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By Hand Delivery

Food and Drug Administration
Office of Food Labeling (HFS-150)
200 C Street, SW
Washington, DC 20204

To whom it may concern:

Enclosed please find for filing a petition pursuant to §403(r)(4) of the Federal Food, Drug, and Cosmetic Act, and 21 C.F.R. §101.70, for a health claim with respect to vegetable oil sterol esters and reduced risk of coronary heart disease. Two copies of the petition (three volumes each) are enclosed.

Correspondence and questions on this petition should be directed to the undersigned or to Daniel Dwyer at Kleinfeld, Kaplan & Becker at 202-223-5120.

Sincerely,

Nancy Schnell

Nancy L. Schnell
Deputy General Counsel

NLS/DLT
Enclosure

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00P-1275

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January 28, 2000

Petitioner: Lipton
Address: 800 Sylvan Avenue
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Attn: Dr. William Franke

Subject: Petition for Health Claim – Vegetable Oil Sterol Esters (VOSE) and Coronary Heart Disease
(Volume I of III)

Food and Drug Administration
Office of Food labeling (HFS-150)
Center for Food Safety and Applied Nutrition
200 C Street, S.W.
Washington, D.C. 20204

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VOSE Health Claim Petition

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VOSE Health Claim Petition

I. Introduction

Pursuant to §403(r) (4) of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. §343(r) (4)) Lipton submits this petition for a health claim about the association between certain foods containing vegetable oil sterol esters (VOSE) and reduced risk of coronary heart disease (CHD). The petition requests that such a claim be permitted for spreads and salad dressings that contain a minimum of 1.6 g of VOSE per reference amount customarily consumed (RACC).

VOSE consist of esterified plant sterols, or phytosterols. These substances are structurally very similar to the main "animal" sterol, cholesterol (see Figure 1). The role of plant sterols, particularly the main dietary plant sterol, β-sitosterol, in interfering with cholesterol absorption and reducing blood cholesterol levels was first recognized in the early 1950's. Since the initial discoveries, many studies in both animals and man have confirmed and amplified the early work. At the same time, research has confirmed the importance of blood cholesterol as a major risk factor for CHD. This research has led to today's public health policies, best exemplified by the National Cholesterol Education Program, that stress dietary control to maintain healthy cholesterol levels as a primary means of reducing individual and population risks for CHD.

FDA has already authorized three new CHD health claims on foods and/or food components (oat foods, psyllium fiber and soy protein), all based on the food's ability to lower cholesterol levels as part of a diet low in saturated fat and cholesterol. In 1987, O.J. Pollak, a pioneer in the research on plant sterols, advocated increasing plant sterol

O.J. Pollak, a pioneer in the research on plant sterols, advocated increasing plant sterol content in margarines as a public health measure to reduce population blood cholesterol levels (Pollak, 1987). He suggested that adding plant sterols to the diet was similar to the addition of iron or B vitamins to foods to correct an imbalance in the American diet. More recently, Pearson and Lester (1998) advocated including sterol containing foods in the arsenal for dietary intervention to reduce the risk of CHD.

Based on the evidence detailed in the following sections, Lipton believes that VOSE - containing spreads and dressings, and the requested health claim, meet the applicable requirements for a health claim set forth in 21 C.F.R. §101.14. Foods meeting the conditions of this petition are useful in reducing the risk of CHD when consumed as part of a diet low in saturated fat and cholesterol. A health claim for these foods would assist consumers in maintaining healthy dietary practices.

II. Preliminary Requirements

A. Vegetable Oil Sterol Esters Are Associated with Reduced Risk of a Major Disease: Coronary Heart Disease

CHD is the leading cause of mortality in the USA (Hoyert, et al., 1999; NCEP, 1993) and most other Western countries. The three major modifiable risk factors for CHD are smoking, elevated blood pressure and high blood cholesterol. The importance of cholesterol as a risk factor for CHD is well established in both animals and humans.

Cholesterol reduction by diet or medication will lower the risk of CHD when used for either primary or secondary prevention (NCEP, 1993).

The FDA has recognized the importance of CHD and the significance of dietary control of blood cholesterol in reducing CHD risk in several preceding regulatory proceedings. For example, the agency has acknowledged that "High blood total and LDL-cholesterol are major modifiable risk factors in the development of CHD" (21 C.F.R. 101.81(b)(1)). It has also acknowledged the importance of dietary components (such as soluble fiber) in helping to lower blood total- and LDL- cholesterol levels (21 C.F.R. 101.81(b)(2)). FDA has also stated that "There is a continuum of mortality risk from coronary heart disease that increases with increasing levels of blood LDL-cholesterol. Individuals with high blood LDL-cholesterol are at greatest risk. A larger number of individuals with more moderately elevated cholesterol also have increased risk of coronary events; such individuals comprise a substantial proportion of the adult U.S. population (21 C.F.R. 101.75(b)(1)).

Vegetable oil sterol esters, and in particular the sterol component, have been the subject of research for their cholesterol lowering properties for nearly 50 years. As detailed in the scientific summary, the evidence that sterols and their esters will reduce blood cholesterol is conclusive, and therefore the general requirements set forth in 21 C.F.R. §101.14(b)(1) are met.

B. Vegetable Oil Sterol Esters are a Food Ingredient that provides Nutritive Value

Vegetable oil sterol esters are part of the plant sterol family. Plant sterols occur widely throughout the plant kingdom and are present in many edible fruits, vegetables, nuts, seeds, cereals and legumes (Weihrauch and Gardner, 1978). The most common sterols of nutritional significance are β-sitosterol, campesterol and stigmasterol (Vahouny and Kritchevsky, 1981). Figure 1 shows the chemical structures of the three most common plant sterols, cholesterol, and an example of a sterol ester. As seen in Figure 1, VOSE are composed of one mole of fatty acid and one mole of sterol.

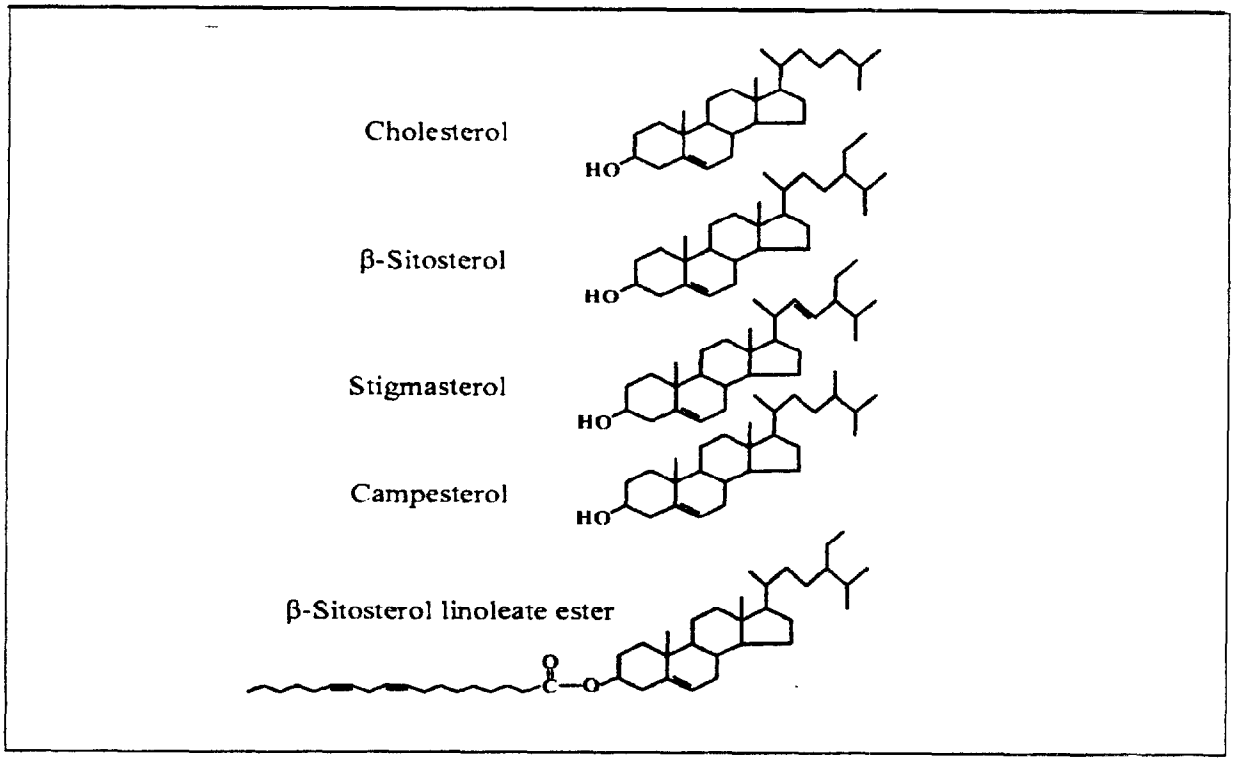

Figure 1 Structures of Common Plant Sterols, Cholesterol and Linoleic Acid Ester of β-Sitosterol (adapted from Moreau et al., 1999)

In this petition, the fatty acid component of the VOSE is predominantly unsaturated fatty acids with an 18-carbon chain length (see specification in Appendix D). Therefore, VOSE are about 60% sterol and 40% fatty acid, by weight, and one gram of sterol is equivalent to about 1.6 g VOSE.

The National Nutrient Database (USDA, 1998) includes sterol composition data for 323 foods. The highest concentrations occur in oil seeds, vegetable oils derived from oil seeds and foods made from these oils, such as margarines and spreads. The plant sterols in foods may occur as either the free sterol or esterified with a fatty acid. In vegetable oils, typically between 25 and 80% of the sterol is in the ester form (Johansson, 1979; Johansson and Appelqvist, 1978; Johansson and Hoffman, 1979; Kochhar, 1983). Method of refinement of the vegetable oil and seed cultivar both influence the relative amount of free and esterified sterol in oils.

Several studies have estimated dietary plant sterol intake. From a population in the Los Angeles area, Nair et al. (1984) found that plant sterol (β-sitosterol and stigmasterol) intake ranged from 77.9 mg/d in the general population to 343.6 mg/d in lacto-ovo vegetarians. The 1991 British diet was estimated to contain about 158 mg/d plant sterols (β-sitosterol, stigmasterol and campesterol) (Morton, et al., 1995). Scandinavian vegetarians consume, on average, 513 mg/d and non-vegetarians 398 mg/d (Meittinen and Tarpila, 1978). Plant sterol intake in the Japanese diet is estimated at 373 mg/d (Hirai, et al., 1986). In an analysis of diets of participants in the Seven Countries Study, deVries, et al. (1997) found plant sterol intake (sitosterol, stigmasterol and campesterol)

ranged from 170 mg/d among U.S. railroad workers to 358 mg/d in Corfu, Greece. In a review, Ling and Jones (1995) estimated average United States intake at 250 mg/d and this could be doubled among vegetarians.

Thus, VOSE, and in particular the sterol component, are a constituent of the diet for various population groups, including Americans.

VOSE provide nutritive value within the meaning of 21 C.F.R. §101.14(b)(3)(i). In this regulation, "nutritive value" is defined as "value in sustaining human existence by such processes as promoting growth, replacing loss of essential nutrients, or providing energy." The agency has stressed that this definition is intended to be flexible, 58 Fed. Reg. 2478, 2488 (January 6, 1993). Thus, for example, nutritive value may include:

- "the usefulness of a food component, consumed as part of the total diet, in reducing the risk, or forestalling the onset, of a chronic disease condition;" 55 Fed. Reg. 5176, 5178 (February 13, 1990);
- "value for cellular functions by providing catalytic support for protective reactions (e.g., inhibiting harmful processes);" 56 Fed. Reg. 60537, 60542 November 27, 1991);
- the value of a food substance where "the claimed effect on disease is associated with the normal maintenance of human existence," for example, "assisting in the efficient functioning of classical nutritional processes and of other metabolic processes necessary for the normal maintenance of human existence." 59 Fed. Reg. 395, 407 (January 4, 1994);

- where the food substance is intended for consumption at higher levels than normally in the diet, "the substance has nutritive value where the mechanism by which the substance affects the risk of disease at these high levels is the same as the mechanism by which it functions as a nutrient at normal dietary levels. Id. at 408.

Lipton has concluded that the effects of VOSE are attributable to the nutritive value of the substance for the following reasons:

(i) VOSE are a naturally occurring component of our diet, and they act through a normal digestive process to inhibit the harmful process of increased cholesterol absorption, resulting in lower LDL-cholesterol levels.

(ii) VOSE aid the control of normal lipid absorption and metabolism, and thereby help (as part of an overall diet) to reduce risk of chronic disease.

(iii) The effects of VOSE are observed at normal dietary levels and at increased consumption levels.

A detailed analysis of the FDA's "nutritive value" requirement and why VOSE meets this requirement is provided in Appendix C. Based on this analysis, the conditions in 21 C.F.R. §101.14(b)(3)(I) are met.

C. Vegetable Oil Sterol Esters Are Generally Recognized As Safe

The use of vegetable oil sterol esters in spreads and dressings up to 1.6 g/serving (about 1.0 g free sterols/serving) is safe and lawful within the meaning of §101.14(b)(3)(ii). The basis of Lipton's conclusion is as follows:

As discussed in section II. B above, plant sterols and their esters are part of the food supply. They are natural components of commonly consumed vegetable oils and other foods. Approximately 250 mg of plant sterols are consumed every day as part of an average U. S. diet. Certain groups of people, in particular vegetarians, may consume twice this amount. Vegetable oil sterols are obtained from the same fraction of edible oils as vitamin E, and are esterified with fatty acids from edible sunflower oil. Esterification is achieved using methods and materials commonly employed by the edible oil processing industry.

As discussed in sections III. A and C below, VOSE are hydrolyzed to free plant sterols and fatty acids in the gastrointestinal tract. Therefore, the extensive human safety information from published clinical studies of plant sterols is pertinent in establishing safe exposure limits for VOSE. There are a number of these studies, including studies of high-doses, (up to 25 g/d), over a long term (up to three years). Over 1800 people have participated in these trials, and some studies involved children and adolescents. No adverse health effects have been reported.

Extensive animal and *in vitro* safety testing has been conducted on vegetable oil sterol esters, with no evidence of any adverse effects (Baker, et al., 1999; Hepburn, et al., 1999; Waalkens-Berendsen, et al., 1999). These data corroborate the animal and human safety data on plant sterols accumulated since the 1950s.

