GR

Original Submission

000001·040

GRAS Notification for Fat Suspensions Enriched with Phytosterols

Volume 1 of 1

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Submitted on behalf of:

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August 15, 2002

000001·041

[redacted]

Cover Letter

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PHOENIX
REGULATORY ASSOCIATES, LTD.
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August 16, 2002 **Via: Courier**

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

Re: Notification of GRAS determination for fat suspensions enriched with phytosterols (vegetable or tall oil source) use in eight different food categories; **GRAS Exemption Claim**

Dear Dr. Gaynor:

Phoenix Regulatory Associates, Ltd. is submitting the enclosed notification on behalf of Teriaka Ltd. In accordance with the Food and Drug Administration's (FDA) proposed policy [62 FR 18938 -18964; April 17, 1997], Teriaka Ltd. is voluntarily notifying the agency that it has determined that the use of its fat suspensions enriched with phytosterols from either a vegetable oil that has been already been notified or a tall oil extracted from an European Pine, is 'generally recognized as safe' (GRAS). This cover letter containing the exemption claim is attached to the document that details Teriaka's basis for its GRAS determination. Three copies of the materials are enclosed as required by proposed 21 CFR 170.36(b).

Teriaka basis for its GRAS determination of its enriched ingredients with phytosterols is scientific procedures as well as substantial equivalence to existing phytosterols that have been self-determined GRAS by their manufacturers or end users. Teriaka has had its pending EU Novel Food Application reviewed by the Finnish Novel Food Board for Safety. The Board stated that it had no safety concerns regarding Teriaka's ingredients. Based on this assessment as well as published literature and data contained in other GRNs that have previously been submitted to FDA for phytosterols, Teriaka has made a self-determination that its ingredients enriched with phytosterols are GRAS for the intended uses stated in this Notification.

The safety of phytosterols and their esters as well as phytostanols and their esters have been well characterized through chemical identity, nonclinical and clinical studies, both published and unpublished, as a part of previous GRAS notifications including McNeil's Benecol® product, Lipton's Take Control® product, Novartis' Phytrol™ now called Reducol™ product that is isolated from tall oil extracted from an American Pine, Archer Daniel Midland's

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product of phytosterol isolated from vegetable oil, Cargill's vegetable oil phytosterol ester product as well as Procter & Gamble's phytosterol ester enriched vegetable oil product.

Teriaka has provided in this notification detailed chemical information about its ingredients containing phytosterols and particularly about the phytosterol from a tall oil source since the phytosterols from the vegetable oil source is produced by Archer Daniel Midland and has been subject to a GRN. Teriaka also provides data on the use and the functionality of the phytosterols as well as intake levels for the various food categories and has conducted an extensive literature search dated from January 2000 to assure itself that all published data about these types of chemicals have been reviewed so that Teriaka could report in this GRN any supporting or adverse findings. Finally, Teriaka has provided both unpublished and published data to demonstrate the safety of its ingredients.

Teriaka provides the following information for the exemption claim as required in proposed 21 CFR 170.36(c)(1) for this notification:

Notifier:	Teriaka Ltd. Leikosaarentie 32 POB 15 FIN-00981 Helsinki, FINLAND Attn: Leena Morander, M.Sc. Product Development Manager
GRAS Substance:	fat suspensions (anhydrous milkfat, hydrogenated coconut oil, rapeseed oil or partially hydrogenated refined palm kernel oil) enriched with phytosterols isolated from either vegetable oil or tall oil extracted from an European Pine
Intended Use:	The ingredients enriched with phytosterols from either a vegetable oil source supplied by Archer Daniels Midland Company or a tall oil source extracted from an European Pine which is substantially equivalent to one produced by Forbes Medi-Tech Inc., will be used in the following food categories: vegetable spread, yogurt, milk-juice beverages, ice cream, cream cheese, snack bars, salad dressings, and white bread products. The amount will provide no more than 1.5 g of the phytosterol ingredient per day to the average consumer when used as directed on the food label.

Basis for GRAS Determination: Scientific Procedures

Teriaka will provide all the data/information used by it to determine that its products and the phytosterol from the European Pine tall oil source are GRAS to FDA directly if requested or for review at the Phoenix's address, 21525 Ridgetop Circle, Suite 240, Sterling, VA 20166. The contact person at Phoenix for such requests is C. K. Gund, Ph.D., President at 703-406-0906, fax: 703-406-9513; email: phoenix@phoenixrising.com.

If you have any questions, please contact either Dr. Gund (see above) or Leena Morander, M.Sc., Product Development Manager, Teriaka Ltd Leikosaarentie 32, POB 15, FIN-00981 Helsinki, Finland, phone: 011-358-9-3198502 or email: leena.morander@paulig.fi.

Sincerely,

C. K. Gund, Ph.D.
President
Enclosure: Three (3) copies of GRAS Notification for Fat Suspensions Enriched with Phytosterols

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Executive Summary

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Executive Summary

By this submission Teriaka, Ltd. (Teriaka) notifies the U.S. Food and Drug Administration (FDA) that it has determined that its ingredients (fat/oil suspensions of various food grade fat/oils (65% - 70%) enriched with phytosterols from either vegetable or tall oil (18% - 30%) and potable water), trade name, Diminicol®, are Generally Recognized as Safe under scientific procedures for their intended uses. Teriaka ingredients will be sold to various manufacturers, based upon their requirements for a particular type of fat or oil composition, to be used as an ingredient in the following food products: fat based spreads (margarine-like spreads); dressing for salads; snack bars (health); milk-based juice beverages; yogurt-like products; ice cream-like products; soft cheeses (i.e., cream cheese-type products); and white bread products. Teriaka's ingredients provide a technical effect as a texturizer (21 CFR 170.3(o)(32) and its ingredients also meet the definition of 'nutritive value' found in 21 CFR 101.14(a)(3) Health claims: general requirements.

Teriaka's ingredient will be included in various formulations in an amount that will provide a recommended total daily intake of 1.5 g phytosterols. The amount of phytosterols per serving of the final food products listed above will either be 0.75 g/serving or 1.5 g/serving (yogurt) and each product's label will contain a declaration of how many servings will provide the total daily amount. Teriaka based this total daily amount on several factors. The clinical trials conducted using Teriaka's product; the total daily levels identified in other GRNs and FMFs (that are mentioned throughout this submission) and submitted to the agency for these types of chemicals; and the interim final rule that the agency published in 2000 allowing a health label claim that allow an association between a diet containing plant sterol/stanols esters and the reduced risk of coronary heart disease.

Executive Summary (continued)

A clinical trial using Teriaka's rapeseed suspension with the tall oil phytosterols confirms that 1.5 g phytosterol per day significantly reduces the level of plasma total and LDL-cholesterol for hyperchloesterolemic subjects compared to the control while a higher level of the phytosterols (3.0 g/d) resulted in no additional improvement of lowering cholesterol [Christiansen, L., et al., 2001]. The results of this study are comparable to clinical trials conducted on other phytosterols, phytostanols and their esters as found in the literature and reported in GRNs and FMFs identified throughout this submission.

The GRAS status of phytosterols from vegetable oil has been notified to the FDA by Archer Daniels Midland Company GRN 000061 (2000) for use as an ingredient in vegetable oil spread, dressings for salad, health drinks, health bars, yogurt-like products, and as a raw material for the manufacturing of vegetable oil derived plant sterol esters. The agency raised no questions regarding ADM's conclusion that the ingredients plant sterols and plant sterol esters are GRAS under the intended conditions of use [US FDA Letter, 2001]. Therefore, Teriaka concludes that for these same uses, its ingredients containing the ADM's phytosterols are GRAS.

The phytosterols from tall oil that are used in Teriaka's ingredients are extracted from pine trees grown in Europe. Based on the chemical analytical and safety data presented in this GRN and the data presented in Novartis Consumer Health Inc. GRN No. 000039 (2000); McNeil's FMF 626 (1999); as well as other GRNs submitted to the agency for both phytosterols from vegetable oil, phytostanols and the ester of both chemicals [GRNs 000048, 2000; 000053, 2000; 000061, 2000; and FMF 625, 1999], Teriaka concludes that its tall oil derived phytosterols ingredient is substantially equivalent to these that have already been notified to the agency and for which the agency provided no objections on the conclusions of the notifiers that their products were GRAS for their intended uses.

Executive Summary (continued)

Teriaka's GRAS determination is based on its own nonclinical and clinical studies, the review of all data contained in the above GRNs and FMFs including various Expert Panels' assessments of the safety of applicable phytochemical products; FDA's letters of no object to the notifiers, as well as its memoranda, where applicable, to a specific submission; the preamble with its extensive reference list used to support the interim final rule that the agency published authorizing a health claim on the relationship between diets containing plant sterols/stanols esters and the reduced risk of coronary heart disease; the conclusion of the Finnish Ministry of Trade and Industry's Novel Food Board that Teriaka's ingredients are considered safe as other comparable products; and the review and reports in this submission of any relevant published literature since January 2000 that might provide additional consensus that phytochemicals and their esters are safe for their intended uses or new data that might subject these chemicals to further safety evaluation.

The notification provides information required by proposed 21 CFR 170.36(c)(2), (3), and (4). Teriaka concludes based on all the data that experts qualified by scientific training and experience to evaluate such ingredients for their safety would reach a consensus that there is a reasonable certainty that Teriaka's ingredients are not harmful under the intended conditions of uses. Therefore, Teriaka has determined that its fat/oil suspensions containing phytosterols from either vegetable or tall oil are GRAS for their intended uses.

I. Introduction

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I. Introduction

Teriaka Ltd. (Teriaka) submits this document to the U.S. Food and Drug Administration (FDA) in accordance with FDA's proposed rule [U.S. FDA, 1997] to notify the agency about the company's conclusion that its fat suspensions enriched with phytosterols ingredients, are Generally Recognized as Safe (GRAS) for their intended uses. Diminicol® is the trade name for these ingredients. An ingredient containing phytosterols isolated from either vegetable or tall oil can be formulated with a different food grade fat or oil depending on its use in the final formulated product. Teriaka will either market its ingredients or license the technology for producing its ingredients.

Diminicol® is a butter-like semisolid mass composed of food-grade fat, plant sterols and water. Teriaka purchases all components used in the production of Diminicol®. The fat ingredients, anhydrous milk fat, low erucic acid rapeseed oil, hydrogenated coconut oil and partially hydrogenated refined palm kernel oil are traditional food grade ingredients commonly used in a wide variety of commercially available food products.

The plant sterol mixtures are either derived from vegetable or tall oil. The vegetable oil derived plant sterols product is purchased from Archer Daniels Midland Company who notified FDA that its vegetable oil derived plant sterols were GRAS for use in vegetable oil spreads, dressings for salads, health bars, health drinks and yoghurt-type products [GRN 000061, 2000]. FDA raised no questions regarding the company's conclusion that this product as well as its sterol esters derived from oil seed, was GRAS under the intended conditions of use [U.S. FDA, 2001].

The other plant sterol derived from tall oil used in Teriaka's product is manufactured by a French company, Derives Resiniques et Terpeniques, (DRT). This phytosterol is substantially equivalent to a product [See Section III] that is

I. Introduction (continued)

manufactured by Forbes Medi-Tech Inc. and was the subject of Novartis Consumer Health, Inc. notification of GRAS to the agency [GRN 000039, 2000]. FDA raised no questions regarding the Novartis' conclusion that this product was GRAS under the intended conditions of use (vegetable oil spreads) [U.S. FDA 2000a and 2000e]. Novartis also submitted a second report to the agency, dated October 12, 2001, stating that its tall oil phytosterols were GRAS for additional uses, such as cereals, food bars, fruit drinks and smoothies and which were to be marketed by Altus Foods Company, a joint venture between Novartis Consumer Health Inc. and Quaker Foods Company [Novartis, 2001].

Teriaka also finds that its tall oil phytosterols are substantial equivalent to Cytellin™, the ethical drug sold by Eli Lilly Research Laboratories in the United States between 1954 and 1982 as an anti-hypercholesterolemic agent.

Teriaka proposes to use its suspension enriched with phytosterols in the following food categories: yellow spreads/margarine, dressing for salads, yogurt-type products, fruit milk beverages, ice cream, soft cheese, snacks (i.e., cream cheese), and white bread and rolls. The total intake of sterols will be 1.5 g/day at normal intake levels.

The self-GRAS determination of Teriaka's fat suspension ingredient containing plant sterols is based on scientific procedures. Over the years, extensive data and information regarding the identification and safety of various plant sterols/stanols and their esterified forms have been published. Although plants containing sterols/stanols have been eaten for centuries, as noted below, there have been several self-determinations of GRAS for phytosterols and their esters by other companies for a variety of intended uses. FDA has reviewed these earlier

I. Introduction (continued)

submissions on phytosterols esters and phytostanol esters [Lipton FMF No. 625, 1999a, 1999b and U.S. FDA 1999b; McNeil FMF 626, 1999 and U.S. FDA 1999a; Cargill GRN 000048, 2000 and U.S. FDA 2000c and 2000g; Procter & Gamble, GRN 000053, 2000 and U.S. FDA, 2000b and 2000c], and provided letters/memoranda for some to the notifiers that the agency has no questions regarding the self determination of GRAS for their intended uses [U.S. FDA, 2001, 2000a through e and 2000g; 1999a and b].

The Ministry of Trade and Industry Novel Food Board of Finland, composed of experts qualified by scientific training and experience, made a determination that to Teriaka's ingredients are safe as other comparable products with added phytosterols [Novel Food Board, 2001]. The Board reviewed all the chemical analytical, use and functionality as well as safety (nonclinical and clinical) data presented in a Novel Food Application submitted by Teriaka for the following food categories: yogurt, milk-juice beverages, yellow spreads (margarine) and soft cheeses.

Teriaka has also filed a Novel Food Application with the European Community for its ingredients. The review by the Scientific Committee on Foodstuffs is ongoing.

