APPENDIX B

APPENDIX B

Summary of Scientific Data

I. The Amendment to the Existing Health Claim Requested by this Petition is Supported by Significant Scientific Agreement

This scientific analysis supports a petition to amend the phytosterol health claim to permit a claim to be made for a food that provides the full daily intake of phytosterols in a single serving. Because a health claim has already been authorized for consumption of phytosterols two or more times per day, this analysis will focus on data supporting the effectiveness of phytosterols when consumed once a day. This analysis concludes that the totality of the publicly available evidence supports the proposed claim and that there is significant scientific agreement among qualified experts that the claim is valid.

FDA's determinations, as reflected in 21 CFR 101.83, that heart disease is a significant public health problem for the population as a whole, that the ability of a substance to reduce total and LDL-cholesterol provides valid evidence that it can help reduce the risk of CHD, and that scientific evidence demonstrates that including phytosterols in the diet helps to lower blood total and LDL-cholesterol levels and may reduce the risk of CHD, are incorporated herein by reference.

A. *Summary of data on which the original health claim was based*

In September 2000, FDA authorized the use, on food labels and in food labeling, of health claims on the association between plant sterol/stanol esters and reduced risk of coronary heart disease (CHD) based on its recognition of two specific facts: (1) heart disease is a significant public health problem for the population as a whole, and (2) plant sterol/stanol esters are efficacious in reducing blood levels of total and LDL-cholesterol (TC and LDLC, respectively) and thus can help reduce the risk of heart disease. 65 FR 54685 (September 8, 2000). In the evaluation of the original petitions, FDA conducted an extensive scientific review. FDA accepted data from 9 human studies on plant sterols and 15 human studies on plant stanols which met the applicable scientific selection criteria, including: (1) data and descriptions of the study design and methods were adequately presented; (2) estimates of intakes of plant sterols or stanols and their esters were provided; (3) blood total cholesterol and other blood lipids related to CHD were directly measured; and (4) the study subjects were representative of the general U.S. population. In the case of criterion (4), these persons were considered to be adults with blood total cholesterol levels less than 300 mg/dl. In all but 4 studies reviewed by FDA, plant sterols/stanols were incorporated into table fats, including margarine, vegetable oil spread and butter, and consumed with meals at a frequency of 2-3 times daily. The majority of the studies tested plant sterols/stanols in the form of esters (of sunflower or canola oil fatty acids). Subjects in studies with plant sterols were normal to mildly hypercholesterolemic adults, and subjects in studies with plant stanols were mildly hypercholesterolemic adults. Studies with familial

hypercholesterolemic children, who are not representative of the general population, were excluded from the review. No lactating or pregnant women were included in any of the studies.

In reviewing the studies supporting the original health claim, FDA said:

> In the studies showing a statistically significant effect of plant sterols or plant sterol esters on blood total and LDL cholesterol levels, subjects were provided with and instructed to consume the daily intake of plant sterols or plant sterol esters in two [citing four studies] or three [citing two studies] servings at different times of the day, or subjects were provided with the plant sterol-containing food and asked to replace from 25 to 50 g of their typical dietary fat intake with an equal amount of the test food over the course of the day's dietary intake, usually during meals [citing three studies]. The agency concludes that, to be consistent with the conditions of the studies on which the claim is based, the daily intake of plant sterol esters should be consumed in at least two servings eaten at different times during the day with other foods. ... FDA is specifying two servings as the target number of servings.

65 FR at 54704-705. Because, as this petition demonstrates, there are now adequate data upon which to base a conclusion that phytosterols may reduce the risk of CHD when consumed once a day, FDA's prior rationale for requiring at least twice-a-day use is no longer applicable. As will be discussed in section I.C. below, this petition includes data from eleven new studies that involve once-a-day use.

B. *Summary of data on effects of phytosterols on blood lipoprotein cholesterol levels published since the original health claim was authorized*

On March 7-9, 2001, thirty-two experts on lipids, nutrition, and heart disease met in Stresa, Italy and discussed the efficacy, safety, and future research required for phytosterols. Their deliberations were essentially similar to those expressed by Law (2000), which concluded that the effects of free and esterified sterols and stanols were similar. In evaluating the efficacy of various sterols and stanols on plasma lipoproteins, the Stresa Workshop participants identified 41 publications from MEDLINE and review articles; these included all but two (Ayesh et al 1999; Denke 1995) publications identified by FDA in the original health claim authorization, and 18 studies published between 2000 and 2002 after the release of the FDA health claim. Of these 18 studies, two were conducted with healthy children and 16 (including 3 studies in which phytosterols were administered once a day) were conducted with adult men and non-pregnant and non-lactating women having normal to mild elevated blood TC and LDLC levels. The Stresa Workshop experts (Katan et al 2003) concluded that the effects of phytosterols (mainly as esters), when used at 2 or 3 portions over the day, are comparable and independent of the background diet. Free phytosterols can have the same effect as esterified forms; however, the matrix and emulsification appear to be important. There is a linear relationship for the percentage reduction in LDLC as a function of dose up to 2 g/day, and there is little additional effect at doses higher than 2.5g/day. Data from meta-analyses by Law (2000) and the Stresa Workshop (Katan et al 2003) both indicate that a regular daily intake of two 1-g servings of phytosterols with meals can result in a reduction in LDL-cholesterol of about 10% (Figure 1).