A well-designed and conducted 13-week study of vegetable oil sterol esters in rats established a no-observed-adverse effect level of 3.9 g free sterols/kg/d, calculated from the highest concentration tested, 5% of the diet as sterols (8.1% of the diet as vegetable oil sterol ester) (Hepburn, et al., 1999). This was equivalent to a dose of 6.6 g vegetable oil sterol esters/kg body weight/day. In a well-designed two-generation reproductive study, 8.1% vegetable oil sterol ester (5% plant sterols in the diet) was considered the no-observed-adverse effect level. This was equivalent to 2.5-9.1 g vegetable oil sterol ester/kg body weight/d, depending on period of diet feeding (1.54-5.62 g/kg/d for vegetable oil sterols) (Waalkens-Berendsen, et al., 1999). Based on these studies, the other available animal and human data on plant sterols and vegetable oil sterol esters, and using an appropriate safety factor, an acceptable daily intake (ADI) of 130 mg/kg/d (as free plant sterols) was established. For a 60 kg individual, this would be 7.8 g sterols/person/d, which is equivalent to 13.0 g vegetable oil sterol esters/person/d.

Using the 90th percentile consumption of spreads by adults and children, the estimated daily intake will not exceed the ADI. For adults, the intended consumers, such consumption of vegetable oil sterols from reduced-fat vegetable oil spread will be in the range of 1.1-2.2 g/d; consumption by children would be lower.

Using the 90th percentile consumption of dressings by adults and children, the estimated daily intake also will not exceed the ADI. For adults, the intended consumers, such consumption of vegetable oil sterols from reduced-fat dressings will be in the range of 1.1-2.2 g/d; consumption by children would be lower.

Using the 90th percentile consumption of both spreads and dressings combined, the estimated daily intake also will not exceed the ADI. For adults, such consumption of vegetable oil sterols would be about 4.4 g/day; consumption by children would be lower. Current labeling of products containing VOSE recommends that individuals consume up to 3 servings a day of spreads and dressings (equivalent to 3.3 g/d of free sterols). Both of these values are below the ADI.

Based on a critical evaluation of all the available information on plant sterols and their esters, and on the advice of a panel of independent experts, Lipton determined that the use of vegetable oil sterol esters meeting appropriate food-grade specifications and produced by current good manufacturing practice (21 C.F.R. 182.1(b)), is safe for use as an ingredient in spreads and dressings in amounts not to exceed 1.7 grams per serving.

Lipton concluded that (1) the data and information relied on are generally available, and (2) there is a consensus among qualified experts about the safety of VOSE for this intended use. Therefore, VOSE are generally recognized as safe (GRAS) for their intended use in spreads and dressings within the meaning of §201(s) of the FD &C Act, 21 C.F.R. §§170.3 and 170.30, and the proposed rules at 62 Fed. Reg. 18960 (April 17, 1997).

Lipton submitted a GRAS notification to the FDA on VOSE on January 11, 1999, and FDA responded on April 30, 1999, stating its conclusion that there is no basis to question Lipton's GRAS determination. A copy of this letter is provided at Appendix D. The FDA's review of Lipton's GRAS notification was thorough, involving requests for additional information and review of studies. Lipton therefore believes that the information submitted in its GRAS notification is sufficient for purposes of demonstrating that VOSE are safe and lawful under 21 C.F.R. §101.14(b)(3)(ii).

III. Summary of Scientific data supporting the claim

A. Introduction/Overview

Plant sterols are naturally present in food in three forms: free sterol, esterified with C12-C18 fatty acids (25-80% of total plant sterols), and conjugated as glucosides (generally in small amounts). The majority of crude vegetable oils contain 100-500 mg plant sterols per 100 g oil, while some oils like rice bran or wheat germ, can contain up to 4%

plant sterols. Reduced- and low-fat spreads currently on the market contain approximately 0.1-0.4% plant sterols. Full fat margarines contain 0.3-0.4%. The plant sterols used by Lipton are obtained from vegetable oil, predominantly soybean, distillate which is the same fraction used as a source of vitamin E. The major sterol components are β-sitosterol, campesterol and stigmasterol, the same sterols commonly consumed in the diet.

This petition focuses on vegetable oil sterol esters, plant sterols esterified with unsaturated fatty acids from sunflower. The solubility of free sterols in oil is only 2%. Solubility of sterol esters in oil exceeds 20% (Jandacek, et al., 1977), thus Lipton esterifies sterols with fatty acids from sunflower to improve solubility. Improved solubility of VOSE creates a palatable product and is associated with more uniform distribution in the product and in the gastrointestinal tract. Esterification is achieved using methods and materials commonly employed by the edible oil processing industry. Plant sterol esters are hydrolyzed in the intestine to free sterols and fatty acids by pancreatic carboxy ester lipase/esterase (Best and Duncan, 1958, Mattson, et al., 1977). In vitro studies have shown that the rate of hydrolysis depends on the fatty acid moiety and that esters of unsaturated fatty acids were much more readily hydrolyzed than esters of saturated fatty acids (Swell et al., 1954; Best and Duncan, 1958). Since plant sterol esters are hydrolyzed to free sterols and fatty acids in the gastrointestinal tract, and free sterols are the active moiety of VOSE, the literature on free plant sterols have a direct bearing on this petition.

There has been considerable research on the effects of plant sterols on blood cholesterol levels. We have limited our scientific summary to the review of full scientific reports, whether published or unpublished, and have focused our review on the effects of plant sterols on blood cholesterol levels. The research on the effects of plant sterols has occurred in three distinct phases:

1) A discovery phase in the 1950's where initial observations of decreased cholesterol absorption, and thus decreased plasma cholesterol, were noted in several animal models and in some early human trials;
2) The 1970's, where β-sitosterol was examined in the form of a drug product to lower cholesterol in humans;
3) The 1990's, where advances in food technology led to the development of several palatable food products with certain forms of esterified plant sterols.

In the 1970's, with the approval of β-sitosterol in the form of a drug, Cytellin (Eli Lilly), there was agreement that this material was safe and effective in lowering cholesterol. An issue with this particular product was compliance, given its potentially unpalatable nature. Kritchevsky (1997) reported that reasons for discontinued use of β-sitosterol preparations were its chalky taste and the need to take the product before every meal.

In the 1990's, esterification of vegetable oil sterols to certain fatty acids and their incorporation into vegetable oil spreads like margarines was found to provide a product that was not only palatable, but also effective in lowering cholesterol levels.

VOSE Health Claim Petition

This section of the petition will summarize the data substantiating the cholesterol lowering effect of VOSE. Since neither the Surgeon General's report on "Nutrition and Health" (1988) nor the National Academy of Sciences report on "Diet and Health"(1989) discussed the effects of plant sterols, we have included literature on plant sterols prior to 1988. We will not recapitulate conclusions reached in prior FDA rulemakings to the effect that decreased levels of blood total- and LDL- cholesterol are associated with decreased risk of developing coronary heart disease. These conclusions include: (1) "High blood total- and LDL-cholesterol are major modifiable risk factors in the development of coronary heart disease." (21 C.F.R. §101.75(a)); (2) that "Diets low in saturated fat and cholesterol are associated with decreased levels of blood total- and LDL-cholesterol, and thus, with decreased risk of developing coronary heart disease." (21 C.F.R. §101.75 (a)(2)); (3) that "There is a continuum of mortality risk from coronary heart disease that increases with increasing levels of blood LDL-cholesterol. Individuals with high blood LDL-cholesterol are at greatest risk. A larger number of individuals with more moderately elevated cholesterol also have increased risk of coronary events; such individuals comprise a substantial proportion of the U. S. adult population." (21 C.F.R. §101.75(a)(2)(b)); and (4) that studies evaluating individuals with elevated cholesterol are appropriate in order to determine whether a health claim will benefit the overall public health.

B. Vegetable oil sterol and sterol ester efficacy in lowering blood cholesterol levels

Over forty years of published research is available describing the effects of plant sterols and sterol esters on cholesterol metabolism. Several excellent reviews have been published which focus on plant sterols and, in particular β-sitosterol (Peterson, 1958; Pollak and Kritchevsky, 1981; Vahouny and Kritchevsky, 1981; Pollak, 1985; Ling and Jones, 1995). The largest of these reviews has over 956 references, citing over 90 studies in humans, and covers plant sterols in both animals and humans; vegetable oil sterols and specifically β-sitosterol as cholesterol "depressants"; effects of sitosterol esters; sterol absorption and excretion; and mechanisms by which sitosterol reduces cholesterol (Pollak and Kritchevsky, 1981). This review concluded that "Sitosterol, alone or combined with dietary measures, offers a non-medical approach to the reduction of plasma cholesterol...." Additionally, the authors went on to point out that the use of β-sitosterol to lower cholesterol levels is close to the ideal approach since it is effective and free of significant side effects or toxicity. Vahouny and Kritchevsky (1981) reported that plant sterols, as analogs of cholesterol, are absorbed with less efficiency than cholesterol and may interfere with the normal absorption of dietary and/or biliary cholesterol. They stated, "For this reason, phytosterols, and in particular β-sitosterol, have been employed as therapeutic agents to compete with normal absorption of cholesterol and thereby ultimately express a hypocholesterolemic effect in man." They also stated there is little question that plant sterols are absorbed less efficiently than cholesterol, and predicted that β-sitosterol "will receive renewed attention as hypocholesterolemic agents, used either alone or in combination with other dietary

measures." Pollak (1985) stated β-sitosterol would be a good alternative to other hypocholesterolemic approaches. He suggested "prophylactic supplementation" of foods with β-sitosterol. He also stated that, while this supplementation would be limited to butter, margarines or similar products for practical reasons, the β-sitosterol provided would affect dietary cholesterol and cholesterol from non-dietary sources available for absorption or re-absorption. Ling and Jones (1995), in the most recent comprehensive review on plant sterols, concluded from studies over the past 40 years that "moderate levels of plant sterols offer advantages as safe and inexpensive primary cholesterol lowering agents in humans." Finally, Pearson and Lester (1998) reported that "the current literature on plant sterol-supplemented margarine shows a consistent and significant effect: the product lowers LDL-cholesterol 7 to 14 percent. This suggests enormous possibilities for population-wide CHD prevention."

These peer-reviewed, published review articles demonstrate that there is significant scientific agreement that plant sterols and sterol esters lower elevated cholesterol levels in both animal models and in human clinical trials. A comprehensive discussion of the effects of the ingestion of plant sterols and sterol esters on cholesterol levels in humans is presented below.

C. Sterols and Sterol Esters Lower Plasma Total- and LDL-Cholesterol but Not HDL-Cholesterol in Humans

A considerable body of published research is available describing the effects of plant sterols and sterol esters on cholesterol metabolism in humans. A summary of this

research is presented in Table 1 and a more extensive review of these studies is presented in Table 2. Eleven of the fifteen studies reported in Tables 1 and 2 appear to meet FDA criteria for evaluating studies for effects on cholesterol levels. Namely, these studies have adequate experimental designs, include appropriate control groups or periods, have an intervention period of sufficient duration (3 weeks), and evaluate subjects that are relevant to the U. S. population (normal to moderate elevation in cholesterol levels). Three studies of short duration also merit consideration, since they were of careful design and show that plant sterol consumption significantly reduced blood cholesterol levels (Pollak, 1953; Beveridge, et al., 1964; Kudchodkar, et al., 1976). In addition another study in children with familial hypercholesterolemia also merits consideration since it shows the effectiveness of plant sterols even in this high-risk subpopulation (Becker, et al., 1993). Even if not considered as primary evidence, these four studies provide supporting evidence of the benefits of plant sterols on cholesterol levels.

(i) Studies of Plant Sterols are relevant to an Evaluation of VOSE.

Several studies included in this group of 15 human trials evaluated the effects of Cytellin, a drug consisting principally of β-sitosterol that had been approved for lowering cholesterol. FDA concluded in the health claim regulation regarding soluble fiber from psyllium that studies evaluating the effects of psyllium as an over-the-counter laxative were appropriate to consider in its evaluation of the nutrient/disease relationship. Important to the agency's conclusion was the fact that the material was essentially the same in both the food and the drug, and the concentrations consumed in food were

Table 1: Summary of human studies on the effects of plant sterols on cholesterol metabolism

Study	Subjects	Study Length	Study Material	Results
Ayesh, et al., Accepted for publication	21 M/F	Males: 3 wks Females: 4 wks	Sterol ester margarine to provide 8.6 g/d sterols	Sterol: TC -18%; LDL -23%; no effect on HDL
Jones, et al., Submitted for publication	15 M	3 wks	Sterol ester or stanol ester margarine to provide 1.8 g/d sterols	Sterol: TC -7.8; LDL –9.3%; no effect on HDL; Chol. absorp. -36%; Chol. syn. +53%. Stanol: TC -3.8; LDL -3.8%; no effect on HDL; Chol. absorp. –25.9%; Chol. syn. +38%
Maki, et al., Submitted for publication	224 M/F	5 wks	Sterol ester spread to provide 0, 1.1 or 2.2 g/d sterols	Sterol: TC -3.9 to -5.6%; LDL -5.9 to -6.9%; TC/HDL -5.5 to -7.3%; apoB -5.1 to -6.4%
Hendricks, et al., 1999	100 M/F	3.5 wks	Sterol ester spread to provide 0.85, 1.61 & 3.22 g/d sterols	Sterol: TC -4.9 to -6.8%; LDL -6.7 to -9.9%; LDL/HDL: -6.5 to -7.9%
Westrate & Meijer, 1998	95 M/F	3.5 wks	Sterol ester spread to provide 3.3 g/d sterols	Sterol: TC -8.3%; LDL -13%; LDL/HDL -14% Stanol: TC -7.3%; LDL -13%; LDL/HDL -12%
Pelletier, et al., 1995	12 M/F	4 wks	Sterol enriched butter to provide 0.74 g/d sterols	Sterol: TC -10%; LDL -15%; LDL/HDL -19%
Miettinen & Vanhanen, 1994	31 M/F	9 wks	Sterol enriched mayonnaise to provide 0.70 g/d sterols	Sterol: TC -3.1(ns); LDL -4.2% (ns); LDL/HDL -4.2% (ns)
Becker, et al., 1993	9 FH children	3 mths	Sterol pastil 6 g/d	Sterol: TC -17%; LDL -19%; LDL/HDL -17%
Vanhanen & Miettinen, 1992	24 M/F	9 wks	Sterol enriched spread to provide 0.72 g/d sterols	Sterol: TC -4.7% (ns)

Table 1: Summary of human studies on the effects of plant sterols on cholesterol metabolism

Study	Subjects	Study Length	Study Material	Results
Lees, et al., 1977	46 M/F adults & children	10 mths	Soy sterol suspension or powder – 18 g/d tall oil sterol suspension or powder – 3-6 g/d	Soy sterol suspension: TC -12%; LDL -19% soy sterol powder: TC -12%; LDL not reported; Tall oil sterol powder: TC -12%; LDL not reported; Tall oil suspension: TC -7%
Kudchodkar, et al., 1976	6 M/F	9-15 days	Sterol as Cytellin (3g tid) 9 g/d	TC -9%
Beveridge, et al., 1964	92 M/F	8 days	Sitosterol in homogenized diet – 160 mg to 20 g/d	Sterol intake above 0.87 g/d significantly reduced TC. At 0.87 g/d: TC -12.7%; 1.3 g/d: TC -15.7%; 5.5 g/d: TC -24.8%; 20.4 g/d: TC-35.4%.
Farquhar, et al., 1956	15M	12-24 wks	Sitosterol: 12-18 g/d	TC -17%
Pollack, 1953	26	1-4 wks	Mixed sterols (75-80% sitosterol): 5-10 g/d	TC - 27.6%

Table 2: Details of human studies on effects of plant sterols on cholesterol metabolism

Study	Design and Treatment	Subjects, Background Diet	Length of Each Feeding Period	Main Results
Ayesh, R., et al., Accepted Food and Tox. Chem. 1999	Design: randomized, double-blind, two period, parallel, placebo controlled Treatments: 0 and 8.6 g/d plant sterol in 40 g/d spread (80% fat) Sterol composition: 46% β-sitosterol; 20% stigmasterol and 26% campesterol	21 males/females Age: 36 ± 2.7 years; BMI: 24 ± 2.98 kg/m^2 Baseline TC: 4.84 ± 0.64; Background diet: fat en %: 40%;	3 weeks for males; 4 weeks for females	TC: 18% ↓; LDLC: 23% ↓; No biologically relevant effects on serum female hormone levels, bacterial profile or metabolic activities of gut microflora.
Jones, P. J., et al., Submitted for publication 1999	Design: randomized, crossover, double-blind, placebo controlled Treatments: 0, 1.84 g/d sterols from sterol ester, and 1.84 g/d sterols from stanol ester in 23 g/d spread	15 males Age: 37–61 years Baseline: TC ~ 6.25 mM; LDLC ~4.4 mM; Background diet: fat en %: ~ 35%; S/M/P: 10/15/10;	3 weeks with 5 week washout phase	As compared to control spread Sterol ester: TC 7.4% ↓; LDLC 9.3% ↓; cholesterol absorption 36.2% ↓; cholesterol synthesis 53.3% ↑. Stanol ester: TC 3.8% ↓; LDLC 3.8% ↓; cholesterol absorption 25.9% ↓; cholesterol synthesis 37.8% ↑. No effect of either ester on HDLC.

