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PETITIONER: McNEIL CONSUMER HEALTHCARE
7050 CAMP HILL ROAD
FORT WASHINGTON, PA 19034

SUBJECT OF PETITION: PLANT STANOL ESTERS

FOOD AND DRUG ADMINISTRATION
OFFICE OF FOOD LABELING (HFS-150)
200 C STREET SW
WASHINGTON, DC 20204

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The undersigned, Gilbert A. Leveille of McNeil Consumer Healthcare, submits this petition pursuant to Section 403 (r)(4) and 403(r)(5)(D) of the Federal Food, Drug & Cosmetic Act with respect to plant stanol esters and the claim that plant stanol esters reduce blood total and LDL-cholesterol levels and may reduce the risk of heart disease.

Attached hereto, and constituting a part of this petition, are the following:

A. Preliminary Requirements
B. Summary of Scientific Data
C. Chemistry and Analyses
D. Proposed Health Claim (Including Qualifying Criteria and Model Health Claim)
E. EPA-Categorical Exclusion
F. Conclusion
G. References
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A. PRELIMINARY REQUIREMENTS

1. Food Substance

The food substances that are the subject of this petition are plant stanol esters. A more detailed description of this food substance is provided in Section C.1. The food substance may be incorporated into a variety of conventional foods as well as into a dietary supplement. Ingestion of plant stanol esters as a regular part of the daily diet, has been shown to reduce blood levels of total and LDL-cholesterol without changing HDL-cholesterol or triglyceride levels, and may, therefore, reduce the risk of heart disease.

2. Eligibility

The food substance covered by the petition, plant stanol esters, meets all of the eligibility requirements for a health claim in 21 CFR §101.14(b):

a. Association of Plant Stanol Esters and a Reduced Risk of Heart Disease

Plant stanol esters have been demonstrated clinically to significantly lower serum total and LDL-cholesterol levels (see Section B - Summary of Scientific Data). FDA has on numerous occasions recognized the relationship between serum cholesterol levels and coronary heart disease (CHD). [See 21 CFR §101.75(b)(1) (dietary saturated fat and cholesterol); 101.77(b)(1) (fruits, vegetables and grain products that contain fiber, particularly soluble fiber); 101.81(b)(1) (soluble fiber from certain foods) and 101.82(b)(1) (soy protein)]. Specifically, in these health claim regulations, FDA has stated:

> **CHD is a major public health concern in the United States. It accounts for more deaths than any other disease or group of diseases. Early management of risk factors for CHD is a major public health goal that can assist in reducing risk of CHD. High blood total and LDL-cholesterol are major modifiable risk factors in the development of CHD.**

Despite decreases in CHD death rates over the past 30 years, CHD remains the leading cause of death for both men and women in the United States (US), accounting for more than 475,000 deaths and contributing to approximately 250,000 additional deaths in 1996. CHD accounts for 50% of all cardiovascular disease related deaths and approximately two-thirds of deaths due to heart disease (National Heart, Lung and Blood Institute, 1998).

In 1995, it is estimated that 2,130,000 hospitalizations and 9,941,000 visits to physicians offices were the result of CHD (National Heart, Lung and Blood Institute, 1998). The prevalence of CHD is estimated at 12,000,000 persons. It is the leading cause of premature, permanent disability in the US labor force, accounting for 19% of disability allowances by the

Social Security Administration. CHD has a significant effect on US health-care costs. For 1999, total direct costs related to CHD are estimated at $53 billion and indirect costs from lost productivity associated with morbidity (illness and disability) and mortality (premature deaths) at $46.7 billion (American Heart Association, 1998).

As stated in the National Cholesterol Education Program (NCEP), Report of the Expert Panel on Population Strategies for Blood Cholesterol Reduction 1990, epidemiological, clinical and experimental evidence clearly demonstrate that the likelihood that a person will develop or die from CHD is directly related to the level of blood cholesterol. The relationship is continuous and the risk increases with rising cholesterol levels. It is also well established that lowering blood cholesterol levels is associated with a decreased risk of CHD (NCEP, 1990). The NCEP in 1993 reaffirmed that an increased blood cholesterol level, specifically high LDL-cholesterol, increases risk for CHD. Conversely, lowering total cholesterol and LDL-cholesterol levels reduces CHD risk (NCEP, 1993). Two approaches can be taken to lower blood cholesterol levels in the American population. One is a clinical approach that identifies individuals at high risk who need intensive intervention efforts. The second is a public health (population) approach that aims to shift the distribution of cholesterol levels in the entire population to a lower range through dietary change. The two approaches are complementary, and together represent a coordinated strategy for reducing coronary risk. **It is estimated that for every 1% reduction in serum cholesterol a 2 - 3% reduction in CHD is seen (NCEP, 1993).**

Data from the Third National Health and Nutrition Examination Survey (NHANES III) estimated that 20% of American adults have high total blood cholesterol (240 mg/dL and above) and an additional 31% have borderline high levels (between 200 and 239 mg/dL) (Sempos et al., 1993). These observations establish that half of the US population is at risk for CHD and that an effective dietary approach to reducing blood cholesterol levels represents a major public health benefit.

b. Plant Stanol Esters are Food Substances that Provide Nutritive Value and are Safe

Plant stanol esters are to be consumed at "other than decreased dietary levels". As required by 21 CFR §101.14(b)(3)(i) and (ii) plant stanol esters provide nutritive value and are safe.

1) Plant Stanol Esters Provide Nutritive Value

Plant stanols and stanol fatty acid esters occur in nature and are ingested as components of food. The main dietary sources of stanols and stanol esters are wheat, rye and corn (Barnes, 1983; Dutta and Appelqvist, 1996; MacMurray and Morrison, 1970). Since virtually

no absorption of the plant stanol component of plant stanol esters occurs and they are excreted unchanged in the stool, dietary intake can be estimated from the amount of stanols excreted in the feces (Lütjohann et al, 1995; Czubayko et al, 1991; Hallikainen and Uusitupa, 1999; Gylling and Miettinen, 1999). Fecal analysis from subjects consuming their usual diets show mean excretion rates of 20 - 30 mg of stanols per day (Gylling and Miettinen, 1994; Czubayko et al, 1991; Gylling and Miettinen, 1999).

The natural occurrence of plant stanol esters and plant stanols in foods has been well documented. Plant stanols have been found in cereal grains, brans, vegetable oils, seeds, seedling sprouts, coffee, and may occur in some vertebrate aquatic animals (Barnes, 1983; Dutta and Appelqvist, 1996; MacMurray and Morrison, 1970).

Plant stanol esters provide nutritive value in the maintenance of healthier cholesterol levels. In addition, plant stanol esters provide energy. A serving of food containing the proposed minimum level of 0.85 g plant stanol esters would provide approximately three calories from the fatty acid moiety.

2) Plant Stanol Esters are Safe and Lawful

Plant stanol esters are generally recognized as safe (GRAS). In February 1999, McNeil Consumer Healthcare submitted information to FDA setting out the basis for the GRAS status of plant stanol esters (see Food Master File 000626 which is hereby incorporated by reference). After reviewing this information, FDA stated it had no questions regarding the safety of this ingredient (see Letter from Alan Rulis, PhD, Director, Office of Premarket Approval, dated May 17, 1999, which is also hereby incorporated by reference).

B. SUMMARY OF SCIENTIFIC DATA

In accordance with 21 CFR §101.70(D) all McNeil clinical trials were conducted under good clinical practices and were administered under Institutional Review Board (IRB) approval and with subject informed consent. For published trials, the methods sections of the respective journal articles describe review boards/ethics committees utilized and/or if subject informed consent was obtained. All unpublished studies were conducted under IRB review and approval and subject informed consent.

1. Significant Scientific Agreement

FDA may authorize a health claim when there is significant scientific agreement among qualified experts that the totality of the publicly-available scientific evidence supports the claimed health benefit. 21 U.S.C. §403 r(3)(B); 21 CFR §101.70(F). The available data on plant stanol esters easily satisfies that standard.

"Significant scientific agreement" has been established through multiple, peer-reviewed, published clinical studies demonstrating the cholesterol lowering effects of the regular dietary use of plant stanol ester. The most compelling clinical data are derived from double-blind, placebo-controlled studies. This methodology is generally accepted within the scientific community as the most rigorous and reliable means to evaluate the health benefits of a food substance. FDA has stated that, in reviewing a health claim petition, the Agency "will give the greatest weight in its evaluation to well-designed studies conducted with human subjects". The clinical data - along with the other supporting data in this petition - provide the "sound body of valid scientific evidence" necessary to support a health claim [58 Fed. Reg. 2504, 2506 (Jan. 6, 1993)].

More than 40 years of extensive research led to the recognition of the cholesterol-lowering potential of plant stanol esters. In a recent review article, Dr. Tu Nguyen of the Mayo Clinic reviewed the available data on the cholesterol-lowering action of plant stanol esters. From his literature review he concludes that levels of 3.4 – 5.1 g per day of plant stanol esters have been shown to reduce LDL cholesterol. In addition, his review states that "The esterification of plant stanols has allowed their incorporation into various foods such as margarine without changing the taste and texture of those foods..... Plant stanol esters appear to be a helpful dietary adjunct to a prudent diet to lower cholesterol." (Nguyen, 1999)

An additional review of the cholesterol lowering efficacy of plant stanol esters and their implications for CHD risk reduction is in press (Cater, in press). Dr. Cater concludes "Plant stanol ester has been shown in over 20 studies to be an effective and safe cholesterol-lowering ingredient" and "Results of studies with plant stanol ester have important

implications for both the clinical and public health approaches to CHD risk reduction." He further notes randomized, placebo controlled studies indicate that 3.4 - 5.1 g per day of plant stanol esters significantly reduce LDL-cholesterol levels by 10 - 20%. These results have been consistently found in a variety of population groups, including young and elderly men, pre- and postmenopausal women, children with and without hypercholesterolemia, type 2 diabetics and people with CHD. Finally he observes, no clinically significant adverse effects have been detected in studies or in Finland were plant stanol ester containing foods have been available since 1995.

An extensive database exists on plant stanol esters, including 23 published clinical trials (17 trials in peer-reviewed publications, three trials in published journal supplements, and three trials as published abstracts) demonstrating significant reductions in serum total and LDL-cholesterol levels. Eleven additional trials have been completed and the results are yet to be published. The substantial body of published reports provides impressive and consistent evidence of the efficacy and safety of plant stanol esters.

2. Overview

Plant stanols, such as sitostanol and campestanol, occur naturally in small quantities in the lipid fractions of cereal grains such as wheat, rye and corn, (Barnes, 1983; Dutta and Appelqvist, 1996; MacMurray and Morrison, 1970) and in vegetable oils such as corn and olive oil (Schuhmann and Schneller, 1996; Boskou, 1996). The average western diet provides 160-360 mg of plant sterols and 20-50 mg of plant stanols daily (Cater and Grundy, 1998). Despite their molecular similarity, absorption of these compounds differs markedly, ranging from 4 - 5% for plant sterols and less than 0.5% for plant stanols. Esterification of plant stanols with fatty acids renders plant stanols readily soluble in foods. This process makes an effective vehicle for delivery of plant stanols to the small intestine. Even though they are virtually unabsorbed, ingestion of 3.4 - 5.1 g plant stanol esters daily leads to reduced absorption of cholesterol and a consistent cholesterol lowering effect which can reduce the risk of CHD.

In trials with adults having elevated serum cholesterol, consumption of at least 3.4 g per day of plant stanol esters, significantly lowered total cholesterol and LDL-cholesterol. Studies in adults with normal serum cholesterol also showed that consumption of more than 3.4 g per day plant stanol esters and up to 5.1 g per day plant stanol esters lowered LDL-cholesterol significantly, up to 14%. Studies in adults with CHD and non-insulin-dependent diabetes mellitus (NIDDM) showed LDL-reductions of 9 - 15%. In children with elevated serum cholesterol, consumption of 5.1 g per day plant stanol esters lowered LDL-cholesterol 15%. Use of 2.5 g per day plant stanol esters lowered LDL-cholesterol 7% in normal healthy

children. In all of these subject types, a consistent pattern of LDL-cholesterol reduction with adequate consumption of plant stanol esters was observed.

In summary, this document will review published and unpublished data that demonstrate consumption of at least 3.4 g per day plant stanol esters produces significant reductions in serum total and LDL-cholesterol levels. A significant number of clinical trials demonstrate that a greater reduction of serum total and LDL-cholesterol levels occurs when up to 5.1 g plant stanol esters is used daily. **The LDL-cholesterol reduction of up to 14% with the ingestion of 5.1 g plant stanol esters daily can potentially reduce the risk of CHD by 28 - 42% in the US. This ingredient can have a significant public health impact in reduction of cardiovascular morbidity and mortality.**

3. Review of Published Studies

A total of 23 published clinical trials provide data on the efficacy and safety of plant stanol esters. Nine of these published trials were conducted in adults with elevated serum cholesterol and are summarized in Appendix H.1; three of the published trials were conducted in adults with normal cholesterol and are summarized in Appendix H.2; three published trials were conducted in adults with CHD which are summarized in Appendix H.3; three published trials were conducted in adults with NIDDM and are summarized in Appendix H.4; six of the published trials were conducted in adults using cholesterol-lowering medications and are summarized in Appendix H.5; two published trials were conducted in children with elevated cholesterol levels and are summarized in Appendix H.6; one published study was conducted in children with normal cholesterol and is summarized in Appendix H.7.

Overall, adequate consumption of plant stanol ester containing foods (3.4 g to 5.1 g daily) significantly reduced total and LDL-cholesterol in published clinical trials. These data demonstrate that plant stanol ester containing foods can have a significant public health benefit by reducing the risk of CHD in the US.

4. Review of Unpublished Studies

The results of 11 clinical trials evaluating the efficacy of plant stanol esters have not been published to date. Summaries of these completed, unpublished clinical studies, including one study in press, are presented in Appendix H.8.

In these studies, the investigators examined the effect of food form or as a dietary supplement, consumption level-response relationship, the frequency of consumption, and the effect of dietary or prescription drug intervention on the effectiveness of plant stanol

esters. The study results indicate that plant stanol esters are effective and that dietary or drug interventions and higher levels of consumption (>5 g per day) are associated with greater reductions in LDL-cholesterol. These data support the conclusion that plant stanol ester-containing foods can have a significant public health benefit by reducing the risk of CHD in the US.

5. Specificity of the Cholesterol-Lowering Effect of Plant Stanol Esters

The clinical trials presented in this health claim petition, support the conclusion that plant stanol esters are responsible for the observed reductions in serum total and LDL-cholesterol. These studies used a variety of plant stanol ester-containing foods and dietary regimens, including diets low in saturated fat and cholesterol, as well as the replacement of similar fats in the diet without other dietary changes. The majority of the studies in this petition use plant stanol ester containing products and control products with similar total fat, saturated fat and cholesterol content. A recent review article has addressed the issue of specificity of the effect. The author concluded that plant stanol esters at a level of 3.4 - 5.1 g per day reduce LDL-cholesterol (Nguyen, 1999).

