studies with β-Sitosterol. *J Ethnopharmacol 28: 221-234.*

NAS (2000) National Academy of Science's Panel on Dietary Antioxidants and Related Compounds. Food and Nutrition Board. Institute of Medicine, Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium and Carotenoids p. 25. National Academy Press, Washington DC.

Nestel P *et al.* (2001). Cholesterol-Lowering Effects of Plant Sterol Esters and Non-Esterified Stanols in Margarine, Butter and Low-Fat Foods. *Eur J Clin Nutr. 55:1084-1090.*

Noakes M *et al.* (2002). An Increase in Dietary Carotenoids when Consuming Plant Sterols or Stanols is Effective in Maintaining Plasma Carotenoid Concentrations. *Am J Clin Nutr. 75: 79-86.*

Ntanios FY *et al.* ((2002). A Spread Enriched with Plant Sterol-Esters Lowers Blood Cholesterol and Lipoproteins without Affecting Vitamins A and E in Normal and Hypercholesterolemic Japanese Men and Women. *J Nutr. 132: 3650-3655.*

Ostlund RE *et al.* (2002). Gastrointestinal Absorption and Plasma Kinetics of Soy $\Delta^5$-Phytosterols and Phytostanols in Humans. *Am J Physiol. Endocrinol Metab 282: E911-E916.*

Pollak, O and Kritchevsky, D. (1981) Sistosterol. Monographs on atherosclerosis. Basel; S. Karger

Piironen V and Lampi, AM (2004), Occurrence and Levels of Phytosterols in Food: Chapter 1 pp1-32: in *Phytosterols as Functional Food Components and Nutraceuticals.* Edited by PC Dutta, Marcel Decker, New York and Basel.

Salen G *et al.* (1985). Lethal Atherosclerosis Associated with Abnormal Plasma and Tissue Sterol Composition in Sitosterolemia with Xanthomatosis. *J Lipid Res. 26: 1126-1133.*

Salen G *et al.* (1989). Increased Sitosterol Absorption, Decreased Removal, and Expanded Body Pools Compensate for Reduced Cholesterol Synthesis in Sitosterolemia with Xanthomatosis. *J Lipid Research. 30: 1319-1330.*

Sanders DJ *et al.* (2000). The Safety Evaluation of Phytosterol Esters. Part 6. The Comparative Absorption and Tissue Distribution of Phytosterols in the Rat. *Food and Chemical Toxicology 38:485-491.*

Shipley RE *et al.* (1958). Sitosterol Feeding: Chronic Animal and Clinical Toxicology and Tissue Analysis. *Circulation 6: 373-382.*

Slesinki RS *et al.* (1999). Development Toxicology of Vegetable Oil-Derived Stanol Fatty Acid Esters. *Reg Toxicol Pharmacol. 29: 227-233.*

Sudhop T *et al.* (2002). Serum Plant Sterols as a Potential Risk Factor for Coronary Heart Disease. *Metabolism. 51: 1519-1521.*

Tamagawa M *et al.* (1992a). Carcinogenicity Study of $\gamma$-Oryzanol in F344 Rats. Food Chem Toxicol 30: 41-48.

Tamagawa M *et al.* (1992b). Carcinogenicity Study of γ-Oryzanol in B6C3F1 mice. *Food Chem Toxicol 30: 49-56.*

Turnbull D *et al.* (1999a). Genotoxicity Evaluation of Wood-Derived and Vegetable Oil-Derived Stanol Esters. *Reg Toxicol Pharmacol. 29:205-210.*

Turnbull D *et al.* (1999b). Short-Term Tests of Estrogenic Potential of Plant Stanols and Plant Stanol Esters. *Reg Toxicol Pharmacol. 29: 211-215.*

Turnbull D *et al.* (1999c). Thirteen-Week Oral Toxicity with Stanol Esters in Rats. *Reg Toxicol Pharmacol. 29: 216-226.*

USDA (1996) Continuing Survey of Food Intakes by individuals-1994-1996 data at: http://www.barc.usda.gov/bhnrc/foodsurvey/pdf/Portion.pdf.

Vanstone CA *et al.* (2002). Unesterified Plant Sterols and Stanols Lower LDL-Cholesterol Concentrations Equivalently in Hypercholesterolemic Persons. *Am J Clin Nutr. 76: 1272-1278.*

Waalkens DH *et al.* (1999). Safety Evaluation of Phytosterol Esters. Part 3. Two-Generation Reproduction Study in Rats with Phytosterol Esters- a Novel Functional *Food. Food Chem Toxicol 37: 683-696.*

Weststrate JA and Meijer (1998). Plant Sterol-Enriched Margarines and Reduction of Plasma Total and LDL-Cholesterol Concentrations in Normocholesterolaemic and Mildly Hypercholesterolaemic Subjects. *Eur J Clin Nutr.52:334-343.*

Weststrate JA *et al.* (1999). Safety Evaluation of Phytosterol Esters. Part 4. Faecal Concentrations of Bile Acids and Neutral Sterols in Healthy Normolipidemic Volunteers Consuming a Controlled Diet Either with or without a Phytosterol Ester-enriched Margarine. *Food Chem Toxicol 37: 1063-1071.*

Whittaker MH *et al.* (1999). Two Generation Reproductive Study of Plant Stanol Esters in Rats. *Reg Toxicol Pharmacol. 29: 196-204.*

Wolfreys AM and Hepburn PA. (2002). Safety Evaluation of Phytosterol Esters. Part 7. Assessment of Mutagenic Activity of Phytosterols, Phytosterol Esters and the Cholesterol Derivative, 4-Cholesten-3-One. *Food Chem Toxicol. 40:461-470.*