Table 2: Details of human studies on effects of plant sterols on cholesterol metabolism

Study	Design and Treatment	Subjects, Background Diet	Length of Each Feeding Period	Main Results
Maki, K. C., et al., Submitted for publication 1999	Design: randomized, double-blind, three parallel arms; Treatments, 0, 1.1 and 2.2 g/d plant sterol in 14 g reduced-fat spread given in two 7 g servings	224 males/females Age: ~58 ± 10 years; BMI: ~27 ± 3.6 kg/m^2 Baseline TC, LDLC, HDLC: ~6.2 ± .08, 4.2 ± .07, 1.35 ± .05 mM; Background diet: fat en %: ~30%; S/M/P: 9.2-10.4%, 11.7–13.5%, 5.2-8.1%; cholesterol: 178-220 mg/d	5 weeks following a 4 week lead in period	Percent Reduction g/d TC LDLC HDLC 1.1 -3.9 -5.9 1.8 2.2 -5.6 -6.9 1.7 Significant reduction in TC, LDLC but not HDLC as compared to controls. TC/HDLC decreased 5.5% to 7.3% for 1.1 and 2.2 g/d group, respectively. apoB decreased 5.1% and 6.4% for 1.1 and 2.2 g/d group, respectively. There were no significant differences in TC, LDLC or HDLC between 1.1 and 2.2 g/d groups. >80% compliance resulted in LDLC reduction of 7.6% and 8.1% for 1.1 and 2.2 g/d group, respectively
Hendricks, H. F. J., et al., Euro. J. Clin. Nutr. 1999	Design: randomized, double-blind balanced incomplete Latin square; 4 cycles/person Five Treatments: Flora, Butter, and 3 levels of soy sterols (3.37%, 6.47% and 13.06%) to provide .85, 1.62 and 3.26 g/d plant sterols in 25 g spread. Phytosterol content of Butter 0.22 g/100 g; Flora 0.30 g/100 g.	100 males/females Age: 37 ± 10 years; BMI: 22.8 ± 2.5 kg/m^2; Baseline TC, LDLC, HDLC: 5.1, 2.97, 1.65 mM Background diet: fat en:%:~ 33%; S/M/P: 13.3-13.5/ 11.2-11.8/5.7-6.2; Cholesterol: 249-257 mg/d	3.5 weeks No washout between periods	TC LDLC HDLC mM Butter 5.27 3.15 1.65 Flora 5.16 3.05 1.64 3.4% 4.94 2.86 1.65 6.5% 4.84 2.77 1.63 13.1% 4.81 2.75 1.61 Significant reduction due to plant sterols of 4.9%-6.8% in TC as compared to Flora; LDLC decreased 6.7%-9.9%; LDLC/HDLC ratio decreased 6.5%-7.8% No significant difference among sterol ester margarine groups. No effect on TG

Table 2: Details of human studies on effects of plant sterols on cholesterol metabolism

Study	Design and Treatment	Subjects, Background Diet	Length of Each Feeding Period	Main Results
Weststrate & Meijer, Euro. J. Clin. Nutr. 1998	Design: randomized, double blind, balanced incomplete Latin square; 4 cycles/person. Treatments: Flora, Benecol (stanol esters) and phytosterol esters from soybean, rice bran, or sheanut. Test spreads substituted for habitual spread and no other dietary changes. 30 g spread containing esterified phytosterols, (g/100g): Flora 0.36; Benecol 9.12; soybean 10.82; rice bran 5.58; sheanut 10.15.	95 male + female Age: 45 ± 12.8 years BMI: 24.2 ± 2.2 kg/m²; Baseline TC, LDLC, HDLC: 5.35,3.54,1.26 mM; Background diet: fat en%:~42; S/M/P: 15-16/14-15.5/8.5-10.8 cholesterol: 225-230 mg/d	3.5 weeks No washout between periods	Soybean phytosterol and Benecol, at ~3.3 g/d, were equally effective in lowering TC and LDLC compared with Flora (TC and LDLC ↓ 7.3%, and 13% with Benecol, and 8.3%, and 13% with soybean sterol). HDLC unaffected, thus LDLC/HDLC ratio decreased 12% for Benecol and 14% for soybean sterol ester. Routine clinical chemistry and hematology unaffected. However, 20% reduction in plasma carotenoids (α+ß carotene and lycopene) noted. Rice bran and sheanut sterol esters had no effect on cholesterol levels.
Miettinen, T. A. and H. T. Vanhanen, Atheroscelrosis 1995	Randomized, double-blind placebo, controlled parallel trial. Four Treatments: a) Control – rapeseed oil, mayonnaise b) a + 700 mg/d sitosterol c) a + 700 mg/d sitostanol d) a + 800 mg/d sitostanol ester	31 subjects 22 male/9 female Age: 45 ± 3 years BMI:25.2 ± 1.2 kg/m² Baseline TC: > 6mM Background diet/day: ~ 2400 kcal and ~ 115 g fat S/M/P: 20/12/10 Cholesterol: ~ 300 mg/d	6 week run-in with control diet then randomized the treatments for an additional 9 weeks	Non significant decrease in TC during sitosterol feeding ~ 3-4 ↓ TC and LDLC. Combined sterol and stanol analyses showed significant reductions in TC (-4.2%) and LDLC (-5.5%) Greater reductions seen in individuals with E4 apolipoprotein phenotype. Sitosterol significantly decreased cholesterol absorption.

Table 2: Details of human studies on effects of plant sterols on cholesterol metabolism

Study	Design and Treatment	Subjects, Background Diet	Length of Each Feeding Period	Main Results
Pelletier, X., et al., Ann. Nutr. Metab. 1995	Design: randomized, crossover controlled feeding. 1 week adaptation Treatments: with vs. without 1.07% sterols in butter (0.74 g/d) phytosterol to cholesterol ration = 1.88 (control = 0.7)	12 males Age: 19-29 ± 22.7 years BMI: 22.3 kg/m²	4 weeks	TC 10%↓, LDLC 15%↓ HDLC/LDLC 25.6% ↑
Becker M., et al., Ped. Pharm. Therap. 1993	Design: sequentially control, treatment 1, and treatment 2 Sitosterol pastil: 2 g tid Sitostanol: 0.5 g tid	9 FH children Age: 9.8-14.6 years TC, LDLC, HDLC 9.57, 7.87, 1.18 mM Background diet: Cholesterol: ~200 mg/d Strict diet	12 weeks	Sitosterol: LDLC 19.5% ↓ at 3 mo, (7.72 vs. 6.23 mM) Sitostanol: LDLC 33% ↓ at 3 mo., (7.72 vs. 5.15 mM) 29% ↓ at 7 mo. (7.72 vs. 5.46 mM)
Vahanen, H. T. and T. A. Miettinen, Clinica. Chemica. Acta. 1992	Randomized double-blind placebo controlled parallel trial. Three Treatments: a) Control – rapeseed oil spread b) A + 722 mg/d sitosterol c) a + 630 mg/d sitostanol	24 subjects male/female Age: 24-44 years Baseline TC: >6.0 mM	6 week run-in with control diet then additional 9 weeks on various treatments	Non significant decrease in TC during sitosterol feeding (5.99 vs. 5.71 mM) or sitostanol feeding (5.66 vs. 5.58mM). Authors state sterol intake should exceed 1 g/d to cause significant reduction in serum cholesterol levels.

Table 2: Details of human studies on effects of plant sterols on cholesterol metabolism

Study	Design and Treatment	Subjects, Background Diet	Length of Each Feeding Period	Main Results
Briones, E. R., et al., Mayo Clin. Proc. 1984	Design: randomly assigned parallel treatments; Treatments: diet alone; colestipol at 20 g/d; colestipol at 30 g/d; sitosterol at 12 g/d; and colestipol at 30 g/d with sitosterol at 12 g/d Sitosterol given in four doses of 3 g before meals and at bedtime	16 Adults; 9 males/7 females Age: ave 51years; range: 23-65 years; Baseline TC: ave: 384.9; range 307-576; Background diet: fat en %: 35; CHO en %: 45; protein en %: 20; cholesterol: < 200 mg/d	Diet alone: 8 wks Colestipol, 20 g/d: 12 wks Colestipol,30 g/d: 12 wks Sitosterol: 12 wks Combination: 12 wks	Sitosterol: TC 8% ↓ (ns for entire group; significant for one group). Colestipol: TC 17% ↓; LDL and HDL followed TC. Combination: mixed results, further decrease in one group, increase in the other. Surprisingly, cholesterol absorption increased (ns) during sitosterol feeding.
Kudchodkar, B. J., et al., Atherosclerosis 1976	Crossover with crossback Treatment: Cytellin (65% sitosterol, 30% campesterol and 5% stigmasterol) 3 g tid	6 subjects 5 male/1 female Age: 29-52 years BaselineTC: 176-337	9-15 days	Sterol feeding resulted in slight to moderate decrease in plasma cholesterol Fecal excretion of cholesterol and neutral metabolism increased significantly 34%-102%.

Table 2: Details of human studies on effects of plant sterols on cholesterol metabolism

Study	Design and Treatment	Subjects, Background Diet	Length of Each Feeding Period	Main Results
Beveridge, J. M. R., et al., J. Nutr. 1964	Short term controlled feeding trial. Sterol intake ranged from ~160 mg to 20 g/day	92 University students – 73 male/19 women 7 day run-in on homogenized diet with 45% of calories from butter. Treatments: same diet as above + 50, 100, 200, 300, 400, 600, 800, 1600, 3200 and 6400 mg sitosterol per 950 kcal 85 subjects completed the trial	7 day run-in 8 day feeding of treatments	Sterol intake above 870 mg/d resulted in significantly lower plasma cholesterol Reduction in cholesterol was 12.74% at 870 mg/d, 24.7% at 5.4 g/d and 35.4% at 20 g/d

Table 2: Details of human studies on effects of plant sterols on cholesterol metabolism

Study	Design and Treatment	Subjects, Background Diet	Length of Each Feeding Period	Main Results
Beveridge, J. M. R., et al., Can. J. Biochem. Physiol. 1958	Parallel, controlled feeding trial. I.Treatments: a) fat free diet b) 60% calories as corn oil c) sitosterol equal to b) d) 30% butterfat e) 30% butterfat + 30% kcal as corn oil f) d + c *Sterol level was 0.741 g/950 kcal	I. 57 male	8 days on fat free then 8 days on treatment	Sitosterol significantly reduced cholesterol 12.9 mg/dl or 9.3% as compared to previous level on fat free diet Sitosterol also prevented significant rise in cholesterol on butter fat diets
	II. Treatments: a) Base diet as in I but with 35% calories as butterfat and 25% calories as corn oil b) Sitosterol equal to a c) Corn oil residue = a 0.18% sitosterol d) Corn oil residue = to a + sitosterol to = b *Sterol level was 0.251 g/950 kcal	II. 52 subjects: 45 male/7 female	8 days Base diet then 8 days on treatment	No effect of sitosterol on cholesterol levels. Authors conclude cholesterol lowering of corn oil more than just sitosterol. Sitosterol levels may be too low or overcome by saturated fat from butterfat
	III. Treatments: a) Base diet as II b) At 25% kcal as corn oil c) a + sitosterol = to b d) a + 25% kcal as hydrogenated corn oil e) d + sitosterol = to b f) a + 25% kcal as coconut oil g) f + sitosterol = to b	III. 62 subjects: 57 male/5 female	8 days Base diet then 8 days on treatment	Sitosterol in the presence of corn oil significantly reduced cholesterol levels. No effect when combined with coconut oil

Table 2: Details of human studies on effects of plant sterols on cholesterol metabolism

Study	Design and Treatment	Subjects, Background Diet	Length of Each Feeding Period	Main Results
Farquhar, J. W., et al., Circulation 1956	Controlled feeding. 12-18 g/d sitosterol in three equal portions before meals	15 men Age: 26-45 years with previous MI BaselineTC: ~ 290 mg/d Background diets: Fat en %: 23-28% for 9 patients, 40-48% kcal from fat for 6 patients; cholesterol: 200-300 mg/d for 9 patients, 900-1000 mg/d for 6 patients	6-12 week run-on; 12-24 weeks on sitosterol; 10-16 weeks on placebo	Feeding sitosterol caused a prompt fall in plasma cholesterol that was sustained for 6 months. Average cholesterol reduction was 17%. A prompt rise in plasma cholesterol was noted following placebo feeding Pre 293 > p < .01 Sitosterol 242 >p < .01 Placebo 288 Changes were similar regardless of background diet and starting cholesterol level.
Pollack, O. J., Circulation 1953	Therapeutic Trial Treatments: 2.5 g/d bid sitosterol mixture; 7 or 10 g/d sitosterol mixture in three doses with meals	26 subjects Baseline TC: 126-414 mg/dl No diet restriction	Varied 7-28 days	Significant reduction due to sitosterol independent of dose. Reduction was minimal in subjects with cholesterol <200 mg/dl. Cholesterol returned to original values after stopping sitosterol intake.

similar to those consumed in drugs. Similar reasons support a conclusion that the Cytellin studies may support a health claim for VOSE in food: 1) the active moieties are similar - Cytellin has been reported to be a mixture of 65% β- sitosterol, 30% campesterol, and 5% stigmasterol and the sterols in Cytellin come from the same source and are essentially the same as the sterols found in VOSE; 2) various dosages have been used ranging from 3-18 g/day, and the lower dosage of Cytellin is similar to doses in some studies of VOSE formulated in vegetable oil spreads; and 3) Cytellin was available to a broad patient population and effectiveness was monitored by physicians. Since Cytellin was safe and effective for a broad population, and since it is appropriate to use Cytellin studies to support the health claim for VOSE in food, these studies provide strong foundational support for a conclusion that the claim is substantiated by significant scientific agreement.