6. Proposed Mechanism for the Cholesterol-Lowering Effects of Plant Stanol Esters

Cholesterol absorption in the intestine is a complicated process. Three major barriers must be overcome before cholesterol is absorbed. The first is the transformation of cholesterol into a soluble form. Solubilization is accomplished by the formation of mixed micelles, which are composed of bile acids, lysolecithin, monoglycerides, and fatty acids. These other lipids function as detergents that facilitate solubilization of cholesterol and facilitate cholesterol transport to close proximity with the intestinal mucosal cell. Cholesterol must be solubilized to be absorbed. Some insoluble unesterified cholesterol molecules never become solubilized; only 40 - 60% of cholesterol in the intestine is actually absorbed.

The second barrier to cholesterol absorption is the diffusion step. Cholesterol must pass from the mixed micelle in the lumen into the intestinal mucosal cell by monomolecular diffusion through a thin, unstirred water layer. The third barrier is esterification. In the mucosal cell, cholesterol must be esterified before it can be incorporated into chylomicrons and secreted into the bile. Thus, cholesterol absorption is tenuous because it requires special mechanisms, with each step in the process being a potential barrier to absorption and a potential target of agents that can interfere with absorption (Cater and Grundy, 1998).

Plant stanol esters decrease the absorption of both exogenous and endogenous cholesterol. After hydrolysis to free stanols and fatty acids, the stanols inhibit absorption by competing with cholesterol for incorporation into mixed micelles. It is unknown if plant stanol esters interfere with other steps in cholesterol absorption.

7. Proposed Recommended Daily Intake of Plant Stanol Esters

A minimum daily intake of 3.4 g plant stanol esters has been shown in clinical studies to significantly reduce total and LDL-cholesterol and likewise, reduce the risk of CHD. By consuming 5.1 g plant stanol esters, a greater LDL-cholesterol reduction can be seen.

Two well-controlled trials demonstrate the efficacy of 3.4 g plant stanol esters daily. In Miettinen et al. (1995), 51 subjects substituted a spread containing 3.4 g plant stanol esters for dietary margarine or butter for six months. Mean LDL-cholesterol was reduced 12.1% when compared to the control spread group. This reduction is statistically significant ($p<0.001$). In a second study, 82 subjects substituted a spread containing 3.4 g per day plant stanol esters for dietary margarine or butter for eight weeks (Nguyen et al., 1999). In this group, the mean decrease in LDL-cholesterol vs. baseline was 4.6%, $p<0.001$. This was a significant decrease when compared with the 0.8% increase in LDL-cholesterol vs. baseline observed in the control spread group, $p=0.01$. In both cases, these reductions are clinically significant since reductions in LDL-cholesterol are associated with reduced risk of cardiovascular disease.

A number of clinical trials have demonstrated the effectiveness and safety of 4.5 - 5.1 g plant stanol esters in adults and children (Miettinen et al., 1995; Gylling and Miettinen, 1999; Nguyen et al., 1999; Gylling et al., 1995). In these clinical studies, subjects were instructed to substitute plant stanol ester products for their usual foods (i.e., substitute plant stanol ester spread for margarine or butter) three times per day for a total of 5.1 g plant stanol esters.

It is proposed that the amount of plant stanol esters required to qualify an individual food or dietary supplement to bear the plant stanol esters and heart disease health claim be established at 0.85 g plant stanol esters per serving. This level is derived by dividing the recommended intake of 3.4 g plant stanol esters by four daily servings. Each serving would, therefore, provide 25% of the minimum recommended daily intake. The most effective benefit can be achieved by ingesting 5.1 g per day plant stanol esters.

a. Level of Intake Where There May Be Potential for Adverse Effects

Animal toxicity studies of plant stanol esters show that there is no evidence of acute toxicity and there is no suggestion of any irritant or allergenic potential. A subchronic toxicity study in rats reported no evidence of systemic toxicity at dietary levels up to 8.5% plant stanol esters in the diet (Turnbull et al., 1999). Overall, the animal toxicology studies demonstrate a no-observed-adverse-effect level (NOAEL) of at least 1.7% plant stanol esters in the diet, corresponding to a daily intake of about 870 mg of plant stanol esters per kg body weight, or about 61 g of plant stanol esters for a 70 kg adult human (Turnbull et al., 1999).

In two clinical studies, subjects consumed larger quantities of plant stanol esters. Plat and Mensink (1998) examined the clinical safety aspects of two plant stanol ester mixtures in normal, healthy volunteers. During this randomized, double-blind, placebo-controlled study, 112 subjects consumed one of three test diets for eight weeks after a four-week canola oil spread and shortening run-in period. The test diets included spread and shortening, or spread and shortening with 6.5 or 6.8 g per day plant stanol esters. At the end of the trial (12 weeks), subjects completed questionnaires about possible adverse events experienced during the trial. Specific information was requested about the occurrence of "physical inconveniences," which included headache, stomach complaints, nausea, abdominal distention, flatulence, diarrhea, intractable constipation, itching, skin eruptions/rashes, fatigue, and dizziness. On the basis of the study questionnaire, there were no significant differences between treatment and control groups in specific complaints, and the reported complaints were not related to dietary treatment and were not considered clinically significant.

A recent clinical trial examined the safety and effectiveness of 5.1 g, 10.2 g and 17 g plant stanol esters consumed daily for two weeks in 90 subjects (McNeil Protocol 98-058). This trial was conducted to assess the tolerability of higher servings of plant stanol esters. Subjects (30 per group) were advised to substitute plant stanol ester-supplemented spreads and/or yogurt for similar amounts of fat normally consumed in their daily diet. Adverse events were monitored throughout the study. The results of the safety analysis showed no statistically significant differences between groups.

It is important to note that study subjects consumed a range of 24 g of spread in the 5.1 g plant stanol ester group to an unusually high intake of 40 g of spread and 20 ounces of yogurt in the 17 g plant stanol ester per day group. No subjects in either the 5 g or the 10 g per day plant stanol esters group withdrew from the study because of adverse events. Five subjects (16.7%) in the 17 g plant stanol esters per day group withdrew from the study because of adverse events; three withdrawals were possibly thought to be related to the

larger amounts of plant stanol esters-containing food products. The most common digestive system events judged likely to be related to the products were diarrhea, eructation and flatulence; most events were mild in intensity. No adverse events were considered serious.

b. Special Population Groups Which May Be Adversely Affected

There is no indication that any special population group may be adversely affected by products containing plant stanol esters. Special population groups that could possibly be adversely affected by plant stanol esters are non-insulin dependent diabetics for reasons of glycemic control, children because of their special nutritional requirements, and individuals with phytosterolemia because of this unusual lipid storage disease. Examination of the published data, however, suggests that these groups would not be adversely affected by products containing plant stanol esters. A brief discussion of these special population groups follows.

1) Studies in Adults with Non-Insulin Dependent Diabetes Mellitus (NIDDM)

In a double-blind, crossover study in 11 men with NIDDM (Gylling and Miettinen, 1994), subjects were randomly assigned to a six-week control period, or a six-week trial period using plant stanol esters (5.1 g/day of plant stanol esters) in canola oil spread after a four-week run-in period where subjects followed their normal diet. Follow-up with the investigator confirmed active collection of safety data, with no side effects or laboratory value abnormalities reported. Serum triglyceride, phospholipid levels and bile acid synthesis were unchanged. There was no significant change in glycosylated hemoglobin or body weight.

Two other trials in diabetics are discussed below in the section on subjects also consuming cholesterol-lowering drugs (Gylling and Miettinen, 1995; Gylling and Miettinen, 1996). No significant changes in glycemic control were observed in the three studies.

2) Studies in Children with Elevated Cholesterol

Gylling et al. (1995) assessed the effects of dietary plant stanol esters in 14 children with familial hypercholesterolemia in a double-blind, randomized, complete crossover study. The children were divided into two groups and received either canola oil spread alone or the spread supplemented with plant stanol esters (5.1 g/day stanol esters, or 140 mg/kg/day) for 6 weeks in random order. The stanol ester spread was well tolerated.

Follow-up with the author yielded no further issues regarding safety. The HDL/LDL ratio in this study increased significantly by 27%. The body weights of the children in the two groups increased only slightly and similarly during the two test periods. HDL-cholesterol levels

increased (not significantly) and triglycerides decreased (not significantly) in the plant stanol ester group. (Compared with spread-only controls, total and LDL-cholesterol declined significantly for children receiving plant stanol esters.)

3) Studies in Children with Normal Cholesterol

A randomized, crossover trial in 72 children (Tammi et al., 1999) reported that plant stanol ester spread (2.5 g/day plant stanol esters in margarine for 3 months) was well tolerated and no adverse clinical effects were observed. The children were participants in the Special Turku Risk Intervention Program or STRIP project, a randomized, prospective trial aimed at reducing the predisposition of children to known arteriosclerosis risk factors. The children were already on a diet low in saturated fat and cholesterol. After consumption of plant stanol ester spread, there were no adverse effects observed. In addition, there was no change in HDL-cholesterol or triglyceride levels. Plant stanol esters reduced total and LDL-cholesterol significantly, from baseline, and serum campesterol was also reduced.

4) Subjects with Phytosterolemia

Phytosterolemia is an extremely rare, inherited lipid storage disease; only 50 people have been identified worldwide. It is associated with elevated absorption and reduced hepatic clearance of plant sterols, resulting in elevated plasma concentrations of these compounds. Lütjohann et al. (1995) studied serum lipids in three of these subjects and concluded these subjects had significantly elevated campesterol and sitosterol levels when compared with control subjects. Measurable levels of campestanol and sitostanol were found in subjects with phytosterolemia as well, presumably derived from the conversion of the absorbed plant sterols. Consumption of 1.5 g sitostanol for four weeks reduced cholesterol absorption 24% and 44% in two subjects with phytosterolemia. Serum sitosterol decreased 10% and 25% in these two subjects while serum sitostanol levels were unchanged (+4% and -13%). Additionally, fecal excretion of cholesterol and plant sterols increased in these two subjects during sitostanol consumption.

c. Use of Plant Stanol Esters with Cholesterol-Lowering Medications

Vanhanen (1994) investigated whether consuming plant stanol esters (2.6 g/day plant stanol esters contained in a canola-based spread) or neomycin for six weeks would result in additional cholesterol-reducing effects in 14 patients already taking the cholesterol synthesis-inhibiting drug pravastatin (40 or 80 mg/day). Serum HDL-cholesterol and triglycerides did not change significantly in this study. Limited laboratory chemistry values were collected, with no reports of change. The addition of plant stanol ester reduced total

and LDL-cholesterol, although not significantly, when compared to the reduction caused by pravastatin alone.

Although the Vanhanen (1994) study failed to demonstrate a significant additive cholesterol-lowering effect with plant stanol esters and pravastatin, Gylling and Miettinen (1996) found an additional cholesterol-lowering effect at higher plant stanol ester consumption levels. In this study, seven NIDDM men consumed the following in four seven-week regimens: (a) canola oil spread, (b) spread plus 5.1 g/day plant stanol esters, (c) spread plus 40 mg/day pravastatin, and (d) spread plus 5.1 g/day plant stanol esters plus 40 mg/day pravastatin. Plant stanol esters had no effect on HDL-cholesterol or triglyceride levels, or on the metabolic parameters related to apoprotein A-1 concentration. Weight and glycemic control were also unchanged. The plant stanol margarines and pravastatin were reported to be "well tolerated without any side effects." Plant stanol esters alone lowered total and LDL-cholesterol by 11% and 14%, respectively; plant stanol esters consumed in addition to pravastatin reduced total and LDL-cholesterol by 35% and 44%, respectively.

Gylling and Miettinen (1995) investigated whether there was an additional effect of plant stanol esters on patients receiving the cholesterol-reducing drug neomycin. Six male non-insulin-dependent diabetic subjects received canola oil spread for a control period of six weeks, followed by 1.5 g/day of neomycin and control spread for four weeks, then 1.5 g/day neomycin plus 5.1 g/day plant stanol esters in spread for an additional four weeks. Plant stanol esters consumed with neomycin was reported to be well tolerated. Glycemic balance, body weight and renal function remained unchanged. HDL-cholesterol did not change, and triglyceride levels were decreased in the plant stanol ester plus neomycin groups as compared to the neomycin alone group. Neomycin alone reduced serum total and LDL-cholesterol by 26% and 29%, respectively. Plant stanol esters combined with neomycin further decreased both serum total and LDL-cholesterol by 12%.

In a study performed in postmenopausal women with a history of previous myocardial infarction, ten women who had used simvastatin (10-20 mg/day) for one year were instructed to use 21 g/day canola oil spread (with 5.1 g plant stanol esters) for 12 weeks, replacing a similar amount of their daily fat intake (Gylling et al., 1997). With consumption of plant stanol ester spread while taking simvastatin, mean serum triglyceride levels were further reduced (not significantly) and HDL-cholesterol values increased (not significantly). Body weights were not affected. Follow-up with the investigator confirmed active solicitation of safety data, with no adverse events reported. Combined with simvastatin, plant stanol esters reduced serum total cholesterol and LDL-cholesterol by an additional 11.3% and 16.5%, respectively.

8. Effect of Increased Plant Stanol Ester Intake on Nutrient Consumption of US Diet

McNeil Consumer Healthcare has conducted an extensive evaluation of the safety of plant stanol esters with respect to fat-soluble vitamin and nutrient status. The results of this evaluation were submitted to the FDA in May 1999. Based on published and unpublished data/literature, extensive product use in Finland (with safety surveillance), animal studies and a review of all data by a panel of qualified experts in the field, McNeil Consumer Healthcare concludes that plant stanol esters do not affect fat soluble vitamin and nutrient status.

Vitamins A, E and the carotenoids, and dietary forms of vitamins D and K, are fat-soluble nutrients which, like cholesterol, have a common requirement for intestinal solubilization from lipid droplets into mixed micelles in order for nutrient absorption by the enterocyte to occur. Plant stanol esters are not known to bind to any substance in the gastrointestinal tract; rather plant stanols specifically block the absorption of cholesterol and plant sterols at the micellular level in the intestine. Plant stanol esters present little potential to affect the absorption of fat soluble vitamins because they do not cause fat malabsorption.