Therefore, based on the use of commercially available food grade fats and phytosterols that have either been determined to be safe for the intended uses (GRN 000061) or found to be substantially equivalent by Teriaka to tall oil phytosterols subject to GRN 000039 and Eli Lilly's product and the total body of evidence concerning phytosterols safety based on general recognition by scientists and experts that have been incorporated into the above mentioned GRNs, the health claim petition and interim final rule as well as this GRN, it is Teriaka's conclusion that its ingredients enriched with phytosterols (vegetable or tall oil source) is considered GRAS for the proposed intended uses.

II Background

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II. Background

Plant sterols are naturally occurring plant compounds and, therefore, all food items of vegetable origin contain a certain amount of plant sterols. The most important sources of plant sterols in the Western diet are vegetable oils and vegetable-oil-based products, such as margarine, although breads and other cereals are also important sources of plant sterols [Morton, G. M., et al., 1995]. At least 44 plant sterols have been identified in plants [Bean, G. A., 1973]. Most frequent plant sterols in nature are β-sitosterol, campesterol and stigmasterol. The structures of plant sterols are similar to that of cholesterol with an extra methyl or ethyl group and a double bond in the side chain.

In 1991, the average British diet contained 186 mg of plant sterols per day, whereas in the 1987 it was only 91 mg/day. The changes in plant sterol intake are largely accounted for by the rising consumption of vegetable oils [Morton, G. M., et al., 1995]. Dietary plant sterol concentrations vary greatly among different populations. In Japan, plant sterol intake has been about 373 mg/day from 1957 to 1982 [Hirai, K., et al., 1986]. In the United States, daily intake of sitosterol and stigmasterol has been studied among Seventh-day Adventists [Nair, P., et al., 1984]. Lacto-ovo vegetarians had the greatest sterol intake with 344 mg/day, non-vegetarians with 231 mg/day and pure vegetarians with 89 mg/day when the daily sterol intake among group of general population non-vegetarians was 78 mg/day [Nair, P., et al., 1984]. The average daily phytosterols intake from natural plants sources in the United States is about 250 mg [Ling, W. H. and Jones, P. J., 1995].

Plant sterols are marketed as capsules, granules or pastille form in health food shops and at chemists in the European Union while in the United States as dietary supplements. The range of recommended daily dosage of beta sitosterol derived from these types of preparations is wide: 0.03-16 grams. In Finland and Germany the manufacturers and distributors recommend plant sterol capsules for cholesterol-lowering purposes and for

II. Background (continued)

benign prostate hyperplasia. In Germany, beta sitosterol is used also for treating rheumatism. The retailed trademarks in Finland and Germany are presented in Appendix 1 for these products.

In Finland, since 1995 when Raisio launched Benecol®, a tall oil derived plant stanol ester margarine product and then later launched fresh cheese, snack bars, salad dressing and yogurt containing this tall oil plant stanol ester ingredient, there have been over 12.5 million packages sold in Finland which is over 125 million daily portions [Benecol News, 2001]. Some or all of these products are also sold in Benelux, the United Kingdom, Ireland, Sweden, Denmark and the United States. In addition, stanol ester milk is on the market in Ireland and the United Kingdom since fall 2001 and meatballs, salads and sausages containing this ingredient has been marketed in Finland since fall 2001. Recently the Raisio group announced that it has signed an agreement with a company who will market and distribute a range of Benecol® products, dairy products, fruit juices and spreadable fats to the United Arab Emirates that will include Saudi Arabia, Kuwait, Yemen, Bahrain and Oman. McNeil Consumer Nutritionals Europe has signed an agreement with a British group to extend Benecol® into the French market with a light dairy spread and one that contains 53% fat [Food Navigator, 2002].

Unilever launched Take Control™, under a different trademark, Flora/Becel pro.activ™, in 1999 in Australia, Brazil, New Zealand and Switzerland and in 2000 in the European Union. Also a margarine product with plant sterol esters from Unilever was launched in Japan in 2001. The safety of Benecol® and Take Control™/Becel pro-active™, products has been tested before, and after placement on the market in general studies, and no risks to human health have been found. The Scientific Committee on Food (EU) has concluded that the use of plant sterol esters in yellow fat spreads, at a maximum level corresponding to 8% of free plant sterol, is safe for human use [European Commission Opinion, 2000].

II. Background (continued)

In Australia, Goodman Fielder has launched the soybean derived plant sterol enriched Logicol™ spread, salad dressing, mayonnaise, milk and breakfast bars.

Sitosterols are also used as a tracer in butter in the European Union. By marking butter with sitosterol, commercial abuses (for instance with customs) are prevented. The permitted amount of sitosterol is 600g/100kg of butter [European Commission Regulation No. 2571, 1997].

From the 1950s to 1980s, Eli Lilly marketed Cytellin™, a drug product formulated with mainly the beta-sitosterol phytosterol, for the treatment of hypercholesterolemia. Several clinical trials were conducted on large doses (25 g/day, sometimes 50 g/day) over several months up to three years to determine the effects of this drug on cholesterol blood chemistry [Pollak, O. J. and Kritchevsky, D., 1981; Pollak, O. J., 1985; and Ling, W. H. and Jones, P. J. H., 1995; Best, M. M., et al., 1955; Best M. M. et al., 1956; Cooper, E. E., 1958; Farquhar, J. W., et al., 1956; Lehmann, J. H., 1957; and Sachs B. A. and Weston, R. E., 1956]. No adverse effects were reported from these studies.

Both vegetable and tall oil phytosterols have been determined GRAS for their intended uses by several companies and are discussed as support throughout in this GRN [Lipton, FMF 625, 1999; McNeil, FMF 626, 1999; ADM, GRN 000061, 2000; Novartis, GRN 000039, 2000 and Novartis Addendum 2001; Cargill, GRN 000048, 2000; Procter and Gamble, GRN 000053, 2000].

III Chemical Identity

000022

A. Identity
B. Name

000023

III. Chemical Identity and Composition

A. Identity

Teriaka's phytosterols enriched fat suspension is a butter-like semisolid mass produced with food grade fat/oil, plant phytosterols, and water. The four types of food grade fat used are:

1) anhydrous milk fat,
2) hydrogenated coconut oil,
3) low erucic acid rapeseed oil (LEAR), or
4) partially hydrogenated refined palm kernel oil.

The plant phytosterols are from either vegetable oils (soybean, cottonseed, corn, sunflower seed, canola or peanuts) or tall oil extracted from pine trees, such as *Pinus pinaster* and *Pinus sylvestris*. Potable water is the remaining ingredient.

B. Common or Usual Names

Depending on the food grade fat used to produce the sterol-enriched ingredient, the common or usual names as follows:

1) vegetable fat mixture with phytosterols (i.e., hydrogenated coconut oil, or partially hydrogenated refined palm kernel oil);
2) vegetable oil mixture with phytosterols (i.e., low erucic acid rapeseed oil); and
3) anhydrous milk fat mixture with phytosterols.

Teriaka's trade name used for these products is Diminicol®.

C. Composition

C. *Composition*

1. Teriaka Product

Vegetable fat (hydrogenated coconut or partially hydrogenated refined palm kernel), oil (rapeseed), or anhydrous milk fat enriched phytosterols ingredients contain phytosterols (source, vegetable or tall oil) in the range of 18% - 30% with an individual oil or fat in the range of 65% - 70%.

Teriaka purchased all the ingredients except potable water from qualified vendors. The water comes from a company, Helsinki Water, Helsinki, Finland that supplies municipal city water supply at Teriaka's manufacturing site.

2. Fats or Oils

The various food grade fats or oils are purchased from reputable manufacturers of such ingredients who have certified to Teriaka as to the food grade quality of the fat or oil.

The hydrogenated coconut oil and partially hydrogenated refined palm kernel oil produced by Karlshamns Ab, SE-37482, Karlshamn, Sweden, contain less than 0.1 mg/kg heavy metals (as Pb). The company has certified to Teriaka that its fats are manufactured using fractionation and isolation procedures common to the oil/fat manufacturing processes and are produced in accordance with ISO standard SS-EN ISO 9001:1994.

The anhydrous milk fat ingredient produced by Corman, Goé-Limburg, Belgium, is defined in the Codex Almentarius Standard: Codex Standard A-2-1973, Rev 1-1999 and in the International Dairy Federation Standard 68A:1977. It contains no more than 0.05 mg/kg heavy metals (as Pb), and is produced using Grade A milk. The manufacturer states that its product is manufactured in accordance with 21 CFR Part 110.

The LEAR oil is produced by Mildola Ltd., Kirkkonummi, Finland, contains less than 0.1 mg/kg, heavy metals (as Pb), <0.1 mg/kg of Pb, <2% erucic acid value and conforms to Codex Standard for Named Vegetable Oils [Codex-Standard 210:1999]. The manufacturer holds the Quality System Certificate for the ISO 9000 standard. The manufacturer has certified to Teriaka that its product is food grade and is manufactured in accordance with 21 CFR Part 110.

3. Vegetable Phytosterols

Vegetable phytosterols are purchased from Archer Daniels Midland Company (ADM), Decatur, Illinois. These vegetable phytosterols were subject to GRN No. 000061. ADM received a letter from FDA dated April 18, 2001, that stated the agency had no questions regarding ADM's "conclusion that the ingredients, plant sterol and plant sterol esters, are GRAS under the intended conditions of use."

4. Tall Oil Phytosterols

The tall oil phytosterols are produced by Les Derives Resiniques et Terpeniques, (DRT) DAX cedex, France and sold under the trade name Beta Sitosterol®. The tall oil, a natural product of pine trees (*Pinus pinaster* and *Pinus sylvestris)*, is isolated by means of the kraft pulping process and is composed of the ether extractable, non lignin, non cellulosic portion of the pine tree. The main components of tall oil are rosin acids, fatty acids and unsaponifiables. The unsaponifiables are predominantly a mixture of phytosterols, mainly β-sitosterol with β-sitostanol, campesterol and campestanol as other major phytosterols/stanols.

DRT's ingredient has the following composition:

Chemical formula	$C_{29}H_{50}O$ (β-sitosterol)
Molecular Weight	414 (β-sitosterol)
CAS Registry Number	83-46-5 (β-sitosterol)
Melting Point	139-142 °C
Desiccation loss	<3.5%
Optional rotation	-24 to -32° in a 2% chloroform solution
Sulphuric ashes	<0.1%
Solubility	Insoluble in water, slightly soluble ethanol, soluble chloroform, ether and carbon disulphide

III. Chemical Identity and Composition (continued)
C. Composition (continued)

DRT had one batch (Batch No. 01032) of its Beta Sitosterol® analyzed for various dioxins and furans. The results indicated 0.51 pg/g as I-TEQ. DRT also had a single batch (Batch No. 1200) analyzed for polyaromatic hydrocarbons using a HPLC method with fluorescent and UV detectors according to French norm NF T 90-115. The results indicate no detectable levels of these compounds at the μg/kg level. Carcinogenic PAHs (as identified by U.S. EPA) levels were analyzed in this batch and none found at the levels given: benzo (a) anthracene (<5μg/kg); benzo (a) pyrene (<1μg/kg); benzo (b) fluoranthene (<5 μg/kg); benzo (k) fluoranthene (<1μg/kg); chrysene (<10 μg/kg); dibenzo (a, h) anthracene (<10 μg/kg); and ideno (1,2,3-cd) pyrene (<10 μg/kg).

Teriaka concludes that there is no safety concern posed by PAHs, dioxins and furans when using DRT's tall oil phytosterol in its phytosterol enriched fat/oil suspensions.

Teriaka reviewed the various specifications identified in Novartis' GRN 000039 (2000) for its tall oil phytosterol and compared them to DRT's tall oil phytosterol. Novartis' tall oil phytosterol ingredient is manufactured by Forbes Medi-Tech, Inc. (Forbes) and supplied to Novartis. FDA concluded that it had no questions regarding Novartis' conclusion that those tall oil phytosterols are GRAS for the intended use as stated in GRN 000039.

III. Chemical Identity and Composition (continued)
C. Composition (continued)

The following Table demonstrates that the phytosterols used by Teriaka as supplied by DRT are substantially equivalent to those supplied by Forbes to Novartis with the exception that the β-sitosterol percentage in the DRT's phytosterols is about 1.1-2 fold greater while the β-sitostanol is slightly lower compared to Forbes' ingredient. The differences are mainly due to the different species used (Forbes, North American pine while DRT, European pine) and the extraction methods. Forbes uses a proprietary technology (patented method) that allows additional sitostanol to be added during the production process. This addition increases the percentage of sitostanols in the Forbes' product. DRT's method uses a three-step extraction process that allows the crystallization of the sterols. Teriaka concludes that these differences are not significant between the two tall oil phytosterol products.

III. Chemical Identity and Composition (continued)
C. Composition (continued)

TABLE 1

Comparison of DRT and Novartis Specification Information

Component	DRT *	Novartis **
Phytosterol content	> 99 %	> 95 %
Sitosterol	75 (75.7-80) [1)]	36-60 %
Sitostanol	11 (11.4-14) [1)]	14-34 %
Campesterol	10 (8.1-9) [1)]	9-18 %
Campestanol	2 (1.1-2) [1)]	2-14 %
Others	2 (1.3-2.5) [1)]	Not specified
Total major sterols	> 95 %	> 86 %
Loss on drying (water)	< 3.5 % [1)]	< 5 %
Solvents (methanol)	< 0.2 % [1)]	< 0.5 %
Residue on ignition	Not specified (N.S.)	< 0.1 ppm
Sulphuric ashes	< 0.1 % [1)]	Not specified
Heavy metals	< 20 ppm [2)]	< 10 ppm
Arsenic	0.21 ppm [3)]	< 5 ppm
Lead	0.07 ppm [3)]	< 0.25 ppm
Total aerobic count	10 CFU/g [4)]	< 10,000 CFU/g
Combined mold & yeasts	15 CFU /g [4)]	< 100 CFU/g
Coliforms	N.S. [4)]	Negative
Enterobacteria	Negative [4)]	N.S.
E. Coli	Negative [4)]	Negative
Salmonella	Negative [4)]	Negative

* DRT analyses of its Beta Sitosterol®

** Novartis GRN 000039, stamped p. 000033, (2000)

[1)] DRT sterol analysis using a GC method with an apolar capillary column is based on six batches (batch numbers 01002, 01007, 91280, 91294, 91291 and 01045).