The Stresa Workshop's meta-analysis also concluded that approximately half of the maximal effect (approximately 11.3%) achievable by the consumption of phytosterols is attainable from consumption of 0.7 to 1.1 g/day.

Subsequent to FDA's authorization of the phytosterol health claim, domestic and international health authorities have also endorsed the use of phytosterols by adults to help reduce the risk of CHD. Thus, the Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol of the National Cholesterol Education Program (NCEP) Adult Treatment Panel (ATP) III (2001), the International Atherosclerosis Society (IAS) (2003), and the American Heart Association (AHA) (Lichtenstein and Deckelbaum 2001, Fletcher et al 2005) have recommended, alongside reductions in the dietary intake of saturated fat and cholesterol, the inclusion of 2 g/d of phytosterols as an additional dietary means to intensify the effectiveness of a cholesterol lowering diet. None of these health organizations has specified a recommended number of times per day that the products should be consumed.

C. *Summary of data on once-a-day use that have been published or prepared since the final rule was published in September 2000*

Although phytosterols were initially incorporated into fat-based foods such as vegetable oil spreads, salad dressings and mayonnaise, a number of other viable food forms have since been demonstrated to be appropriate vehicles including orange juice (Devaraj et al 2004) and lemonade (Spilberg et al 2003); low-fat dairy products such as milk or yogurt (Hyun et al 2005, Pineda et al 2005, Clifton et al 2004, Noakes et al 2005, Thomsen et al 2004, Volpe et al 2001); chips, bread, cereal and pasta (Clifton et al 2004, Hayes et al 2004, Salo and Wester 2005); meat products (Matvienko et al 2002, Tapelea et al 2004); and chocolate (deGraaf et al 2002). In most of the studies in which the non- and low-fat food carriers have been tested, they were used with meals with a frequency of 2-3 times daily. Thus, Berger et al (2004) concluded that, if phytosterols are consumed with a meal to stimulate biliary flow, they can effectively lower LDLC regardless of the type of background diet and the form of food carrier.

In a 2005 review on the history and development of plant sterol and stanol esters for cholesterol-lowering purposes, Thompson and Grundy (2005) observed, citing one single study in which stanols were consumed at lunch (Plat et al 2000), that the effectiveness of phytosterols appeared to be similar with once-daily or divided dosage and independent of the fat content of the food vehicle. In total, 11 studies, including one which is in press, have reported once-a-day use of phytosterols in the range of 1-3 g/day. Three of the studies (Plat et al 2000, Matvienko et al 2002, Volpe et al 2001) were included in the meta-analysis published by the Stresa Workshop (Katan et al 2003). Ten studies reported significant reductions in levels of serum TC and LDLC at a magnitude comparable to data generated from twice- and thrice-a-day use. The eating occasion was specified in 10 studies; the food carriers of phytosterols were consumed either at lunch (4 studies), with the main meal or a further non-specified meal (4 studies), at breakfast or before breakfast (3 studies). No study explicitly reported an intake of plant sterol-enriched products at dinner. A recent study, in which a yogurt providing 2 g of plant sterols was ingested at breakfast, showed a significant 7.8% decrease in LDLC (Hyun et al 2005). Such an LDLC-lowering effect is comparable to the 8.8% decrease reported with consumption at lunch of 2 g

plant sterols incorporated in a yogurt-drink (Pineda et al 2005). Another study in which the impact of intake occasion was tested showed that doses of 2.8 and 3.2 g of plant sterols provided in a single-administration yogurt drink significantly reduced LDLC by more than 5% (5.1 and 6.9%, respectively), when consumed at least 30 minutes before breakfast, on an empty stomach. However, when the same yogurt drink was ingested with a meal at lunch time, the LDLC-lowering was more pronounced (9.5% and 9.3% for doses of 2.8 and 3.2 g of plant sterols, respectively), suggesting that consumption together or directly after a meal may be needed for optimal efficacy (Doorbos et al 2006). Indeed, the key step of cholesterol absorption inhibition, the main mechanism of action of plant sterols, is believed to be at the level of dietary mixed micelles. Since micelles are formed in the small intestine after stimulation of bile secretion by food entering the digestive tract, consumption with a meal may be required to effectively stimulate bile flow and allow an optimal competition between plant sterols and cholesterol for absorption.

In contrast with the studies of Hyun et al (2005) and Doornbos et al (2006), which showed a significant LDLC-lowering impact of plant sterol consumption at or before breakfast, one study failed to show a significant reduction in LDLC with ingestion of on average 1.7 g phytosterols at breakfast (AbumWeis et al 2006). Among the factors that could possibly explain the absence of a significant effect on plasma total and LDLC in their study, the authors discussed the plant sterol dosage (an average of 1.7 g/day, based on body weight), compared to doses of 2.5 and 2.7 g in the once-a-day studies of Plat et al (2000) and Matvienko et al (2002), respectively, in which plant sterols were ingested at lunch time. The determination of plant sterol intake according to body weight in the study of AbumWeis et al differs from the approach used in most studies in which plant sterols are supplemented (a fixed dose for all participants), and resulted in different intakes between individuals, ranging from 1.0 to 1.8 g/d. Since Hyun et al (2005) observed a significant LDLC-lowering impact following ingestion at breakfast of a single dose of yogurt providing 2 g/d of plant sterols; it is unlikely that a breakfast dose of 2 g/d would not be sufficient to reduce LDLC. Although the dose used by Doornbos et al was higher (2.8 or 3.2 g/d), the significant LDLC reduction observed despite the fact that plant sterols were consumed on an empty stomach further support the ability of plant sterols to lower LDLC when ingested in a single dose in the morning. To explain the absence of a significant impact of plant sterols taken at breakfast in their study, AbumWeis et al also mentioned the existence of a circadian rhythm of cholesterol synthesis. They hypothesized that a circadian rhythm would exist for the absorption of cholesterol as well, and that this would affect the efficacy of plant sterols during daytime (AbumWeis et al 2006). However, to our knowledge no circadian rhythm of cholesterol absorption has been demonstrated so far, and no data are available at present that could confirm a preferred time of ingestion for optimal plant sterol efficacy.