Despite significant reductions seen in serum cholesterol, plant stanol ester ingestion has not been accompanied by clinically significant changes in fat soluble vitamin status in human clinical trials. Several studies on plant stanol esters included a specific evaluation of fat soluble vitamin status. The findings of many of these studies have been reported in published literature and/or presented at scientific meetings and symposia attended by numerous participants with backgrounds in the fields of nutrition, lipidology, cardiology, and medicine. These studies clearly demonstrate that plant stanol esters do not affect fat soluble vitamin/nutrient status.

Short- and long-term human clinical studies have consistently demonstrated that there is no significant effect on fat-soluble vitamin and nutrient status with plant stanol ester consumption. In addition, widespread use of plant stanol ester spread in Finland for over four years and follow-up in the FINRISK public health survey has revealed no fat-soluble vitamin related issues.

C. CHEMISTRY AND ANALYSES

1. Description of Plant Stanol Esters

a. Chemical Identity for Plant Stanol Esters

The substance of this petition is a mixture of plant stanols esterified to food grade fatty acids. The stanols are primarily sitostanol and campestanol and may be derived from hydrogenated plant sterol mixtures or extracted from plant sources. The principal plant stanol esters are fatty acid esters of the plant 5α-stanols, sitostanol and campestanol. Sitostanol (24-ethylcholestan-3β-ol, CAS No. 19466-47-8) is formed by the hydrogenation of the Δ^5-mono-unsaturated plant sterol, sitosterol (24-ethylcholest-5-en-3β-ol, CAS No. 83-46-5), and also by the complete hydrogenation of the $\Delta^{5,22}$-di-unsaturated plant sterol, stigmasterol (24-ethylcholest-5,22-dien-3β-ol, CAS No. 83-48-7), hence the alternative name "stigmastanol." Campestanol (24-methylcholestan-3β-ol) is formed by the hydrogenation of the Δ^5-mono-unsaturated plant sterol, campesterol (24-methylcholest-5-en-3β-ol, CAS No. 474-62-4).

Synonyms for sitostanol are β-sitostanol, 5,6-dihydro-β-sitosterol, 24-α-ethylcholestanol, dihydrositosterin, fucostanol, spinastanol, stigmastanol. Sitosterol is often referred to in the scientific literature as β-sitosterol.

The fatty acids (e.g., oleic acid, $C_{18}H_{34}O_2$) are derived from appropriate food grade products.

b. Chemical Structure of Principal Plant Sterols and Plant Stanols

The structure of sitostanol, of its immediate precursors sitosterol and stigmasterol, of campestanol, and of its immediate precursor campesterol, are shown below in Figure 1. (The structure of cholesterol is shown for comparison.)

c. Chemical Structure of Plant Stanol Esters

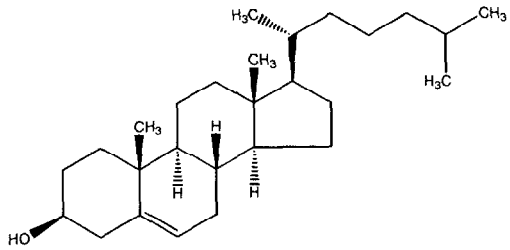
The mixture of plant stanols (primarily sitostanol and campestanol) resulting from the hydrogenation of mixed plant sterols is esterified using methyl esters of fatty acids up to C-24. Figure 2 shows the structures of sitostanol and campestanol fatty acid esters.

d. Physical Characteristics

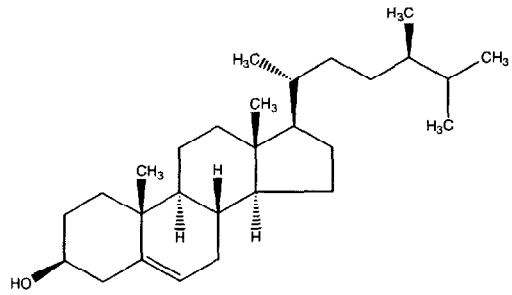
The physical characteristics of any batch of plant stanol esters are determined primarily by the fatty acid profile of that batch, which, in turn, is dependent on the fatty acid source. Plant stanol esters exhibit a polymorphic behavior similar to that of normal crystallizing triglyceride

fats. Thus, the basic procedures used for handling and producing products made from plant stanol esters are those used for normal fats. Carbon chain length and the degree of unsaturation of the fatty acids affect the melting point of the esters, i.e., melting points decrease with increasing molecular weight of saturated fatty acid until a minimum is reached at the C_{14}-C_{16} region after which the melting points increase.

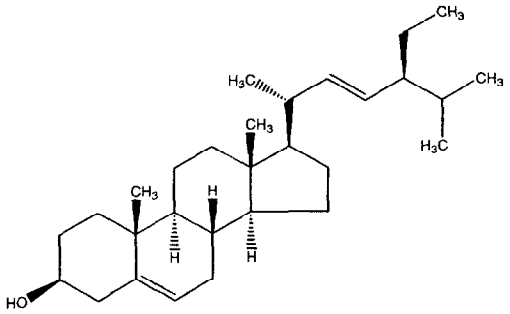
Figure 1: Sterol and Stanol Structures



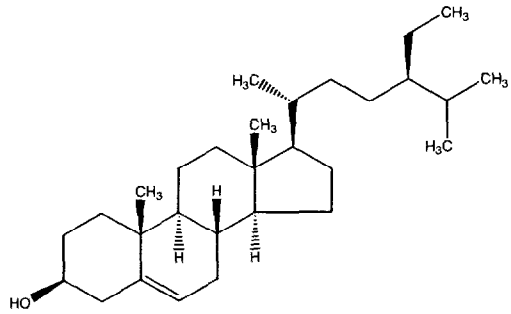
Cholesterol



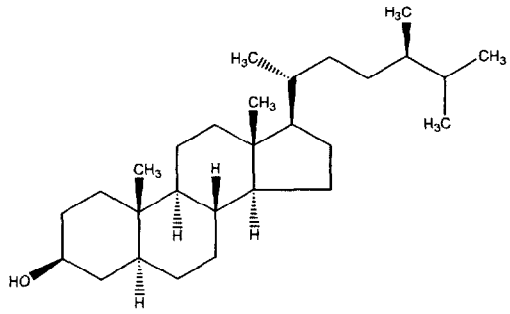
Campesterol



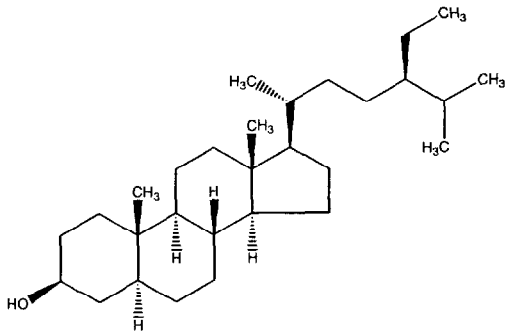
Stigmasterol



Sitosterol

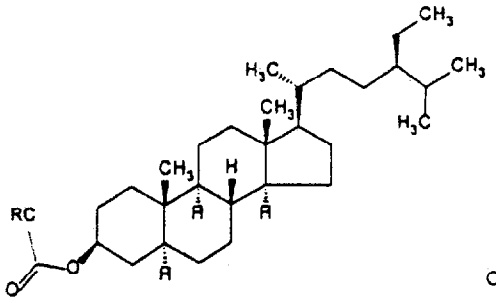


Campestanol

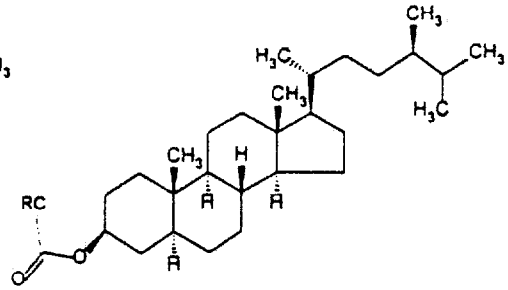


Sitostanol

FIGURE 2. STRUCTURES OF PLANT STANOL ESTERS



SITOSTANOL FATTY ACID ESTER



CAMPESTANOL FATTY ACID ESTER

(R= aliphatic, up to C-23)

The physical characteristics of plant stanol esters derived predominantly from canola oil, for example, are as follows:

Table C-1. Physical Properties of Plant Stanol Esters with Fatty Acids Derived from Canola Oil	
Property	**Description**
Appearance/physical form	whitish, waxy solid at room temperature
Taste	bland, no obvious flavor
Solubility	insoluble in water soluble in non-polar solvents soluble in oils and fats
Melting Point Range	25.7 to 38.8°C

Physically, stanol esters derived from wood and vegetable oil are virtually indistinguishable as demonstrated by their melting point curves (Raisio BRG 1404-PCT).

e. Caloric Value

The caloric value of plant stanol esters manufactured by esterification with canola oil, as measured by bomb calorimeter, is 42.71 MJ/kg (10.2 kcal/g).

f. General Description of Manufacturing Process

Plant stanols are typically produced by hydrogenation of commercially available plant sterol blends. The plant stanols so obtained are converted to their fatty acid esters by an esterification process using fatty acid alcohol esters obtained from food-grade refined, bleached and deodorized vegetable oils, fats, or their blends (Miettinen et al., 1991).

2. Marker Analysis for Foods Qualifying for the Proposed Health Claim

To determine the amount of plant stanol esters in a single serving of food or dietary supplement, the most accurate and rugged analytical approach is to quantify plant stanol esters on the basis of "Plant Stanol" content. Since no AOAC method is available for this analysis, McNeil Consumer Healthcare has developed and validated analytical methods to quantify plant stanol esters on the basis of "plant stanol" content for all marketed products

containing plant stanol esters. These methods will be submitted to a peer-reviewed scientific journal.

To determine the plant stanol content of a food or dietary supplement containing plant stanol esters, a uniform analytical approach has been developed for quantitative and routine plant stanol analysis across all products. Analysis of plant stanols in food matrixes is achieved using a direct saponification procedure in which the stanol ester food is spiked with Internal Standard (cholestanol) and saponified directly with ethanolic potassium hydroxide (McNeil Consumer Healthcare, Analytical Method 88-AM-601). For dietary supplements, the plant stanol ester core is first extracted with organic solvent followed by direct saponification as described for plant stanol ester foods (McNeil Consumer Healthcare, Analytical Method 88-AM-701).

Following saponification, plant stanols are extracted with hexane, derivatized to trimethylsilyl ethers and analyzed by gas chromatography. Individual Plant Stanols (sitostanol, campestanol) are quantified against Internal Standard and Plant Stanol content calculated from the sum of sitostanol plus campestanol concentrations. Method validation testing across both food and dietary supplement forms has demonstrated that analysis of plant stanol esters as plant stanols is linear (r^2>0.999), accurate (98-102% plant stanol recovery), precise (<2.0% Relative Standard Deviation) and rugged between laboratories. A representative method validation report including statistical evaluation of the analytical method (accuracy, precision, linearity) is included (McNeil Consumer Healthcare, Method Validation Report MV-88-AM-601).

D. MODEL HEALTH CLAIM

1. Description of Food Eligible to Bear the Claim

a. Minimum Level of Plant Stanol Esters Per Serving

This petition defines a level of 0.85 g plant stanol esters per serving as the minimum amount for the use of the proposed claim. To obtain the amount of plant stanol esters which has been demonstrated to lower LDL-cholesterol, consumption of at least four servings of foods containing this amount per serving, would provide the recommended daily amount. Consumption of six servings per day would provide approximately 5.0 g of plant stanol esters, a more effective amount. A serving of food containing 0.85 g of plant stanol esters would contribute 25% of the recommended level for significant cholesterol reduction.

b. Disqualifying Nutrient Levels and Exemption for Small Servings

In accordance with 21 CFR 101.14 (a)(5), foods qualifying to bear the health claim must not exceed 20% or more of the daily recommended value (DRV) for the disqualifying nutrients of total fat, saturated fat, cholesterol, and sodium per serving of food, per "reference amount customarily consumed" (RACC) and, for small servings (less than 2 tablespoons or 30 grams), per 50 grams of the food. Foods eligible to bear the health claim sought by this petition will meet this requirement on a "per serving" and a "per RACC" basis. However, in a limited number of situations involving the use of stanol ester in foods consumed in small quantities, McNeil believes it is important to waive the "per 50 gram" criteria for the disqualifying level of fat for these small servings. To do otherwise would preclude the use of the health claim on the leading product on the US market containing plant stanol ester – Benecol spread. According to 101.14 (e)(3), FDA may permit a health claim despite the fact that a disqualifying level of a nutrient is present in the food based on a finding that such a claim will assist consumers in maintaining healthy dietary practices. McNeil believes a limited exemption from this criterion is necessary and justified for the following reasons:

1. Products qualifying for this exemption would be small servings only and would continue to comply with the fat levels in §101.14(a)(5) on a "per serving" and "per RACC" basis.

2. The health claim will encourage regular consumption of foods containing stanol ester and will instruct consumers on the appropriate number of servings necessary to achieve their health benefits. The claim, therefore, will help consumers develop a dietary approach that will result in significantly lower cholesterol levels and an accompanying reduction in the risk of heart disease. This substantial benefit outweighs any possible negative dietary consequences of foods qualifying for this narrow exemption.

3. Benecol foods are promoted as foods to be used in place of other similar foods. In the case of spreads, for example, Benecol spreads can be used as an alternative to butter, margarine or other spreads and, therefore, will not increase the overall level of fat in the diet while providing the cholesterol-lowering benefits of plant stanol esters.

In sum, McNeil believes this limited exemption from the "per 50 gram" criteria for fat will promote the availability of plant stanol ester in foods sold in small serving sizes that can be easily incorporated into the daily diet. The use of the claim on these foods will enable consumers to maintain a healthy dietary pattern that provides the substantial health benefits of plant stanol esters.

c. Exemption from Minimum Nutrient Content Requirement

The scientific evidence shows the cholesterol-lowering effect of plant stanol esters, is specific to this substance and is not dependent upon the effects of other components of a plant stanol ester-enriched diet. Due to the nature of plant stanol esters, the presence of 10% or more of the Reference Daily Intake or the Daily Reference Value for vitamin A, vitamin C, iron, calcium, protein, or fiber, stated in 101.14 (e)(6), has no relationship to the ingredient's health benefit. Individual foods containing plant stanol esters make up a relatively small portion of the daily diet. Consumers today have the nutrition information necessary to select the total daily diet that meets their nutritional needs without relying on foods containing plant stanol ester to provide the six designated nutrients. McNeil believes that the importance of communicating the valuable benefits of plant stanol ester outweighs the need of these foods to contribute certain nutrient levels and justifies an exemption in this case. Therefore, this petition requests an exception be made to this requirement as permitted by 21 CFR part 101 subpart E.

2. Proposed Claims

The following model claims characterizing the relationship between plant stanol ester and heart disease are proposed for foods that meet the previously described qualifying criteria:

* A diet with at least 3.4 g plant stanol esters per day reduces cholesterol and may reduce the risk of heart disease. 5 g of plant stanol esters per day is more effective in reducing cholesterol and may further reduce the risk of heart disease. This product contains ___ g plant stanol esters per serving.