It is also appropriate to rely on studies of sterols in reaching a conclusion about a health claim for VOSE because sterols are the active component of VOSE. As previously mentioned, sterol esters are hydrolyzed in the gastrointestinal tract by carboxy ester lipase/esterase (Best and Duncan, 1958; Mattson, et al., 1977). *In vitro* studies have shown that the rate of hydrolysis depends on the fatty acid moiety of the ester compound and esters of unsaturated fatty acids are much more readily hydrolyzed than esters of saturated fatty acids (Swell et al., 1954). Several research teams have shown the physiological equivalence of free sterol and certain sterol esters in affecting cholesterol metabolism. Early research by Best and Duncan (1958) indicated that cholesterol esters of acetate and oleate elevated liver cholesterol in rats similarly to free

cholesterol. Esters of benzoate, palmitate and stearate did not raise cholesterol as much as free cholesterol. This study also showed that sitosterol acetate and oleate were as effective as free sitosterol in lowering liver cholesterol levels associated with cholesterol feeding, whereas esters of propionate and palmitate were less effective. Mattson and others (1977) reported that oleate esters of sitosterol, campesterol, stigmasterol, and mixed sterols were equally effective in reducing cholesterol absorption in rats. Sitosterol esters of oleate, decanoate and succinate were also equally effective in reducing cholesterol absorption. It has also been reported that sitosterol and sitosterol oleate decrease cholesterol absorption in humans by 33%-42% (Mattson, et al., 1982). This accumulated research indicates that VOSE are hydrolyzed in the intestinal lumen to fatty acids and free sterols, and accordingly studies of the effectiveness of plant sterols in blood cholesterol reduction are relevant to the evaluation of this petition.

(ii) Literature Summary – Clinical Studies

This section summarizes the available literature on studies involving human subjects. As early as the 1950's, 5-10 g/d of a "plant sterol mixture" comprised of 75-80% sitosterol was reported to be effective in lowering total blood cholesterol levels in humans. In 26 subjects, using various amounts of the "sterol mixture" (5 g in divided doses; 7 or 10 g divided in three doses) for various lengths of time (8 days to 8 months), Pollak (1953) reported an average reduction of over 27% in total cholesterol levels.

In the first fairly large trial evaluating the effects of plant sterols on cholesterol metabolism, Beveridge et al., (1964) reported the effects of various levels of plant

sterols in 92 university students fed a homogenized formula diet. This study was of short duration (8 days) but included levels of plant sterol ranging from 50 mg to 6.4 g/950 kcal or approximately 160 mg to over 20 g/d. These diets resulted in a range of molar ratios of plant sterol to cholesterol of .37 to 47.8. Significant reductions in plasma cholesterol levels were observed when plant sterol intake was greater than 300 mg/950 kcal, 870 mg/d or a ratio of plant sterols to cholesterol of 2.2 or higher. The dose response effects are very instructive. The results indicate a curvilinear response with decreased incremental effects reported above 1.6 mg/950 kcal or 5.4 g/d.

In another short-term study, Kudchodkar and others (1976), in one of the reported studies of Cytellin, reported a 9% reduction in plasma cholesterol levels in six subjects (4 subjects had cholesterol levels of around 200 mg/dl or less) given 9 g/d Cytellin for 12 or 15 days.

Briones and colleagues (1984) examined the effects of sitosterol alone and in combination with colestipol, a bile acid sequestrant. Sixteen patients with relatively high cholesterol levels (304-576 mg/dl) were asked to modify their diet for eight weeks, take two different doses of colestipol (20 and 30 g/d) for 12 weeks each, take 12 g/d sitosterol for 12 weeks and take a combination of sitosterol (12 g/d) and colestipol (30 g/d) for 12 weeks. One-half of the patients started with colestipol and the other half started with sitosterol. In one group sitosterol caused a significant ($p<.02$) reduction in total plasma cholesterol of 7.6%. In the other group sitosterol decreased plasma cholesterol 8.0%, but this was not statistically significant. Cholesterol absorption was

determined in a subset of four subjects. In this small group cholesterol absorption increased slightly with sitosterol. This finding is contrary to most of the available literature and may be a chance result of the small group size.

In nine children with familial hypercholesterolemia, Becker, et al. (1993) evaluated the effects on cholesterol levels of adding 6 g/d β-sitosterol to the diet for three months. Total- and LDL-cholesterol levels were reduced by 17.1% and 19.5%, respectively ($p<.01$ for both) after the β-sitosterol feeding period. There was no change in HDL-cholesterol levels.

Farquhar and colleagues (1956) reported the effects of β-sitosterol on cholesterol levels of young men with a previously documented myocardial infarction. Fifteen subjects were given 12 to 18 g β-sitosterol divided in three doses for a period of 12 to 24 weeks. The average reduction in serum cholesterol was about 17% (293.1 to 242.8 mg/dl) which was highly significant. The drop in cholesterol was noted in 2-3 weeks and no tendency to return to pre-treatment values was observed through as long as six months. A prompt rise in cholesterol was reported within three weeks of removal of β-sitosterol.

In another study, the effects of various forms of Cytellin in four therapeutic trials were reported (Lees, et al., 1977). Four forms of sterol preparation were used: 1) soy sterol powder; 2) soy sterol suspension; 3) tall oil sterol powder; and 4) tall oil sterol suspension. The number of subjects per preparation varied from 6 to 31. The average daily dose ranged from 3 to 18 g/d. The duration of treatment ranged from 8 weeks to 3

years. The average reduction in total cholesterol levels ranged from 5 to 12%. While LDL-cholesterol was not presented for all treatments, the authors point out that reduction in LDL-cholesterol closely followed the drop in total cholesterol (total- and LDL-cholesterol reductions of 12% and 17%, respectively were presented for the soy sterol suspension group).

The above mentioned reports provide historical support that plant sterols, and specifically sitosterol, significantly lower plasma cholesterol. In many of the studies relatively high doses of sitosterol were fed to obtain the results. The plant sterol preparation used in many of these studies has been reported to be unpalatable and obviously better delivery systems for plant sterols were needed. VOSE formulated in foods such as vegetable oil spreads appear to be a solution to the palatability issues seen in the early research.

Research from the last decade, examining the effects of plant sterols and sterol esters on cholesterol metabolism, show marked improvements in experimental design, statistical analysis and characterization of subjects. More data about the different types of cholesterol-laden particles are included in the more recent reports. Additionally, the more recent reports control dietary variables to a greater extent. Also many of the studies used a vegetable oil spread as the delivery vehicle for the plant sterols, a much more palatable option than used before. All of the recent studies meet criteria previously cited by FDA as studies of adequate quality to determine an effect on cholesterol levels. These studies were well designed, include appropriate control groups, the intervention

period was of a sufficient duration (greater than 3 weeks), and subjects were relevant to the U. S. population (normal to moderate elevation in cholesterol levels).

Pelletier et al., (1995) reported that the ingestion of 740 mg/d of soybean sterols incorporated into butter significantly decreased plasma total- cholesterol and LDL-cholesterol 10.2% and 15.2 % respectively, with no effect on HDL-cholesterol. These results were obtained in a cross-over experiment with two, four-week feeding periods with twelve normocholesterolemic men (4.3 ± 0.16 mM total cholesterol). The overall intake of fat as a percent of energy was 36.4% during both the control period and during the sterol-feeding period. The cholesterol intake during the control period was 436 mg/d and 410 mg/d during the sterol-feeding period. This research team designed the diets to have a plant sterol to cholesterol ratio of 2.0, which has repeatedly been shown to effect cholesterol levels in various animal models. It is important to indicate that the HDL-/LDL- cholesterol ratio improved over 25% since the major effect of soybean sterols was to lower the LDL- cholesterol fraction.

Effects of feeding less than 1 g/d plant sterols have been variable. While all the studies do show decreased plasma cholesterol levels during the ingestion of plant sterols, in some studies the differences were not statistically significant. T. A. Miettinen and H. T. Vanhanen published several papers on the effects of plant sterols and stanols on cholesterol levels. Their experiments examined the effects of adding 50 g/d of a rapeseed oil spread in which various levels of plant sterols had been incorporated. In one study (Miettinen and Vanhanen, 1994), they reported that 50 g/d of rapeseed oil

spread provided 700 mg/d of sitosterol which was associated with about a 4% decrease in cholesterol level as compared to the rapeseed oil spread control group. This statistically non-significant result would not be unexpected for two reasons. First, the level of sitosterol fed is below levels shown to be effective in previous experiments. Beveridge and colleagues (1964) indicated that sitosterol levels of over 870 mg/d were necessary to see a significant effect. Another reason for the lack of statistical significance is the small number of subjects fed sitosterol, namely nine.

In another study by Vanhanen and Miettinen (1992) the addition of an additional 600 mg of plant sterols also showed a small insignificant decrease (3.1%) in cholesterol levels. Close reading of this paper indicates that the sitosterol level was around 435 mg/d, again too low to be effective in statistically significantly lowering cholesterol based on previous work. The authors indeed point out the need to feed higher levels of plant sterols to ensure effectiveness. In these studies by Miettinen and Vanhanen, the lack of statistical significance is a function of the magnitude of the effect and the sample size used. Other important variables affecting sterol effectiveness in lowering cholesterol are the solubility of the plant sterol in the delivery vehicle, usually a spread or butter, and the solubility of the sterol in the digestive tract. From the results above, it appears a fat-based food like butter is a superior vehicle to deliver plant sterols by making them more soluble and thus more effective in interfering with cholesterol absorption in the digestive tract. Fortunately, VOSE have an increased solubility in a variety of spread vehicles as compared to plant sterols.

In the 1990's, VOSE were being successfully incorporated into spreads. Several of these products were evaluated for effects on cholesterol metabolism. Ayesh and colleagues (1999) reported that in 21 adults, consumption of 8.6 g/d of sterols from 40 g/d spread containing sterol esters reduced total- and LDL-cholesterol 18% and 23%, respectively ($p<.001$, for both) after 21 days in males and 28 days in females. In another study submitted for publication, Jones et al., (1999, submitted for publication) reported the effects on blood cholesterol levels in 15 adults of consumption of 1.84 g/d of sterols from either sterol ester or stanol ester delivered via 23 g/d of spread. Both sterol- and stanol-ester lowered cholesterol significantly after three weeks. As compared to the control group, the additional reduction in total- and LDL-cholesterol for the sterol ester group was 7.4% and 9.3%, respectively ($p<.05$ for both). Comparable reductions for the stanol ester group were 3.8% and 4.8%, respectively ($p<.05$ for both).

The latest published reports on the effects of vegetable oil sterols on cholesterol metabolism are by far the largest and the most relevant to the products encompassed by this petition. Weststrate and Meijer (1998) reported the effects of vegetable oil sterol ester-enriched margarines on cholesterol levels in 95 individuals in the Netherlands. The baseline average total-, LDL- and HDL-cholesterol levels were 5.35 ± 1.06, 3.54 ± 0.97, and 1.26 ± 0.35 mM, respectively. In this randomized double-blind placebo–controlled trial, an incomplete Latin square design was utilized with five treatments and four periods. Subjects were given VOSE enriched margarine (30 g/d) to replace spreads habitually used. Each feeding period lasted 3.5 weeks. The vegetable oil sterol used in this trial was esterified with fatty acids from sunflower oil. A commercially

available high polyunsaturated fatty acid (PUFA) margarine (0.36% sterols) was used as a control. As compared to the control group, ingestion of vegetable oil sterols (about 3 g/d) significantly decreased total- and LDL-cholesterol 8.3 and 13.0%, respectively ($p<.001$ for both). There were no effects on HDL-cholesterol and thus the LDL-/HDL-cholesterol ratio deceased significantly (-14.0%; $p<.001$). An additional analysis, separating the subjects into tertiles of beginning cholesterol levels, was also reported. The cholesterol lowering effects of ingestion of vegetable oil sterols were similar in the low tertile (total cholesterol: 4.16±0.50 mmol/L), medium tertile (total cholesterol: 5.41±0.30 mmol/L) and the high tertile (total cholesterol: 6.54±0.61 mmol/L) groups. The background diet of this group was approximately 42% calories as total fat, about 16% of calories as saturated fat, and cholesterol intake was 230-240 mg/d. The diets remained remarkably constant during the different periods. This research also examined the effects of two other sterol sources, namely rice bran oil and sheanut oil. Sterols from these sources did not have a significant effect on cholesterol levels. The authors speculated that the lack of response may be due either to the different esters used (ferulic, cinnamate and acetic acid) or due to the difference in structure of the sterols, which in rice bran and sheanut are 4,4'-dimethylsterols and not the 4-dimethylsterols present in soybean oil.

In a very recent publication, Hendriks, et al., (1999) reported the effects of feeding three different levels of vegetable oil sterol ester incorporated in spreads having the same fatty acid composition. This large study (100 subjects) in the Netherlands used a similar design as that of Weststrate and Meijer (1998), namely a randomized, double-blind

placebo controlled balanced incomplete Latin square design with five treatments and four periods. Each period was 3.5 weeks. The treatments were butter (0.22% sterols), a commercially available high PUFA spread (0.30% sterols) and three spreads containing three different concentrations of vegetable oil sterol ester: 3.37, 6.47, and 13.06 % w/w. The vegetable oil sterols were esterified to sunflower oil and the degree of esterification was 82%. The spreads provided sterol intakes of 0.83, 1.61 and 3.24 g/d, respectively. Baseline total-, LDL-, and HDL-cholesterol were 5.10 ± 0.97 mM, 2.97 ± 0.83 mM and 1.65 ± 0.38 mM, respectively. Total- and LDL-cholesterol levels significantly decreased after ingestion of vegetable oil sterol ester enriched spreads as compared to the commercially available spread or butter ($p<.001$). As compared to the commercially available spread, the decrease was 4.9, 5.9 and 6.8 % for total-cholesterol and 6.7, 8.5 and 9.9 % for LDL-cholesterol for vegetable oil sterol intakes of .83, 1.61 and 3.24 g/d, respectively. There were no effects on HDL-cholesterol. Thus, there was a significant reduction in the LDL-/HDL-cholesterol ratio of 6.5, 7.9 and 7.8 % for vegetable oil sterol intake of .83, 1.61, and 3.24 g/d, respectively. There was a trend for further reduction in cholesterol levels with increased VOSE intake but there were no statistically significant differences between the three levels of vegetable oil sterol intake regarding the blood cholesterol lowering effects. The percentage of energy from total fat and saturated fat ranged from 33-34% and 13.3-13.5%, respectively. The cholesterol intake ranged from 244 to 257 mg/d. Thus, the diet in this trial was similar to the average American diet of 257 mg/d (USDA, 1999).