Various doses and times of ingestion during the day have been reported in the once-a-day studies, with some variability in LDLC lowering. Overall, there is no indication that ingestion at a given time during the day should be preferred. However, available data do suggest that ingestion at meal time is an important factor for optimal efficacy. Taking into consideration the amount of phytosterols consumed and the eating occasion, the results of these 11 studies as a whole, in conjunction with the existing literature and authoritative recommendations on their usage, indicate that a comparable reduction in serum levels of LDLC, at a magnitude of ≈ 10%,

can be expected from consuming phytosterols at 2 g/day once-a-day or 1 g per portion twice-a-day together with a main meal.

II. Evaluation of Individual Datasets in Support of the Relationship between Once-a-Day Intake of Phytosterols and Reduced Risk of Heart Disease

A detailed description of studies which evaluated the effects of consuming phytosterols once-a-day on serum lipids and lipoproteins is presented below. A summary of the research is presented in Table 1 and a more extensive summary of these studies is presented in Table 2. Although male and female adults of various ages were enrolled in the studies, no pregnant or lactating women were included.

A. *Plat et al (2000)*

As part of their ongoing research in the biochemistry and nutrition of phytosterol metabolism, the research group of R.P. Mensink at Maastricht, the Netherlands conducted a randomized, double-blind, placebo-controlled and crossover feeding study in which adult men (n=11) and women (n=28) having normal BMI and normal to moderately elevated levels of blood TC and triglycerides (TG) were assigned to consume 2.5 g plant stanols (as esters) either at lunch or spaced in 3 meals (0.42 g at breakfast, 0.84 g at lunch and 1.25 g at dinner, in proportion to cholesterol content of the meals of their habitual diets). Each of the three treatments (placebo control, once-a-day, thrice-a-day) lasted 4 weeks without a washout. Fasting blood samples were drawn once at the beginning and twice at the end of the third week of each treatment period for analysis.

Results of this study showed that, compared with the control treatment period, reductions in TC (6.3% with the once-a-day treatment and 6.6% with the thrice-a-day treatment, $p<0.001$ for both), low density lipoprotein cholesterol (LDLC) (9.4% with once-a-day and 10.4% with thrice-a-day, $p<0.001$ for both), and TC/HDLC ratio (5.6% for both, $p< 0.01$ for once-a-day, $p<0.001$ for thrice-a-day) were comparable whether the plant stanol-containing margarine was consumed once-a-day at lunch or thrice-a-day with 3 meals. No changes in high density lipoprotein cholesterol (HLDC) and TG were observed. The magnitudes of effects on serum lipids were consistent with existing literature.

The researchers also examined the status of fat-soluble antioxidants and reported more pronounced, although not statistically different, reductions in the thrice-a-day treatment than in the once-a-day treatment.

The quality of this study is rated as high. It had a randomized, double-blind, placebo-controlled and crossover design which is the optimal design for human dietary intervention studies. In particular, the inclusion of a control feeding treatment arm within the cross-over design provided high-quality correction for individual variation. The study addressed adequately the inclusion/exclusion criteria, subject characteristics, composition of background diets, composition and manner of the administration of the test margarine, compliance, and data collection and analysis. As the subjects included smoking and non-smoking adult men and

women (pre-menopausal with some contraceptive users; and post-menopausal) the study findings should be applicable to all adults.

B. *Volpe et al (2001)*

This study was conducted in two parts. Participants were adult male and female outpatients with primary moderated hypercholesterolemia attending the Lipid Clinics of Rome and Milan Universities. The subjects were instructed to consume the AHA Step 1 diet before and during the study. The dietary intake was assessed with 3-day food records at the beginning and the end of each treatment period.

Part 1 had a randomized, double-blind, placebo-controlled cross-over design and enrolled 21 men and 9 women. Phytosterols were administered through a low fat yogurt drink containing 1 g plant sterols (presumably as esters) in 100 ml consumed once-a-day. Compared with the baseline serum values at the beginning of the 4 week feeding period, TC, LDLC and LDLC/HDLC decreased by 6.7% (p=0.0005), 11.1% (p= 0.0009) and 15.1% (p=0.05), respectively, when subjects consumed the phytosterol containing yogurt drink, and decreased by statistically insignificant amounts (2.3%, p=0.3; 4.9%, p=0.2; and 8.7%, p=0.3, respectively) when subjects consumed the placebo yogurt drink. HDLC and TG levels were unchanged.