* 3.4 g plant stanol esters per day may reduce the risk of heart disease.
  5 g plant stanol esters may be more beneficial in reducing the risk of heart disease. This product provides ____ g of plant stanol esters per serving.

* 3.4 g plant stanol esters per day has been demonstrated to lower LDL (bad) cholesterol and may reduce the risk of heart disease. 5 g plant stanol esters per day has been shown to further lower LDL (bad) cholesterol and may further reduce the risk of heart disease. This product contains ___ g of plant stanol esters per serving.

3. Claim Summary and Generalizability

The information presented in this petition provides significant scientific evidence that plant stanol esters reduce total serum cholesterol and LDL-cholesterol in the US population by a clinically significant amount and therefore reduces the risk of CHD. All of the references deal specifically with plant stanol esters and do not include free sterols, stanols, or sterol esters.

4. Promotion of Dietary Change by the Claim

The proposed health claim will effectively aid consumers in selecting an overall diet that provides sufficient levels of plant stanol esters to provide substantial health benefits. Based on the scientific evidence submitted with this petition, the minimum recommended amount of plant stanol ester that must be consumed to have a clinically significant effect on serum total cholesterol and LDL-cholesterol levels is 3.4 g per day. This amount of plant stanol ester can be readily consumed by eating four servings a day of any food containing at least 0.85 g plant stanol esters. Maintaining a diet that includes 3.4 – 5.0 g of plant stanol esters in the diet is a practice that consumers are fully capable of understanding and following, and is very similar to the dietary approach that must be followed under other health claim regulations [see 21 CFR §§101.81 (soluble fiber) and 101.82 (soy protein)].

Information provided to consumers through labeling, advertising and other promotional/educational materials states that foods containing plant stanol esters should be eaten along with a diet low in saturated fat and cholesterol. These materials recommend that consumers use plant stanol ester food products in place of similar foods in the diet. By incorporating plant stanol esters into a greater variety of food products, consumers will find it easier to incorporate plant stanol esters into their diets on a daily basis. It is likely that the claim will also attract and encourage a greater number of consumers to use products containing plant stanol esters who have not done so before.

For these reasons, McNeil believes the proposed health claim will effectively promote positive dietary changes that will result in substantial health benefits for consumers.

E. EPA - CATEGORICAL EXCLUSION

McNeil Consumer Healthcare claims a categorical exclusion under 21 CFR 25.32 (p) for an environmental assessment (EA) and environmental impact statement (EIS). Under the environmental impact consideration regulations, actions involving the issuance of a health claim petition do not require the preparation of an EA or EIS.

F. CONCLUSION

Plant stanol esters effectively lower serum total and LDL-cholesterol. This has been demonstrated in multiple, well-designed, peer-reviewed published studies. The data referenced in this submission meet all requirements for establishing the "significant scientific agreement" standard.

FDA has acknowledged the existence of the relationship between lowering serum LDL-cholesterol levels and a reduction in the risk for cardiovascular disease. Consequently, the data presented clearly establish that plant stanol ester containing foods and supplements reduce LDL-cholesterol levels and the risk of cardiovascular disease.

I, Gilbert A. Leveille, Worldwide Vice President, Regulatory and Scientific Affairs, hereby certify that to the best of my knowledge that the petition is a representative and balanced submission that includes both favorable and unfavorable information that is known to me to be pertinent to the evaluation of the proposed health claim.

Signed this 10th Day of February, 2000.

Gilbert A. Leveille

Yours very truly,

McNeil Consumer Healthcare

By Gilbert A. Leveille

Worldwide Vice President, Regulatory and Scientific Affairs

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H. APPENDICES

1. Published Studies in Adults with Elevated Serum Cholesterol

Study 1 (Miettinen et al., 1995)

In a long-term study of the safety and efficacy of plant stanol esters conducted in Finland, 153 mildly hypercholesterolemic subjects were instructed to consume 24 g/day of canola oil spread or spread containing plant stanol esters for 12 months, without other changes in diet (Miettinen et al., 1995). After an initial six-week run-in period in which all subjects were advised to replace 24 g/day of their normal dietary fat with a canola oil spread, subjects were randomized to receive either control spread or spread containing 5.1 g/day plant stanol esters for six months. At the end of six months, those consuming plant stanol esters were randomly assigned to continue spread containing 5.1 g/day plant stanol esters or 3.4 g/day plant stanol esters for an additional six months. Based on measured spread consumption, average plant stanol ester intakes were 4.4 g/day (in the 5.1 g/day target group) and 3.1 g/day (in the 3.4 g/day target group). After the end of the one-year intervention period, subjects resumed their usual diets for two months ("home diet period").

After one year of use, subjects consuming 4.4 g/day plant stanol esters had significantly reduced mean total and LDL-cholesterol levels by 10% and 14%, respectively compared to the control spread. Subjects who lowered their plant stanol ester intake to 3.1 g/day for six months did not demonstrate further cholesterol reductions, but generally did maintain the lower cholesterol levels that were achieved after the initial six-month period of 4.4 g/day plant stanol esters. Total and LDL-cholesterol levels returned to baseline two months after subjects discontinued the study spreads. Importantly, no significant change in HDL-cholesterol, serum triglycerides, or body weight was observed.

Study 2 (Hallikainen and Uusitupa, 1999)

Hallikainen and Uusitupa (1999) investigated the effect of two reduced-fat spreads containing either wood or vegetable-oil derived plant stanol esters on serum total and LDL-cholesterol concentrations as part of a low-fat, low-cholesterol diet. Fifty-five mildly, hypercholesterolemic subjects participated in this randomized, double-blind, parallel design, placebo-controlled trial. All subjects consumed a high fat diet (36 - 38% of energy) for the four-week run-in period. Subsequently, subjects were randomly assigned to consume 25 g/day of reduced fat (35%) canola oil spread (control spread) or one of two reduced fat[1/] (40%) plant stanol ester spreads (wood or vegetable oil-derived stanol esters) as part of a low-fat (26% of energy) diet for the eight-week experimental period.

[1/] The spread fat content (40%) includes the plant stanol esters; the spread therefore contains only 32% absorbable fat, since stanols (8% of the spread) are not absorbed.

Total cholesterol was significantly reduced in both the wood and vegetable stanol ester groups, providing 3.7 or 3.9 g/day plant stanol esters, by 11% and 8%, respectively when compared with the placebo group. LDL-cholesterol was also significantly reduced by 24% in the wood stanol ester group and by 18% in the vegetable stanol ester group compared to the run-in treatment period. There was no change in HDL-cholesterol or triglyceride levels during the study.

Study 3 (Weststrate and Meijer, 1998)

In 1998, Weststrate and Meijer examined the effectiveness of various vegetable oil-derived plant sterol esters blended in spread, with plant stanol ester spread as a positive control. Ninety-five normal, healthy subjects with mild hypercholesterolemia were randomly assigned to four of five treatment periods (3.5 weeks each) with spreads containing 5.5 - 11% plant sterols/stanols: soybean oil sterol esters (25% campesterol, 46% sitosterol), ricebran oil sterol esters (14% campesterol, 47% 24-methylcycloartenol + cycloartenol), sheanut oil sterol esters (38% α- + β-amyrin, 55% other shea sterols), Benecol (plant stanol esters; 10% campestanol, 82% sitostanol, in ester form), or control spread containing 0.36% plant sterols. Subjects were instructed to replace their usual spread(s) with the test spreads at lunch and dinner with no other changes in diet.

Consuming the spread containing 4.2 g/day plant stanol esters reduced total and LDL-cholesterol levels by 7% and 12%, respectively versus the control. There was no change in HDL-cholesterol or triglyceride levels.

Study 4 (Gylling and Miettinen, 1999)

A double-blind, complete crossover study was conducted at the University of Helsinki (Gylling and Miettinen, 1999). In this study, 23 mildly hypercholesterolemic, postmenopausal women were allocated to study periods where they consumed 25 g per day of test product containing 5.4 g per day of either wood or vegetable oil-derived plant stanol esters, replacing a similar amount of dietary fat in their normal diet. After six weeks, subjects consumed the other spread for an additional six weeks. Following an eight-week home-diet wash-out period, 21 of the subjects were randomly assigned to consume either butter or 4.1 g per day plant stanol esters in butter for an additional five weeks.

In this study both the wood and vegetable stanol ester spreads lowered total cholesterol by 4% and 6%, respectively. LDL-cholesterol was also reduced by 8% and 10% when compared with baseline values in both the wood and vegetable stanol ester groups. The group consuming butter containing 4.1 g per day plant stanol esters also reduced their total

and LDL-cholesterol levels by 8% and 12%, respectively compared to butter alone. There was no change in HDL-cholesterol or triglycerides.

Study 5 (Vanhanen et al., 1993; Blomqvist et al., 1993 collectively)
In a randomized, double-blind study performed in Finland with modestly hypercholesterolemic subjects, 34 subjects received 5.8 g per day of plant stanol esters in a canola oil mayonnaise preparation and 33 subjects received a mayonnaise alone over a six-week period (Vanhanen et al., 1993; Blomqvist et al., 1993 collectively).

Plant stanol esters reduced both total and LDL-cholesterol by 6.5% and 9%, respectively when compared with the run-in treatment period. There was no change in HDL-cholesterol or triglyceride levels.

Study 6 (Vanhanen et al., 1994)
The effects of plant stanol esters on serum cholesterol levels in small groups (seven or eight subjects) of hypercholesterolemic subjects were investigated by Vanhanen et al. (1994). Following a six-week run-in period in which subjects consumed canola oil-based mayonnaise alone, subjects were randomized to continue to consume canola oil mayonnaise or to consume canola oil mayonnaise containing 1.4 g per day plant stanol esters for nine weeks followed by six weeks of consumption of a mayonnaise with 3.4 g per day plant stanol esters.

In this study, consumption of plant stanol esters reduced total and LDL-cholesterol levels by 9% and 15%, respectively compared to the control. In addition, the stanol ester group had no change in HDL-cholesterol or triglyceride levels when compared to the control group.

Study 7 (Miettinen and Vanhanen, 1994)
A double-blind, placebo-controlled trial was conducted in subjects with primary hypercholesterolemia and different apolipoprotein E phenotypes to examine the effects of small amounts of sitosterol, sitostanol and sitostanol esters on serum lipids and cholesterol metabolism (Miettinen and Vanhanen, 1994). Following a six-week run-in period in which subjects consumed canola oil mayonnaise alone, subjects were randomized to one of four groups for an additional nine-week period: canola oil mayonnaise alone (control); canola oil mayonnaise containing 0.7 g per day of sitosterol; canola oil mayonnaise with 0.7 g per day of sitostanol; or canola oil mayonnaise plus 0.8 g per day of sitostanol as 1.4 g per day of stanol esters.

The results of this study demonstrate that total and LDL-cholesterol levels were significantly decreased up to 4% in the stanol/sitosterol-supplemented groups combined versus the

control group. The greatest reduction was achieved in the group receiving stanol esters. There was no significant change in triglycerides in any of the study groups. There was also no change in HDL-cholesterol levels in any of the groups with the exception of the stanol ester group who experienced a small but significant increase in HDL-cholesterol.

Study 8 (Andersson et al., 1999)
A double-blind, randomized study was conducted in 68 men and women with cholesterol levels > 194 mg/dL (Andersson et al., 1999). This study had a four-week run-in period where all subjects received a low-fat margarine to be consumed with bread three times daily. After this period, subjects were randomized to receive either low-fat spread containing 3.4 g per day plant stanol esters with a controlled, low-fat diet; control spread with a controlled, low-fat diet; or to continue their normal diet with the addition of a spread containing 3.4 g per day plant stanol esters for eight weeks.

In this study total and LDL-cholesterol was reduced in all three treatment groups. The group consuming spread containing plant stanol esters with the controlled low-fat diet experienced the greatest reduction in total and LDL-cholesterol by 19% and 15%, respectively versus the end of the run-in period. Total and LDL-cholesterol was reduced by 9% and 12%, respectively in the group following a normal diet and consuming spread containing plant stanol esters. The group consuming the controlled low-fat diet and the control spread achieved a total and LDL-cholesterol reduction of 8% and 12%, respectively. This study suggests that 3.4 g per day plant stanol esters in conjunction with a normal or controlled, low-fat diet can significantly lower serum cholesterol levels. Also, there was no change in HDL-cholesterol or triglyceride levels during the study.

Study 9 (Nguyen et al., 1999)
In a randomized, double-blind, multi-center trial conducted in the US, 318 men and women (mean age = 53 years) consumed either European spread[2/] containing 5.1 g per day plant stanol esters, reformulated US spread containing 5.1 g per day plant stanol esters, reformulated US spread containing 3.4 g per day plant stanol esters, or reformulated canola oil spread without plant stanol esters (control) for eight weeks (Nguyen et al, 1999).

In this study, all plant stanol ester preparations demonstrate significant reductions in total and LDL-cholesterol concentrations compared to the control canola oil spread. The US spread containing 5.1 g per day plant stanol esters lowered total and LDL-cholesterol 6.4%

[2/] Four test spreads were used in Nguyen et al. (1999). The European formulation was similar in composition to the plant stanol ester (5.1 g/day) product used by Miettinen et al. (1995). The two reformulated spreads differed from each other in the amount of plant stanol esters and from the European formula in fatty acid composition. The reformulated spreads had a lower percentage of saturated and a higher percentage of polyunsaturated fatty acids. The control spread was similar to the reformulated spreads in fatty acid composition.

and 10.1%, respectively when compared to baseline. Subjects consuming the 5.1 g per day plant stanol esters European spread received a 4.7% reduction in total cholesterol and a 5.2% reduction in LDL-cholesterol compared to baseline. The 3.4 g per day plant stanol ester US group showed a 4.1% reduction in both total and LDL-cholesterol levels compared to baseline. In addition, there was no significant change in HDL-cholesterol or triglycerides in any group.