A large randomized, double-blind controlled clinical trial involving 224 subjects in the United States evaluating the effects of two levels of vegetable oil sterol ester as part of a NCEP Step I diet was recently completed (Maki, et al., 1999, submitted for publication). After a four-week diet stabilization period, subjects were randomized to one of three treatments for a five-week intervention period. The treatments were: 1) a control spread with no added vegetable oil sterol esters; 2) vegetable oil sterol ester enriched spread providing 1.1 g sterol per day and 3) vegetable oil sterol ester enriched spread providing 2.2 g sterol per day. All the spreads were reduced fat products. Subjects consumed 14 g/d spread, separated into two 7 g servings as part of the NCEP Step I diet. Baseline total-, LDL- and HDL-cholesterol were approximately 6.1, 4.1, and 1.3 mM, respectively. After five weeks, total- and LDL-cholesterol, total-cholesterol:HDL-cholesterol ratio, and apolipoprotein B levels were all significantly reduced in the groups consuming vegetable oil sterol ester spreads as compared to the control group ($p<.001$). In the group fed 1.1 g/d sterol as sterol ester, total- and LDL-cholesterol were reduced by 3.9% and 5.9% ($p<.01$ for both), respectively as compared to the control group. Compared to the control group, in the group fed 2.2 g/d sterol as sterol ester, total- and LDL-cholesterol were reduced by 5.6% and 6.9% ($p<.01$ for both), respectively. Apolipoprotein B levels were also reduced, as compared to the control group, by 5.1% and 6.4% ($p<.01$ for both) in the groups fed 1.1 and 2.2 g/d sterol as sterol ester, respectively. Compared to the control group, total-cholesterol:HDL-cholesterol ratio was reduced by about 5.5% and 7.3% ($p<.01$ for both) in the low and high intake groups, respectively. No significant differences were seen comparing the two levels of sterol intake. Total- and LDL-cholesterol and total-

cholesterol:HDL-cholesterol ratio in the groups fed sterol ester spread were significantly lower ($p<.01$), as compared to the control group, after two weeks of intervention.

From the well designed clinical trials described above, it can be concluded that providing 1 g/d sterol in free form or 1.6 g/d sterol in ester form will significantly lower total- and LDL-cholesterol levels. Cholesterol reduction with sterol consumption of less than 1 g/d, whether as free sterols or as sterol esters, was shown to be not statistically significant in two trials (Miettinen and Vanhanen, 1994; Vanhanen and Miettinen, 1992) and statistically significant in two other trials (Pelletier, et al., 1995; Hendriks, et al., 1999). However, sterol consumption of greater than 1 g/d has consistently been shown to lower total- and LDL-cholesterol levels (Maki, et al., submitted for publication; Hendriks, et al., 1999; Weststrate and Meijer, 1998). While the current data do not describe a precise clear dose response effect, there is a tendency for greater cholesterol reduction with increased VOSE intake. Taken together, the data indicate that 1.6 g/d sterol ester (about 1 g/d sterol) could be expected to lower total- and LDL-cholesterol levels at least 5% and 7% or more, respectively. This level of sterol intake was found to have no impact on HDL-cholesterol levels and thus improves the LDL-:HDL-cholesterol or total-:HDL-cholesterol ratio significantly (Maki, et al., submitted for publication; Hendriks, et al., 1999). Although this level was associated with decreased absorption of certain fat-soluble components of the diet (carotenoids), this decrease in absorption led to plasma levels which were well within the variation observed between seasons (which occurs due to such factors as fresh fruit and vegetable availability). These changes in carotenoid levels are not regarded as biologically significant (Maki, 1999, submitted for publication; Hendricks, et al., 1999).

D. Studies in Animal Models Support Effects of Sterols and VOSE in Lowering Cholesterol Levels

FDA has repeatedly stated that animal studies are helpful in supplementing data from human clinical trials and that animal studies are especially helpful in elucidating mechanism of action. An extensive body of literature exists evaluating the effects of plant sterols and sterol esters in various animal models of lipid and cholesterol metabolism. Many of these studies examine the mechanism by which sterols and sterol esters lower cholesterol levels. Collectively, these data provide significant support to the human clinical data presented above.

The following section summarizes the considerable body of evidence in a variety of animal models of the effects of plant sterol/sterol esters on cholesterol metabolism. The major studies evaluating the effects of feeding vegetable sterols on cholesterol metabolism are summarized in Table 3. A more extensive description of each study is presented in Table 4.

The first report of the effects of plant sterols on cholesterol levels was performed in chickens. Peterson (1951) reported, in a variety of diets, that 1.0% soy sterols in the diet prevented the rise in plasma and liver cholesterol associated with increased cholesterol (1.0%) added to the diet. The effects were rather dramatic; when cholesterol was added to the diet, plasma and liver cholesterol increased from 85 to 553 mg/dl and 1.01 to 5.21 dry weight % as compared to the control diet, respectively.

Table 3: Summary of animal studies on the effects of plant sterols on cholesterol metabolism

Study	Animal	Study Length	Study Material	Results
Moghadasian, et al., 1999	Male apoE knock-out mice	20 wks	2% plant sterol from tall oil in 9% fat and 0.15% cholesterol diets	Sterol: VLDL/LDL -37%; LDL -20% (ns); Atherosclerotic lesion size decreased by half
Ntanios, et al., 1999	Male/female hamsters	90 days	+/- 0.5 or 1.0% plant sterols from tall oil or soybean oil in .25% cholesterol diets	Soybean: TC -25% in males; -34% to -44% in females; Tall oil: TC -14% to -31% in males; -15% and -31% in females
Laraki, et al., 1991	Wistar rats	3 wks (+1 wk adaptation)	24 or 96 mg/d phytosterol in 8% butter diets with 24 mg/d cholesterol	Phytosterols: TC -13.6% and -22% for the low and high phytosterol level, respectively. The high phytosterol group had a 22.2% lower LDL-C
Bhattacharyya, et al., 1984	Adult male rhesus monkeys	4 wks to 7 mths	+/- 2% plant sterols +/-0.05% triparanol in semi-synthetic, low cholesterol diet	Plant sterols: cholesterol absorption decreased 40-50%
Uchida, et al., 1984	CRJ:CD-1 male mice	15 days	+/- 1.0% phytosterols	Phytosterols: cholesterol absorption, liver cholesterol and bile acid excretion decrease, fecal sterol excretion increase
Hirai, et al., 1984	Female Wistar rats	18 days	+/- 5.0% phytosterols	Phytosterols: TC -68.4%; LDL/VLDL drop dramatically
Sugano, et al., 1982	Male Wistar rats and JAC ddy mice	31 to 40 days	+/- 0.5% sitosterols; 0.1%, 0.5% and 2.0% sitosterols	Sitosterol: LivC -17.5%, -23.3%, and -31.5% for 0.1%, 0.5%, 2.0% sitosterol, respectively
Bhattacharyya, et al., 1981	Male rhesus monkeys	4 wks to one year	+/- 2.0% phytosterols and +/- 0.05% triparanol in low and high cholesterol diets	Phytosterols: TC -6 to -43%; cholesterol absorption -29.7 to -52.6%
Chandler, et al., 1979	Male chicks	19-24 days	1% sitosterol +/- 1% cholesterol 1% stigmasterol +/-1% cholesterol	Sito: TC -57%; LivC -49% Stig: TC -36% (ns); LivC -9% (ns)

Table 3: Summary of animal studies on the effects of plant sterols on cholesterol metabolism

Study	Animal	Study Length	Study Material	Results
Mattson, et al., 1977	Male rats	3 days	Sitosterol, stigmasterol and various esters 2-4% of fat	Sterol and sterol esters significantly reduced cholesterol absorption 19.7% to 37.7%
Sugano, et al., 1977	Male Wistar rats	14-23 days	0.5% or 1% sitosterol or sitostanol +/- 0.5% cholesterol	TC -10% to -5% depending on fat source and presence of cholic acid; LivC -61% to -82%
Bartov, et al., 1969	Male chicks	14 days	1-3% soy sterols +/- 1% cholesterol	TC -32% to -66%; LivC -42% as compared cholesterol fed groups
Best & Duncan, 1958	Male rats	14 days	5% sitosterol, various sterol esters, + 1% cholesterol	Sitosterol and sitosterol acetate or oleate: LivC -76% to -83%; other esters higher: LivC -32% to -58%
Peterson, et al., 1954	Male chicks	3 wks	1.5% soy sterols +/- 1% cholesterol with various fats	Soy sterols: TC -73 to -87% as compared to cholesterol fed groups
Pollack, 1953	Male rabbits	14 days	Sitosterol 1, 3, 5,6,7 g/d +/- 1g/d cholesterol	Sitosterol: TC -62 to -90%; atheroscelrosis eliminated by 3 g/d and higher
Peterson, et al., 1953	Male chicks	28 wks	1.3% soy sterols +/- 1% cholesterol	Soy sterols: TC –86%; LivC –72% as compared to cholesterol fed groups: decreased incidence of atheroscelrosis
Peterson, et al., 1952	Chicks M/F	5 wks 6 wks 5 wks	1% soy sterol +/- 1% cholesterol; 0.25%-3% sitosterol +/- cholesterol; 1% stigmasterol, 1% ergosterol, and 1.38% soy caprate +/- 1% cholesterol;	Soy sterols: TC -74 to -82% as compared to cholesterol fed groups Sito: TC -35 to -91%: LivC -13 to -83% as sitosterol increased; Sito, stig, ergo: TC -78 to -88% Caprate ester: TC -39%
Peterson, et al., 1951	Chicks M/F	5 wks	1% soy sterols +/- 1% cholesterol	Soy sterol: TC -80%; LivC -67% as compared to cholesterol fed groups

Table 4: Details of animal studies on effects of plant sterols on cholesterol metabolism

Study	Design and Treatment	Animals	Length of Each Feeding Period	Main Results
Moghadasian, M. H., et al., Circulation 1999	a) Picolab mouse diet: 9% fat b) a + 1.0% probucol c) a + 2.0% plant sterols from tall oil	24 apoE deficient knockout mice	20 weeks	VLDL/LDL significantly lowered 78% in probocol group and 37% in sterol group. HDLC lower in probocol group and slightly increased in sterol group. Probocol caused a 175% increase in average lesional area while sterol decreased lesional area by 50%
Ntanios, F. Y., et al., Can. J. Physiol. Pharmacol. 1999	a) basal semi-purified diet +0.25% cholesterol b) a + 0.5% tall oil sterol c) a + 1.0% tall oil sterol d) a + 0.5% soybean oil sterol e) a + 1.0% soybean oil sterol Tall oil sterol contained 64% β-sitosterol, 16% sitostanol and 16% campesterol; soybean oil sterol contained 65% sitosterol, 20% campesterol and 15% dihydrobrassicasterol.	120 Golden Syrian male and female hamsters	90 days	Total cholesterol percentage decrease compared to control: Soybean 0.5% 1.0% Tall 0.5% 1.0% M -25 -25 -14 -31 F -34 -44 -15 -31 HDLC also lowered in most groups. Fractional synthesis rates of cholesterol were greater in tall oil groups as compared to soybean groups though both groups were not different from the control group. Soybean oil sterols possibly more effective in females.

Table 4: Details of animal studies on effects of plant sterols on cholesterol metabolism

Study	Design and Treatment	Animals	Length of Each Feeding Period	Main Results
Laraki, L., et al., Ann. Nutr. Metab. 1991	a) basal diet (8% butter) + 12 mg/d cholesterol b) basal diet (8% butter) + 24 mg/d cholesterol c) b + 24 mg/d phytosterol d) b + 96 mg/d phytosterol Phytosterol used was 72.5% β-sitosterol, 20.5% campesterol and 7% stigmasterol	48 Adult Wistar rats	3 wks (+1 wk adaptation)	Body weights lower in phytosterol groups compared to high cholesterol group but similar to low cholesterol diet group. No effects on liver weight. High cholesterol group had 37% higher TC and 56% higher LDL-C than low cholesterol group. Phytosterol groups had lower TC than comparable control group 13.6% ↓ and 22% ↓ for the low and high phytosterol level, respectively. The high phytosterol group had a 22.2% lower LDL-C
Bhattacharyya, A. K., et al., Atheroscelrosis 1984	a) semi-synthetic low cholesterol diet b) a) + 0 .05% triparanol c) a) + 2% plant sterols d) b + c Major endpoint: cholesterol absorption	11 Adult male Rhesus monkeys; high and low responders to an atherogenic diet	a) 7 mths b) 4 wks c) 14 wks d) 4 wks	With plant sterols cholesterol absorption decreased more than 50% in high responders and about 40% in low responders regardless of triparanol administration
Uchida, K., et al., J. Lipid Res. 1984	a) basal diet (JCL-CA-1) b) a + diosgenin c) a + 1.0% phytosterols Phytosterols were composed of 77% β-sitosterol, 16% sitostanol, 6% campesterol and 1% campestanol.	20 CRJ:CD-1 male mice	15 days	No effect of sterols on body weight or food intake. With phytosterol feeding: fecal total sterol excretion ↑, cholesterol excretion ↑, total bile acid excretion ↓, cholic acid derived bile acid excretion ↓, chenodeoxcholic derived bile acid excretion↓↓, biliary cholesterol ↓ , liver cholesterol ↓, cholesterol absorption ↓ and coprostanol excretion, bile acid pool size and plasma cholesterol were unchanged.