Although the study had a cross-over design, the authors did not make use of the "self as control" design to obtain an estimate of the phytosterol effect. Instead, two sets of treatment effects (expressed as mean and SD) with references to their respective run-in "baseline" time points (i.e., sterol vs. baseline and placebo vs. baseline) were reported. By using this statistical approach in analyzing the data, the reported reductions in TC, LDLC and LDLC/HDLC were higher than those which would be expected based on the literature (Katan 2003) for an intake of 1 g/day, and were more comparable to an intake of 2.5 g/day. However, based on the reported values of the two sets of treatment effects it is possible to approximate the "true" treatment effect of phytosterols on each dependent variable (by subtracting the reported mean of the placebo treatment (in reference to its baseline) from the reported mean of the sterol treatment (in reference to its baseline). The "true" statistical significance of the treatment effect, however, cannot be determined as the difference of means does not always equal to the mean of differences. With this approach of data transforming, the reductions in TC, LDLC and LDLC/HDLC from the consumption of 1g plant sterols/day are estimated to be comparable to the literature values at 4.4%, 6.2% and 6.4%, respectively.

Part 2 of the study was an open label trial designed not only to estimate the magnitude of effect but also to obtain information on safety and tolerability of the sterol treatment. This trial enrolled 7 men and 4 women who participated in the first trials. After having been on a background diet similar to that used in the first trial, the subjects consumed two servings of the sterol containing yogurt drink for 8 weeks. No treatment effects on liver enzymes (AST, ALT), serum levels of sex hormones (FSH, SHGB, estradiol, testosterone) or vitamins A and E were found. Because the researchers did not report the timing or manner in which the two servings of phytosterol containing yogurt drinks were consumed, it can only be surmised that they were not consumed at one sitting as customary consumption pattern of yogurt drinks suggests. Therefore, no clear conclusion can be made as whether the doses were taken once or twice a day, and the

data are not useful for the current discussion. Further, because there was not a placebo drink period, the reported reductions of TC, LDLC and TC/HDLC (11.2%, 15.6% and 13.3%, estimated through differences of the means by authors of this document), which are higher than what can be expected based on literature data, cannot be considered as true effects.

The quality of Part 1 of this study is rated as medium because of the statistical approach used, and also because the eating occasion was not described precisely. The data are applicable to hypercholesterolemic adult subjects without other pathology. The quality of Part 2 of this study is rated as low primarily because of the lack of a placebo control.

C. *Matvienko et al (2002)*

In a study having a randomized, triple blind (to the supplier of the test food items, the researchers and study subjects), placebo-controlled parallel arm design, Matvienko et al evaluated the effects of 2.7g plant sterols (2/3 as esters, 1/3 in free form) delivered through 7 ground beef based food items, one consumed on each day of the week, on serum TC and LDLC in mildly hypercholesterolemic, slightly overweight male college students. The study subjects consumed the experimental food items as part of a prepared and supervised lunch, and were asked to maintain their habitual diet patterns except not to consume red meat other than that provided in the experimental lunch and to limit egg consumption to 2-3 eggs/week. The two parallel treatments lasted 4 weeks and each arm had 17 subjects.

At the end of the 4-week study period, serum TC and LDLC concentrations and TC/HDLC decreased 9.3%, 14.6% and 9.1% ($p<0.001$), respectively, from baseline in subjects consuming 2.7 g/day phytosterols, whereas values of these markers of subjects in the control group remained unchanged. As estimated by the authors of the present document, the placebo-adjusted decreases in TC, LDLC, and TC/HDLC observed with the phytosterol treatment were 8.4%, 13.4%, and 9.1%, respectively. The intake of phytosterols did not affect HDLC and TG levels. These findings are consistent with the literature reported values for comparable amounts of phytosterols delivered in table spread form and consumed 2-3 times daily. Neither the proportion of absorbance in large and small LDL nor the LDL particle diameter was significantly influenced by treatment. No subject changed from LDL pattern A to pattern B or vise versa in the treatment group. On the other hand, contrary to literature (Hendriks et al 1999), no significant association was found between the percentage of change in serum LDLC concentration and total phytosterol intake in a range of doses from 0.025 to 0.0425 g/kg body weight. The authors concluded that lean ground beef is an excellent vehicle for delivering a dose of plant phytosterols to effectively lower plasma TC and LDLC concentrations and improve TC/HDLC, and thus lower the risk of heart disease in mildly hypercholesterolemic young adults.

The quality of this study is rated as high. The study has adequately addressed issues of inclusion/exclusion, subject characteristics, composition of test materials, compliance, and data collection and analysis. Although the participants were young college men there is a good basis to believe, based on knowledge of the effects of phytosterols, that the findings are applicable to men and women older in age (Law 2000). The lack of a run-in period with the placebo beef product is a shortcoming of this study. However, because the baseline data for the two treatment groups were similar and because there were no numeric changes in variables of control subjects

before and after treatment, this shortcoming in design did not compromise the integrity of the study data.