Table H-1 Published Studies in Adults with Elevated Serum Cholesterol

Citation	Study Design	Product, Amount of Plant Stanols, *Plant Stanol Esters*[a]; Duration	N Enrolled	Mean Age (range) Gender	Study Results
Miettinen et al., N Engl J Med 1995	R, Db, PC	Canola oil spread; 52 weeks Spread + stanol esters, 2.6 g/d stanols, ***4.4 g/d stanol esters***; 26 weeks Then randomized to: Spread + stanol esters, 2.6 g/d stanols, ***4.4 g/d stanol esters***; 26 weeks or Spread + stanol esters, 1.8 g/d stanols, ***3.1 g/d stanol esters;*** 26 weeks	51 102 51 51	50 y (25 - 64 y) 58% F, 42% M	**Population:** Subjects with mild hypercholesterolemia **Run-in:** 6 weeks canola oil spread. **Results:** After one year, 4.4 g/d stanol ester spread group reduced total and LDL-cholesterol 10% and 14%, respectively vs. control spread (p <0.001). There was no significant change in HDL-cholesterol or triglyceride levels. The 3.1 g/d plant stanol ester spread group reduced total and LDL cholesterol 8% and 10%, respectively vs control spread (p < 0.001).
Hallikainen and Uusitupa, Am J Clin Nutr 1999	R, Db, PC	Step 1 Diet + low-fat canola spread; 8 weeks Step 1 Diet + spread + stanol esters, 2.3 g/d stanols, ***3.9 g/d wood stanol esters***; 8 weeks Step 1 Diet + spread + stanol esters, 2.2 g/d stanols, ***3.7 g/d vegetable oil stanol esters***; 8 weeks	17 18 20	(20 - 60 y) 35 F, 20 M	**Population:** Subjects with hypercholesterolemia **Run-in:** 4 week high fat diet **Results:** Both wood and vegetable stanol esters decreased total (11% and 8% vs. low-fat canola spread, p<0.05) and LDL-cholesterol (24% and 18% vs. run-in, p<0.01). There was no significant change in HDL-cholesterol or triglyceride levels.

a: Consumption originally reported in terms of stanols was converted as follows: 1 g plant stanols = 1.7 g plant stanol esters

Abbreviations: Alk Phos=alkaline phosphatase, ALT=alanine aminotransferase, AST=aspartic acid aminotransferase, CO=crossover, DB=double blind, F=female, GGT=gamma glutamyltransferase, Hb=hemoglobin, Hct=hematocrit, M=male, Mayo=mayonnaise, MI=myocardial infarction, NS=not statistically significant, OL=open label, PC=placebo controlled, Plt= platelet, R=randomized, y=year.

Table H-1 Published Studies in Adults with Elevated Serum Cholesterol

Citation	Study Design	Product, Amount of Plant Stanols, *Plant Stanol Esters*[a]; Duration	N Enrolled	Mean Age (range) Gender	Study Results
Weststrate and Meijer, Eur J Clin Nutr 1998	R, Db, PC, CO	Control spread Rice bran oil sterol spread Sheanut oil sterol spread Soybean sterol ester spread Canola oil spread + stanol esters, 2.5 g/d stanols, ***4.2 g/d stanol esters;*** 3.5 week periods for each spread	95	45 ± 13 y	**Population:** Normal subjects or subjects with mild hypercholesterolemia **Results:** Plant stanol ester spread reduced total and LDL-cholesterol 7% and 12%, respectively. Soybean oil sterol spread lowered total and LDL-cholesterol 8% and 13%, respectively. Sheanut or ricebran oil spreads were not effective. There was no significant change in HDL-cholesterol or triglyceride levels.
Gylling and Miettinen, Metabolism 1999	R, Db, PC, CO	Canola spread + stanol esters, 3.2 g/d stanols, ***5.4 g/d vegetable oil stanol esters***; 6 weeks; then Canola spread + stanol esters, 3.2 g/d stanols, ***5.4 g/d wood stanol esters***; 6 weeks; then Butter; 5 weeks; then Butter + stanol esters, 2.4 g/d stanols, ***4.1 g/d wood stanol esters****; 5 weeks	24 21	53 y (50 - 55 y) 23 F	**Population:** Postmenopausal women with moderate hypercholesterolemia **Results:** Both wood and vegetable stanol esters significantly lowered total cholesterol (4% and 6%) and LDL-cholesterol (8% and 10%) vs. baseline. Butter increased total and LDL-cholesterol. Plant stanol esters in butter reduced total and LDL-cholesterol 8% and 12% compared to butter alone. There was no significant change in HDL-cholesterol or triglyceride levels. *Stanols esterified to butter fatty acids, not canola oil.
Vanhanen et al., J Lipid Res 1993 Blomqvist et al., Nutr Metab Cardiovas Dis 1993	R, Db, PC	Canola oil mayonnaise; 6 weeks Mayonnaise + stanol esters, 3.4 g/d stanols, ***5.8 g/d stanol esters***; 6 weeks	33 34	43 ± 2 y 48 ± 2 y 20 F, 47 M	**Population:** Subjects with hypercholesterolemia **Run-in:** 4 week canola oil mayonnaise. **Results:** Stanol ester reduced total and LDL-cholesterol by 6.5% and 9%, respectively, vs. run-in. There was no significant change in HDL-cholesterol or triglyceride levels.

a: Consumption originally reported in terms of stanols was converted as follows: 1 g plant stanols = 1.7 g plant stanol esters

Abbreviations: Alk Phos=alkaline phosphatase, ALT=alanine aminotransferase, AST=aspartic acid aminotransferase, CO=crossover, DB=double blind, F=female, GGT=gamma glutamyltransferase, Hb=hemoglobin, Hct=hematocrit, M=male, Mayo=mayonnaise, MI=myocardial infarction, NS=not statistically significant, OL=open label, PC=placebo controlled, Plt= platelet, R=randomized, y=year.

Table H-1 Published Studies in Adults with Elevated Serum Cholesterol

Citation	Study Design	Product, Amount of Plant Stanols, *Plant Stanol Esters*[a]; Duration	N Enrolled	Mean Age (range) Gender	Study Results
Vanhanen et al., Clin Sci 1994	R, Db, PC	Canola oil mayonnaise; 15 weeks Mayonnaise + stanol esters, 0.8 g/d stanols, ***1.4 g/d stanol esters;*** 9 weeks; then Mayonnaise + stanol esters, 2 g/d stanols, ***3.4 g/d stanol esters;*** 6 weeks	8 7	47 y (33 - 60 y) 4 F, 11 M	**Population:** Subjects with hypercholesterolemia **Run-in:** 6 weeks canola oil spread. **Results:** Control group-adjusted total and LDL-cholesterol decreased slightly with low stanol intake. Cholesterol absorption efficiency decreased from 28.7% to 23.4%. Control group-adjusted total and LDL-cholesterol decreased 9% and 15% with high stanol ester intake. The stanol ester group had no change in HDL-cholesterol or triglyceride levels when compared to the control group.
Miettinen and Vanhanen, Atherosclerosis 1994	R, Db, PC	Canola oil mayonnaise; 9 weeks Mayonnaise + stanol esters, 0.7 g/d sterols; 9 weeks Mayonnaise + stanol esters, 0.7g/d stanols; 9 weeks Mayonnaise + stanol esters, 0.8 g/d stanols, ***1.4 g/d stanol esters;*** 9 weeks	8 9 7 7	45 ± 3 y 9 F, 22 M	**Population:** Subjects with primary hypercholesterolemia **Run-in:** 6 weeks canola oil mayonnaise. **Results:** Total and LDL-cholesterol decreased 4% in stanol/sitosterol-supplemented groups combined (n=23) vs. control. There was also no change in HDL-cholesterol levels in any of the groups with the exception of the plant stanol ester group which experienced a small but significant increase in HDL-cholesterol.
Andersson et al., Eur Heart J Supplements 1999	R, DB, PC	Home diet + low-fat spread + stanol esters, 2 g/d stanols, ***3.4 g/d stanol esters***; 8 weeks Low-fat diet + low-fat control spread; 8 weeks Low-fat diet + low-fat spread + stanol esters, 2 g/d stanols, ***3.4 g/d stanol esters***; 8 weeks	21 21 19	55 y (30 - 65 y) 33 F, 28 M	**Population:** Adult subjects with mild hypercholesterolemia. **Run-in:** 4 week low-fat control spread. **Results:** Stanol ester spread and low-fat diet reduced total and LDL-cholesterol by 19% and 15%, respectively. Stanol ester spread and usual diet reduced total and LDL-cholesterol by 9% and 12%, respectively. Control spread and low-fat diet reduced total and LDL-cholesterol by 8% and 12%, respectively. There was no significant change in HDL-cholesterol or triglyceride levels.

a: Consumption originally reported in terms of stanols was converted as follows: 1 g plant stanols = 1.7 g plant stanol esters

Abbreviations: Alk Phos=alkaline phosphatase, ALT=alanine aminotransferase, AST=aspartic acid aminotransferase, CO=crossover, DB=double blind, F=female, GGT=gamma glutamyltransferase, Hb=hemoglobin, Hct=hematocrit, M=male, Mayo=mayonnaise, MI=myocardial infarction, NS=not statistically significant, OL=open label, PC=placebo controlled, Plt= platelet, R=randomized, y=year.

Table H-1 Published Studies in Adults with Elevated Serum Cholesterol

Citation	Study Design	Product, Amount of Plant Stanols, *Plant Stanol Esters*[a]; Duration	N Enrolled	Mean Age (range) Gender	Study Results
Nguyen et al., Mayo Clinic Proc 1999	R, DB, PC	Control spread; 8 weeks US reformulated spread, 3 g/d stanols, ***5.1 g/d stanol esters***; 8 weeks European formulated spread, 3 g/d stanols, ***5.1 g/d stanol esters***; 8 weeks US reformulated spread; 2 g/d stanols, ***3.4 g/d stanol esters***; 8 weeks	80 77 79 82	53 y (23 - 77 y) 161 F, 157 M	**Population:** Adult subjects with total cholesterol ≥200 mg/dL and ≤280 mg/dL who are not using any other cholesterol reducing medications or products. **Run-in:** 4 week canola oil spread **Results:** There were significant % reductions in total and LDL-cholesterol compared to baseline for all stanol ester groups. Total Chol. / LDL-Chol. Control group: + 0.5% / + 0.1% 5.1 g/day US group: 6.4% / 10.1% 5.1 g/day European group: 4.7% / 5.2% 3.4 g/day US group: 4.1% / 4.1% There were no significant changes in HDL-cholesterol or triglyceride levels in any group. **NOTE:** "European formulation" was similar in fatty acid composition to the product used by Miettinen et al. (1995). Reformulated products had a lower percentage of saturated fatty acids and a higher percentage of polyunsaturated fatty acids, compared with the European formulation.

a: Consumption originally reported in terms of stanols was converted as follows: 1 g plant stanols = 1.7 g plant stanol esters

Abbreviations: Alk Phos=alkaline phosphatase, ALT=alanine aminotransferase, AST=aspartic acid aminotransferase, CO=crossover, DB=double blind, F=female, GGT=gamma glutamyltransferase, Hb=hemoglobin, Hct=hematocrit, M=male, Mayo=mayonnaise, MI=myocardial infarction, NS=not statistically significant, OL=open label, PC=placebo controlled, Plt= platelet, R=randomized, y=year.

2. Published Studies in Adults with Normal Cholesterol

Study 1 (Plat and Mensink, 2000)

Plat and Mensink (2000) examined the clinical effectiveness of two plant stanol ester preparations in normocholesterolemic, healthy volunteers. During this randomized, double-blind, placebo-controlled study, 112 subjects consumed one of three test diets for eight weeks after a four-week canola oil spread and shortening run-in period. The test diets included spread and shortening, or spread and shortening with 6.5 or 6.8 g per day plant stanol esters derived either from wood or vegetable oil. Subjects were instructed to replace their usual spread at breakfast and lunch with test spread and to replace cooking fat at dinner with test shortening.[3/]

Both plant stanol ester groups reduced LDL-cholesterol by about 14%. Also, there was no change in HDL-cholesterol or triglyceride levels in these groups.

Study 2 (Niinikoski et al., 1997)

In a randomized, double-blind study, 12 normocholesterolemic subjects consumed 3.7 g per day plant stanol esters in canola oil spread, while 12 control subjects were given canola oil spread with no added plant stanol esters for five weeks (Niinikoski et al., 1997).

Consuming spread containing plant stanol esters providing 3.7 g per day plant stanol esters reduced total cholesterol levels by 15.7% compared to the home diet. HDL-cholesterol levels were unchanged for both the plant stanol ester and control groups.

Study 3 (Puska et al., 1998)

Puska et al. (1998) conducted a community based intervention study in several villages in Finland involving 1333 individuals. A group of subjects were given a dietary survey that included an interview and a 24-hour dietary recall. The 200 subjects that consumed plant stanol ester spread and reported using more than 5 teaspoonfuls a day (4 g plant stanol esters) achieved a 16% reduction in total cholesterol and all the villages collectively reduced their total cholesterol by 9%.

[3]/ Shortening is the product used in the Netherlands for pan frying; the gravy formed is used as a sauce in the main meal.

Table H-2 Published Studies in Adults with Normal Serum Cholesterol

Citation	Study Design	Product, Amount of Plant Stanol, *Plant Stanol Esters*[a]; Duration	N Enrolled	Mean Age (range) Gender	Study Results
Plat and Mensink, Atherosclerosis 2000	R, Db, PC	Canola oil spread and shortening; 8 weeks Spread and shortening + vegetable stanol esters, 3.8 g/d stanols, ***6.5 g/d stanol esters***; 8 weeks Spread and shortening + wood stanol esters, 4 g/d stanols, ***6.8 g/d stanol esters***; 8 weeks	42 36 34	(18 - 65 y) 71 F, 41 M	**Population:** Normocholesterolemic healthy subjects **Run-in:** 4-week canola oil spread and shortening. **Results:** HDL-cholesterol and triglyceride levels were unchanged. LDL-cholesterol levels were significantly reduced by about 14% in both stanol ester groups. Apo-B was significantly reduced by stanol esters.
Niinikoski et al., Scand J Nutr 1997	R, Db, PC	Canola oil spread; 5 weeks Spread + stanol esters, 2.2 g/d stanols, ***3.7 g/d stanol esters***; 5 weeks	12 12	36 y (24 - 52 y) 16 F, 8 M	**Population:** Normocholesterolemic healthy men and women **Results:** Stanol ester reduced total cholesterol from 197 mg/dL (5.1 mmol/L) to 166 mg/dL (4.3 mmol/L), a change of 31 mg/dL (0.8 mmol/L) vs. home diet, $p<0.05$. Control spread reduced total cholesterol 11.5 mg/dL (0.3 mmol/L) vs. home diet. Stanol-ester specific effect was 0.5 mmol/L. HDL-cholesterol levels remained unchanged.

a: Consumption originally reported in terms of stanols was converted as follows: 1 g plant stanols = 1.7 g plant stanol esters

Abbreviations: Alk Phos=alkaline phosphatase, ALT=alanine aminotransferase, AST=aspartic acid aminotransferase, CO=crossover, DB=double blind, F=female, GGT=gamma glutamyltransferase, Hb=hemoglobin, Hct=hematocrit, M=male, Mayo=mayonnaise, MI=myocardial infarction, NS=not statistically significant, OL=open label, PC=placebo controlled, Plt= platelet, R=randomized, y=year.