Table 4: Details of animal studies on effects of plant sterols on cholesterol metabolism

Study	Design and Treatment	Animals	Length of Each Feeding Period	Main Results
Hirai, K., et al., J. Nutr. Vitaminol. 1984	Exp. I unrelated to phytosterols Exp. II a) basal diet + 1.0% cholesterol + 0.25% cholic acid + 10% butter b) a + 10% cod liver oil c) a + 5.0% phytosterol Phytosterols undefined	12 Female Wistar rats	18 days	Body weights and food intake similar. Liver weights lower during phytosterol feeding probably due to decreased lipid content. Phytosterol: TC 68.4% ↓ from comparable control group. LDL + VLDL decreased dramatically with phytosterol feeding
Sugano, M., et al., J. Nutr. Sci. Vitaminol. 1982	Exp. I a) basal diet + 10% safflower oil b) basal diet + 9.5% butter fat c) b + 0.5% sitosterol	Male Wistar rats	31 days	No effects on serum cholesterol levels. Presence of sitosterol significantly decreased liver cholesterol: Exp. I: 27.5% ↓ (3.71 to 2.69 mg/g);
	Exp. II a) basal diet + 10% butter fat b) a + 0.5 % sitosterol	Male Wistar rats	35 days	Exp. II: 31.0% ↓ (4.26 to 2.94 mg/g)
	Exp. III a) basal diet + 10% butter fat b) a + 0.1% sitosterol c) a + 0.5% sitosterol d) a + 2.0% sitosterol	Male Wistar rats	35 days	Exp. III: 17.5% ↓, 23.3% ↓, and 31.5% ↓ for 0.1, 0.5, 2.0% sitosterol, respectively
	Exp. IV a) basal diet + 10% safflower oil b) basal diet + 9.5 % butter fat c) b + 0.5% sitosterol Sitosterol used was 93% β-sitosterol and 7% campesterol	JAC ddy mice	40 days	Exp. IV: 54% ↓ (8.89 to 4.09 mg/g) and 55% ↓ in liver triglyceride (69.4 to 30.9 mg/g) Apolipoprotein data presented but did not show a clear pattern of effect of sitosterols

Table 4: Details of animal studies on effects of plant sterols on cholesterol metabolism

Study	Design and Treatment	Animals	Length of Each Feeding Period	Main Results
Bhattacharyya, A. K., et al., J. of Lipid Res. 1981	a) semi-synthetic low cholesterol diet (0.02 mg/kcal) b) semi-synthetic high cholesterol diet 0.15 mg/kcal) c) a + 0.05% triparanol d) b + 0.05% triparanol e) a + 2% plant sterols f) b + 2% plant sterols Plant sterols were 65% β-sitosterol, 35% campesterol and 5% stigmasterol.	11 Adult male Rhesus monkeys; high and low responders to an atherogenic diet	a) 7 months b) one year c) 4 wks d) 4 wks e) 14 wks f) 14 wks	Plant sterols in the high cholesterol diet reduced TC 43% (230 to 132 mg/dl) in high responders and 13% (142 to 123, ns) in low responders. Plant sterols in the low cholesterol diet reduced TC 17% (ns) in high responders and 6% (ns) in low responders. Plant sterols reduced cholesterol absorption in high responders on both high- and low-cholesterol diets (73.0 to 42.8% and 85.4 and 40.3%, respectively). Plant sterols reduced cholesterol absorption in low responders on both high- and low-cholesterol diets as well (58.6 to 41.2% and 75.6 and 42.8%, respectively).
Chandler, R. F., et al., J. Pharm. Sci. 1979	a) Base diet: (undefined) b) a + 1.0% cholesterol c) a + 1.0% test material d) b + 1.0% test material 3 test materials were evaluated: 1. cholestyramine 7 chicks/group 2. β-sitosterol 11 chicks/group 3. stigmasterol 9 chicks/group	White Leghorn cockerels Age: one day old	14 days	Cholestyramine and β sitosterol, but not stigmasterol decreased plasma and liver cholesterol associated with cholesterol feeding. Plasma cholesterol on diets b,c,d, were 331, 114 and 99 mg/dl for β sitosterol; 538, 343, 134 mg/dl for stigmasterol. No difference in macroscopic examination of hearts. Authors conclude similar antihypercholesterolemia activity of cholestyramine and β sitosterol.

Table 4: Details of animal studies on effects of plant sterols on cholesterol metabolism

Study	Design and Treatment	Animals	Length of Each Feeding Period	Main Results
Sugano, M., et al., J. Nutr. 1977	Base diet containing 5% cholesterol with various fat sources, various sterols, various levels of sterols, some diets contained cholic acid	Male Wistar rats (85-115 g)	Varied 14-23 days	No difference in food intake and weight gain Rise in plasma cholesterol from increased cholesterol and/or cholic acid in the diet prevented by 0.5% and 1.0% sitosterol in the diet. Similar effects seen for liver cholesterol. Feeding 0.5% to 1.0% sitosterol resulted in less than 4% sitosterol of the total sterol content of adipose tissue or adrenal glands and 1.0% or less of sterol composition of aortas. Fecal recovery of sitosterol ranged between 85-92%
Mattson, F.H., et al., J. Nutr. 1977	Measured cholesterol absorption as affected by β-sitosterol, campesterol, stigmasterol or a mixture of sterols in both free and various ester forms 15mg cholesterol given + various sterols	Sprague-Dawley Male rats (200-650 g)	48 hour collection of lymph from cannulated thoracic duct	↑ Plant sterol resulted in exponential decrease in cholesterol absorption: % decrease = 41log10 S+19 where S is % of dietary fat as plant sterol expressed as free sterol. 2% plant sterol on a molar basis significantly reduced cholesterol absorption 20-30%. Acetate, oleate, decanoate, succinate esters as effective as free sterol.

Table 4: Details of animal studies on effects of plant sterols on cholesterol metabolism

Study	Design and Treatment	Animals	Length of Each Feeding Period	Main Results
Bartov, I., et al., Poul. Sci. 1969	I. a) Base diet: b) a + 4% soy bean oil c) a + 10% coconut oil d) a + 3.0% soy sterols (89% free sterol, 5% sterol esters; 54% sitosterol, 24% campesterol, 22% stigmasterol) II. e) a + 1.0% cholesterol f) d + 3.0% soy sterols g) c + 1.0% soy sterols h) c + 0.51% cholesterol b) g + 1.0% soy sterols III. a) Base diet with no added fat b) a + 3.0% soy sterols c) a + 1.0% cholesterol d) c + 3.0% soy sterols e) a + 2.0% cholesterol every other day with diet a f) a + 2% cholesterol <u>or</u> 6% soy sterols on alternate days	New Hampshire White leghorn male chicks Age: one day old Rhode Island Red White leghorn chicks 10 chicks/group	14 days	Soy sterols completely inhibited the rise in plasma and liver cholesterol associated with cholesterol feeding regardless of fat type in the diet. No effect of soy sterols on plasma cholesterol on Base diet. Again soy sterols prevented the rise in plasma and liver cholesterol associated with simultaneous cholesterol feeding. Feeding soy sterols on alternate days with cholesterol (diet f) resulted in a higher plasma and liver cholesterol than diet a (209 vs.108 mg/dl; 1.1 vs. 3.9 mg/dl respectively) and diet e (209 vs.138 mg/dl; 11.1 vs. 4.4 mg/dl; respectively)

Table 4: Details of animal studies on effects of plant sterols on cholesterol metabolism

Study	Design and Treatment	Animals	Length of Each Feeding Period	Main Results
Best, M. M. and C.H. Duncan, J. Nutr. 1958	a) Base diet was Harlan rat-mouse mash + 5% cottonseed oil + 1.0% cholesterol b) a + 5% beta-sitosterol c) a + 5.51% sitosterol acetate d) a + 5.68% sitosterol propionate e) a + 7.87% sitosterol palmitate f) a + 9.57% sitosterol oleate (only 61% of sterol esterified)	Male Holtzman rats (~300 g) 4 to 8 rats per group	14 days	Plasma cholesterol unaffected by treatment however, liver cholesterol was reduced by sitosterol and by all sterol esters. Propionate and palmitate esters groups had higher liver cholesterol than the group receiving free sitosterol. Authors propose rate of hydrolysis of ester explain differential effects.
	II. Harlan rat-mouse mash + 5% cottonseed oil +10% cholesterol + 0.5, 1.0 and 2.0% of sterol as sitosterol, sitosterol acetate or sitosterol oleate (only 61% of sterol esterified)	4 to 6 rats per group	14 days	Liver cholesterol decreased curvilinearly regardless of ester used
Peterson, D. W., et al., 1954	a) Base diet + 4% cottonseed oil + 1.0% cholesterol + the following: b) 1.5% soy sterols c) 0.3% cholic acid + 1.5% soy sterols d) 4% oleic acid e) 4% oleic acid + 1.5% soy sterols	Male, Single Comb, White Leghorn chicks Age: 3 weeks old 9 chicks per group	6 weeks	Cholic acid dramatically increased plasma cholesterol levels. Presence of soy sterols dramatically decreased effects of cholic acid on plasma cholesterol. In the presence cholesterol in the diet, soy sterol (1.5%) also decreased plasma cholesterol on oleic acid or cottonseed oil diets.

Table 4: Details of animal studies on effects of plant sterols on cholesterol metabolism

Study	Design and Treatment	Animals	Length of Each Feeding Period	Main Results
Pollack, O. J., Circulation 1953	a) Purina Rabbit Chow + greens (N=10) b) a + oil/alcohol vehicle 15 cc (N=10) c) a + 1 g/d cholesterol (N=5) d) a + 1.5 g/d cholesterol (N=5) e-k) a + 1,2,3,5,6,7,10 g/d mixed sterols (75-80% sitosterols) (N= 5 each) l) c + 1 g/d mixed sterols (N=10) m) c + 3 g/d mixed sterols (N=10) n) c + 5 g/d mixed sterols (N=10) o) c + 6 g/d mixed sterols (N=10) p) c + 7 g/d mixed sterols (N=10)	Male rabbits 2600 gm	14 days	Cholesterol fed groups had elevated blood cholesterol levels. Also nine of the ten rabbits had evidence of atheroscelrosis. In the absence of cholesterol in the diet, no effect of mixed sterol feeding on blood cholesterol. None of the 35 rabbits fed mixed sterols had evidence of atheroscelrosis. Evidence of atheroscelrosis was absent in groups fed cholesterol plus 3, 5, 6, 7 g/d mixed sterols.

Table 4: Details of animal studies on effects of plant sterols on cholesterol metabolism

Study	Design and Treatment	Animals	Length of Each Feeding Period	Main Results
Peterson, D. W., et al., 1953	Three treatments: a) control b) control + 1.0% cholesterol c) control + 1.3%, mixed soy sterols (N=44/group)	Single Comb, White Leghorn chicks Age: 2 weeks old	28 weeks blood alternate weeks; after 4 weeks 10 chicks/diet sacrificed and at 10, 15, 24, 28 weeks for evaluation for atheromatous plaque	Animals fed diet c) had significantly lower cholesterol levels, liver cholesterol levels and decreased development of atheroscelrosis than chicks fed diet b) and only slightly higher than chicks fed diet a)
Peterson, D. W., et al., 1952	Base diet: 4% cottonseed oil I. + 1.0% cholesterol +/- 1.0% sterols II. Experiment 2 + 1.0% cholesterol +/- 1.0% soy sterols +/- .5% lecithin III. + 1.0% cholesterol + soy sterols at .25, .5, 1.0, 2.0 and 3.0% of the diet IV. + 1.0% cholesterol + 1.0% sitosterol or β sitosterol, or ergosterol, or stigmasterol V. + 1.0% cholesterol + 1.33% soy sterol caprate	Single Comb, White Leghorn chicks Age: 2-3 weeks old	5 weeks 5 weeks 6 weeks	I. Significant decrease in plasma cholesterol with addition of soy sterols II. Significant decrease in plasma cholesterol by soy sterol with/without addition of lecithin. III. Dramatic curvilinear dose response of soy sterol reduction in plasma and liver cholesterol IV. All sterol containing diets reduced plasma and liver cholesterol V. Only free sterols reduced plasma and liver cholesterol – caprate esters ineffective

Table 4: Details of animal studies on effects of plant sterols on cholesterol metabolism

Study	Design and Treatment	Animals	Length of Each Feeding Period	Main Results
Peterson, D. W., Proc. Soc. Exp. Biol. Med. 1951	I. a) Standard stock diet (N=7) b) a + 4% cottonseed oil (N=6) c) b + 1.0% cholesterol (N=6) d) c + 1.0% soy sterols (N=7) e) d but cholesterol and soy sterols mixed separately (N=7) f) d + 1.0% soy sitosterols (N=7)	Single Comb White leghorn chicks Age: 14 days old	4 weeks	Soy sterols and soy sitosterols dramatically reduced plasma and liver cholesterol as compared to cholesterol fed animals (see diet d-f vs diet c). Diet / TC mg/dl / Liver TC dry wt% a) 91 1.12 b) 85 1.01 c) 553 5.21 d) 109 1.69 e) 133 1.99 f) 109 1.65 No effect of mixing cholesterol and soy sterols separately thus concluding effect of soy sterols not due to complex formed during heating and mixing of diet.
	II. g) a + 4.5% cottonseed oil (N=9) h) g + 1.0% cholesterol (N=9) i) h + 1.3% soy sterols (N=9)	Male chicks	10 weeks	Comparably results in experiment II in that soy sterols prevented the rise in plasma and liver cholesterol. Similar effects found through 10 weeks.

Addition of soy sterols resulted in plasma and liver cholesterol levels of 109 mg/dl and 1.69 dry weight %, respectively. This was equivalent to an 80% reduction of plasma cholesterol and a 68% reduction of liver cholesterol. Further work by Peterson's group replicated their initial findings (Peterson et al., 1952; Peterson et al., 1953; Peterson 1954). The cholesterol lowering effect of soy sterols was reported in the presence of lecithin (Petersen, et al., 1952), cholic acid (Peterson, et al., 1954) and in the presence of unsaturated fatty acids like oleic acid (Peterson, et al., 1954). Later, Bartov and others (1969) reported, also in chicks, a 66% reduction in plasma cholesterol associated with feeding 3.0% soy sterols in cholesterol (1.0%) supplemented diets. They also reported a 32% reduction in plasma cholesterol and a 42% reduction in liver cholesterol associated with feeding 1.0% soy sterols in cholesterol (0.5%) supplemented diets.

Chandler and others (1979) compared the effects of cholestyramine and plant sterols, specifically β-sitosterol and stigmasterol, in chicks. Again the addition of 1.0% cholesterol to the diet dramatically increased plasma and liver cholesterol levels. The addition of 1.0% cholestyramine reduced serum cholesterol 66% from 331 to 114 mg/dl ($p<.05$) and liver cholesterol 44% (14.8 to 8.4 g/100g of lipid; $p<.05$). The addition of 1.0% β-sitosterol reduced serum cholesterol 57% from 349 to 150 mg/dl ($p<.05$) and liver cholesterol 48% (1.62 to 8.3 mg/100 gm of lipid; $p<.05$). The addition of 1.0% stigmasterol reduced serum cholesterol 36% ($p<.05$) and liver cholesterol 9% (NS). Thus, the authors concluded that stigmasterol significantly reduces serum and liver cholesterol but was considerably less effective than cholestyramine or β-sitosterol.

The effects of soy sterols on cholesterol metabolism have also been reported in rats. Best and Duncan (1958) fed various sources of sitosterol in the diet along with 1.0% cholesterol and 5.0% cottonseed oil for 14 days. While there was little effect of sterol feeding on serum cholesterol, there were significant effects on liver cholesterol. With cholesterol feeding, there was a four-fold increase in liver cholesterol. Feeding acetate and oleate cholesterol esters also led to a four-fold increase in liver cholesterol. Thus, these cholesterol esters were readily cleaved in the intestines releasing free cholesterol for absorption. Benzoate, palmitate and stearate esters of cholesterol only caused a slight rise in liver cholesterol. The addition of 5.0% β-sitosterol completely prevented the rise in liver cholesterol associated with adding cholesterol to the diet. This group also showed that not all sitosterol esters deliver the same degree of cholesterol lowering effect. These researchers suggested that specificity of pancreatic cholesterol esterase might explain the different effects of the various cholesterol and sitosterol esters.