D. *Salo and Wester (2005)*

In a review article, Salo and Wester reported the results of three different studies testing different food product vehicles for plant sterols. The studies tested the effects of plant sterols incorporated in a ready-made meal, pasta or yogurt drink. The results obtained from the ready-made meal and the pasta study were not yet reported in an original publication, but only in the review article; however, the results of the yogurt drink experiment were previously reported in a German dietetic journal (Ernährungs-Umschau 2004). Reductions of serum TC (6.8%, $p<0.001$) and LDLC (10.1%. $p<0.01$) were observed in subjects having normal to slightly elevated serum total cholesterol levels who consumed, once-a-day, 2 g plant stanols (as esters) which were incorporated into a low fat ready-made meal (added into either the meat or mayonnaise) and consumed at lunch. These results were obtained in a double blind experiment in 60 subjects (presumably 30 each in the control and intervention arms) consuming a habitual background diet. A similar design was used in the two other studies: one enrolled 60 subjects who consumed, once-a-day, 2 g plant stanols (as esters) incorporated into a 100% durum wheat pasta and consumed at lunch for two weeks; and another one enrolled 40 subjects who consumed, once-a-day, 2 g plant stanols (as esters) incorporated into a low fat yogurt drink (65 ml) and consumed with a main meal of the day for 6 weeks. Compared to the control arms, serum TC and LDLC were reported to reduce by 8.0% and 10.9%, respectively, with pasta, and 10.4% and 12.6%, respectively, with the yogurt drink. These decreases in TC and LDLC were apparently obtained from comparison to baseline. From the German publication reporting the yogurt study we understand that the 12.6% decrease in LDLC with the yogurt drink was related to baseline; the placebo-adjusted lowering effect was calculated as 11.3%. No changes in HDLC and TG were reported for all three trials.

Subjects were reported to be adults aged 12-65 years with no additional characteristics given. The researchers did not report actual values of the lipoprotein cholesterols at all points of the studies, the composition of the background diet, or compliance. The incomplete reporting of this study makes it impossible for another researcher to replicate the study. The quality of this study is therefore rated as low.

E. *Hyun et al (2005)*

The efficacy of a plant sterol-enriched, low fat yoghurt was investigated in a double-blind, placebo-controlled, parallel arm study. Fifty-one normo- and mildly hypercholesterolemic (total cholesterol > 4.5 mmol/L) Korean subjects were divided in two groups. They were randomly assigned to receive either 150 mL of a placebo yogurt or a plant stanol-enriched yogurt providing the equivalent of 2 g/d free plant stanols. The yogurt was consumed at breakfast, for 4 weeks, and was replacing an equivalent amount of energy in the traditional low-fat Korean diet. The 4-week treatment phase was preceded by 2-week run-in period during the usual dietary pattern of the subjects was evaluated. The subjects were interviewed by a dietitian every week during the treatment phase, and dietary intake was evaluated by semi quantitative food frequency questionnaire and 24-hour recall at the beginning and end of the treatment period.

After 4 weeks of consumption, the plant stanol-enriched yogurt lowered plasma total and LDL-cholesterol by 5.8 and 9.7%, respectively, relative to baseline concentrations, while the placebo non-significantly reduced total and LDLC by 1.3 and 1.9%, respectively. From these data, the reduction ascribed to the plant stanol yogurt can be calculated from the difference between the effects of the two treatments: 4.5% for total cholesterol, and 7.8% for LDLC, which is in the range of effects reported with intakes of plant sterols of about 2g/d (Katan et al 2003). The plant stanol-enriched yogurt also reduced plasma levels of oxidized LDL by 5.4% compared to baseline, and this effect was significantly different from that of the placebo.

This study shows that 2 g plant sterols incorporated into a "once-a-day" low-fat yogurt consumed at breakfast significantly lowers plasma total and LDLC. These results also show that plant sterols may help protect against cardiovascular diseases in part by decreasing the oxidation of LDL particles. The quality of this study is rated as high. The design and its application were appropriate; food intake was monitored; data analysis was done correctly, and the results are applicable to normo- and mildly hypercholesterolemic individuals. Although the Korean subjects were consuming a low-fat diet, the results may be applicable to US individuals, as plant sterols have been previously shown to reduce total and LDLC both as part of a low-fat diet (Cleghorn et al 2003) and a typical Western diet (Temme et al 2002).

F. *Pineda et al 2005*

The efficacy of a plant stanol-enriched yogurt drink was investigated in a double-blind, placebo-controlled, parallel arm study. Thirty-two men and women with LDLC higher than 120 mg/dL (3.1 mmol/L) were divided in two groups. They were randomly assigned to receive either one 65 ml-bottle of a placebo yogurt or a plant stanol-enriched yogurt drink providing the equivalent of 2 g/d free plant stanols. The yogurt drink was consumed after a main meal (presumably lunch or supper), but the exact time in the day was not specified. The treatment phases were 3 weeks in duration. The subjects received dietary instructions before the start of the treatment phase.

After 3 weeks of consumption, the plant stanol-enriched yogurt drink lowered plasma total and LDL-cholesterol by 6.7 and 10.3%, respectively, relative to baseline concentrations, while the placebo non-significantly reduced total and LDLC by 2.4 and 1.5%, respectively. From these data, the reduction ascribed to the plant stanol yogurt drink can be calculated from the difference between the effects of the two treatments: 4.3% for total cholesterol, and 8.8% for LDLC. These placebo-adjusted reductions in LDLC are in the range of effects reported with intakes of plant sterols of about 2g/d (Katan et al 2003).

This study shows that 2g plant sterols incorporated into a "once-a-day" yogurt drink consumed with a main meal significantly lowers plasma total and LDLC. The quality of this study is rated as medium-high. The double-blind, parallel arm design is appropriate. However, there was no run-in period. Although dietary intakes were not reported, the patients were given dietary instructions. Data analysis was done correctly. The results are applicable to mildly hypercholesterolemic individuals.