Table H-2 Published Studies in Adults with Normal Serum Cholesterol

Citation	Study Design	Product, Amount of Plant Stanol, *Plant Stanol Esters*[a]; Duration	N Enrolled	Mean Age (range) Gender	Study Results
Puska et al., Postgraduate Medicine Special Report 1998	OL	Community based intervention Self-selected stanol ester spread consumption	1333 200	49 y (20 - 70 y)	**Population:** A subset of Finnish national survey was selected for a dietary survey. The subjects were interviewed and completed a 24-hour dietary recall. **Results:** The mean cholesterol reduction was 16% ($p<0.001$) in the winning village and 9% ($p<0.001$) in average among participating villages. About 200 subjects used Benecol®. Those who reported using more than 5 teaspoonfuls stanol ester spread/day (3.4 g plant stanol esters) had a 21.3% reduction in total cholesterol.

a: Consumption originally reported in terms of stanols was converted as follows: 1 g plant stanols = 1.7 g plant stanol esters

Abbreviations: Alk Phos=alkaline phosphatase, ALT=alanine aminotransferase, AST=aspartic acid aminotransferase, CO=crossover, DB=double blind, F=female, GGT=gamma glutamyltransferase, Hb=hemoglobin, Hct=hematocrit, M=male, Mayo=mayonnaise, MI=myocardial infarction, NS=not statistically significant, OL=open label, PC=placebo controlled, Plt= platelet, R=randomized, y=year.

3. Published Studies in Adults with Coronary Heart Disease

Study 1 (Gylling et al., 1997)

Gylling et al. (1997) conducted a randomized double-blind, crossover study in 22 postmenopausal women who had suffered a myocardial infarction at least three months prior to selection for the study. Subjects were assigned to ingest 21 g per day unsupplemented canola oil spread for seven weeks, or spread plus 5.1 g per day plant stanol esters for seven weeks, followed by the switching of the spreads for another seven weeks. There was no washout period between treatment periods.

In this study, the spread containing plant stanol esters reduced total and LDL-cholesterol levels by 8% and 15%, respectively compared to the control spread. Also, there was no change in HDL-cholesterol and triglyceride levels.

Studies 2 and 3 (Gylling et al., 1997; Gylling and Miettinen, 1998)

Two other trials in adults with CHD are discussed in Appendix H.5 which summarizes published studies in adults consuming plant stanol esters in addition to cholesterol-lowering drugs (Gylling et al., 1997; Gylling and Miettinen, 1998).

Table H-3 Published Studies in Adults with Coronary Heart Disease

Citation	Study Design	Product, Amount of Plant Stanol, *Plant Stanol Esters*[a]; Duration	N Enrolled	Mean Age (range) Gender	Study Results
Gylling et al., Circulation 1997	R, Db, PC, CO,	Canola oil spread; 7 weeks Canola oil spread + stanol esters, 3 g/d stanols, ***5.1 g/d stanol esters***; 7 weeks	22	51 y (48 - 56 y) 22 F	**Population:** Postmenopausal women with previous myocardial infarction. **Results:** Canola oil spread + stanol ester lowered total- and LDL-cholesterol by 8% and 15%, respectively, vs. control spread. There was no significant change in HDL-cholesterol or triglyceride levels.
Gylling et al., Circulation 1997	OL	Data listed in Table 8-5, Published Studies in Adults Consuming Cholesterol-Lowering Drugs			
Gylling and Miettinen, J Am Coll Cardiol 1998 (Abstract)		Data listed in Table 8-5, Published Studies in Adults Consuming Cholesterol-Lowering Drugs			

a: Consumption originally reported in terms of stanols was converted as follows: 1 g plant stanols = 1.7 g plant stanol esters

Abbreviations: Alk Phos=alkaline phosphatase, ALT=alanine aminotransferase, AST=aspartic acid aminotransferase, CO=crossover, DB=double blind, F=female, GGT=gamma glutamyltransferase, Hb=hemoglobin, Hct=hematocrit, M=male, Mayo=mayonnaise, MI=myocardial infarction, NS=not statistically significant, OL=open label, PC=placebo controlled, Plt= platelet, R=randomized, y=year.

4. Published Studies in Adults with Non-Insulin Dependent Diabetes Mellitus

Study 1 (Gylling and Miettinen, 1994)

A double-blind, crossover study was performed on 11 men with NIDDM (Gylling and Miettinen, 1994). After a four-week run-in period with subjects following their normal diet, subjects were randomly assigned to a control period using canola oil spread or a trial period using 5.1 g per day of plant stanol esters in canola oil spread for six-week periods in a crossover design.

The results of this study demonstrate that spread containing plant stanol esters reduced total and LDL-cholesterol levels by 6% and 9%, respectively.

Studies 2 and 3 (Gylling and Miettinen, 1996; Gylling and Miettinen, 1995)

Two other trials in diabetics are discussed in Appendix H.5 which summarizes studies in adult subjects also consuming cholesterol-lowering drugs in addition to plant stanol ester-containing spread preparations (Gylling and Miettinen, 1996; Gylling and Miettinen, 1995).

Table H-4 Published Studies in Adults with Non-Insulin Dependent Diabetes Mellitus

Citation	Study Design	Product, Amount of Plant Stanol, *Plant Stanol Esters*[a], Duration	N Enrolled	Mean Age (range) Gender	Study Results
Gylling and Miettinen, Diabetologia 1994	R, Db, PC, CO	Canola oil spread; 6 weeks Canola oil spread + stanol esters; 3 g/d stanols, ***5.1 g/d stanol esters***; 6 weeks	11	58 y 11 M	**Population:** NIDDM subjects with hypercholesterolemia **Results:** Stanol ester reduced total and LDL-cholesterol 6% and 9%, respectively. Cholesterol absorption dropped from 25% to 9%, p<0.001.
Gylling and Miettinen, J Lipid Res 1996		Data listed in Table 8.5, Published Studies in Adults Consuming Cholesterol-Lowering Drugs			
Gylling and Miettinen, Atherosclerosis 1995		Data listed in Table 8.5, Published Studies in Adults Consuming Cholesterol-Lowering Drugs			

a: Consumption originally reported in terms of stanols was converted as follows: 1 g plant stanols = 1.7 g plant stanol esters

Abbreviations: Alk Phos=alkaline phosphatase, ALT=alanine aminotransferase, AST=aspartic acid aminotransferase, CO=crossover, DB=double blind, F=female, GGT=gamma glutamyltransferase, Hb=hemoglobin, Hct=hematocrit, M=male, Mayo=mayonnaise, MI=myocardial infarction, NS=not statistically significant, OL=open label, PC=placebo controlled, Plt= platelet, R=randomized, y=year.

5. Published Studies in Adults Consuming Cholesterol-Lowering Drugs

Study 1 (Vanhanen, 1994)

Vanhanen (1994) investigated whether consuming 2.6 g per day plant stanol esters (contained in a canola-based spread) or neomycin for 6 weeks would result in additional cholesterol-reducing effects in 14 patients already taking the cholesterol synthesis-inhibiting drug pravastatin (40 or 80 mg per day).

The results of this study demonstrate that the addition of a spread containing plant stanol esters reduced total and LDL-cholesterol, although, not significantly, as compared to the reduction caused by pravastatin alone. HDL-cholesterol and triglyceride levels did not change significantly.

Study 2 (Gylling and Miettinen, 1996)

Although the Vanhanen (1994) study failed to demonstrate a significant additive cholesterol-lowering effect with plant stanol esters and pravastatin, Gylling and Miettinen (1996) found an additional, although not strictly additive, cholesterol-lowering effect at higher plant stanol ester consumption levels. In this study, seven men with NIDDM consumed: (a) canola oil spread (control), (b) spread plus 5.1 g per day plant stanol esters, (c) control spread plus 40 mg/day pravastatin, and (d) spread plus 5.1 g per day plant stanol esters plus 40 mg/day pravastatin. Each treatment period was four weeks long.

Spread containing plant stanol esters lowered total and LDL-cholesterol by 11% and 14% respectively. Additionally, a further reduction of both total (8%) and LDL-cholesterol (10%) was achieved when a plant stanol ester product was consumed in addition to pravastatin when compared with pravastatin alone. HDL-cholesterol and triglyceride levels did not change. In subjects taking a HMG-CoA reductase inhibiting drug, plant stanol ester consumption will produce an additional reduction in total and LDL-cholesterol.

Study 3 (Gylling and Miettinen, 1995)

Gylling and Miettinen (1995) investigated if there was an additional effect of plant stanol esters on subjects already receiving the cholesterol-reducing drug neomycin. While neomycin is not routinely used for cholesterol reduction because of its systemic toxicity, six male, NIDDM subjects in this study received canola oil spread for a control period of six weeks, followed by 1.5 g per day of neomycin and control spread for four weeks, then 1.5 g per day neomycin plus 5.1 g per day plant stanol esters in spread for an additional four weeks.

The results of this study demonstrate that plant stanol esters in addition to neomycin treatment significantly reduced both total and LDL-cholesterol compared with neomycin treatment alone. LDL-cholesterol levels were reduced in the plant stanol ester/neomycin group by 37.3% compared to the control, while neomycin taken alone reduced LDL-cholesterol levels by 29.3% compared to the control. There was no change in HDL-cholesterol or triglyceride levels.

Study 4 (Gylling et al., 1997)
In a study performed in postmenopausal women with a history of previous myocardial infarction, ten women who had used simvastatin (10-20 mg/day) for the previous one year were instructed to use 21 g per day canola oil spread with 5.1 g per day plant stanol esters for 12 weeks, replacing a similar amount of their daily fat intake. (Gylling et al., 1997).

The results of this study demonstrate that the addition of a spread containing plant stanol esters to simvastatin treatment reduced total and LDL-cholesterol by 11% and 16%, respectively compared to simvastatin alone. HDL-cholesterol and triglyceride levels were not changed in this study. These results demonstrate an additional cholesterol-lowering effect in subjects already stabilized on statin therapy.

Study 5 (Gylling and Miettinen, 1998)
Two open-label trials evaluating subjects consuming spread containing plant stanol esters have been published as abstracts (Gylling and Miettinen, 1998; Vuorio et al., 1998). Gylling and Miettinen (1998) investigated spread containing 5.1 g per day plant stanol esters in CHD subjects taking simvastatin who had either high or low cholestanol/cholesterol ratios for 12 weeks.

The results of this study demonstrate that the plant stanol esters reduced serum cholesterol concentrations more consistently in statin-treated, coronary subjects with high cholestanol/cholesterol ratios.

Study 6 (Vuorio et al., 1998)
Vuorio et al. (1998) conducted a study in subjects from 19 families with incidence of the familial hypercholesterolemia North Karelia deletion mutation (FH-NK). This study involved four subject groups, including FH-NK individuals already following a simvastatin therapy regimen.

The results of this study demonstrate that the plant stanol ester spread significantly reduced LDL-cholesterol levels: 17% in FH-NK children, 13% in unaffected family members, and 20%

in FH-NK family members on simvastatin. The group of FH-NK parents showed a non-significant decrease of 12% in LDL-cholesterol.

Table H-5 Published Studies in Adults Consuming Cholesterol-Lowering Drugs

Citation	Study Design	Product, Amount of Plant Stanol, *Plant Stanol Esters*[a]; Duration	N Enrolled	Mean Age (range) Gender	Study Results
Vanhanen, Eur J Clin Pharmacol 1994	R, Db, PC	Familial hypercholesterolemics: Pravastatin; 6 weeks Pravastatin + neomycin; 6 weeks Other hypercholesterolemics: Pravastatin + canola oil mayonnaise; 6 weeks Pravastatin + mayonnaise + stanol esters; 1.5 g/d stanols, ***2.6 g/d stanol esters***; 6 weeks	 6 6 7 7	53 y 18 F, 8 M	**Population:** Subjects with hypercholesterolemia **Run-in:** 3 week canola oil mayonnaise. **Results:** Stanol ester caused no further reductions in total and LDL-cholesterol levels over and above the reduction caused by pravastatin and canola oil mayonnaise only. There was no significant change in HDL-cholesterol or triglyceride levels.
Gylling and Miettinen, J Lipid Res 1996	R, Db, PC, CO	Canola oil spread Spread + stanol esters, 3 g/d stanols, ***5.1 g/d stanol esters*** Pravastatin + spread Pravastatin + spread + stanol esters; 3 g/d stanols, ***5.1 g/d stanol esters***, 7 week periods for each spread	8	60 y 8 M	**Population:** Subjects with NIDDM and hypercholesterolemia **Run-in:** 4 week canola oil spread **Results:** Stanol ester spread lowered total and LDL-cholesterol 11% and 14%, respectively. Stanol ester lowered total cholesterol to 200 mg/dL (<5.2 mmol /L) in 3/8 subjects. Serum campesterol- and sitosterol-to-cholesterol ratios were reduced 46% and 43%, respectively, and cholesterol absorption was reduced 68% vs. the control period. Pravastatin + stanol ester reduced total and LDL-cholesterol further (8% - 10% vs. pravastatin alone). There was no significant change in HDL-cholesterol or triglyceride levels.

a: Consumption originally reported in terms of stanols was converted as follows: 1 g plant stanols = 1.7 g plant stanol esters

Abbreviations: Alk Phos=alkaline phosphatase, ALT=alanine aminotransferase, AST=aspartic acid aminotransferase, CO=crossover, DB=double blind, F=female, GGT=gamma glutamyltransferase, Hb=hemoglobin, Hct=hematocrit, M=male, Mayo=mayonnaise, MI=myocardial infarction, NS=not statistically significant, OL=open label, PC=placebo controlled, Plt= platelet, R=randomized, y=year.