[2)] Analysis of heavy metals was conducted by an ICP/MS method, a modification of NF EN ISO 11885, with a limit of detection, 0.1 mg/kg.

[3)] DRT arsenic and lead values are based on 3 batches (Lot Numbers 11034, 11034, 11035). The method is an ICP-MS with a microwave digestion using nitric acid; limits of detections between 0.05 mg/kg – 0.5 mg/kg.

[4)] Analyses based on one batch, nr 91110. Method used was 32 Eur Ph 1997 (2-6-12). Also analyzed for *Clostridia*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* with negative results reported. N.S. = Not Stated but DRT measured *E. coli* instead of coliforms because this organism is the most representative of all coliforms.

To further confirm that the two tall oil phytosterols ingredients are substantially equivalent, Teriaka had Lot Q4050301A of Forbes' wood sterol analyzed by the University of Helsinki, Department of Applied Chemistry and Microbiology and compared the results to that of DRT's tall oil phytosterol. The table below shows that the profiles of Forbes' and DRT's phytosterols are equal except for the sitostanol content that is lower in Forbes' product.

TABLE 2

Comparison of Forbes' Phytosterols and DRT's Phytosterols Composition

Sterol Component	Forbes Medi-Tech Wood Phytosterols Lot Q4050301 A [1]		DRT Tall Oil Phytosterols [2]
	Supplier information	*Analyzed at UH*	*Supplier information*
Campesterol	4-25	8.4	6-9
Campestanol	0-14	1.1	0-2
Sitosterol	36-79	76.1	75-80
Sitostanol	6-18	7.6	10-14
Others [3]	Not mentioned	3.4	2.5
Total	> 95	96.4	99

1) The first column is Forbes Medi-Tech Inc. information provided with a 60 kg lot purchased by Teriaka. The second column contained the values obtained based on the analysis at the University of Helsinki, Department of Applied Chemistry and Microbiology of the same lot.
2) DRT's information is based on six batches, Lot Numbers 01002, 01007, 91280, 91294, 91291, and 1045, that were purchased by Teriaka.
3) Others include, e.g., stigmasterol and avenasterols.

Teriaka also reviewed the other GRN and FMF submissions to determine the levels of various sterols/stanols and their esters in their products and compared them to DRT's tall oil phytosterols product. Teriaka also included Eli Lilly's Cytellin™ drug product because it contains a comparable concentration of β-sitosterol to that of DRT's product. From the data contained in the various GRNs and FMFs, Table 3 compares, where possible, what sterols/stanols are present and at what levels in comparison to DRT's product.

III. Chemical Identity and Composition (continued)
C. Composition (continued)

TABLE 3

Percent of Sterols/Stanols in Various Products

Chemical	Lipton Sterol Esters[1] (%)	McNeil Stanol Esters [2] (%)	Novartis Tall Oil Sterols[3] (%)	Cargill & P&G Vegetable Oil Sterol Esters[4] (%)	ADM Vegetable Oil Sterols [5] (%)	Eli Lilly Cytellin™ Tall Oil Sterols[6] (%)	DRT Tall Oil Phytosterols[7] (%)
Phytosterols	~95	>94	>95	>95	>90	80-90	>99
β-sitosterol	40-55 46*	4**	38-60 76.1***	40-45	40-58	80	75-80 75-80****
Campesterol	20-28 23-25*	- 3**	9-18 8.4***	20-25	20-30	7	6-9 6-9****
Stigmasterol	14-23 16-18*			20-25	14-22		
Bassicasterol	0-6 2-4*			<1	0-6		
D5-Avenasterol	0-4 4*			~1	0-4		
Other (minor Sterols)	0-4 4*	6**	3-4***	0-4			<3.0 2.5****
β-sitostanol	0-4 1*	~62 64**	14-34 7.6***	2	0-5	10	10-14 10-14****
Campestanol	0-2	<32 23**	2-24 1.1***	1	0-2	2	0-2 0-2****
Total Major Sterols			>86	>85			>95
Unsaturated Sterols		<6.0					

1) Lipton FMF 625 prestamped page 14 gives a general distribution of phytosterols, a vegetable oil sterol ester, and not Lipton's product actual composition because those values were deleted from the FMF received under FOI. The values designated with * came from Cargill Inc. GRN 000048, stamped page 24 (2000).
2. McNeil Consumer Healthcare FMF 626 (1999), provides limited information on the breakdown of stanol components and the value designated with ** came from Cargill Inc. GRN 000048, prestamped page 16, June (2000).
3. Novartis Consumer Health, Inc., GRN 000039, prestamped page 33 (2000), and the values designated *** came from Teriaka Ltd GRN submitted August 2002.
4. Cargill Inc., GRN 000048, prestamped pages 24 and 26 (2000).
5. Archer Daniels Midland Company, GRN 000061, prestamped page 12 (2000).
6. Novartis Consumer Healthcare, GRN 000039, prestamped page 54 (2000). Cytellin™, repeated therapeutic load 9,000-30,000 mg/day.
7. Teriaka Ltd. GRN, submitted August 2002, including information designated with **** taken from Table 2 above in this Part of Teriaka's GRN.

Both vegetable and tall oil derived phytosterols contain many of the same sterols but vegetable derived phytosterols have other sterols not identified in the tall oil derived ingredient. DRT's tall oil derived phytosterols ingredient is nearly identical to the Eli Lilly drug product that was marketed in the United States and Canada and also nearly identical to the Novartis' tall oil derived phytosterols if one compares the values presented in Table 2 above as well as those noted in Table 3. As stated above, the phytosterols ratios from a particular plant source depends on time of harvest, environmental factors as well as the species from which they are extracted. Teriaka concludes that the DRT product is substantially equivalent to the Forbes' product that is currently being marketed and that was notified in Novartis' GRN 000039, (2000).

5. Potable Water

Tap water from Helsinki Water, Helsinki, Finland's municipal city plant is used. Helsinki Water analyzed the water bimonthly to assess coliforms (0 CFU/100 mL), heterotrophic plate count at 22° C (0 CFU/mL) and at 37° C (0 CFU/mL). The analysis, measuring several other components (i.e., nitrogen, various metals, alkalinity, oxygen, ammonium nitrogen, etc.) in accordance with Finnish water standards and the water is classified potable.

D. Proposed Food Grade Specification

D. *Proposed Food Grade Specifications*

1. DRT's Phytosterols Food Grade Specifications

Teriaka and DRT have proposed the following food grade specifications for DRT's phytosterol ingredient.

Phytosterol content	>99%
Beta sitosterol	75 – 80%
Beta sitostanol	10 - 14%
Campesterol	6 - 9%
Campestanol	0 - 2%
Others*	<3.0%
Loss on Drying (water)	<3.0%
Solvents (methanol)	< 0.2 %
Sulphuric ash	<0.1%
Heavy metal (as Pb)	<20 ppm
Arsenic	<0.25 ppm
Lead	<0.1 ppm
Total aerobic count	<10,000 CFU/g
Combined molds & yeasts	<100 CFU/g
Coliforms	negative
E. coli	negative
Salmonella	negative

* Includes brassicasterol, stigmasterol, Δ-7-stigmasterol, Δ-7-avenasterol, capestadiene and sitostadiene (dehydrated forms of campesterol and sitosterol).

2. Teriaka's Food Grade Specifications for Diminicol®

As stated above, Teriaka's Diminicol® product may be composed of several different fat/oil ingredients (partially hydrogenated refined palm kernel oil, hydrogenated coconut oil, low erucic acid rapeseed oil, or anhydrous milk fat) and two different phytosterols ingredients from either vegetable oil or tall oil. Teriaka will market its products based on the final food product manufacturer's requirements. Teriaka analyzed its products containing the highest concentration of a fat/oil (~60%) and of the phytosterols (~30%) to establish food grade specifications, i.e., most extreme case. Below are the proposed food grade specifications for the Diminicol® products.

III. Chemical Identity and Composition (continued)
D. Proposed Food Grade Specifications (continued)

TABLE 4

Proposed Food Grade Specifications for Diminicol®

Specification	Value
Appearance	Homogeneous, slightly marbleized
Flavor	Bland, mildly oily
Color	Off-white
Melting Range	70° C - 85° C
Structure at RT and <8° C	Soft, spreadable
Fat Content (%)	65-70
Phytosterol content (%)	18-30
Total Major Sterols (%)	18-30
Heavy Metals (as Pb) [1)]	<10 mg/kg
Lead [2)]	<1 mg/kg
Cadmium [2)]	<1 mg/kg
Arsenic [2)]	<1 mg/kg
Mercury [3)]	<1 mg/kg
Total Plate Count	<100 CFU/g
Yeast and Molds	<100 CFU/g
Coliforms	<10 CFU/g

1) FCC IV, 1997, Method II
2) Atomic Absorption graphite furnace with microwave digestion, prEN 14084
3) Cold-vapor atomic absorption spectrometry, SFS 5229

The phytosterol ratios differ depending on the source, i.e., vegetable oil or tall oil. Based on an approximate 30% phytosterol concentration in the Diminicol® products, the food grade specifications for the phytosterols are shown in Table 5 below.

TABLE 5

Proposed Food Grade Specifications for Phytosterol Ratio in Diminicol®

Phytosterol	Source of Oil	
	Vegetable	Tall
β-sitosterol (%)	40.0 - 58.0	75.0-80.0
Campesterol (%)	20.0 – 30.0	6.0 – 9.0
Stigmasterol (%)	14.0 – 22.0	
Brassicasterol (%)	0 – 6.0	
β-sitostanol (%)	0 - 5.0	11.0 – 14.0
Campestanol (%)		0 – 2.0
Others (%)		<3

Teriaka also analyzed for the major nutrients of its Diminicol® products that contained approximately 65% fat/oil and 30% phytosterols. These values are shown in Table 6.

TABLE 6

Major Nutrient Values of Diminicol®

Nutrient	Value (per 100 g)
Protein (g)	0.2 ± 0.1
Carbohydrate (g)	0
Lipids (g)	65 ± 0.1
Energy, kJ (kcal)	2430 (590)

III. Chemical Identity and Composition (continued)
D. Proposed Food Grade Specifications (continued)

Teriaka has obtained Halal approval for its products. The products will be labeled to store at <8° C or freeze. The shelf life of the products is 12 months when stored as instructed.

E. Manufacturing Process

000041

E. Manufacturing Process

1. [redacted]® Manufacturing Process

Tall oil is a natural product from pine trees isolated during the kraft pulping process. It is composed of the ether extractable non-lignin and non-cellulosic portion of the pine tree. The main components of tall oil are rosin acids, fatty acids and the unsaponifiables. The rosin acids are diterpene carboxylic acid; the fatty acids are predominantly C18 straight chains; and the unsaponifiable are predominantly a mixture of phytosterols.

[redacted]

2. Teriaka's Manufacturing Procedure

The full line processing of the phytosterol enriched ingredients by Teriaka Ltd. is conducted in accordance with 21 CFR Part 110.

F. Stability

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F. Stability

DRT specifies that its [redacted]® product has a 3-year shelf life when stored in a sealed container.

Teriaka Ltd. conducted a chemical, sensory and microbiological stability study on its sterol enriched ingredients under cool storage conditions. Eight different Diminicol® ingredients (four different fats/oils (anhydrous milk fat, rapeseed oil, coconut fat and partially hydrogenated refined palm kernel oil) with either 18% or 30% plant sterol concentration by weight) were stored at 4-8° C for 12 months in food grade polyethylene bags. The reference samples for sensory evaluation were stored at –20 to -25° C.

The Food Chemistry Division of the University of Helsinki conducted the chemical analyses for plant sterol and plant sterol oxide concentrations at 3, 6, 9, and 12 months using a capillary gas chromatographic method with saponification extraction and derivatization steps that was published by Toivo, J., et al. (1998). The GC was used for identification while quantification for the sterol oxides was determined by an internal standard method by GC-FID.

The Environmental Laboratory of the City of Helsinki, Finland conducted the following analyses: peroxide value, acid value, pH, and water activity after preparation and at 1 month intervals up to 3 months and after that at 3 month intervals up to 12 months.

The sensory evaluations were performed by the National Veterinary and Food Research Institute EELA using the IDF Standard 99C:1997 method after preparation and at 1-month intervals up to 3 months and after that at 3-month intervals up to 12 months. The National Veterinary and Food Research Institute EELA evaluated the nutritional values of the different ingredients initially.

III. Chemical Identity and Composition (continued)
F. Stability (continued)

The Environmental Laboratory of the City of Helsinki, Finland performed the following microbiological analyses: total plant count, total coliforms, hemolytic bacteria 37 ° C, hemolytic bacteria 55° C, and yeasts and molds initially and at 1-month intervals up to 3 months and after that at 3-month intervals up to 12 months.

The plant sterol and plant sterol oxide concentrations in the ingredient samples did not change during the 12-month cool storage period although the plant sterol oxide concentration was greater in the ingredient samples with 30% plant sterols. The peroxide values of all the ingredient samples were relatively low, less than 10 meq/kg throughout the study and the water activity and pH did not change during the 12-month period. The sensory properties changed most in the ingredient samples containing rapeseed oil and anhydrous milk fat while the hard fat ingredient samples maintained their sensory quality throughout the entire storage period. Teriaka concludes that all ingredient samples meet a sensory shelf life of 12 months despite some deterioration in the quality. All microbiological values were under the detection levels of the methods used and did not change during the storage time.