G. *Doornbos et al (2006)*

The impact of fat content of the carrier and eating occasion on the effectiveness of plant sterols was tested in a randomized, double-blind, placebo-controlled study with 5 parallel arms. Each arm consisted of a 4-week run-in period to acclimatize the study subjects and a 4-week yogurt drink consuming period. The 5 study arms were: (I) placebo control, (II) drink A (0.1% dairy fat, 2.2% total fat) consumed at lunch, (III) drink A consumed ≥ 0.5 hour before breakfast, (IV) drink B (1.5% dairy fat, 3.3% total fat) consumed at lunch, and (V) drink B consumed ≥ 0.5 hour before breakfast. Drink A provided 3.2 g plant sterols, and drink B, 2.8g, in the form of plant sterol esters. A total of 184 moderately hypercholesterolemic men and women (age 57±2 years) completed the study. The results showed that the cholesterol-lowering effect was independent of the fat content of the carrier when consumed once a day. When subjects consumed the plant sterols at least 30 minutes before breakfast, significant reductions of TC and LDLC were observed. Compared with placebo, drinks A and B decreased LDLC by 5.1 and 6.9%, respectively. However, when the same products were consumed once-a-day at lunch, the reductions in LDLC were more pronounced (reaching 9.5 and 9.3%, respectively). HDLC and TG levels were unaffected in all cases. The authors concluded that for optimal efficacy, a plant sterol-enriched single-dose yogurt drink should be consumed with a meal.

The quality of this study is rated as high. It was well designed and executed. The study has adequately addressed issues of inclusion/exclusion, subject characteristics, composition of test materials, compliance, and data collection and analysis. A run-in period was included before the treatment phases and, baseline characteristics of the subjects were similar between groups. The data are applicable to mildly hypercholesterolemic subjects.

H. *AbuMweis et al (in press)*

At the Experimental Biology meeting 2005, AbuMweis et al reported that plant sterol preparations when taken as a single dose at breakfast failed to reduce blood cholesterol (AbuMweis 2005). The study has been described in a manuscript accepted for publication in the Journal of Nutrition. Below is a description of the study details.

The study had a randomized, crossover, placebo-controlled single blind design. The 5 study treatments were (I) control, (II) free plant sterols, (III) plant sterols esterified to fatty acids from sunflower oil, (IV) plant sterols esterified to fatty acids from fish oil, and (V) free plant sterols in combination with fatty acids from fish oil. The 4 forms of plant sterols were incorporated into margarine and consumed under supervision, to ensure compliance, at breakfast. Thirty overweight (average BMI = 28.5), hypercholesterolemic (average TC=5.9 umol/L, LDLC-3.8 mmol/L) men and postmenopausal women with an average age of 59 years completed the study. The experimental diets were provided to the subjects, and each diet was consumed for 29 days. Each diet phase was followed by a 2 to 4 week washout period during which subjects consumed their habitual diets.

The dose of plant sterols in each of the four plant sterol treatment phases was based on body weight (22 mg/kg). This manner of determining plant sterol intake differs from the approach used in most studies in which plant sterols are supplemented at a fixed dose, and resulted in a

wide range of doses, from 1.0 to 1.8g/d, with an average of 1.7g/d. The addition to the diet of free sterols, free sterols plus fish oil sterols esterified to fish oil or sterols esterified to sunflower oil for 4 weeks had no significant effect on endpoints and percentage changes of TC, LDLC, TG, and Apo A1 concentrations compared to control. However, HDLC was higher ($p<0.05$), and thus TC/HDLC was lower ($p<0.05$) after subjects consumed the diets containing the sterols, independent of preparation, relative to the control diet period intervention. No significant effect of dietary perturbations on apoB, Lp (a) or CRP levels were identified.

In contrast with the studies of Hyun et al (2005) and Doornbos et al (2006), which showed a significant LDLC-lowering impact of plant sterol consumption at or before breakfast, the study of AbumWeis et al failed to show a significant reduction in LDLC with ingestion of 1.7 g phytosterols at breakfast (AbumWeis et al 2006). Among the factors that could possibly explain the absence of a significant effect on plasma total and LDLC in their study, the authors discussed the plant sterol dosage (an average of 1.7 g/day, with a range from 1.0-1.8g, depending on body weight), compared to doses of 2.5 and 2.7 g in the once-a-day studies of Plat et al (2000) and Matvienko et al (2002), respectively, in which plant sterols were ingested at lunch time. While the three doses tested in these three studies all fall within the dose-response range of the Stresa meta-analysis, only the once-a-day responses observed in the studies of Plat et al (2000) and Matvienko et al (2002) were consistent with the predicted LDLC reduction. The large range of doses used in the study of AbumWeis et al, with some participants receiving doses of plant sterols as low as 1g/d should not be discarded as a possible explanation for the absence of a significant impact. On the other hand, since Hyun et al (2005) observed a significant LDLC-lowering impact following ingestion at breakfast of a single dose of yogurt providing 2g/d of plant sterols, it is unlikely that a breakfast dose of 2g/d would not be sufficient to reduce LDLC. Other factors may also have contributed. It is known that cholesterol synthesis follows a circadian rhythm and is high in the evening hours. Although no circadian rhythm as been observed for cholesterol absorption so far, AbumWeis et al hypothesized that cholesterol absorption may follow a circadian rhythm opposite to that of cholesterol synthesis, and would be low in the early morning. Consequently, a further lowering of absorption by phytosterols would not lead to a significant decrease in blood cholesterol. However, no data are available at present that could confirm this hypothesis and the existence of a preferred time of ingestion for optimal plant sterol efficacy.