Table H-5 Published Studies in Adults Consuming Cholesterol-Lowering Drugs

Citation	Study Design	Product, Amount of Plant Stanol, *Plant Stanol Esters*[a]; Duration	N Enrolled	Mean Age (range) Gender	Study Results
Gylling and Miettinen, Atherosclerosis 1995	Sequential PC	Canola oil spread; 6 weeks Spread + neomycin; 4 weeks Spread + neomycin + stanol esters, 3 g/d stanols, ***5.1 g/d stanol esters***; 4 weeks	6	63 y 6 M	**Population:** Subjects with hypercholesterolemia and NIDDM **Results:** Stanol ester + neomycin significantly reduced total and LDL-cholesterol vs. neomycin treatment only. (LDL-cholesterol: neomycin + stanol ester, -37.3% vs. control; neomycin alone, - 29.3% vs. control). There was no significant change in HDL-cholesterol or triglyceride levels.
Gylling et al., Circulation 1997	OL	Simvastatin; 12 weeks Simvastatin + spread + stanol esters, 3 g/d stanols, ***5.1 g/d stanol esters***; 12 wks	10	Ages unknown 10 F	**Population:** Postmenopausal women with previous myocardial infarction. **Results:** Simvastatin + stanol ester lowered total and LDL-cholesterol by 11% and 16%, respectively, vs. simvastatin alone. There was no significant change in HDL-cholesterol or triglyceride levels.
Gylling and Miettinen, J Am Coll Cardiol 1998 (Abstract)	OL	Spread + stanol esters, 2 g/d stanols, ***3.4 g/d stanol esters;*** 12 weeks High cholestanol/cholesterol subjects Low cholestanol/cholesterol subjects	 15 15		**Population:** Hypercholesterolemic subjects treated with simvastatin. **Results:** Stanol ester spread reduced cholesterol more consistently in statin-treated subjects with high cholestanol/cholesterol ratios.
Vuorio et al., Circulation 1998 (Abstract)	OL	Spread + stanol esters, Amount and duration unknown	56		**Population:** Families with FH-NK deletion mutation **Results:** Stanol ester spread significantly reduced LDL-cholesterol: 17% in FH-NK children, 13% in unaffected family members, and 20% in FH-NK family members on simvastatin treatment. Stanol ester spread non-significantly reduced LDL-cholesterol by 12% in FH-NK parents.

a: Consumption originally reported in terms of stanols was converted as follows: 1 g plant stanols = 1.7 g plant stanol esters

Abbreviations: Alk Phos=alkaline phosphatase, ALT=alanine aminotransferase, AST=aspartic acid aminotransferase, CO=crossover, DB=double blind, F=female, GGT=gamma glutamyltransferase, Hb=hemoglobin, Hct=hematocrit, M=male, Mayo=mayonnaise, MI=myocardial infarction, NS=not statistically significant, OL=open label, PC=placebo controlled, Plt= platelet, R=randomized, y=year.

6. Published Studies in Children with Elevated Cholesterol

Study 1 (Gylling et al., 1995)

Gylling et al. (1995) assessed the effects of dietary plant stanol esters in 14 children with familial hypercholesterolemia in a double-blind, randomized study with a complete crossover design. The children were divided into two groups and received either canola oil spread alone or spread supplemented with 5.1 g/day stanol esters for six weeks.

The results of this study demonstrate that plant stanol esters reduced both total and LDL-cholesterol by 11% and 15%, respectively compared to the control spread. HDL-cholesterol and triglyceride levels did not change significantly in this study.

Study 2 (Williams et al., 1999)

Another crossover trial in children was published in 1999 (Williams et al.,1999). In this study, 19 healthy, preschool children (ages 2-5 years) were randomly assigned to use 5.1 g/day plant stanol esters as a spread or to use wheat bran cereal for four weeks. These children had a total cholesterol level ≤ 300 mg/dL at baseline. After a two-week wash-out period, the treatments for the two groups were switched.

The results of this study demonstrate that after using a spread containing 4.5 g/day plant stanol esters for four weeks, total cholesterol levels were reduced by 12% and LDL-cholesterol levels were reduced by 16%. HDL-cholesterol and triglyceride levels were not significantly changed in this study.

Table H-6 Published Studies in Children with Elevated Serum Cholesterol

Citation	Study Design	Product, Amount of Plant Stanol, *Plant Stanol Esters*[a]; Duration	N Enrolled	Mean Age (range) Gender	Study Results
Gylling et al., J Lipid Res 1995	R, Db, PC, CO	Canola oil spread; 6 weeks Spread + stanol esters, 3 g/d stanols, ***5.1 g/d stanol esters***; 6 weeks	14 + 1	9 y (2 - 15 y) 7 F, 8 M	**Population:** Children with familial hypercholesterolemia (14 heterozygous, 1 homozygous) **Results:** Stanol esters reduced total and LDL-cholesterol by 11% and 15%, respectively vs. control spread. There was no significant change in HDL-cholesterol or triglyceride levels.
Williams et al., J Amer Coll Nutr 1999	CO, OL	Spread + stanol esters, 3 g/d stanols, **5.1 g/d stanol esters** Rice bran cereal, 3 serv/d; 4 week periods separated by a 2 week washout	19	(2 - 5 y)	**Population:** Healthy children with total cholesterol ≤ 300 mg/dL and triglycerides ≤ 300 mg/dL. **Results:** After 4 weeks of stanol ester spread intake, total cholesterol was reduced 12% (p=0.02) and LDL-cholesterol was reduced 16% ($p < 0.05$). The dietary fiber did not significantly affect total or LDL-cholesterol levels. There was no significant change in HDL-cholesterol or triglyceride levels.

a: Consumption originally reported in terms of stanols was converted as follows: 1 g plant stanols = 1.7 g plant stanol esters

Abbreviations: Alk Phos=alkaline phosphatase, ALT=alanine aminotransferase, AST=aspartic acid aminotransferase, CO=crossover, DB=double blind, F=female, GGT=gamma glutamyltransferase, Hb=hemoglobin, Hct=hematocrit, M=male, Mayo=mayonnaise, MI=myocardial infarction, NS=not statistically significant, OL=open label, PC=placebo controlled, Plt= platelet, R=randomized, y=year.

7. A Published Study in Children with Normal Cholesterol

A randomized, crossover trial in 72 children[4/] reported that 2.5 g/day plant stanol esters in margarine for three months was well tolerated and no adverse clinical effects were observed (Tammi et al., 1999). The children were participants in the Special Turku Risk Intervention Program (STRIP) project, a randomized, prospective trial aimed at reducing the predisposition of children to known arteriosclerosis risk factors. The children were already on a diet low in saturated fat and cholesterol.

The results of this study demonstrate that plant stanol ester spread reduced total and LDL-cholesterol levels by 5.4% and 7.5%, respectively compared to baseline. HDL-cholesterol and triglyceride levels were unchanged in this study.

[4/] Eighty-one children were enrolled in this study; 72 completed both arms of the cross-over phase.

Table H-7 A Published Study in Children with Normal Serum Cholesterol

Citation	Study Design	Product, Amount of Plant Stanol, *Plant Stanol Esters*[a]; Duration	N Enrolled	Mean Age (range) Gender	Study Results
Tammi et al., Ped Res 1999 (Abstract)	R, Db, PC, CO	Low-fat, low cholesterol diet + control spread Spread + stanol esters, 1.46 g/d stanols, ***2.5 g/d stanol esters;*** 12 week periods separated by a 6 week washout	72 72	6 y	**Population:** A subgroup of children in the STRIP project intervention group **Results:** Sitostanol ester spread reduced total and LDL-cholesterol 5.4% and 7.5%, respectively from baseline. There was no significant change in HDL-cholesterol or triglyceride levels.

a: Consumption originally reported in terms of stanols was converted as follows: 1 g plant stanols = 1.7 g plant stanol esters

Abbreviations: Alk Phos=alkaline phosphatase, ALT=alanine aminotransferase, AST=aspartic acid aminotransferase, CO=crossover, DB=double blind, F=female, GGT=gamma glutamyltransferase, Hb=hemoglobin, Hct=hematocrit, M=male, Mayo=mayonnaise, MI=myocardial infarction, NS=not statistically significant, OL=open label, PC=placebo controlled, Plt= platelet, R=randomized, y=year.

8. Unpublished Studies in Adults

Study 1 (Hallikainen et al., in press)

Hallikainen et al. (in press) conducted a single-blind, crossover study in which 22 hypercholesterolemic adult subjects consumed spread containing 0, 1.4 g, 2.7 g, 4.1 g, and 5.4 g/day plant stanol esters for four weeks in a randomized order.

Serum total cholesterol and LDL-cholesterol decreased vs. control in a dose-dependent fashion. Total and LDL-cholesterol levels were reduced 2.8% and 1.7%, respectively in the 1.4 g/day plant stanol ester dose period compared to baseline. Serum cholesterol lowering occurred with the 2.7 g, 4.1 g and 5.4 g/day plant stanol ester periods. Total cholesterol significantly decreased 6.8%, 10.3% and 11.3%, respectively; and LDL-cholesterol significantly decreased 5.6%, 9.7% and 10.4%, respectively compared to control. This study demonstrates that approximately 3.4 g/day plant stanol esters can significantly reduce both total and LDL-cholesterol levels up to 10% compared to the control group. No significant change in HDL-cholesterol or triglyceride levels was observed.

Study 2 (McNeil Protocol 98-058)

A randomized, open-label trial in 90 adults evaluated plant stanol esters consumed in the form of spread and yogurt at daily intakes of 5.1 g, 10.2 g and 17 g plant stanol esters for two weeks (McNeil Protocol 98-058). The objective of this trial was to assess the tolerability of higher than recommended daily amounts of plant stanol esters. This study involved significant dietary changes for subjects in the 10.2 g/day plant stanol ester and 17 g/day plant stanol ester groups due to the large amount of study product they needed to consume.

Total cholesterol was lowered 6.9%, 10.5% and 12.3% with 5.1 g, 10.2 g and 17 g/day plant stanol esters, respectively compared to baseline. LDL-cholesterol was lowered 11%, 15.7% and 18.2% with 5.1 g, 10.2 g and 17 g/day plant stanol esters, respectively compared to baseline. This study suggests that increasing amounts of plant stanol esters may result in further reduction in total and LDL-cholesterol concentrations although no placebo arm was included in this trial.

Study 3 (McNeil Protocol 98-043)

After completion of the pivotal, placebo-controlled US trial in 1998 (Nguyen et al., 1999), one study site (Mayo Clinic) offered the study participants the option of continuing to use plant stanol ester spread for up to one year (McNeil Protocol 98-043).

In this one year extension study, consumption of 5.1 g/day plant stanol ester spread significantly lowered total and LDL-cholesterol levels approximately 9% and 11%,

respectively compared to baseline in 45 subjects. This cholesterol lowering efficacy was sustained over the 52 week study period. There were no significant changes in HDL-cholesterol compared to baseline.

Study 4 (McNeil protocol 97-035)
A randomized, double-blind, placebo-controlled trial was conducted to determine the effect of plant stanol ester spread in adult subjects currently taking a stable dosage regimen of one of the following HMG Co-A reductase inhibitors: Lipitor™, Mevacor®, Pravachol®, or Zocor® (McNeil protocol 97-035). Subjects who had been stabilized on a statin drug for at least 90 days with an LDL-cholesterol >130 mg/dL (N = 141) were randomly assigned to consume control spread or plant stanol ester spread containing 5.1 g/day plant stanol esters for eight weeks.

Subjects using plant stanol ester spread along with the statin drug significantly reduced total cholesterol levels by an additional 11.4% and LDL-cholesterol by an additional 16.7% while subjects using placebo spread reduced total cholesterol by an additional 4.5% and LDL-cholesterol by an additional 6.7%. There were no significant changes in HDL-cholesterol or triglyceride levels. This study suggests that plant stanol esters have an additive effect to cholesterol-lowering medications, such as statins and confirm published studies previously cited in this petition (Gylling and Miettinen, 1995; Gylling and Miettinen, 1996; Gylling et al., 1997).

Study 5 (McNeil Protocol 99-080)
The effects of plant stanol esters incorporated into softgels on serum cholesterol levels were investigated in a randomized, double-blind, placebo-controlled trial involving 160 adults (McNeil Protocol 99-080). Subjects were randomized to consume either placebo softgels or softgels containing plant stanol esters three times a day for three weeks. Half of the subjects were instructed to take the softgels with food, while the other half were given no instruction with regard to food intake. Targeted daily consumption of plant stanol esters was 4.5 g/day.

The results of this study demonstrate that after consuming softgels containing 4.5 g/day of plant stanol esters for three weeks, total and LDL-cholesterol levels were reduced by 4.9% and 7.4%, respectively compared to baseline. These reductions are significant compared to control.

Study 6 (Mensink, Spread Study)

Mensink evaluated the effect of changing the frequency of consumption of plant stanol esters on cholesterol lowering in a placebo-controlled, double-blind study with a crossover design (Mensink, Spread Study). In this trial, 39 healthy, normocholesterolemic subjects were randomized to control spread or 4.2 g/day plant stanol esters used once or three times per day for four-week periods. The three times per day group consumed .70 g plant stanol esters with breakfast, 1.4 g plant stanol esters with lunch and 2.1 g plant stanol esters with dinner.

The resulting decreases in cholesterol levels did not significantly differ between the plant stanol ester groups. Both groups were significantly decreased versus control. Consuming the plant stanol ester spread once daily significantly lowered total and LDL-cholesterol by 6.3% and 9.4%, respectively compared to the control spread. The plant stanol ester spread taken three times daily significantly lowered total and LDL-cholesterol by 6.6% and 10.4%, respectively compared to the control spread. Therefore, this study suggests that consuming 4.2 g/day plant stanol esters lowers total and LDL-cholesterol up to 7% and 10%, respectively compared to control group. Furthermore, the cholesterol lowering efficacy was not affected by the different consumption frequencies. HDL-cholesterol and triglyceride concentrations were not changed during the study periods.

Study 7 (Nguyen et al., Sitostanol Feasibility Study)

Nguyen et al. (Sitostanol Feasibility Study) conducted an eight-week, open-label study in adult residents of Olmsted County, Rochester, MN to determine how plant stanol ester spread is used in a free living setting and to evaluate if the method of use affects the cholesterol lowering response. Five-hundred adults were randomly assigned to use spread containing 1.7 g plant stanol esters per serving in a "spread use only" or a "general purpose use" fashion for two months. The "spread use only" group was instructed to follow the label directions to consume three servings a day. The "general purpose use" group was allowed to consume/use as much as they wanted to.

The cholesterol-lowering efficacy was maintained in the "non-research setting" in the free-living subject population. The effect of plant stanol esters on serum cholesterol levels was greater in subjects with total cholesterol ≥ 200 mg/dL. In these subjects, it was demonstrated that < 3.4 g/day, 3.4 - 5.1 g/day and ≥ 5.1 g/day plant stanol esters significantly reduced LDL-cholesterol by 6.7%, 8.9% and 11%, respectively compared to baseline. In subjects with total cholesterol < 200 mg/dL, it was demonstrated that < 3.4 g/day plant stanol esters nonsignificantly increased LDL-cholesterol by 1.7% and 3.4-5.1 g/day plant stanol esters nonsignificantly decreased LDL-cholesterol by 1.5%. The consumption of ≥ 5.1 g/day plant stanol esters significantly lowered LDL-cholesterol by 9%

compared to baseline in this group. In this study, the method of use, either "spread use only" or "general purpose use", did not affect the total and LDL-cholesterol response.

Study 8 (Scott)
In a single-blind study, 79 adult subjects with LDL-cholesterol > 130 mg/dL were randomly assigned to control spread with or without a Step I diet or plant stanol ester spread (5.1 g/day plant stanol esters) with or without following a Step I diet for six weeks (Scott).