A second study was conducted to determine the microbiological and chemical quality of Teriaka's various sterol enriched ingredients (anhydrous milk fat, rapeseed oil, hydrogenated coconut oil, partially hydrogenated refined palm kernel oil) with either 18% or 30% sterols (source, vegetable or tall oil) when they were stored at ambient room temperature (<25° C >20 °C) during working hours and working days and then stored at <6° C during the weekends and outside working hours for a period of three weeks.

III. Chemical Identity and Composition (continued)
F. Stability (continued)

The results of this study demonstrated that even though there was no growth in any of the microorganisms tested (total plate count, total coliforms, hemolytic bacteria, and yeast and mold), pH increased in 7 of 8 samples over the three weeks and water activity increased in six samples but decreased in two samples. Therefore, Teriaka will recommend on its label that its sterol-enriched ingredients be stored at refrigerated or frozen temperatures.

G. Other Properties
-Sterol Oxidation

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G. Other Properties – Sterol Oxidation

Cholesterol is susceptible to oxidation and cholesterol oxidation products, called oxysterols, can be formed by physical processes involving reactive oxygen and free radical species [Smith, L. L., 1996], or enzymatically, by specific cytochrome P450 monooxygenases [Mayes, P. A., 1993]. Consequently, cholesterol oxidation products, as present in the human body, may be derived from absorption of oxidized sterols present in the food, as well as endogenous origin. As reported in FMF 626, stamped page 000029 (1999), certain oxidized cholesterol chemicals have been shown to be cytotoxic, artheriogenic, mutagenic and/or carcinogenic [Smith, L. L. and Johnson, B. H., 1989]. Two recent studies reported that oxysterols, especially 7β-hydroxycholesterol, may be associated with the increased risk of arteriosclerosis [Salonen, J. T., et al., 1997 and Zieden, B. and Diczfalusy, U., 1997]. For example, in Lithuania where the mortality in coronary heart disease is high, the mean plasma concentration of 7β-hydroxycholesterol was 12 ± 5 ng/ml in men [Zieden, B. and Diczfalusy, U., 1997]. Although not documented, oxidized plant sterols may have a similar artheriogenic effect because of their structural similarity to cholesterol oxides.

Plant sterols may be oxidized even more easily than cholesterol [Li, W. and Przybylski, R., 1995 and Plat, J. et al., 2001]. Oxidation products of plant sterols are analogous to oxidation products of cholesterol as shown in Figure 1 on the next page.

III. Chemical Identity and Composition (continued)
G. Other Properties – Sterol Oxidation (continued)

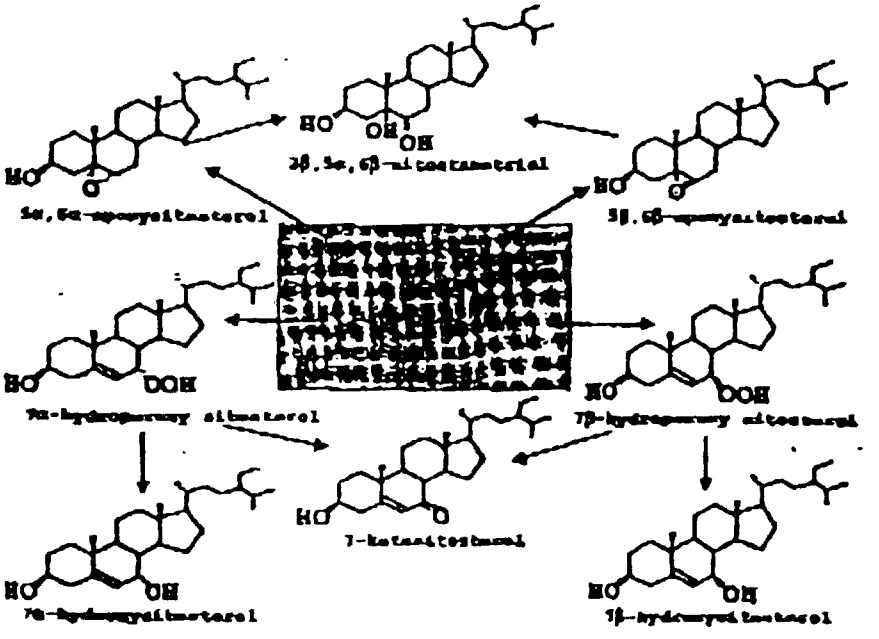


Figure 1: Possible *in vivo* oxidation of plant sterols, either by enzymatic or non-enzymatic mechanisms [Plat, J., et al., 2001].

After stereospecific peroxidation of sitosterol to 7α- and/or 7β-hydroperoxy sitosterol, reduction of these compounds results in the formation of 7α- or 7β-hydroxysitosterol, while dehydration of the hydroperoxide leads to the formation of 7-ketositosterol. Further, 5α, 6α-epoxysitosterol and 5β, 6β-epoxysitosterol are formed by epoxidation of sitosterol. Epoxysterols at C5-C6 are rapidly converted into their triol end-products, such as 3β,5α,6β-sitostanetriol. Comparable products can be formed from campesterol and other plant sterols.

At least two earlier phytosterol submissions to the agency discussed the possible oxidization of plant sterols during the manufacturing process or at the end use of the phytosterol-containing product [See McNeil FMF 626, stamped page 000029, 1999 and Procter & Gamble GRN 000053, stamped page 000024, 2000]. McNeil in its FMF raised possible concerns with the oxidation of sterol products although reported that certain oxidized sterols were not mutagenic in the test strains of *S. typhimurium* while naturally air-aged oxidized cholesterol was [McNeil FMF 626, stamped page 000029, 1999]. McNeil also reported on a published report that showed a wood-based plant sterol blend that has been stored for a year at room temperature contained about 750 ppm oxidized plant sterols [McNeil FMF 626, stamped page 000029, 1999].

Procter & Gamble conducted an assessment on heating its cooking oil that was enriched with phytosterols esters (<13.3% w/w) to determine whether the temperature (~290° F) and time (12 minutes) used in home cooking would increase the oxidized sterol chemicals. The resulting chromatograms of the control oil (unheated) and the heated oil were nearly identical [Procter & Gamble, GRN 000053, stamped pages 24-25, 31-39, 2000].

Teriaka reviewed the recent literature related to this issue and found that plant sterols in food can oxidize under frying conditions [Adcox, C., et al., 2001]. Small amounts of oxidized plant sterols have been found in both fried and non-fried foods (e.g., fried potatoes, wheat flour, vegetable oils and coffee) [Dutta, P.C., 1997]. Oxidized plant sterols occur in vegetable fat-rich processed foods at ppm levels. French fries prepared in rapeseed oil/palm oil blend, sunflower oil, and high-oleic acid sunflower

oil and high-oleic acid sunflower oil contained total sterol oxides at levels 2.4, 2.8 and 4.0 ppm, respectively [Dutta, P. C., 1997]. Italian infant milk formulas and milk cereals contained 7-ketositosterol at levels 0.7-5.0 μg/g lipid [Zunin, P., et al., 1998].

Recently, it was shown in rats that oxidized plant sterols are absorbed from the diet and incorporated into mesenteric lymph [Grandgirard, A., et al., 1999]. Absorption rate of oxidized plant sterols in humans has not been reported, however. For healthy humans, the absorption rate of non-oxidized plant sterols is usually less than 5% of dietary levels [Ling, W. H. and Jones, P. J. H., 1995]. In a study by Plat, J., et al. (2001) the percentage of oxidized sitosterol in serum of rare phytosterolemic patients was around 1.4%. Total serum oxidized sitosterol concentration varied from 4.21 μg/ml to 4.49 μg/ml at different time points in phytosterolemic patients. Oxidized campesterol molecules were also found, but exact concentrations could not be calculated. The investigators could not detect any oxidized plant sterols in pool serum from non-phytosterolemic patients and patients suffering from cerebrotendinous xanthomatosis. The detection limits for different plant sterols oxidation products varied from 0.41 μg/ml to 0.83μg/ml.

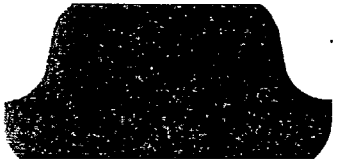
It was also reported in the literature that oxidized sitosterol and campesterol cause cellular damage *in vitro* in culture macrophage-derived cell lines similar to oxysterols, although less severe [Adcox, C., et al., 2001]. The observations by these investigators of the cellular damage by the oxidized sterols were reduced protein content of the cell and cell viability with increased lipid intake of the cell.

III. Chemical Identity and Composition (continued)
G. Other Properties – Sterol Oxidation (continued)

To determine whether storing Diminicol® products over a twelve month period at 4 – 8 °C temperature would increase the major oxidation products of the phytosterols used in Teriaka's products, Teriaka had the University of Helsinki analyze representative samples of its Diminicol® products containing either 18% and 30% phytosterols from tall oil with the various fat/oils identified above in this part. The products, pre and after twelve months, were analyzed for oxidized sterols using a GC-MS method after they were purified and derivatized. The limit of detection for 7-keto compounds was 10 μg/g and that of the other oxidation products 5 μg/g.

The plant sterol oxides identified were 7α-hydroxysitosterol, 7β-hydroxysitosterol, 5,6α-epoxysitosterol, 5,6β-epoxysitosterol, 7-keto sitosterol, and total plant sterol oxides. The results of this study showed that the concentrations of these plant sterol oxides, in the 23-54 μg/g FW range, remained stable during twelve months storage.

Teriaka finds that the presence of these sterol oxides in its Diminicol® products are much lower than those that have been reported in the literature and summarized in FMF 626 [McNeil FMF 626 stamped page 000029, 1999] and, therefore, concludes that the oxides do not present a concern for safety for its products.



IV Intended Use

IV. Intended Use

A. Background

Phytosterols and phytostanols are found in fruits, a variety of plants, vegetable oils, nuts, seeds, cereals and legumes [Weilrauch, J. L. and Gardner, J. M., 1978]. Morton, G. M., et al. (1995) reported that in addition to the primary sources of phytosterols in the British diet, breads and other cereals were also important sources. Consumers in the United States ingest approximately 250 mg/day from these natural sources [Ling, W. H. and Jones, P. J. H., 1995].

Plant sterols/stanols and their esters are added to food products because these chemicals have the ability to decrease serum cholesterol concentrations in humans by blocking the cholesterol update in the small intestine. The sterols have a greater affinity for mixed micelles than dietary and biliary cholesterol in the small intestine and displace cholesterol from the micelles, thereby reducing the absorption of cholesterol [Jones, P. J. H., et al., 1997 and von Bergmann et al. 1999]. The unabsorbed cholesterol is excreted in feces. As a result, the serum concentrations of total and LDL-cholesterol decrease.

Moghadasian and Frohlich (1999) reviewed 16 recently published studies, in which various plant sterol/stanol ester mixtures were used to reduce serum cholesterol levels. On average, there was a 13% reduction in LDL-cholesterol and a 10% reduction in total cholesterol concentration following plant sterol or stanol ingestion. Most recent studies have been made with plant stanols, which are saturated forms of plant sterols. The results demonstrated that these plant stanols were effective in reducing cholesterol [Miettinen, T. A., 1999 and Plat, J. and Mensink, R. P., 2000].

IV. Intended Use (continued)
A. Background (continued)

Teriaka conducted clinical trials to determine if its ingredient (rapeseed oil suspension enriched with tall oil phytosterols) would reduce cholesterol. The results from a double blind, randomized, placebo-controlled study with a 6-week run-in and a 6-month experimental period, using 155 hypercholesterolemic subjects randomly assigned to three experimental groups showed that 1.5 g/day significantly reduced plasma total and LDL-cholesterol concentrations by 7.5 and 11.6%, respectively [Christiansen, L. et al., 2001].

In addition, the effects of unsaturated forms of plant sterols on cholesterol concentrations have also been reported to be effective in reducing cholesterol and neither serum HDL cholesterol nor triglyceride concentrations are significantly affected [Jones, P. J. H., et al, 1998 and 1999; Weststrate, J. A., and Meijer, G. W., 1998, and Hendriks, H. F. J., et al., 1999].

Plant sterols are safe to use because they are absorbed at such low levels. For healthy humans, the absorption rate of plant sterols is usually less than 5% of dietary levels [Ling, W. H., and Jones, P. J. H., 1995]. Plant sterols have already been used for human consumption for many years in various forms: as a part of natural diets, as supplements, and in recent years also as components of food. In several studies, no apparent harmful effects have been observed even in long-term human consumption of plant stanol esters [Miettinen, T. A., et al., 1995; Hallikainen, M. A., and Uusitupa, M. I. J., 1999; Nguyen, T. T., et al., 1999; Vuorio, A. F., et al., 2000] or plant sterol esters [Weststrate, J. A., and Meijer, G. W., 1998 and Hendriks, H. F. J., et al., 1999 and 2001]. No adverse side effects have occurred even with large doses of sterols (up to 18 g/d) were consumed for

up to 3 years [Lees, R. S., et al., 1977]. In children, transferrin concentration was increased but all other laboratory values remained unchanged during sitostanol therapy [Becker, M., et al., 1993]. No significant adverse effects on gastrointestinal function have been observed in children during consumption of plant stanol esters [Williams, C. L., et al., 1999 and Tammi, A., et al., 2000].

B. Food Categories and Intended Effect

Teriaka Ltd. (Teriaka) is proposing to market its food grade Diminicol® products to be used as food ingredients or license it processing procedure to food companies that manufacturer the following types of food products: fat based spreads (margarine-like spreads); dressing for salads; snack bars (health); milk-based juice beverages; yogurt-like products; ice cream-like products; soft cheeses (i.e., cream cheese-type products); and white bread products. Teriaka's products, when incorporated into final fabricated food products, contribute nutritive value within the definition of 21 CFR 101.14(a)(3) and (b)(3)(i) as well as function as a texturizer as described in 21 CFR 170.3(o)(32).