Sugano and others (1977) also reported on the effects of sitosterol on cholesterol metabolism in rats. The addition of sitosterol to the basal diet had no effect on plasma or liver cholesterol. However, rats fed sitosterol (0.5%) and cholesterol (0.5%) had 17% lower plasma cholesterol ($p<.01$) as compared to animals fed only cholesterol (0.5%). The cholesterol lowering effects of sitosterol were also reported in the presence of cholic acid. In the presence of 0.5% cholesterol in the diet, the addition of 0.5% cholic acid significantly increased plasma cholesterol (188 to 426 mg/dl). Adding 0.5% sitosterol to the diets with added cholesterol and cholic acid caused a 55% reduction ($p<.01$) in plasma cholesterol (426 to 166 mg/dl) and a 61% reduction in liver cholesterol

(43.6 to 17.0 mg/g). Adding 1.0% sitosterol decreased plasma cholesterol 71% (426 to 120 mg/dl) and liver cholesterol over 60% (43.5 to 16.3 mg/g). Additionally, in the presence of 20% lard and 0.5% cholesterol, 0.5% sitosterol reduced plasma cholesterol 10% and liver cholesterol 62%. Laraki et al., (1991) examined two ratios of plant sterol to cholesterol in the diet with Wistar rats. They found that feeding 24 mg/d of both plant sterol and cholesterol resulted in a 13.6% decrease in plasma cholesterol. Increasing plant sterols to 96 mg/d while keeping cholesterol at 24 mg/d reduced plasma cholesterol 22%. Hirai and others (1984) reported liver cholesterol decreased over 68% in rats fed 5.0% plant sterol. In another study in rats it was reported that feeding sitosterol at 0.1, 0.5 and 2.0% of the diet for over thirty days decreased liver cholesterol 17.5, 23.3, and 31.5%, respectively (Sugano, et al., 1982).

The effects of plant sterols have also been reported in hamsters and mice. Ntanios, et al., (1998) reported that feeding 0.5% or 1.0% plant sterols from vegetable oil (65% β-sitosterol, 20% campesterol and 15% dehydrobrassicasterol) or tall oil (64% β-sitosterol, 16% campesterol and 21% sitosterol) significantly reduced plasma cholesterol in hamsters fed 0.25% cholesterol. After 90 days, a 25% reduction ($p<.05$) of plasma total cholesterol, as compared to respective controls, was reported in male hamsters fed 0.5% and 1.0% vegetable oil sterols. In female hamsters fed 0.5% and 1.0% vegetable oil sterols, the total cholesterol reduction was 34 and 44% ($p<.05$ for both) respectively. As compared to the respective controls, male hamsters fed 0.5% or 1.0% of tall oil sterols had reductions in total cholesterol of 14 and 31% ($p<.05$ for both), respectively. In female hamsters fed 0.5% and 1.0% tall oil sterols, the total cholesterol reduction was

15 and 31% ($p<.05$ for both), respectively. While these authors reported that vegetable oil sterols might be more effective in female hamsters, it is important to note that vegetable oil sterols significantly reduced cholesterol levels in both male and female hamsters. Sugano et al., (1982) reported that liver cholesterol and triglyceride decreased over 50% in mice fed 0.5% plant sterols for 40 days. After fifteen days of feeding 1.0% plant sterols to mice, Uchida et al., (1984) reported that cholesterol absorption decreased over 60%, liver cholesterol decreased over 25% and bile acid excretion decreased 43% while fecal sterol excretion increased 50% compared to the control group.

In a very recent study, Moghadasian and others (1999) reported the effects of feeding plant sterols (69% β-sitosterol, 16% sitostanol, and 15% campesterol) on cholesterol metabolism and indicators of atherosclerosis in apoE-deficient mice. ApoE-deficient knockout mice have been extensively used to study effects of hypercholesterolemia and other pathogenic aspects of atherosclerosis. Mice were fed a "western-type" diet with 9% fat and 0.15% cholesterol, with or without 2.0% plant sterols. As compared to the control group, mice fed 2.0% plant sterols had a significant 37% reduction in VLDL-+LDL-cholesterol concentrations and a 50% decrease in average lesional area, an important measure of atherosclerosis in this model.

Several research groups have reported results of sterol dose response studies in animals. Peterson and others (1952) reported the effects on cholesterol levels in chickens fed 1.0% cholesterol with various levels of soy sterols (.25% to 3.0% of the

diet). They reported a curvilinear relationship of plasma cholesterol and soy sterol content of the diet. A maximum effect was seen at a ratio of soy sterol to cholesterol of 2:1 to 3:1. In animals fed a ratio of 2:1, plasma cholesterol levels decreased over 90% and liver cholesterol decreased over 80% as compared to the group with cholesterol in the diet but without added soy sterols. Pollak (1953) reported on the effect of adding various levels of mixed sterols to the diets of rabbits fed cholesterol (1.0 g/d) on blood cholesterol levels and measures of atherosclerosis. Adding 1.0 g/d mixed soy sterols resulted in over 55% reduction in total cholesterol levels as compared to the group fed 1.0 g/d cholesterol alone (800 versus 337 mg/dl). None of the animals fed 1.0 g/d mixed sterols showed gross anatomic alterations indicative of atherosclerosis. Only twenty percent of the animals fed this diet showed presence of foam cells in the aorta, a very early indication of atherosclerosis. The addition of 3.0 g/d mixed sterols resulted in a reduction of total cholesterol of about 70% (800 versus 246 mg/dl). None of the animals fed this level of sterols showed any indication of atherosclerosis. The addition of 5.0 g/d mixed soy sterols resulted in total cholesterol reduction of about 85% (800 versus 123 mg/dl) as compared to the animals fed 1.0 g/d cholesterol. The feeding of 6.0 or 7.0 g/d mixed soy sterols virtually eliminated the rise in cholesterol associated with cholesterol feeding and none of the animals in either of these groups showed any signs of atherosclerosis. Thus in rabbits, decreases in cholesterol levels were seen up to a ratio of 5:1 sterol to cholesterol in the diet. At this ratio and higher, the effect of feeding cholesterol on plasma cholesterol levels was virtually eliminated. This group also fed 1.0 to 10 g/d of sitosterol to rabbits without cholesterol added to the diet and observed no significant change in cholesterol levels and no gross or microscopic

anatomic alterations in the aorta or carotid arteries. This indicates that sitosterol has no negative effects on atherosclerosis even when fed at very high levels. In contrast, 90% of rabbits fed as little as 1 g/d cholesterol showed indications of atherosclerosis of the aorta.

In a series of experiments in Rhesus monkeys, it was reported that the addition of 2.0% plant sterols decreased plasma total cholesterol as much as 43% and decreased cholesterol absorption as much as 52% (Bhattacharyya and Eggen, 1981; Bhattacharyya and Eggen, 1984). These researchers separated monkeys into two groups based on their response to dietary cholesterol in previous experiments and fed both a low and a high cholesterol diet with and without plant sterols. In the high cholesterol diet (0.15 mg/kcal) plasma cholesterol levels decreased ($p<.025$) from 230 to 132 mg/dl (42.6% reduction) in high responders after six weeks. In low responders the decrease was from 142 to 123 mg/dl (13.3% reduction). In the low cholesterol diet (0.02 mg/kcal) plasma cholesterol decreased 17% in high responders and 6% in low responders. Cholesterol absorption decreased during plant sterol feeding. On the high cholesterol diet, cholesterol absorption decreased from 73.0% to 42.8% for the high responders and 58.6% to 41.2% for the low responders. While on the low cholesterol diet, cholesterol absorption decreased from 85.4% to 40.3% in the high responders and 67.1% to 42.8% in low responders. Interestingly, the authors point out the addition of plant sterols caused cholesterol absorption to be the same in both low and high responders regardless of the amount of cholesterol in the diet.

Several research groups have evaluated the effects of various fatty acid sterol esters on cholesterol metabolism. As early as 1952, Petersen et al., evaluated the effects of soy sterol caprate and reported that adding soy sterol caprate ester to cholesterol supplemented diets did not have the same effect as adding non-esterified soy sterols. Chicks fed 1.0% cholesterol had plasma and liver cholesterol of 860 mg/dl and 12.7% dry weight, respectively. The addition of 1.0% soy sterols significantly reduced plasma cholesterol to 128 mg/dl and liver cholesterol to 2.33% dry weight. The addition of 1.38% soy sterol caprate (equivalent to 1.0% soy sterol) resulted in plasma cholesterol of 521 mg/dl and liver cholesterol of 8.45% dry weight, a result intermediate to the control and soy sterol group.

Best and Duncan (1958) reported the effects of various fatty acid esters of sitosterol on cholesterol metabolism in rats. They fed esters of acetate, propionate, palmitate and oleate. While there was little effect of sterol or sterol ester feeding on plasma cholesterol, significant effects were noted in liver cholesterol. Feeding 1.0% cholesterol resulted in liver cholesterol of 1268 mg/100g. The addition of 5.0% free sitosterol resulted in an 80% reduction in liver cholesterol (255 mg/100g). Sitosterol acetate (5.51%) and sitosterol oleate (9.57%) gave similar results as free sitosterol, namely 75-80% reduction in liver cholesterol (300 and 211 mg/100g), respectively. Sitosterol propionate (5.67%) and sitosterol palmitate were less effective and resulted in a 58% and 32% reduction in liver cholesterol, respectively. These authors concluded that pancreatic esterase preferred very short chain fatty acids and unsaturated long chain fatty acids. Reported relative rates of hydrolysis were: cholesterol palmitate, 1; oleate,

13.6; and acetate 32.7. Thus, for VOSE to be effective in lowering cholesterol levels, care must be given to ensure fatty acids used are readily enzymatically hydrolyzed in the intestine to release sitosterol and thus decrease cholesterol absorption.

From this section, it is clear that plant sterols and sterol esters, once hydrolyzed to free sterol, inhibit cholesterol absorption in various animal models. Exactly how sterols/sterol esters affect cholesterol absorption is not fully understood but it is clear that the decreased cholesterol absorption does lead to a decrease in liver and eventually plasma cholesterol levels in animal models.

E. *Suggested Mechanism of Action for Sterols and Sterol Esters*

Several mechanisms have been proposed by which plant sterols reduce blood cholesterol levels. Reports by M. Sugano and co-workers and I. Ikeda and co-workers have been invaluable in helping to understand how plant sterols reduce plasma cholesterol levels. It is well accepted that plant sterols inhibit cholesterol absorption (Mattson, et. al., 1977; Pollak and Kritchevsky, 1981; Vahouny and Kritchevsky, 1985; Ling and Jones, 1995). It has been hypothesized that plant sterols may inhibit intestinal absorption of cholesterol by:

1) inhibition of bile salt cholesterol micellar formation;
2) competition with cholesterol at brush border uptake;
3) changing micellar solubilization; and/or
4) competition for intracellular esterification and/or incorporation into chylomicron.

Certain plant sterols, in particular sitosterol and campesterol, are more hydrophobic than cholesterol and have a higher affinity for micelles, and thus, they can reduce the solubility of cholesterol (Armstrong and Carey, 1987; Compassi, et al., 1997). The presence of plant sterols in the intestine provides favorable conditions for displacement of cholesterol from micelles (Ling and Jones, 1995). Heineman et al., (1991) reported an almost 50% decrease in cholesterol absorption after addition of β-sitosterol using the validated technique of intestinal intubation. Mattson and others (1982) showed that both β-sitosterol and β-sitosterol oleate significantly decreased cholesterol absorption 33-42%. The free sterol was somewhat better than the ester but these authors indicated that the ester form has the advantage of its ready incorporation into dietary fat. More recently Jones, et al., (1999, submitted for publication) reported consumption of 1.8 g/d sterol ester reduced cholesterol absorption over 30% in humans.

Jones (1998) reported that the enhanced solubility of sterol esters might increase their solubility in bile salt micelles compared to sterols in powder or other forms. He claimed that such an increase in bilary solubility of plant sterols might further decrease the solubility of cholesterol in the micelle and thus further lower cholesterol absorption.

In further support of the importance of solubility, Ikeda and coworkers (1988) pointed out that solubilization of cholesterol in bile salt micelles is a requirement for its efficient absorption. They showed that binary mixtures of sitosterol and cholesterol resulted in less stable micelles. This instability led to sterol precipitation at a lower total sterol concentration than with cholesterol alone. This group also reported that the cholesterol

lowering effects of sterol were not due to competition for absorption at the brush border membrane, competition for intracellular esterification or to affects via mucin. Compassi et al., (1997) supported the conclusions of the work by Ikeda et al., (1988) that cholesterol lowering effects were not due to differential absorption at the brush border membrane. However, Compassi et al., (1997) provided evidence that the differential absorption of cholesterol and sitosterols was due to different rates of intracellular esterification and incorporation into the lipoprotein particle.

Lower cholesterol levels may also be due to increased cholesterol and neutral sterol excretion. At least in animals, increased excretion of cholesterol and fecal sterols is dependent on the dose of sitosterol. With increased excretion of cholesterol and its metabolites, conversion of cholesterol to bile acids in the liver is increased, causing additional drain on blood cholesterol levels (Ling and Jones, 1995).

While there is still some debate on the exact mechanism by which plant sterols decrease cholesterol absorption and thus plasma cholesterol levels, it is generally agreed that plant sterols do indeed decrease cholesterol absorption.

F. Projected benefits of cholesterol lowering via sterols or sterol esters

Approximately 52 million adults in the U.S. have a blood cholesterol level higher than desired and would be candidates for dietary modifications according to NCEP guidelines (NCEP, 1993). It is well accepted that for every 1 percent decrease in mean total cholesterol levels in the population, the risk of a CHD–related incident in that

population decreases by 2 percent. Thus, reductions in total blood cholesterol of about 5% reported in the previous section, associated with consumption of about 1 g/d of sterol ester, could have a significant impact on CHD risk in the population. Weststrate and Meijer (1998) reported that based on Law et al., (1994) the reductions in LDL-cholesterol seen in their study (~8% total-cholesterol and 13% LDL-cholesterol) would decrease CHD risk by about 40% at age 40 years and about 15% at age 70 years. Using changes in ratios of cholesterol fractions (~14% change in LDL- to HDL-cholesterol ratio) and using the work of Stampfer, et al. (1991) the authors reported a decreased CHD risk of nearly 20%.

Further, in addition to reducing CHD risk, it has also been reported that for each 5% reduction in LDL-cholesterol in the total population, as many as 7 million people would no longer need cholesterol-lowering drugs (NCEP, 1994; Sempos, 1994; Law et al., 1994).