Both plant stanol ester spread groups demonstrated significant cholesterol reductions compared to the control spread group. More specifically, the subjects consuming the plant stanol ester spread as the sole intervention in this study showed 6.7% and 9.9% reductions in total and LDL-cholesterol, respectively compared to baseline. The addition of a Step I diet to plant stanol ester spread use resulted in greater reductions of 8.1% and 13.1% in total and LDL-cholesterol, respectively compared to baseline. HDL-cholesterol and triglyceride levels were not significantly different among the study groups.

Study 9 (Mensink, Yogurt Study)
The effects of plant stanol esters incorporated into yogurt on serum cholesterol levels of healthy, normocholesterolemic subjects were investigated by Mensink (Yogurt Study). Following a three-week run-in period in which subjects consumed placebo yogurt, subjects were randomized either to continue to consume the placebo yogurt or to consume yogurt containing plant stanol esters for four weeks. Targeted plant stanol consumption was 5.1 g/day plant stanol esters.

Preliminary results of this study showed that consumption of plant stanol esters reduced total and LDL-cholesterol by 7.2% and 11.3%, respectively from baseline. In addition, there were no significant changes in HDL-cholesterol or triglyceride levels.

Study 10 (Grundy and Cater)
An additional dose response trial was conducted in eight subjects (Grundy and Cater). This study was conducted in a randomized, crossover fashion with either placebo spread, spread containing 3.4 g/day plant stanol ester, spread containing 4.1 g/day plant stanol esters and spread containing 5.8 g/day plant stanol esters.

In this study, subjects receiving 3.4 g/day plant stanol esters had a mean total cholesterol reduction of 8% and a mean LDL-cholesterol reduction of 12%. The 4.1 g/day plant stanol ester group reduced total cholesterol by 10% and LDL-cholesterol by 13%. The 5.8 g/day plant stanol ester group reduced total cholesterol by 10% and LDL-cholesterol by 14%. All

reductions were statistically different when compared with mean changes in subjects consuming placebo spread.

Study 11 (Tateno)
In a randomized, double-blind trial conducted in Japan, 105 Japanese men and women (mean age 47 years) consumed daily either 5.1 g plant stanol esters, 3.4 g plant stanol esters or a placebo spread for four weeks (Tateno).

Mean total and LDL-cholesterol levels were reduced by 5.5% and 7.3%, respectively in the 5.1 g/day plant stanol ester group, and 6.3% and 9.5%, respectively in the 3.4 g/day plant stanol ester group when compared with baseline values.

Table H-8 Unpublished Studies

Citation	Study Design	Product, Amount of Plant Stanols, *Plant Stanol Esters*[a]; Duration	N Enrolled	Mean Age (range) Gender	Study Results
Hallikainen et al., in press	R, SB, PC, CO	Spread + stanol esters, 0, 0.8, 1.6, 2.4, and 3.2 g/d stanols, ***0.0, 1.4, 2.7, 4.1, and 5.4 g/d stanol esters***; 4 week periods for each spread	22	51 y 14 F, 8 M	**Population:** Adult subjects with hypercholesterolemia **Results:** There were % reductions in total and LDL-cholesterol compared to baseline for all stanol ester groups. Total Chol. / LDL-Chol. 1.4 g/day period: 2.8% / 1.7% 2.7 g/day period: 6.8%* / 5.6%* 4.1 g/day period: 10.3%* / 9.7%* 5.4 g/day period: 11.3%* / 10.4%* *Significant reductions compared to control. There were no significant changes in HDL-cholesterol or triglyceride levels in any period.
McNeil Protocol 98-058	R, OL	Spread + stanol esters, 3 g/d stanols, ***5.1 g/d stanol esters***; 2 weeks Spread + stanol esters, 6 g/d stanols, ***10.2 g/d stanol esters***; 2 weeks Spread and yogurt + stanol esters, 10 g/d stanols, ***17 g/d stanol esters***; 2 weeks	30 30 30	50 y (22 - 78 y) 62 F, 28 M	**Population:** Adult subjects (minimum 20 y) with total cholesterol ≥200 mg/dL and ≤300 mg/dL who are not using any cholesterol-reducing medications or products. **Results:** There were significant % reductions in total and LDL-cholesterol compared to baseline for all stanol ester groups. Total Chol. / LDL-Chol. 5.1 g/day group: 6.9% / 11% 10.2 g/day group: 10.5% / 15.7% 17 g/day group: 12.3% / 18.2%

a: Consumption originally reported in terms of stanols was converted as follows: 1 g plant stanols = 1.7 g plant stanol esters

Abbreviations: Alk Phos=alkaline phosphatase, ALT=alanine aminotransferase, AST=aspartic acid aminotransferase, CO=crossover, DB=double blind, F=female, GGT=gamma glutamyltransferase, Hb=hemoglobin, Hct=hematocrit, M=male, Mayo=mayonnaise, MI=myocardial infarction, NS=not statistically significant, OL=open label, PC=placebo controlled, Plt= platelet, R=randomized, y=year.

Table H-8 Unpublished Studies

Citation	Study Design	Product, Amount of Plant Stanols, *Plant Stanol Esters*[a]; Duration	N Enrolled	Mean Age (range) Gender	Study Results
McNeil Protocol 98-043	OL	Spread + stanol esters, 3 g/d stanols, ***5.1 g/d stanol esters***; 52 weeks	45	Not available	**Population:** Adult subjects (minimum 20 y) who participated and completed the initial study at the Mayo Clinic (Nguyen et al., 1999), including 12 weeks of spread use, and used >66% of the spread during weeks 5 to 12. **Results:** Total and LDL-cholesterol concentrations were significantly reduced approximately 9% and 11%, respectively compared to baseline levels. This cholesterol lowering efficacy was sustained over the study period. There were no significant changes in HDL-cholesterol compared to baseline.
McNeil Protocol 97-035	R, DB, PC	Control spread; 8 weeks Spread + stanol esters, 3 g/d stanols, ***5.1 g/d stanol esters***; 8 weeks	77 71	56.3 y (33 - 78 y) 60 F, 88 M	**Population:** Adults subjects (minimum 20 y) with LDL-cholesterol >130 mg/dL who are currently taking a stable dose regimen of one of the following HMG Co-A reductase inhibitors: Lipitor™, Mevacor®, Pravachol®, or Zocor®. **Results:** There were significant % reductions in total and LDL-cholesterol compared to baseline for the stanol ester group. There were non-significant reductions in the control group compared to baseline. Total Chol. / LDL-Chol. Control group: 4.5% / 6.7% 5.1 g/day group: 11.4% / 16.7% There were no significant changes in HDL-cholesterol or triglyceride levels.

a: Consumption originally reported in terms of stanols was converted as follows: 1 g plant stanols = 1.7 g plant stanol esters

Abbreviations: Alk Phos=alkaline phosphatase, ALT=alanine aminotransferase, AST=aspartic acid aminotransferase, CO=crossover, DB=double blind, F=female, GGT=gamma glutamyltransferase, Hb=hemoglobin, Hct=hematocrit, M=male, Mayo=mayonnaise, MI=myocardial infarction, NS=not statistically significant, OL=open label, PC=placebo controlled, Plt= platelet, R=randomized, y=year.

Table H-8 Unpublished Studies

Citation	Study Design	Product, Amount of Plant Stanols, *Plant Stanol Esters*[a]; Duration	N Enrolled	Mean Age (range) Gender	Study Results
McNeil Protocol 99-080	R, DB, PC	Control softgel; 3 weeks Softgel + stanol esters, 3 g/d stanols, ***4.5 g/d stanol esters***; 3 weeks	80 80	Not available	**Population:** Adult subjects (minimum 20 y) with total cholesterol ≥ 220 mg/dL and ≤ 300 mg/dL who are not using any other cholesterol-reducing medications or products. **Results:** The % reductions in total and LDL-cholesterol levels from baseline for the stanol ester group were significant compared to control. Total Chol. / LDL-Chol. Control softgels: 1.9% / 2.2% Stanol ester softgels: 4.9% / 7.4%
Mensink, Spread Study	PC, CO, DB	Spread + stanol esters 1x or 3x daily, 2.5 g/d stanols, **4.2 g/d stanol esters**; 8 week periods	39	28 F, 11 M	**Population:** Healthy, normocholesterolemic adults with total cholesterol ≤ 251 mg/dL and who are not using lipid lowering medications or diets. **Results:** There were significant % reductions in total and LDL-cholesterol compared to control group for both stanol ester groups. These efficacy results did not differ significantly between the two groups. Total Chol. / LDL-Chol. stanol ester spread 1x daily: 6.3% / 9.4% stanol ester spread 3x daily: 6.6% / 10.4% Stanol ester spread administered in different frequencies did not affect cholesterol-lowering efficacy. HDL-cholesterol and triglyceride levels were not changed.

a: Consumption originally reported in terms of stanols was converted as follows: 1 g plant stanols = 1.7 g plant stanol esters

Abbreviations: Alk Phos=alkaline phosphatase, ALT=alanine aminotransferase, AST=aspartic acid aminotransferase, CO=crossover, DB=double blind, F=female, GGT=gamma glutamyltransferase, Hb=hemoglobin, Hct=hematocrit, M=male, Mayo=mayonnaise, MI=myocardial infarction, NS=not statistically significant, OL=open label, PC=placebo controlled, Plt= platelet, R=randomized, y=year.

Table H-8 Unpublished Studies

Citation	Study Design	Product, Amount of Plant Stanols, *Plant Stanol Esters*[a]; Duration	N Enrolled	Mean Age (range) Gender	Study Results
Nguyen et al., Sitostanol Feasibility Study	R, OL	Spread + stanol esters, 3 g/d stanols, ***5.1 g/d stanol esters***; 8 weeks "Spread use only" or "General purpose use"	500	(19 - 90 y)	**Population:** Randomly selected adult residents (minimum 18 y) of Olmsted County, Rochester, MN. **Results:** There were significant % reductions in LDL-cholesterol from baseline in subjects with total cholesterol ≥ 200 mg/dL for all groups consuming stanol ester. No. Stanol Ester Servings/Day — LDL-Chol. 0 (0 g/day stanol ester) — + 14.7% <2 (<3.4 g/day stanol ester) — 6.7% 2-3 (3.4-5.1 g/day stanol ester) — 8.9% ≥3 (≥ 5.1 g/day stanol ester) — 11.0% There were % reductions in LDL-cholesterol from baseline in subjects with total cholesterol < 200 mg/dL for groups consuming at least 2 servings/day of stanol ester spread. No. Stanol Ester Servings/Day — LDL-Chol. 0 (0 g/day stanol ester) — + 3.2% <2 (<3.4 g/day stanol ester) — + 1.7% 2-3 (3.4-5.1 g/day stanol ester) — 1.5% ≥3 (≥ 5.1 g/day stanol ester) — 9.0%* *Significant reduction compared to baseline.

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Table H-8 Unpublished Studies

Citation	Study Design	Product, Amount of Plant Stanols, *Plant Stanol Esters*[a]; Duration	N Enrolled	Mean Age (range) Gender	Study Results
Scott	R, SB, PC	Control spread; 6 weeks Step I diet + control spread; 6 weeks Spread + stanol esters; 3 g/d stanols, ***5.1 g/d stanol esters***; 6 weeks Step I diet + spread + stanol esters, 3 g/d stanols, ***5.1 g/d stanol esters***; 6 weeks	20 20 19 20	51.6 y (26 - 70 y) 46 F, 33 M	**Population:** Adult subjects (20 - 70 y) with LDL-cholesterol > 130 mg/dL who are not currently taking lipid-lowering medication. **Results:** There were significant % reductions in total and LDL-cholesterol compared to baseline for all stanol ester groups. Total Chol. / LDL-Chol. Control Spread 0.8% 3.2% Diet + control spread 3.8% 7.8% Stanol ester spread 6.7% 9.9% Diet + stanol ester spread 8.1% 13.1% HDL-cholesterol and triglyceride levels were not significantly different among the study groups.
Mensink, Yogurt Study	R, DB, PC	Control yogurt; 4 weeks Yogurt + stanol esters, 3 g/d stanols, ***5.1 g/d stanol esters***; 4 weeks	30 30	36.1 y 44 F, 16 M	**Population:** Healthy, normocholesterolemic subjects. **Run-in:** 3 week control yogurt. **Results:** Preliminary results demonstrate that plant stanol ester yogurt reduced total and LDL-cholesterol by 7.2% and 11.3%, respectively from baseline. There were no significant changes in HDL-cholesterol or triglyceride levels.
Grundy and Cater	R, CO, OL, PC	Control spread; 6 weeks Spread + stanol esters, 2, 3 or 4 g/d stanols, ***3.4 ,5.1 or 6.8 g/d stanol esters***; 6 week periods for each spread	12	unknown	**Population:** Adult subjects with LDL-cholesterol >130 mg/dL and <220 mg/dL who have not taken hypolipidemic medications. **Results:** Study is complete. Analysis is in progress.

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Abbreviations: Alk Phos=alkaline phosphatase, ALT=alanine aminotransferase, AST=aspartic acid aminotransferase, CO=crossover, DB=double blind, F=female, GGT=gamma glutamyltransferase, Hb=hemoglobin, Hct=hematocrit, M=male, Mayo=mayonnaise, MI=myocardial infarction, NS=not statistically significant, OL=open label, PC=placebo controlled, Plt= platelet, R=randomized, y=year.

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Citation	Study Design	Product, Amount of Plant Stanols, *Plant Stanol Esters*[a]; Duration	N Enrolled	Mean Age (range) Gender	Study Results
Tateno	R, DB, PC	Control spread; 4 wks Control spread + stanol esters, 2 g/d stanols, ***3.4 g/d stanol esters*** Spread + stanol esters 3 g/d stanols, ***5.1 g/d stanol esters***	35 34 36	47.6 y 65 F, 36 M	**Population:** Adult subjects with total cholesterol 210 -280 mg/dL.. **Results:** 5.1 g/d plant stanol esters reduced total and LDL- cholesterol levels by 5.5% and 7.3%; 3.4 g/d plant stanol esters reduced total and LDL-cholesterol levels by 6.3% and 9.5% compared to baseline.

a: Consumption originally reported in terms of stanols was converted as follows: 1 g plant stanols = 1.7 g plant stanol esters

Abbreviations: Alk Phos=alkaline phosphatase, ALT=alanine aminotransferase, AST=aspartic acid aminotransferase, CO=crossover, DB=double blind, F=female, GGT=gamma glutamyltransferase, Hb=hemoglobin, Hct=hematocrit, M=male, Mayo=mayonnaise, MI=myocardial infarction, NS=not statistically significant, OL=open label, PC=placebo controlled, Plt= platelet, R=randomized, y=year.