As stated in the agency's preamble to the interim final rule permitting the use of a health claim related to the ingestion of plant sterols/stanols esters with a reduction in the risk of coronary hearth disease, the evaluation of the data cited indicates that it is the free sterol that is the active moiety in reducing serum cholesterol [65 FR at 54690, 2000]. Teriaka's notified GRAS ingredients contain either vegetable derived or tall oil derived phytosterols, the active moieties that assist in the reduction of serum cholesterol, as described elsewhere in this GRN.

IV. Intended Use (continued)

C. Consumer Exposure

Teriaka's ingredients will be included in various formulations in an amount that will provide a recommended total daily intake of 1.5 g phytosterols with each food product's label giving the number of servings to obtain this intake level. For most of the food categories listed above, the amount of phytosterols per serving will by 0.75 g except for yogurt, which will have 1.5 g/serving.

Teriaka ingredients will substitute for other phytosterols and phytostanols as well as their esters in the following categories that have already been subject to various GRNs submitted to the agency: fat based spreads (margarine-like spreads); dressing for salads; snack bars (health); milk-based juice beverages; and yogurt-like products [Lipton FMF 625, 1999; McNeil FMF 626, 1999; ADM GRN 000061, 2000; Novartis GRN 000039, 2000; Cargill GRN 000048, 20000; and Novartis submission dated October 12, 2001]

Teriaka concludes that the other three food categories (cream cheese, ice cream-like products and white bread products) do not add significantly to the daily consumption. This conclusion is based on the values calculated for U.S. population total mean per capita and 90th percentile per capita for all food categories listed above using a propriety software program to combine intake data from the USDA's 1994-1996 and 1998 Continuing Survey of Food Intakes by Individuals (CSFII) with the proposed fortification level. The consumption data averaged over two days of the most recent CSFII survey overestimates daily intake actually eaten over a life-time. For the U.S. population, the values calculated were 1.6 g/day for the mean per capita and 3.6 g /day for 90th percentile per capita.

V Safety

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A. Overview

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V. Safety Information

Because of the extensive amount of published and unpublished nonclinical studies on the phytosterol/stanols and their esters, Teriaka did not conduct additional nonclinical studies except for the one using obese Zucker rats to determine whether the ingredient produced the same effect (reduction in cholesterol uptake) as has been observed in humans. The safety of Teriaka's phytosterol isolated from vegetable oil has been addressed in Archer Daniels Midland Company GRN No. 000061, (2000), Procter and Gamble, GRN No. 000053, (2000); Cargill, GRN No. 000048, (2000) as well as Lipton, FMF 625, (1999) and the FDA letters and memoranda addressing each GRN and the FMF [U.S. FDA, 2000 a through d].

The safety of a phytosterol isolated from tall oil has been addressed in Novartis GRN No. 000039, McNeil FMF No. 626, (1999) and the FDA letters and memoranda [U.S. FDA, 2000a; U.S. FDA, 1999]. Additional information on the safety of phytosterols, particularly sitostanol, campestanol and stigmastanol, were provided as part of the overall safety on plant stanol esters [McNeil, FMF 626, 1999]. Therefore, Teriaka concludes that its ingredient containing either phytosterols from vegetable oil or tall oil is safe based on the following: (1) the fats and oils used are food grade; (2) The phytosterols from vegetable oil have already been determined by Archer Daniel Midland to be safe for its intended uses; (3) Teriaka's phytosterols ingredient derived from tall oil is substantially equivalent to the tall oil phytosterols manufactured by Forbes Medi-Tech, Inc. and determined by Novartis to be GRAS for the intended uses subject to its GRN and its additional submission dated October 21, 2001, as well as substantially equivalent to Eli Lilly's drug, Cytellin™; (4) extensive published literature on nonclinical and clinical data described in this GRN on both Teriaka's ingredients as well as related phytosterols from the above-mentioned GRNs, other related phytostanols and esters of both chemicals, as well as those summarized in the preamble of the interim final rule for a health claim related to the relationship between a diet containing esters of sterols/stanols and the reduction in risk of coronary heart disease; and (5) the conclusion of Finland's Ministry of Trade and Industry, Novel Food Board

Scientific Committee that Teriaka's products are safe for the intended uses as presented in Teriaka Novel Food submission (i.e., yogurt, spreads, fresh cheese, and milk-based fruit juices).

Based on several previous clinical studies and toxicity tests of the added mixture of plant sterols, the plant sterol enriched ingredient, Diminicol®, is unlikely to give rise to nutritional, microbiological, toxicological, or allergenicity problems. Therefore, Teriaka concludes that its ingredient is considered safe for use at the recommended consumption levels in the proposed food categories as part of the daily diet of healthy and hypercholesterolemic consumers.

A. Overview

1. Biological Equivalence of Free and Esterified Sterols

In human gastro-intestinal tract, plant sterol esters are hydrolysed to free phytosterols and fatty acids by pancreatic carboxyl esters lipase [Miettinen, T. A., et al., 1999, Normen, L., et al., 2000]. Therefore, phytosterol esters are biologically and physiologically equivalent to free phytosterols. Based on this fact Teriaka refers to studies that tested vegetable oil derived plant sterol esters in this safety evaluation as well as studies that tested free phytosterols and were reported in previous GRNs or FMFs. Teriaka refers also to studies carried out with phytostanol esters, because one of the raw materials for the production of the phytostanols oil esters is tall oil.

Compositions of phytosterol mixtures derived from tall oil or vegetable oil differ from each other because of natural variation, different species used, and environmental conditions related to the plant's growth. Both tall oil

derived and vegetable oil derived phytosterols have their typical composition that cannot be completely avoided, as is the case for the currently marketed phytosterol enriched products. Therefore, Teriaka's process that produces a suspension of the phytosterols and fat/oil does not change the biological equivalence and will not alter the safety profile established through extensive published and unpublished studies on phytosterols/stanols and their esters that have been reported previously in the GRN's, FMF's and preamble to FDA interim final rule on the stanol/sterol esters health claims as mentioned above.

2. Other Reviews

In a review by Miettinen, T.A. (2001) the author prefers the use of plant stanol esters rather than free stanols to enrich food products, because plant sterol esters increase their own absorption to a larger extent. However, the study by Ostlund and co-workers (2001) supports the view that the efficiency of phytosterol (unsaturated form) absorption is lower than what has been reported earlier. They noted that the plasma half-life for stanols was also significantly shorter than that for sterols. Ostlund et al. (2001) concluded that the differences in the absorption and turn-over are not consistent due to interactions of sterols and stanols.

3. Effects of Plant Sterols on Cholesterol and Bile Acid Metabolism

In general, the serum cholesterol level is kept constant by a balance in the rates of production and removal of this lipoprotein from the circulation [Brown, M. S., et al., 1981]. For example, in the situation of very low

cholesterol absorption the level of cholesterol in serum is ensured by increased endogenous production of cholesterol in liver via VLDL-cholesterol. Most Western diets contain an excess of cholesterol and thus there is an excess of cholesterol in blood circulation as well.

Plant sterols inhibit the absorption of cholesterol by interfering with the incorporation of cholesterol into the mixed micelles [Ling, W. H. and Jones, P. J. H., 1995]. Plant sterols themselves are absorbed much less than cholesterol (sitosterol 5% vs. cholesterol 50% [Salen, G., et al., 1970; Lütjohann, D., et al., 1995; Heinemann, T., et al., 1993; and Miettinen, T. A., et al., 2000]. Plant sterols are not converted to bile acids in humans to any significant degree. On the other hand, it is known that they can be metabolized in the mammal liver into the C21 bile acids [Salen, G. et al., 1970; Boberg, et al., 1990; and Ling, W. H. and Jones, P. J. H., 1995].

Scientists have hypothesized that the action of phytosterols in the intestine results in less intestinal chylomicron cholesterol entering the liver resulting in an up regulation of hepatic cholesterol synthesis and probably LDL-receptor activity. Despite an increase in synthesis of cholesterol in the liver, the net result is lowered serum LDL-cholesterol concentration. In other words, plant sterols do not shift the balance between endogenously produced and absorbed cholesterol abnormally, but rather normalizes the elevated serum cholesterol concentration and metabolism.

4. Effect Of Plant Stanol/Sterols Esters On Cholesterol

In several studies, no apparent side effects have been observed even in long-term human consumption of plant stanol esters (Miettinen, T. A., et al., 1995; Hallikainen, M. A. and Uusitupa, M. I. J., 1999; Nguyen, T. T., et al., 1999; Vuorio, A. F., et al.; 2000) or plant sterol esters (Westrate, J. A., and Meijer, G. W., 1998 and Hendricks, H. F. J., et al., 1999).

Recently, effects of plant stanol esters were studied in 11 colectomized patients focusing the absorption of stanols, biliary secretion and intestinal hydrolysis of stanol esters. In this study by Miettinen and coworkers (2000), it was found that effective inhibition of cholesterol absorption and reduction of serum cholesterol concentration were achieved already after one-day consumption of stanol esters. Small amounts of stanols were recovered in serum and bile, but stanols were effectively secreted through bile without changes in the composition of biliary or fecal bile acids.

Maki and coworkers (2001) documented that a plant sterol ester enriched margarine is beneficial adjunct to dietary treatment of hypercholesterolemia providing 7.6 – 8.1 % additional cholesterol reduction to the effect of National Cholesterol Education Program Step 1 diet. As to the carotenoids in this 4+ 5+ 5 week study using 40 subjects trans beta-carotene concentration was reduced significantly after correction for cholesterol concentration, but serum concentrations of fat-soluble vitamins and carotenoids were generally within reference range prior to and after the intervention.

It has been debated whether plant sterol and stanol esters produce an effect when a person is placed on a low cholesterol diet. A recent study by Mussner and coworkers with 63 healthy subjects supported the view that the improvements in the lipid profile are more pronounced in subjects with high dietary intake of cholesterol. These results are slightly contradictory to the results of the study by Maki described above [Mussner, M. J., et al., 2002].

Another question has been raised regarding whether plant sterol esters and stanol esters are equal in their ability to reduce cholesterol absorption and thus in cholesterol lowering properties as well. Using an advanced dual stable isotope technique, Jones, P. et al. (2000) came to the conclusion that the saturation of plant sterols might affect the efficacy of esterified sterols.

5. Effect Of Non-Esterified Sterols On Cholesterol

A study by Tikkanen, M. J., and co-workers (2001) is one of the first studies using a low-fat food matrix enriched with plant sterols. The investigators used non-esterified tall oil derived sterols (comparable to DRT's phytosterol product). The net effect on total and LDL-cholesterol carried out in 78 mildly to moderately hypercholesterolemic subjects was 5 % and 8 % reduction, respectively.

In an Australian study the cholesterol lowering effects of plant sterol esters were compared to non-esterified stanols in combination with different food matrixes (margarine and low-fat foods). Median LDL-cholesterol was reduced by 13.6 % using sterol esters and 8.3 % using non-esterified stanols. In the second part of the study the plant sterol esters were found effective when added to milk fat based spread. None

of the four measured carotenoids were altered significantly and tocopherols measured in the plasma increased significantly ($p<0.044$) [Nestel, L., et al., 2001].

6. Risk Groups

a. Vitamin A deficiency

Teriaka reviewed the literature to determine, if possible, whether the continuous use of sterol/stanols and their esters might present a health risk for people who are at risk of vitamin A deficiency or have sitosterolemia.

The absorption of carotenes is reduced by plant sterol use. Carotene are precursors of vitamin A and, therefore, individuals who have a low intake of vitamin A or increased need for vitamin A may be at risk of vitamin A deficiency because of plant sterol use. However, vitamin A deficiency is very rare in the United States. A more detailed discussion of the effect of plant sterol/stanols and their esters on various vitamins as studied in both animal and human is provided below.

b. Sitosterolemia

Sitosterolemia is a rare inherited lipid storage disease; about 40 cases of sitosterolemia have been reported in the medical literature [Lütjohann, D. and von Bergmann, K., 1997]. In people with sitosterolemia, the absorption of cholesterol and especially plant sterols from the small intestine is unusually high [Lütjohann, D.,

and von Bergmann, K., 1997]. As a consequence, serum plant sterol (sitosterol and campesterol) concentrations are increased by up to 1560 μmol/l (normal ranges are 4-14 μmol/l). Serum sitostanol and campestanol concentrations are also high [Salen, G., et al., 2000]. Moreover, sterol elimination is impaired and cholesterol synthesis is lowered. A high serum concentration of plant sterols results in xanthomatosis and early coronary atherosclerosis and haemolysis [Lütjohann, D., and von Bergmann, K., 1997]. Therefore, people with sitosteroleumia should not eat food rich in plant sterols.

c. Subjects heterozygotes for phytosterolemia

Two recent studies [Kwiterowitch, P., et al., 2000 and Salen, G., et al., 2000] have now verified that plant sterol ingestion by heterozygotes for phytosterolemia increases the serum plant sterols to a similar magnitude than that observed in normal subjects. These heterozygotes were expected to respond similarly to the homozygotes for phytosterolemia who become atherosclerotic early in life due to exceptionally high serum levels of plant sterols.

d. Risk of drug and plant sterol interaction

The risk of drug and plant sterol interaction may occur in relation to the use of fat-soluble drugs. According to the literature, plant sterols in theory inhibit the absorption of fat-soluble drugs from the small intestine. Some examples of fat-soluble drugs are isotretinoin (a structural analogue of vitamin A) used in acne treatment,

corticosteroids and hormones [Roe, D., 1989]. There is no indication, based on the structure of plant sterols and moderate effects on serum fat-soluble vitamin levels, that such interaction is greater than for other food items known to affect the absorption of fat-soluble agents, e.g., fiber-rich foods (psyllium seeds, etc.) or fat substitutes. A patient on a drug regimen has a responsibility to identify to his/her primary care physician any other drugs prescribed as well as any dietary supplements, OTC products and these types of sterol enhanced food products that may or may not affect the efficacy of a drug regimen.

e. Combination of cholesterol lowering medication and plant sterol enrichment of the diet

Plant sterol ester containing spread has been shown to have incremental reduction of serum total and LDL-cholesterol in combination to statin treatment [Blair, S. N., et al., 2000]. Furthermore, plant sterol ester supplementation has been shown to be safe and effective measure to decrease elevated cholesterol levels in combination to fibrate treatment as well [Nigon, F., et al., 2001].

f. Potential allergenicity of plant sterols

Chemically and physically, plant sterols belong to the lipid group. Lipids are known as non-allergenic compounds while proteins and some hapteins are the components know to be allergenic [Haahtela, T., et al., 1993]. In clinical studies with plant sterols, a few subjects have reported occasional skin reactions but no other

effects have been reported [Hallikainen, M. A., et al., 2000; Hendriks, H. F. J., et al., 1999; Nguyen, T. T., et al., 1999; Plat, J., et al., 1999; and Vuorio, A. F., et al., 2000]. Teriaka concludes that there is no need for further evaluation of allergenicity, since the plant sterol components are protein-free in practice. Any allergenicity reaction will be a result of the other ingredients that are part of the formulation of the final food product.