The quality of this study is rated as high. The study addressed adequately the design, inclusion/exclusion criteria, subject characteristics, composition of background diets, composition of the test foods compliance, and data collection and analysis. The doses of plant sterol used are described, but the determination of the dose differs from the typical approach used in most studies testing plant sterol efficacy. In terms of applicability of the results, the findings of this study are generalizable to most overweight, hypercholesterolemic individuals in the US. Indeed, 64% of US adults age 20 and over are overweight http://www.cdc.gov/nchs/fastats/overwt.htm. Moreover, over half and 46% of the US population have TC and LDLC above 200 mg/dl (5.1 mmol/L) and 130 mg/dl (3.3 mmol/L), respectively, (AHA 2006).

I. *The Emmi Studie Benecol (2004)*

The effects of a plant sterol-enriched yogurt were tested in a placebo-controlled, double-blind study. Forty hypercholesterolemic (TC 220-330 mg/dL or 5.6-7.7 mmol/L) volunteers aged 40-65 years old consumed for 6 weeks either 150 g of a placebo yogurt or 150 g of a yogurt providing 2 g/d of plant stanols. The yogurt was consumed with or after a meal. After 6 weeks of treatment, TC and LDLC were reduced by 6% and 10.5%, respectively, compared to baseline. TC and LDLC reduction in the placebo group were 0.7 and 2.8%. The reductions in LDLC observed with the plant stanol-enriched yogurt were significant compared to placebo. By subtracting the placebo from the plant stanol yogurt impact, the placebo adjusted reduction in LDLC can be evaluated at 7.7%.

The design of the study is appropriate. However, no run-in period was reported. Although the subjects were apparently asked to write food diaries, no data on nutrient intakes were provided. Plasma lipid concentrations at baseline and at the end of the treatment are not available. The incomplete reporting of this study makes it impossible to replicate. The quality of this study is therefore rated as low.

III. Evaluation of Quantity, Overall Strength and Consistency, and Relevance of Evidence in Support of the Relationship between Once-a-Day Intake of Phytosterols and Reduced Risk of Heart Disease

To our knowledge there are eleven studies in which phytosterols were consumed once-a-day. The phytosterol-containing food items were consumed at lunch in 4 studies, at breakfast in two studies, before breakfast in one study, with or after a main meal in three studies, and at an unspecified time of the day in one study. Only adults were enrolled in the studies, but the ages of subjects covered a very wide range, from young male college students to older male and female adults. It appears that sufficient numbers of subjects were enrolled in each study to allow sufficient statistical power to detect a treatment effect.

A. *Quantity of evidence*

The number of studies and the number of individuals tested in studies in which phytosterols were used once-a-day at lunch or with a main meal are sufficient to comfortably generalize to the adult male and female populations.

A wide range of plant sterol intakes were tested in different intake occasions (before or at breakfast in 3 studies, lunch in 5 studies, with a main meal in 2 studies, and unspecified in 1 study). In studies where plant sterols were consumed with a main meal, this could have been either at lunch or dinner. Therefore, intake of plant sterols at all meals has presumably been tested.

B. *Consistency of evidence*

There are three studies (Plat et al 2000, Matvienko et al 2002, and Doornbos et al 2006) of high quality and two studies of lower quality (Salo & Wester 2005) in which phytosterols were consumed at lunch. Three studies of medium or low quality tested the impact of plant sterol intake at an unspecified meal time (Volpe et al 2001, Emmi et al 2004, Salo & Wester 2005). The reductions in LDLC levels reported in these studies were consistent with each other and with literature findings in which phytosterols were consumed with meals two to three times daily.

There are 3 studies, published (Doornbos et al 2006, Hyun et al 2005, AbuMweis et al 2006) of high quality in which phytosterols were consumed during or before breakfast. Hyun et al reported a 7.8% LDLC decrease following ingestion of 2g/d at breakfast. In the study of Doornbos et al, a significant, greater than 5% decrease in LDLC was observed following an intake of 2.8 or 3.2 g/day of sterols consumed at ≥ 0.5 hours before breakfast. These two studies demonstrate that ingestion of 2g/d plant sterols or more in the morning results in a significant LDLC lowering effect. The results of Doornbos et al also emphasize that consumption with a meal may be required to obtain optimal efficacy. This may be directly linked to the mechanism of action of plant sterols, which requires an adequate solublization and interaction of endogenous cholesterol and exogenous phytosterols (Trautwein et al 2003) into dietary mixed micelles. On the other hand, one study showed no effect when an average 1.7g/day of sterols were consumed at breakfast (AbuMweis et al 2006). These, results are inconsistent with those of Hyun et al and Doornbos et al. Overall, the data available at present do not allow a conclusion that a given time during the day should be preferred over others for once-a-day plant sterol ingestion.

C. *Relevance of evidence*

LDLC is the most studied risk factor for cardiovascular disease and the main standard based on which the NCEP estimates risk and recommends therapy, including the use of 2g/day of stanols/sterols as part of the multifaceted lifestyle approach, known as Therapeutic Lifestyle Changes (TLC), to reduce risk for CHD. According to ATPIII, TLC are the foundation of primary prevention, and an integral part of the secondary prevention. Thus, LDLC is the primary focal point of this petition, though other factors are considered as appropriate.