Submission End

THOMAS O. HENTELEFF
RICHARD S. MOREY
KINSEY S. REAGAN
PETER R. MATHERS
ANTHONY L. YOUNG
ANNE V. MAHER
BONNIE A. BEAVERS
DANIEL R. DWYER
GLENN E. DAVIS
STACY L. EHRLICH
JENNIFER A. DAVIDSON
STACEY L. VALERIO
JONATHAN M. WEINRIEB
CARRIE C. HORN

OF COUNSEL:
WILLIAM J. HARDY

LAW OFFICES
KLEINFELD, KAPLAN AND BECKER, LLP
1140 NINETEENTH STREET, N.W.
WASHINGTON, D. C. 20036-6606
TELEPHONE (202) 223-5120
FACSIMILE (202) 223-5619
www.kkblaw.com

WEST COAST OFFICE:
ONE MARKET STREET
STEUART TOWER, SUITE 1450
SAN FRANCISCO, CA 94105-1313
TELEPHONE (415) 538-0014
FACSIMILE (415) 538-0016

VINCENT A. KLEINFELD
1907-1993

ALAN H. KAPLAN
1930-2001

AM

September 1, 2005

RECEIVED
SEP 2 2005
OFFICE OF
FOOD ADDITIVE SAFETY

Via Overnight Mail

Antonia Mattia, Ph.D.
Division of Biotechnology and GRAS Notice Review
Office of Food Additive Safety (HFS-255)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740

**RE: GRAS notification for plant phytosterols for use in egg products including egg whites, and egg substitutes**

Dear Dr. Mattia:

On August 18, 2005, we submitted the above-referenced GRAS notification to FDA on behalf of Michael Foods, Inc. By e-mail of August 22, 2005, to Dr. Felicia Williams, we noted that the expert panel report included with the notification was not signed by the panel members because a minor correction had been made to the text on the signature page. We informed Dr. Williams that we would obtain a signed copy of the signature page and forward it to FDA to complete the record.

Enclosed are four copies of the signed signature page (page 22) of the expert panel report. Please substitute this for the corresponding page in your copy of the report.

Thank you for your assistance with this matter.

Sincerely,

(b)(6)

Daniel R. Dwyer

Counsel to Michael Foods, Inc.

cc:
Felicia B. Williams, Ph.D. (HFS-255)
Robert C. Post, Ph.D., Labeling and Consumer Protection Staff, FSIS, USDA
Tawana Duncan, Labeling and Consumer Protection Staff, FSIS, USDA

## CONCLUSION

Based on a critical evaluation of the publicly available data and information summarized above, the Expert Panel members, whose signatures appear below, have individually and collectively concluded that plant derived phytosterols, meeting the specifications cited above, are safe for addition to egg products including egg whites, and egg substitutes at levels up to 1000 mg phytosterol per 50 grams egg product (1134 mg/recommended serving).

It is also our opinion that other qualified and competent scientists, reviewing the same publicly available toxicological and safety information, would reach the same conclusion. Therefore, we have also concluded that these ingredients, when used as described, are GRAS based on scientific procedures.

(b)(6)

James W. Barnett, Jr., Ph.D., DABT — 8/22/05 Date

(b)(6)

W. Gary Flamm, Ph.D., FACT, FATS — 8/24/05 Date

(b)(6)

Stanley M. Tarka, Jr., Ph.D. — 8/29/05 Date



000038

THOMAS O. HENTELEFF
RICHARD S. MOREY
KINSEY S. REAGAN
PETER R. MATHERS
ANTHONY L. YOUNG
ANNE V. MAHER
BONNIE A. BEAVERS
DANIEL R. DWYER
GLENN E. DAVIS
STACY L. EHRLICH
JENNIFER A. DAVIDSON
STACEY L. VALERIO
JONATHAN M. WEINRIEB
CARRIE C. HORN

OF COUNSEL:
WILLIAM J. HARDY

LAW OFFICES
KLEINFELD, KAPLAN AND BECKER, LLP
1140 NINETEENTH STREET, N.W.
WASHINGTON, D.C. 20036-6606
TELEPHONE (202) 223-5120
FACSIMILE (202) 223-5619
www.kkblaw.com

WEST COAST OFFICE:
ONE MARKET STREET
STEUART TOWER, SUITE 1450
SAN FRANCISCO, CA 94105-1313
TELEPHONE (415) 538-0014
FACSIMILE (415) 538-0016

VINCENT A. KLEINFELD
1907-1993

ALAN H. KAPLAN
1930-2001

AM

January 30, 2006

Via Overnight Mail

REC'D JAN 31 2006

Antonia Mattia, Ph.D.
Division of Biotechnology and GRAS Notice Review
Office of Food Additive Safety (HFS-255)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740

**RE: GRAS notification for plant phytosterols**
**GRN No. 181**

Dear Dr. Mattia:

The above-referenced GRAS notification was originally submitted by Michael Foods, Inc. for the use of plant phytosterols in egg products including egg whites, and egg substitutes. Owing to revised product development plans, Michael Foods hereby amends the notification to cover egg substitutes and other similar or related products that are not "egg products" within the meaning of 9 CFR 590.5. The effect of this change is that the GRAS notification covers only such products containing eggs that are regulated by FDA and excludes egg products regulated by the Food Safety and Inspection Service of USDA.

Thank you for your assistance with this matter.

Sincerely,

(b)(6)

Daniel R. Dwyer

Counsel to Michael Foods, Inc.

cc:
Felicia B. Williams, Ph.D. (HFS-255)
Robert C. Post, Ph.D., Labeling and Consumer Protection Staff, FSIS, USDA
Tawana Duncan, Labeling and Consumer Protection Staff, FSIS, USDA

000046

000046