B. Nonclinical Information

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B. Nonclinical Information

Over the years, there have been extensive toxicological tests conducted on plant sterols, stanols and their esters. Plant sterols are non-toxic compounds that cause no toxicological risks [Shipley, R. E., et al., 1958; Sugano, M., et al., 1977; and Malini, T. and Vanithakumari, G. A., 1990]. In a plant sterol study using β-sitosterol, subcutaneous administration showed no evidence of gross or microscopic lesions in liver or kidney, and the biochemical parameters remained normal [Malini, T. and Vanithakumari, G. A., 1990]. In recent toxicological studies, the toxicity of plant stanols esters that were the subject of FMF 626 has been studied in rats and no toxic effect were observed [Turnbull, D., et al., 1999b; Turnbull, D., et al. 1999c; Slesinski, R. S., et al., 1999; and Whittaker, M. H., et al., 1999]. In addition, toxicological studies on plant sterols isolated from tall oil, were reported in GRN No. 000039 (2000) and those results provide a basis of safety for the phytosterols isolated from this source of plant.

1. Acute Studies

a. β-Sitosterol from tall oil (DRT's product)

Some acute toxicological studies using [redacted]® derived from tall oil (the subject of this GRN) were conducted by its manufacturer, Les Dérivés Résiniques & Terpéniques Granel S.A. According to a guinea pig maximization test, [redacted]® is non-sensitizing for guinea pigs. In another study this product was reported to be a non-irritant substance when applied to the skin of rabbits for four hours. In an acute toxicity test, mortality in rats was not observed over a fourteen-day period of time with 2 g/kg dose of this product.

b. Teriaka's Diminicol® study on rats

Teriaka conducted an oral feeding study using its ingredient, Diminicol®, with a genetic obese strain of Zucker female rats [Summanen, J., et al., 2000, unpublished]. Forty female rats were housed in groups of 4 per cage for adaptation of 12 hour light-dark cycle and then were divided into five subgroups at 7 to 9 weeks. At the beginning of the study, the serum cholesterol values were measured. All groups of rats were fed with different experimental ingredients for 12 weeks. The dietary regimens mixed in standard rat chow for each groups were the following: Group 1: standard rat chow; Group 2: high cholesterol diet using 18% unsalted butter and 1% cholesterol; Group 3: powdered form plant sterols using 0.5% β-sitosterol; 1% cholesterol and 18% unsalted butter; Group 4: Teriaka's product (MPS) (containing 0.5% β-sitosterol, 2.1% rapeseed oil and 0.4% water), 1% cholesterol and 18% unsalted butter; and Group 5: rapeseed oil 2.1%, 1% cholesterol and 18% butter. In all groups the average daily consumption of chow was 25 g and each group was allowed water *ad libitum*.

At the end of 12 weeks, all rats were anaesthetized with carbon dioxide, decapitated and blood collected. After decapitation the liver was dissected out, inspected macroscopically and weighed. There were no significant changes in serum triglyceride, blood glucose, serum GOT and GPT values or body and liver weights.

The powder form plant sterol supplemented diet lowered the serum cholesterol by 25% ($p<0.05$) and the MPS diet by 35% ($p<0.001$) compared with the high cholesterol diet.

An interesting observation was that the plant sterol supplements produced a marked reduction in serum ubiquinone Q9 levels suggesting a possible effect on the isoprene synthesis. Unlike the powder form plant sterol, MPS as well as the plain rapeseed oil decreased serum baseline diene conjugation values suggesting that they protect against oxidative stress-induced lipid peroxidation in rats. The lipid peroxidation diminishing effect is probably due to some anti-oxidation components in rapeseed oil. Although it is unclear whether the decrease in serum ubiquinone Q9 levels in rats may be directly related to humans, one could reasonably infer, since steroid synthesis in rats and humans follow similar pathways, that this would result in a decrease of the production of the total steroid synthesis chain and hence also the reduction of cholesterol synthesis in the liver. Overall these findings show that Teriaka's suspension enriched with phytosterols prevents cholesterol absorption in this genetic strain of obese Zucker female rats.

c. Other acute studies

A 10 day study in Wistar male rats using a tall oil derived sterol ingredient at 1000 mg/kg/day showed no significant changes in food intake or body weight gain [Novartis GRN No. 000039, 2000]. In

addition, Novartis' GRN reported on an acute study conducted on β-sitosterol (Eli Lilly Research Laboratories' (Eli Lilly) drug, Cytellin™) given as a single dose via gavage (5 g/kg triturated in sesame oil) to Albino mice. No toxicity was observed [Novartis GRN No. 000039, 2000].

2. Sub-Chronic Toxicity

Diets containing plant sterols esters were tolerated well and did not produce any general organ or systemic toxicity when fed to rats at doses as high as 8.1% of the diet (equivalent to 6.6 g/kg bw/day) for a period of 90 days. In addition, plant sterol esters (Lipton FMF 625, 1999) did not produce any effects on food and water consumption of body weight gain in rats [Hepburn, P. A., et al., 1999]. In a 13-week rat oral toxicity study using wood- or vegetable oil-derived stanol esters at a dose of 50 mg/100 g body weight, the investigators observed no toxicity [McNeil FMF 626, 1999 and Turnbull, D., et al., 1999b]. In addition a 60-day subcutaneous injection of β-sitosterol in rats was well tolerated and no changes in body tissues were found except at the dose of 1 mg/100 g bw that resulted in histopathological lesions in the kidney [Malini, T., and Vanithakumari, G. A., 1990].

Several subchronic studies were conducted by Novartis on its tall oil derived sterol ingredient and the results showed that there were no effects on body weight gain or food intake for all species (rat, mouse, hamster, and rabbit), even at an extremely high dose (i.e., 3340 mg/kg/day, mouse) [Novartis GRN 000039, 2000].

As reported in Novartis GRN 000039 (2000), there were four subchronic studies conducted on Eli Lilly's sterol drug product in rats, (5% Formula 226 for 8 months or 1% or 5% sitosterol in diet for 18 months) and in dogs, (1000 mg/kg/d of Formula 226 for 8 months or 500 mg/kg/day or 1000 mg/kg/day for 18 months). No toxicity was observed [Novartis GRN 000039, 2000].

Finally a 13-week feeding study in Wistar-derived Alph:Ap$_f$SD rats was fed a diet containing phytosterols up to 5% (3911 mg/kg/day or 4204 mg/kg/day in male and female, respectively). The results showed no toxic effects [Lipton FMF 625, 1999 and Hepburn, et al., 1999].

3. Genotoxicity

No cyctotoxicity effects, gene mutation or chromosome aberrations were found in a study performed on rats with wood and vegetable oil derived stanol esters. [McNeil, FMF 626, 1999 and Turnbull. T., et al., 1999c]. Both vegetable oil sterols and their esters have been evaluated by the Ames test as well as a cytogenetic assay and no evidence of genotoxicity was observed [Lipton FMF 625, 1999]. Moreover there was no evidence of genotoxicity observed when the sterol from a tall oil was tested either by *in vitro* or *in vivo* assays [Novartis GRN No. 000039, 2000].

4. Reproductive And Developmental Toxicity

No adverse effects were found either on reproduction or development in rats with plant stanols (soy or tall oil) or stanol esters [McNeil FMF 626, 1999; Slesinski, R.S., et al., 1999; Whittaker, M. H., et al. 1999 and Turnbull, D., et al., 1999a] or plant sterol esters [Waalkens-Berendsen, D. H., et al., 1999 and Lipton FMF 625, 1999].

5. Metabolism Of Plant Sterols In Animals

A large majority of plant sterols are not absorbed in the small intestine and this will consequently increase the concentration of plant sterols in the large intestine before being excreted in the feces. Plant sterols also increase the concentration of cholesterol in the large bowel since they reduce the absorption of both biliary and dietary cholesterol in the small intestine. For the small amount of plant sterols absorbed, elimination takes place via the biliary route and appears to be more rapid than that of cholesterol [Ling, W. H., and Jones, P. J., 1995]. According to Salen, G., et al. (1970) about 20% of the absorbed beta sitosterols are converted to cholic and chenodeoxycholic acids.

In a recent animal study [Ratnayake, W. M., et al., 2000a], the life span of stroke-prone spontaneously hypertensive rats was altered by a variety of dietary fats. Phytosterols, specifically campesterol and β-sitosterol, in the dietary oils and in liver and brain tissues were inversely correlated with the life span, indicating that the differential effects of vegetable oils might be ascribed, at least partly, to their different phytosterol contents. In another recent study, vegetable oils high in phytosterols made erythrocytes less deformable and shorten the life span of stroke-prone spontaneously hypertensive rats [Ratnayake W. M., et al., 2000b]. However, the results of these studies indicate the effects of different oils and further studies are needed to determine whether the effects on life span and erythrocytes of rats could be attributed to the high content of phytosterols in vegetable oils, and whether these results have a direct correlation to humans. Teriaka concludes that these two studies without confirmation in humans do not adversely affect the safe use of its phytosterol ingredients or of any other products containing sterol/stanols and their esters.

6. Chronic Toxicity

Plant sterols have been shown to have no toxic effects in chronic toxicity studies performed on animals. Oral administration of large daily doses of β-sitosterol to rats (5% in diet), rabbits (4000 mg/day), or dogs (1000 mg/kg/day) for up to 2 years showed no evidence of toxicity [Shipley, R. E., et al., 1958 and Novartis GRN No. 000039, 2000]. Sugano, M., et al. (1977) demonstrated no negative effects on growth and weight of major visceral tissues in rats fed with plant sterols and stanols.

7. Effect On Sex Hormones In Animals

Based on previous long-term animal studies with plant sterols, the expected consumption levels of the present food products enriched with plant sterols when used as directed will not cause any risk of estrogenic effects. There have been some animal studies that indicate at a high level of use plant sterols, especially sitosterol, may have estrogenic activity [Malini, T. and Vaaanithakumari, G. A., 1991; Ling, W. H. and Jones, P. J. H., 1995; MacLatchy, D. L. and Van der Kraak, G. J., 1995; and Mellanen, P., et al., 1996]. Both sitosterol and sitostanol seemed to have weak estrogenic effects at relatively high doses *in vitro* and sitosterol also *in vivo* in rainbow trout but not in rats [Mellanen, P., et al., 1996]. Two other reports indicate, at least in animals, when there is a high level of plant sterol intake, especially sitosterol, estrogenic activity is exhibited [MacLatchy, D. L., and Van der Kraak, G. J., 1995; and Mellanen, P., et al., 1996]. However, earlier animal studies, such as these, have to be interpreted with caution, because the administration routes (subcutaneous, intravenous) are not relevant to the oral ingestion route by humans.

Moreover, recent feeding studies in rats using plant sterol esters and plant stanols and their esters showed no evidence of estrogenic or uterotrophic activity [Baker, V. A., et al., 1999 and Turnbull, D., et al., 1999d]. Plant sterols did not bind to the estrogen receptors and did not stimulate transcriptional activity of the human oestrogen receptors in a recombinant yeast strain (*in vitro* assay) [Baker, V.A., et al., 1999]. As discussed below in the clinical summary, these earlier reported estrogenic effects in animals are not supported by the clinical studies and, therefore, do not present a toxic concern for the use of sterol/stanols and their esters, including Teriaka's ingredients enriched with phytosterols, in food products.

8. Other Studies

It has been reported that the plant stanols or sterols do not have an effect on reabsorption or on bile acid synthesis in hamsters [Trautwein, E. A., et al., 2001]. These results were reported as an abstract only. However, this finding is in agreement with findings of Miettinen, T. A., et al. (2000) in colectomized patients described below in the Clinical Part of this Safety Section.

A study in 80 female rats showed that coprostanol, a breakdown product of cholesterol, and cholesterol excretion were significantly enhanced when given plant sterols, and, therefore, plant sterols may have an unfavourable effect on intestine bacterial activity [Qulliot, D., et al., 2001]. These results were reported as an abstract only. Fecal cholesterol directly or indirectly is thought to alter the balance of gut microflora (meaning both bacterial count or bacterial activity) so that more carcinogenic metabolites may be formed or enhanced endothelial cell proliferation take place

[Cruse, J.P., et al, 1979; Giovannucci, E. and Goldin, B., 1997; Goldin, B. R. and Gorbach, S. L., 1976]. Elevated levels of plant sterols in feces have been hypothesized to operate oppositely, i.e., favorable effect on bacterial activity, and thus, supposedly, less enhanced cell proliferation [Awad, A. B., et al., 1997; Janezic, S. A. and Rao, A. V., 1992]. Human studies testing phytosterols confirm that no changes in bile acid concentration of feces [Weststrate, J. A., et al., 1999 and Miettienen, T. A., et al., 2000] or in short chain fatty acid and bacterial activity take place [Ayesh, R., et al., 1999].