The magnitude of the LDLC lowering effect reported in the five high quality studies in which phytosterols were consumed once-a-day at a specified meal (either breakfast or lunch) is physiologically meaningful and achievable under once-a-day intake conditions. According to available scientific data, there is no reason to believe that phytosterol efficacy will vary according to time during the day, as long as they are consumed together with a meal. Therefore, it is expected that levels of LDLC reduction following the consumption of phytosterols once-a-day at breakfast, lunch or dinner are consistent with the original health claim authorized by FDA in 2000.

D. *Specific conclusions*

- 2 g/day of phystosterols is effective for once-a-day use in all adults.

- The LDLC reduction curve in response to 2 g/day of phytosterols consumed once-a-day is consistent with the LDLC reduction curve in response to 0.7 - 4g/day consumed 2 or 3 times a day reported in the literature. An intake of 2g/day as recommended by NCEP ATPIII and consumed once-a-day with a meal can produce a significant and meaningful LDC reduction in the vicinity of 10%. Beyond this dosage a much greater benefit is not expected.

- For optimal efficacy, plant sterol-enriched foods should be consumed at meal time.

- The LDLC reduction effect of once-a-day consumption of 2 g of phytosterols at breakfast, lunch or dinner is expected to be comparable to that of twice-a-day consumption of 2 g of phytosterols divided equally into two servings and consumed with meals.

- Considered as a whole, the strength of the evidence provides a high level of comfort among qualified scientists that the relationship between phytosterols and heart disease based on once-a-day use at meals is scientifically valid. Enclosed with this petition are letters from three independent experts qualified by scientific training and experience to evaluate health claims. These experts are Penny M. Kris-Etherton, Ph.D., RD (Pennsylvania State University); Ernst J. Schaefer, M.D. (Tufts University); and Peter J. Jones, Ph.D. (University of Manitoba). Each of these experts supports the proposed amendment to the health claim and concludes that it is supported by significant scientific agreement. (Appendix D.)

E. *Overall conclusion*

A phytosterol intake of 2 g/d is effective for once-a-day use at meal time in all adults. The studies supporting this conclusion are of high quality; reflect consistency with each other, with the scientific literature, and with recommendations of authoritative health agencies. The studies include sufficient numbers of subjects to provide a high degree of confidence that results are relevant to the target population. Accordingly, the amended health claim proposed by this petition, is supported by the totality of publicly available scientific evidence, including evidence from well-designed studies conducted in a manner which is consistent with generally recognized scientific procedures and principles. Further, there is significant scientific agreement, among experts qualified by scientific training and experience to evaluate such a claim, that the claim is supported by such evidence.

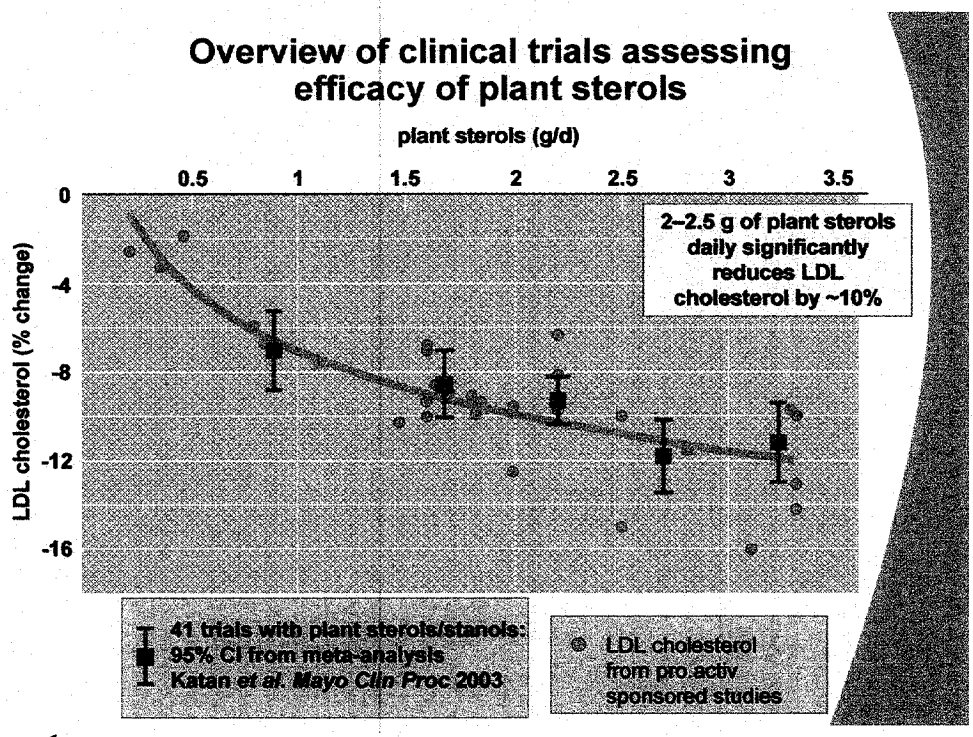
Overview of clinical trials assessing efficacy of plant sterols
plant sterols (g/d)
0.5
1
1.5
2
2.5
3
3.5
0
-4
-8
-12
-16
LDL cholesterol (% change)
2–2.5 g of plant sterols daily significantly reduces LDL cholesterol by ~10%
41 trials with plant sterols/stanols: 95% CI from meta-analysis Katan et al. Mayo Clin Proc 2003
LDL cholesterol from pro.activ sponsored studies

Figure 1.