C. Clinical Studies

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C. *Clinical Studies*

1. Studies Using Teriaka's Diminicol®

In a clinical trial, the intake of a spread (rapeseed oil source) enriched with plant sterols (tall oil source) significantly reduced plasma total and LDL-cholesterol concentrations. The double blind, randomized, placebo-controlled study with a 6-week run-in and a 6-month experimental period was used. There were 155 hypercholesterolemic subjects randomly assigned to three experimental groups. The control group used a placebo margarine-like spread and the two test groups consumed Diminicol®, Teriaka's sterol ingredient in a margarine-like spread that contained either 1.5 g/d or 3.0 g/d plant sterols. The subjects consumed test spreads as a part of their normal diet [Christiansen, L., et al., 2001].

Plasma total and LDL-cholesterol concentrations were significantly reduced by 7.5 and 11.6% (0.46 and 0.62 mmol/l) respectively in test groups consuming the spread enriched with plant sterols compared to the control group. There were no differences in effects between groups consuming either 1.5 grams or 3.0 grams per day of plant sterols. No effect on HDL-cholesterol or triacylglycerol concentrations occurred. There were minor changes in serum concentrations of retinol, α-tocopherol, α-, and β-carotene during the study but these changes were not significant between the test and control groups [Christiansen, L. et al., 2001].

A second clinical study (unpublished) found that 1 gram daily dose of Diminicol® (canola oil suspension enriched with tall oil phytosterols) in cooked and baked products was not effective in lowering serum cholesterol content when using Teriaka's enriched plant sterol ingredient.

An eight-week, single-blind, cross-over feeding study with a controlled diet was conducted with 13 healthy volunteers aged 19-56 years that consumed 1 gram of plant sterols for four weeks (experimental diet) and a similar diet without plant sterols (control diet) for four weeks. In addition, all the vitamins and carotenoids remained within normal ranges throughout the study (serum concentrations of β-carotene, vitamin A, vitamin E, lycopene and Ubiquinol-10) [Isoherranen, M., et al., 2000].

A third clinical study (unpublished) was conducted using Teriaka's enriched plant sterol ingredient, Diminicol®, (canola oil suspension enriched with tall oil phytosterols). A double blind, randomized study with a 2-week run-in period and a 12-week experimental period was used. After the run-in period 34 hypercholesterolemic subjects consumed four test spreads for three weeks in a randomized order. These four spreads provided daily 0 g, 0.8 g, 1.6 g and 2.4 g of the added plant sterols derived from tall oil. Otherwise subjects followed a standardized background diet, which was parallel to the composition of their normal diet except for the fat modification due to the use of the test spreads [Sarkkinen, E., et al., 2001].

There were no significant differences in the mean serum total, HDL-and LDL-cholesterol concentrations between the different test periods. Compared to the control-spread period, the test spread reduced serum total cholesterol concentrations by 0.4% with 0.8 g/day, 4.1% with 1.6 g/day, and 4.0% with 2.4 g/day. Increasing the dose of plant sterols significantly increased the response of serum total cholesterol (4.1% maximum reduction) and LDL-cholesterol (4.6% maximum reduction).

Triacylglycerol concentrations were significantly lower after Diminicol® 1.6 g/day period than after the Diminicol® 0.8 g/day period. The differences in HDL-cholesterol changes between the test periods were not significant [Sarkkinen, E., et al., 2001].

The mean serum vitamin E concentration was significantly lower with 1.6 g/day and 2.4 g/day compared to the control period ($p<0.05$). In addition, the serum vitamin E concentration was significantly lower with the 1.6 g/day compared to the 0.8 g/d ingestion ($p<0.01$). When the serum vitamin E concentration was proportioned to the serum total cholesterol concentration, there were no significant differences among the various levels of plant sterol ingested. No significant differences among the test periods were found in serum vitamin A, α-, β-carotene or lycopene concentrations nor their ratios to the serum total cholesterol concentrations.

Teriaka concludes that the serum level of alpha-tocopherol and beta-carotene tend to decrease as a consequence of plant sterol reducing the absorption of cholesterol from the gut, resulting in reduced serum concentrations of cholesterol. Thus, the changes in levels of vitamins A and E or their precursors are not statistically significant when the changes are adjusted for the change of total and LDL-cholesterol levels, except for beta carotene [Gylling, H., et al., 1999; Nguyen, T. T., et al., 1999; Tammi, A., et al., 2000; and Vuorio, A. F., et al., 2000]. Altogether, the decrease of serum vitamin levels as a result of plant sterol use in the present foods is biologically insignificant in healthy or hypercholesterolemic individuals.

The clinical and biological importance of decreased serum concentrations of beta-carotene is difficult to ascertain because the role of carotenoids, other than their pro-vitamin A activity, is not established with reasonable certainty [Sierksma, A., et al., 1999]. Furthermore, it has been suggested that decreased serum beta-carotene concentrations are not of essential concern, because of the recent reports of harmful effects following beta-carotene supplementation [Gylling, H., et al., 1999; Rapola, J. M. et al., 1997 and Omenn, G. S., et al., 1996].

2. Other Studies

a. Children consuming plant sterols enriched food products

In the studies made with healthy children and children with heterozygous familial hyperchloesterolemia (HFHC) daily intake of plant stanols varied from 1.5 g to 3 g [Becker, M., et al., 1993, Williams, C. L., et al., 1999; Vuorio, A. F., et al., 2000; and Tammi, A., et al., 2000]. There were no reported side effects in the HFHC studies except as noted below and these are considered not serious [Becker, M., et al., 1993; Becker, M., et al., 1992; and Vuorio, A. F., et al., 2000]. In healthy children there were no reported significant adverse effects on gastrointestinal function [Williams, C. L., et al., 1999] or changes in haematologic variables, serum creatinine concentrations, or lactate dehydrogenase and γ-glutamyl transferase activities [Tammi, A., et al., 2000].

The results of a study of children with severe familial hypercholesterolemia [Becker, V. A., et al., 1993] found no abnormalities in sonographs of the liver and gallbladder after both sitosterol (6 g/d) and sitostanol (1.5 g/d) capsule were taken. There were also no changes found in stool pattern. Sitosterol administration decreased alanine aminotransferase activity, alkaline phosphtase activity and carotene concentrations as compared with baseline values. Sitostanol slightly increased plasma transferrin levels and decreased plasma bile acid concentrations, but all values were within normal ranges. No changes were observed in hemoglobin, calcium, iron, phosphate, lipase, and creatine kinase concentrations or in leucocytes or platelet counts with either sitosterol or stiostanol. Similar results were noted in Becker, M., et al. (1992) study of these chilren taking sitosterol (6 g/d) for three months, although hemoglobin concentrations decreased by 5% and alkaline phosphatase activity by 19% when compared with a dietary run-in period. Nonetheless, all values remained within normal ranges.

From the reported studies it appears that, like adults, the dietary use of plant stanols affects the absorption of fat-soluble vitamins or their precursors in children. In healthy children the concentration of α-tocopherol, α- and β-carotene in serum decreased as a consequence of plant stanol use, but when α-tocopherol concentration was proportioned to serum total or LDL-cholesterol concentrations, no changes occurred [Tammi, A., et al., 2000]. In this and a study by Vuorio, A. F., et al. (2000) whose subjects

were families (both adults and children with familial hypercholstesterolemia caused by the FH-North Karelia mutation), β-carotene-to-cholesterol/LDL-cholesterol ratio was reduced, α-carotene-to-cholesterol ratio decreased but serum retinol and vitamin D concentrations were not changed significantly by stanol ester intake.

In the study of Tammi, A., et al. (2000) the serum sitostanol concentration was below the detection limit 0.05 μmol/l in 65 of the 77 healthy children before use of stanol ester margarine. The mean serum sitostanol concentration in the 12 children with measurable concentrations was 0.19 μmol/l. After the three month intake of plant stanols (1.5 g/d), the serum sitostanol concentration was measurable in 73 of the 74 children (0.67 μmol/l). However, the mean concentration was only approximately 1:6000 of the concentration of serum cholesterol, even though the children ingested 10-fold greater amounts of sitostanol than cholesterol. In the study of Becker, M., et al. (1993) whose subjects were HFHC children, plant sterol administration did not cause any increase in serum sitosterol concentration.

b. Tolerability and safety clinical study

Recently, Davidson, M., et al. (2001) studied the safety and tolerability of three levels of plant sterol esters administered in reduced-fat spread and salad dressing. Eighty-four free-living men

and women consumed reduced-fat spread and salad dressing providing 0.0 g/day (n = 21), 3.0 g/day (n = 21), 6.0 g/day (n = 19) or 9.0 g/day (n = 23) of phytosterols as esters for an eight-week treatment period. Side effects (flatulence, gastroesophageal reflux, discoloration of feces, appetite changes, leg cramps, low white blood cells and skin rash) did not differ among the groups during the study, and there were no product-related serious adverse events. There were no changes in clinical laboratory values in response to phytosterol intake. Blood concentrations of all fat-soluble vitamins remained within normal reference ranges, and there were no differences in serum vitamin responses among the four groups. Alpha- and trans-beta-carotene levels were reduced in the 9.0 g/day group vs. control ($p < 0.05$), but all carotenoid values remained within normal ranges throughout the study. The results of the study indicate that phytosterol esters were well tolerated and showed no evidence of adverse effects at a daily intake of up to 9.0 g of phytosterols for eight weeks.

c. Effect on fat-soluble vitamins and carotenoids

Relas, H., et al. (2001) studied the effect of plant sterols on the absorption of beta-carotene after a standardized meal. The investigators' conclusion was that plant stanol esters did not interfere with the absorption of beta-carotene at a detectable level. However, it is important to note that the concentration of this provitamin and its active form in circulation is more informative for the systemic effects and safety than the level of absorption.

Therefore, the safety evaluation focuses on studies that analyzed for serum concentration of fat-soluble vitamins and carotenoids. These studies are reviewed below and summarized in the Table 7 provided below.

The dietary use of plant sterols seems to affect the absorption of fat-soluble vitamins or their precursors, especially beta-carotene and vitamin E. [See Table 7.] The concentrations of vitamin E and beta-carotene in serum decreased as a consequence of either plant stanol ester [Gylling, H., et al., 1999; Hallikainen, M. A. and Uusitupa, M. I. H., 1999; Miettinen, T. A., 1999; Tammi, A., et al., 2000; and Vuorio, A. F., et al., 2000] or plant sterol (partly esterified) [Weststrate, J. A. and Meijer, G. W., 1998 and Hendriks, H. F. J., et al., 1999]. LDL-cholesterol particles act as carriers of vitamin E and beta-carotene in blood, so when the concentration of LDL-cholesterol decreases as a result of plant sterol use, the concentrations of vitamin E and beta-carotene will consequently decline.

When vitamin E (alpha-tocopherol) levels have been compared to serum cholesterol or total lipids levels, no changes in concentrations have occurred in studies made with plant stanol esters [Gylling, H., et al., 1999; Hallikainen, M. A., and Uusitupa, M. I. J., 1999; Hallikainen, M. A., et al., 2000; Tammi, A., et al., 2000; and Vuorio, A. F., et al., 2000] or sterol esters [Hendriks, H. F. J., et al., 1999]. Reduced beta-carotenes compared to cholesterol following plant stanol ester consumption have been

reported [Gylling, H., et al., 1999; Miettinen, T. A. and Gylling, H., 1999; Nguyen, T. T., et al., 1999; Tammi, A., et al., 2000; and Vuorio, A. F., et al., 2000], but neither Hallikainen, M. A., et al. (1999 and 2000) observed any significant change in cholesterol standardized beta-carotene with plant stanol ester consumed as part of a NCEP step 2 diet or other standardized diet and nor did Andersson, A., et al. (1999) nor Plat, J., et al. (2000). Alpha-carotene-to-cholesterol ratios have decreased in children with plant stanol ester therapy [Vuorio, A. F., et al., 2000]. Lipid-standardized plasma (alpha- + beta-) carotenoids concentrations have decreased with plant sterol therapy [Weststrate, J. A., and Meijer, G. W., 1998, and Hendriks, H. F. J., et al., 1999] but not in a study by Sierksma, A., et al. (1999). However, Plat, J. and Mensink, R. P. (2001) reported that there was a significant decease in serum beta-carotene when plant stanol ester mixtures were ingested. Furthermore, lipid standardized lycopene concentrations have decreased with plant sterol therapy [Weststrate, J.A., and Meijer, G. W., 1998; Sierksma, A., et al., 1999 and Novartis, GRN 000039, 2000] but not in a study of Hendriks, H. F. J., et al. (1999).

Vitamin A (retinol) and D levels did not changed significantly during the use of plant sterols or stanols [Gylling, H. and Miettinen, T. A., 1999; Gylling, H., et al., 1999; Hallikainen, M. A. and Uusitupa, J. I. J., 1999; Hendriks, H. F. J., et al., 1999; Nguyen, T. T., et al., 1999; and Tammi, A., et al., 2000]. There

was no effect of plant sterols on vitamin K absorption as reported in one study [Hendriks, H. F. J., et al., 1999]. Also in a one year study the ingestion of 2-3 g per day of plant sterols (sitostanol esters) did not affect vitamin D, retinol concentrations, or alpha-tocopherol/cholesterol proportion, but did reduce serum beta-carotene levels [Gylling, H., et al., 1999].

Plasma beta-carotene concentrations correlates with plasma cholesterol concentrations [Palli, D., et al., 1999]. Decrease in beta-carotene concentrations with phytosterol therapy is expected since, as stated above, beta-carotene is transported by lipoproteins and therefore a decrease in the amount of LDL-cholesterol particles consequently decreases the plasma-lipid soluble antioxidant concentrations [Plat, J. and Mensink, R. P., 2001; and Mensink, R. P., et al., 2002]. Decreases in plasma lipid-soluble antioxidant concentrations can also be avoided by following a simple dietary change similar to that described in the paragraph below.

A recent intervention study demonstrated that consuming an additional daily serving of a high-carotenoid vegetable or fruit when consuming spreads containing sterol or stanol esters maintains plasma carotenoid and lycopene concentrations while lowering LDL-cholesterol concentrations significantly [Noakes, M., et al., 2002]. It also should be noted that despite the reduction of serum beta-carotene concentration in previous studies, the decreased concentrations reported have been within normal range [Gylling, H., et al., 1999 and Davidson, M .H., 2001]. Teriaka

emphasizes that the serum retinol concentrations, a biomarker of the nutritionally essential metabolite beta-carotene, has not been changed. This has been confirmed in children as well [Tammi, A., et al., 2000].

Importantly, in studies whose subjects ingested Diminicol® spreads, there have been no significant changes in beta-carotene or lycopene concentrations. In a six-month clinical trial [Christiansen, L. et al., 2001] there were only minor, non-significant changes in serum concentrations of retinol, alpha-tocopherol, alpha-, and beta-carotene for 155 hypercholesterolemic subjects. There were also no significant differences between control and test groups. In the study of Sarkkinen, E., et al. (2001) there were no significant changes in lycopene concentrations nor in their ratios to the serum total cholesterol concentrations when the subjects were given Diminicol® spreads.

In fact, the health effects of slightly decreased serum beta-carotene and lycopene concentrations may not be relevant. Earlier studies found that the proportion of major coronary events in men with a previous myocardial infarction who smoked was not decreased when supplemented with beta-carotene, and in fact, the risk of fatal coronary heart disease increased in these subjects [Rapola, J. M., et al., 1997]. Another study that provided beta carotene and vitamin

A supplementation to smokers, former smokers and workers exposed to asbestos had no benefit and may have had an adverse effect on the incidence of lung cancer and on the risk of death from lung cancer, cardiovascular disease which led to the discontinuation of carotene and vitamin A supplementation earlier than planned [Omenn, G. S., et al., 1996].

V. Safety Information (continued)
C. Clinical Studies (continued)

TABLE 7

Blood α/α+β-carotene and α-tocopherol concentrations (crude value and proportions related to blood lipid levels) at the end of plant sterol/stanol enriched margarine periods and at the end of control period or at baseline in different studies.

	Crude value α-carotene or α+β-carotene (μmol/l) Mean (SD)	Proportion α-carotene or α+β-carotene/ lipid levels[ab] Mean (SD)	Crude value α-tocopherol (μmol/l) Mean (SD)	Proportion α-tocopherol/ lipid levels[ab] Mean (SD)
Weststrate & Meijer, 1998 (n=100)				
1.5-3.3 g plant sterols	186 7g/l *	31.8 7g/mmol *[b]		
control period	243 7g/l *	39.2 7g/mmol *[b]	-	-
Gylling & Miettinen, 1999				
3.18 g plant stanols (n=23)	1.10 (0.13)	0.19 (0.02)[a]	38.1 (1.7)	6.62 (0.25)[a]
control group (n=23)	1.63 (0.17)	0.27 (0.03)[a]	41.7 (1.9)	6.94 (0.33)[a]
Gylling et al. 1999 (n=102)		Mean (S.E.M)		Mean (S.E.M)
2-3 g plant stanols	-	0.12 (0.01)[a]	-	6.34 (0.13)
baseline	-	0.16 (0.01)[a]	-	6.43 (0.12)
Hallikainen & Uusitupa, 1999 (n=18)				
2.31 g plant stanols	1.22 (0.97)	0.24 (0.23)[a]	45 27 (6.97)	8.50 (0.90)[a]
baseline	1.66 (1.10)	0.27 (0.21)[a]	51.49 (8.17)	7.86 (0.81)[a]
Hendriks et al. 1999 (n=60)				
1.61 g plant sterols	0.52 (0.51)*	0.10 (0.11)*[b]	25.8 (5.2)	4 51 (0.65)[b]
3.24 g plant sterols	0.40 (0.28)*	0.07 (0.05)*[b]	25.6 (5.3)	4.47 (0 61)[b]
control period	0.57 (0.47)*	0.10 (0.09)*[b]	28.1 (5.3)	4 59 (0.72)[b]
Sierksma et al. 1999 (n=76)				
0.8 g plant sterols	217 μg/l *	35.7 μg/mmol *[b]	-	-
control period	226 μg/l *	35.8 μg/mmol *[b]	-	-
Hallikainen et al. 2000 (n = 22)				
0 8 g plant stanols	0.61 (0.64)	0.10 (0.11)	43.35 (6.43)	6.91 (0.74)
1:6 g plant stanols	0.60 (0.50)	0.11 (0.10)	42.13 (6.79)	6.99 (0.72)
2.4 g plant stanols	0.55 (0.45)	0.10 (0.09)	41.89 (7.27)	7.21 (0.74)
3.2 g plant stanols	0.55 (0.46)	0.10 (0.10)	40.58 (6.17)	7.06 (0.66)
control period	0.64 (0.56)	0.10 (0.11)	46.58 (7.65)	7.18 (0.74)

[a] per total cholesterol
[b] per total cholesterol + triacylglycerols
* α+β carotene

d. Effect of sex hormones in humans

The absorption of phytosterols from the small intestine into the blood circulation is known to be relatively small [Pollak, O. J., 1985] and it is very limited compared with that of cholesterol, of which about 30-70 per cent is absorbed [Lütjohann, D. and von Bergmann, K., 1997]. Elimination of plant sterols from the body is mainly in the feces and is initially very rapid. Only traces of sterol are being excreted at days after dosing [Sanders, D. J., et al., 2000]. The absorption rate of beta-sitosterol is 4% [Sanders, D. J., et al., 2000]. Because plant sterols are absorbed in such a low level in humans, the systemic effects in human body, including effects on sex hormones are negligible. However, there are human studies reporting conflicting results on the effects of plant sterols on human sex hormone levels, as discussed below.

In a study of Ayesh, R., et al. (1999) the effect of a margarine enriched with vegetable oil phytosterol esters (8.6 g/day) on female sex hormones was determined in 12 healthy males and 12 healthy females. A significant reduction in progesterone levels (ANCOVA, p= 0.021) was found in the test margarine group. No other differences in serum female sex hormone levels between the intervention group and control group were found either in women or men, although estrone and estradiol levels were decreased by 14 % and 36%, respectively. Using the *t*-test, no differences between the control and intervention group were considered significant.

The investigators noted that the level of progesterone varies significantly during the luteal phase of the cycle. A blood sample collected for sex hormone analysis close to ovulation or in the luteal phase, gave variable results from one cycle to another because every follicle and corpus luteum has a different output of estrogens and androgens and small variations in cycle length affects the values quite substantially. This may explain the reduction in serum progesterone levels and could also offer an explanation as to the fluctuation of estradiol values observed in the test margarine group.

In another study, no effects on sex hormone levels were observed when soy-oil derived phytosterols (2 grams per day) were eaten in low-fat yoghurt [Volpe, R., et al., 2001]. In the study with a 8-week active period, there were no significant changes in serum follicle-stimulating hormone, sex-hormone binding globulin, estradiol and testosterone values in men and women between the values before and after the treatment period [Volpe, R., et al., 2001].

[Hendriks, H. F. J., et al., 2001] investigated the long-term effects of daily consumption of 1.6 grams of plant sterols in a spread by healthy male and female adults. The study subjects consumed plant sterol-enriched spread for 52 weeks. As a result, there was no effect of plant sterols on male and female hormone levels.

Finally, in a study using Teriaka's Diminicol® spreads, there were no significant differences in serum FSH, LH, prolactin and sex-hormone binding globulin concentrations when three different phytosterol doses (0.8 g/day, 1.6 g/day, or 2.4 g/day) were given in either men or women [Sarkkinen, E. S., et al., 2001].

The perceived problem in the human clinical trials on hormonal effects of plant sterol has been the small statistical power due to small sample sizes and wide natural variation (seen as large standard deviations) in sex hormone concentrations. However, Teriaka concludes from these existing human clinical trials that the magnitude of the effect of plant sterols on serum sex hormone levels does not exceed the magnitude of natural variation in hormone levels. According to existing human data, the absorption of plant sterols in humans occurs at such a low level that absorbed plant sterols have no systemic effects on human sex hormone levels either in males or females even with ingesting a high dose (up to 8.6 grams) of plant sterols [Ayesh, R., et al., 1999].

e. Other studies

Products of bacterial metabolism of cholesterol and primary bile acids have been implicated in colon cancer development, as discussed earlier in this section. However, plant sterols have not been found to increase the concentration of fecal bile acid in human [Weststrate, J. A., et al., 1999]. In fact, the concentration of secondary bile acids was decreased by vegetable oil plant sterols.

With a 25-fold increase (daily intake of 8.6 g) in plant sterols intake, the fecal neutral sterol-metabolite concentration increased less than twofold. The concentration of the putative mutagenic substance, 4-cholestan-3-one (metabolite of cholesterol and plant sterols) was increased slightly to about 2 mg/g, that is well within the normal range. The daily intake of 8.6 g plant sterols has not been found to affect the bacterial profile or the metabolic activities of the gut microflora [Ayesh, R., et al., 1999].

Based on the above discussion and the results of the various clinical studies, Teriaka concludes the use of its phytosterol ingredients are safe when eaten in the proposed food products for a total daily intake of 1.5 g.

D. Safety of Cumulative Intake

D. Safety of Cumulative Intake of Plant Sterols

Studies have shown that up to 18 g/day intake of plant sterols is safe. Studies with large amounts of plant sterols have not raised any specific health concerns for healthy adults even when consumed for longer periods of time. Lees, A. M., et al. (1997) reported on patients taking sitosterol (18 g/d) for an average of 18 months. The plasma concentration of sitosterol did not increase compared to 3-6 gram intake of beta-sitosterol. No other adverse side effects were observed other than mild constipation when 18 g/d was ingested [Lees, R. S. and Lees, A. M., 1976 and Lees, A. M., et al., 1997].

Numerous clinical studies using Eli Lilly's drug, Cytellin™ (major component, β-sitosterol) were conducted on both healthy volunteers and patients with cardiovascular problems with no reported serious adverse events [Best, M. M., et al., 1955; Best M. M. et al., 1954; Cooper, E. E., 1958; Farquhar, J. W., et al., 1956; Joyner, C. and Kou, P. T., 1955; Lehmann, J. H., 1957 and Sachs B. A. and Weston, R. E., 1956] even when the drug was taken over several months at 25 grams daily with an increased to 50 grams a day at times [Best, M. M. et al., 1955]. Therapeutic levels for this drug ranged from 9,000 to 30,000 mg/day

In a 13-week oral toxicity study in rats, a no-observed-adverse-effect-level (NOAEL) of 870 mg plant stanol esters per kg body weight for plant stanol esters derived from either vegetable or tall oils was established. The NOAEL correlates to approximately 61 grams per day for a 70 kg person [McNeil FMF 626, 1999 and U. S. FDA 1999a].

V. Safety Information (continued)
D. Safety of Cumulative Intake of Plant Sterols (continued)

In a 13 week rat feeding study, a NOAEL of 3900 mg/kg bw /day in males was established for vegetable oil sterol esters. Using this value and a safety factor of 30, Lipton calculated an acceptable daily intake for free vegetable oil sterols to be 130 mg/kg/day [Lipton, FMF 625, 1999 and U.S. FDA, 1999b]. For a 70 kg person, this ADI translates to 9.1 grams/day.

Based on the above toxicological data that show phytosterols and phytostanols and their esters present no toxic concern at fairly high doses and based on the clinical data that demonstrate that there are no observed adverse effects in the ingestion of beta sitosterol, Teriaka concludes that the values for the total per capita in g/day for both the mean (1.6 g/day) and 90th percentile (3.6 g/day) for all of its food categories listed in this GRN fall well within the ADI values calculated for either esters of the phytosterols/stanols as well as the clinical data discussed above that reported no adverse side effects even at very high doses for long periods of time.

Therefore, Teriaka concludes that its ingredients containing either the vegetable or tall oil derived phytosterols are safe for their intended uses.

VI References

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VI. References

Item

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VII Appendices

000116

Plant sterol products retailed in Finland and Germany.

FINLAND	Manufacturer	
Kolestop®	Hankintatukku Ltd	Daily dosage (6 tablets) contains 2000 mg of plant sterols of which 1527 mg beta-sitosterol and 473 mg beta-sitostanol. For cholesterol reduction.
Prostan®	Leiras Ltd	Daily dosage (4-6 tablets) contains 836-1254 mg beta-sitosterol. For mild prostate troubles.
Sitosan®	Hankintatukku Ltd	Daily dosage (6 tablets) contains 1620 mg beta-sitosterol. For cholesterol reduction.
Tri Tolosen Kasvisteroli®	Oy Bio-Vita Ab	Daily dosage (3-5 tablets) contains 1500-2500 mg of plant sterols. For cholesterol reduction.

GERMANY		
Azuprostat®	Azupharma GmbH	Daily dosage (2-6 capsules) contains 130-390 mg beta-sitosterol. For benign prostate hyperplasia
Flemun® -Kapseln	intermuti pharma GmbH	Daily dosage (3-6 capsules) contains 30-60 mg beta- sitosterol. For rheumatism
Harzol®	Hoyer Gmbh & Co.	Daily dosage (3-6 capsules) contains 30-60 mg beta-sitosterol. For benign prostate hyperplasia
Prostasal®	TAD Pharmazeutis-ches Werk GmbH	Daily dosage (3-6 capsules) contains 30-60 mg beta- sitosterol. For benign prostate adenoma before and after prostate operation.
Sito-Lande®	Synthelabo Arznei-mittel GmbH	Daily dosage (3-6 pastilles or 1-9 granulates) contains 1760-15840 mg beta-sitosterol. For - hyperlipoproteinemia (especially type IIa
Sitosterin Prostata-Kapseln	intermuti pharma GmbH	Daily dosage (3-6 capsules) contains 30-60 mg beta- sitosterol. For prostate hyperplasia.
Triastonal® Kapseln	intermuti pharma GmbH	Daily dosage (3-6 capsules) contains 30-60 mg beta-sitosterol. For benign prostate hyperplasia.

Method to produce Diminicol®

3/21/2002

LM, approved

Teriaka

Submission End