G. Summary and conclusions

From the data presented, it can be concluded that consumption of 1.6 g/d VOSE (equivalent to 1.0 g/d sterol) will significantly reduce total- and LDL-cholesterol by about 5% and 7%, respectively. These reductions are clinically significant; accomplished in the U.S. population, a 10-15% reduction in CHD risk could be expected. Consumption of higher levels of VOSE, about 3 g/d (which appears to be optimum), has been reported to decrease total cholesterol about 10% and LDL-cholesterol by 13%

(Weststrate and Meijer, 1999). This could lead to a 20-25% reduction in CHD risk if accomplished in the population.

The cholesterol lowering effect of VOSE have been reported in diets with fat intakes comparable to the American diet (~34% calories from fat and ~300 mg/d cholesterol; Hendricks, et al., 1999) and diets both higher in fat (~40% calories as fat; Weststrate and Meijer, 1998) and lower in fat (~30 % calories as fat; Maki, et al., 1999, submitted for publication). The effects are similar regardless of various baseline cholesterol levels from 5.1 mM in Hendriks, et al., (1999) to 6.2 mM in Maki, et al., (submitted for publication) and 7.9 mM in Becker, et al., (1993). Results from various animal models, in mice, rats, hamsters and monkeys, all indicate that sterols and sterol esters inhibit cholesterol absorption, thus decreasing liver and plasma cholesterol levels. The animal data supplement the human clinical data and support the conclusion that plant sterols and sterol esters significantly reduce cholesterol levels in a variety of diets and species. Taken together, these data support a conclusion that the cholesterol lowering effect of VOSE is robust, and relatively unaffected by fat in the diet.

In addition to the extensive data presented in this section, further support of the petitioned health claim is presented in Appendices G through L, which contain statements from various experts in the field of lipids and health. These experts (whose *curricula vitae* are provided in the respective Appendices) agree that there is significant scientific agreement that 1.6 g/d VOSE (1.0 g/d sterol) will significantly reduce total- and LDL-cholesterol levels. Barbara Howard, PhD, a former chair of the Nutrition Study

Section for NIH, states in Appendix H, "data linking cholesterol reduction with consumption of vegetable oil sterol esters are strong and consistent. There is significant scientific agreement that cholesterol lowering by vegetable oil sterol esters can reduce the risk of CHD." Fergus Clydesdale, PhD, Professor and Head of the Department of Food Science at the University of Massachusetts, member of Food and Nutrition Board and current Chair of the Food Forum of the Institute of Medicine, states in Appendix I, that "the association between vegetable oil sterol esters and reduced risk of CHD is supported by a broad range of clinical experience in a wide variety of subjects over many years. The cholesterol-reducing properties of phytosterols, particularly beta-sitosterol, is well established." He also states that "data linking cholesterol reduction with consumption of vegetable oil sterol esters are reproducible and robust. These data indicate that the association between vegetable oil sterol esters and reduced risk of CHD meets the standard of significant scientific agreement." Sachiko St. Jeor, PhD, RD, a member of the 1995 U. S. Dietary Guidelines Advisory Committee and current member of the Nutrition Committee of the American Heart Association (AHA), states in Appendix J that the proposed health claim "is in line with standards which serve as the basis of other health claims and the scientific evidence presented thoroughly address issues of efficacy in reducing blood cholesterol levels."

David Kritchevsky, PhD, a renowned expert in cholesterol and lipid metabolism, states in Appendix K, "Studies of vegetable oil sterols confirm that they do in fact reduce cholesterol levels when formulated into everyday foods such as vegetable oil spreads." He also states, "Considering all of the studies together, it is my opinion that the data

linking cholesterol reduction with consumption of vegetable oil sterol esters are reproducible and sound."

Thomas Pearson, MD, PhD, MPH, the Chair of the Department of Community and Preventive Medicine at the University of Rochester School of Medicine and Dentistry, former member of the Nutrition Committee of the AHA and current Chair of the Population Committee of AHA, states in Appendix L that " vegetable oil sterol esters use is associated with a reduced risk of coronary heart disease, as supported by a large number of metabolic studies and randomized clinical trials. The cholesterol-reducing properties of phytosterols such as beta-sitosterol are well established, based on decades of research experience. Vegetable oil sterol esters appear to act similarly as unesterified phytosterols, as they appear to be released in the gut during digestion."

Ernest Schaefer, MD, Professor of Medicine and Nutrition at Tufts University School of Medicine, Director of the Lipid and Heart Disease Prevention Program at New England Medical Center and Chief of the USDA Lipid Metabolism Laboratory at Tufts University in Boston states in Appendix M that " data indicate that the association between vegetable oil sterol esters and reduced risk of CHD is supported by significant scientific agreement. Further, I believe that others in my professional field, reviewing the same data, would reach a similar conclusion." He closes his statement by saying, "I am confident that a health claim about the association between vegetable oil sterol esters and reduced risk of CHD would assist consumers in maintaining healthy dietary practices."

In summary the information presented above indicates that:

1) ingestion of plant sterols decrease total- and LDL-cholesterol levels in humans with no effects on HDL-cholesterol levels;
2) the effectiveness of plant sterols is predictive of the effectiveness of VOSE;
3) adequate and well-controlled studies in humans, as well as studies in animals, show that VOSE decreases total- and LDL- cholesterol levels with no effects on HDL-cholesterol levels;
4) independent scientists qualified by training and experience to evaluate the issue, conclude that VOSE are effective in lowering cholesterol.

Accordingly, based on the totality of publicly available scientific evidence (including evidence from well-designed studies conducted in a manner which is consistent with generally recognized scientific procedures and principles), there is significant scientific agreement among experts qualified by scientific training and experience to evaluate such claims, that the claim is supported by such evidence.

IV. Nature of the Food Eligible to Bear the Claim

At this time petitioner is requesting that the claim for VOSE be permitted on labeling of vegetable oil spreads and dressings for salads. The only reason for this limitation is that, at this time, the GRAS determination for VOSE covers only these foods.

Both spreads and dressings are condiments and are used to enhance the flavor or texture of other foods. As condiments, these foods offer versatility in use uniquely different from foods previously approved for health claims based on their cholesterol lowering properties. During the 1994/1996 USDA Continuing Survey on Food Intakes by Individuals (CSFII) over half of all adults consumed either a spread or a dressing or both on at least one of two survey days (See Appendix G). The widespread use of these foods offers the potential for minimal disruption to normal eating behaviors, thus facilitating the incorporation of these foods into the normal diet.

Because VOSE are lipophilic, their incorporation into foods requires the presence of some oil. Both spreads and dressing are traditionally oil based foods, yet offer formulation flexibility in the quantity needed to provide organoleptically desirable products.

Although spreads and dressings are widely consumed, they are usually consumed only in small quantities. Mean intake of spreads and dressings from the 1994/1996 CSFII study was 11.4 g/d and 40 g/d, respectively. These intake levels are very close to the single reference amount customarily consumed (RACC) amount for spreads and dressings, 1 and 2 tablespoons, respectively. Thus, we are requesting that the qualifying level of VOSE be the same as the level per serving, to assure that consumers who consume a small quantity of these foods will still obtain the benefit of the added VOSE. (In order to realize additional benefit from consuming VOSE at greater than the qualifying level, labeling may encourage consumption of up to 2-3 servings per day.)

A detailed specification for VOSE appears in Appendix E. The analytical method used for determining the level of VOSE in food is contained in Appendix F. The analysis of VOSE is based upon a standard triglyceride or cholesterol determination that uses sample saponification, followed by heptane extraction including an internal standard. The extract is analyzed by gas chromatography using a non-polar stationary phase capillary column and a flame ionization detector. This method has been shard with FDA lab staff and appears in its entirety in Appendix F.

V. Summary of Evidence Supporting Limited Exemptions.

Lipton hereby requests an exemption from the 13 g fat disqualifying level for health claims (21 C.F.R. §101.14(a)(4)) and, for dressings for salads, from the requirement that a serving contain at least 10% of the Reference Daily Intake or the Daily Reference Value of dietary fiber, protein, vitamin A, vitamin C, calcium or iron (21 C.F.R. §101.14(e)(6)).

A. Exemption from Total Fat Requirement and Low Fat CHD Criteria

For a health claim, the disqualifying level for fat is 13 grams per reference amount, per serving, and for foods with reference amounts of 30 g or 2 tablespoons or less, per 50 g. (21 C.F.R. §101.14(a)(4)). Since the products that are the subject of this health claim petition - spreads and salad dressings - both have reference amounts less than or equal to 30 g and 2 tablespoons, the disqualifying level is calculated on a 50 g basis. Spread products typically contain fat levels above the disqualifying level.

Under 21 C.F.R. §101.14(e)(3), a health claim on foods containing these levels of fat would be prohibited "unless FDA has permitted a claim ...based on a finding that such a claim will assist consumers in maintaining healthy dietary practices, and ... the label bears a disclosure statement ... highlighting the nutrient that exceeds the disqualifying level." These criteria can be met for foods made with vegetable oil sterol esters, and an exemption from the disqualifying levels for fat is justified for following reasons:

i. Spreads and dressings are appropriate vehicles for the ingredient.

ii. The use of the ingredient in spreads and dressings is not expected to result in increased consumption of fat and will be low in saturated fat and cholesterol.

iii. The requested exemption is necessary in order for consumers to obtain important labeling information about how vegetable oil sterol esters will assist in maintaining healthy dietary practices.

1. Spreads and dressings are appropriate vehicles for the ingredient

The use of vegetable oil sterol esters in both spreads and salad dressings is reasonable because these foods are used as flavorings and condiments on other foods (including breads and vegetables), all of which are consistent with a healthy diet. Spreads and dressings are typically consumed in small quantities and their consumption is self-limiting because of their intense flavor contribution. Based on USDA's 1994/1996 CSFII, mean per capita intake in the U. S. population was 5.97 g for dressings and 2.28

g for spreads. Among consumers, mean intakes were 40.0 g and 11.4 g respectively for dressings and spreads (see Appendix G).

Moreover, VOSE are derived from vegetable oils, are soluble in oil, and are naturally present in highest levels in dietary oils. Therefore, VOSE use in vegetable oil-based foods like spreads and dressings is consistent with the basic nature of the ingredient.

2. Foods bearing this health claim will not increase fat consumption

The use of this ingredient in spreads and dressings is not expected to result in increased consumption of fat. Consumption of vegetable oil sterol ester-containing spreads and salad dressings is intended to substitute for pre-existing consumption of similar foods. Therefore, consumers would not be expected to experience a net increase in fat consumption.

It is unlikely that consumers would eat vegetable oil sterol ester-containing spreads and salad dressings in addition to pre-existing levels of these foods because (1) the nature of the foods (i.e., oil-based condiments) tends to make their consumption self-limiting; (2) average consumption of these types of foods is already relatively low; and (3) the target consumers (i.e., cholesterol-conscious adults) would normally be disinclined to consume higher levels of fat.

The minimum effective level of VOSE is about 1.6 grams per day, which would be provided in a single serving of food. Since the average consumption of spreads and

dressings is 11.4 g/d and 40.0 g/d, respectively (CSFII, Appendix G), substitution of VOSE-containing spreads and dressings would not result in a significant change in levels of fat consumption. No increase in fat intake was observed in the large clinical trials where subjects were asked to replace their usual intake of spreads with spreads containing sterol esters (Weststrate and Meijer, 1998: Hendricks, et al., 1999; Maki, et al., 1999 submitted for publication).

Saturated fat and cholesterol are the key dietary ingredients that appear to contribute to CHD risk. Lipton proposes that spreads and dressings eligible for the proposed health claim must meet the nutrient content requirements in 21 C.F.R. §101.62 for a "low cholesterol" and a "low saturated fat" food. Thus, foods bearing the proposed CHD health claim will meet the same standards for cholesterol and saturated fats as foods eligible for previously approved CHD claims.

3. Exemption required to convey information on the healthful properties of the product

The requested exemption is necessary in order for consumers to obtain important labeling information about how VOSE will assist in maintaining healthy dietary practices. The proposed health claim would provide the only source of labeling information on the relationship between VOSE and risk of heart disease, since this information cannot be provided via nutrient content claims or structure-function claims.

4. FDA's Three Factors

In 1995, the FDA outlined three factors as being important to a decision as to whether to grant an exemption from the disqualifying levels. (60 Fed. Reg. 66206, 66222; Dec. 21, 1995.)

Public health significance of the disease

The first factor is whether the disease that is the subject of the petition is of such public health significance, and the role of the diet so critical, that the use of a disqualifying level is not appropriate. This factor strongly favors Lipton's request for an exemption. Coronary heart disease is of the highest public health significance, and the role of the diet is critical to reducing risk. Since VOSE help reduce blood cholesterol levels, and thereby help reduce CHD risk, an exemption from the disqualifying level is appropriate.

Adequate availability of foods qualifying for the health claim

The FDA's second factor is whether there would be adequate availability of foods that would qualify for a health claim for VOSE if an exemption to the disqualifying level were not granted. Without an exemption from the disqualifying level for fat, spreads would be ineligible for this claim. Thus, if an exemption were not granted, the number of foods eligible for this health claim would be so limited that it would not be possible to meet the public health goal of the claim.

Relevance of the disqualifying nutrient to the target population

The FDA's third factor focuses on the extent to which the population to which the health claim is targeted is at risk for the disease or health-related condition associated with the disqualifying nutrient. Because spreads and salad dressings are normally consumed at

low levels, and because the consumption of VOSE-containing spreads and dressings would normally substitute for consumption of non-VOSE containing spreads and dressings, there is unlikely to be a significant increase in fat intake that would pose a meaningful risk to the target population. On the contrary, products containing vegetable oil sterol esters have been shown to reduce blood cholesterol without concomitant changes in dietary fat intake (Weststrate and Meijer, 1998; Hendricks, et al., 1999; Maki et al., 1999 submitted for publication). Further, labeling information on VOSE-containing products will inform consumers about fat levels in the products, and consumers can take these into consideration as they decide whether or not to include the products in their diets.

In conclusion, for the reasons discussed above, Lipton believes that an exemption from the disqualifying level for fat is justified for products eligible for the proposed health claim.

B. Exemption for Dressings for Salads from 10% DV Nutrient Requirement

The regulation found at 21 C.F.R. §101.14(e)(6), makes a food ineligible to be labeled with a health claim unless it contains 10% or more of the RDI or DRV for vitamin A, vitamin C, iron, calcium, protein, or fiber per reference amount, prior to any nutrient addition (sometimes referred to as the "jellybean" rule). Spread products meet this requirement because they contain 10% of the RDI for vitamin A (through fortification that is for purposes of ensuring nutritional equivalence to margarine). However, salad dressings would normally not meet the 10% requirement. Dressings are typically not

fortified, and their serving sizes are so small as to prevent them being a good source of any of these nutrients.

Salad dressings containing VOSE are not the type of foods intended to be precluded from bearing a health claim by the "jellybean rule". This rule was meant to apply to foods that provide calories but no meaningful levels of nutrients, such as jellybeans and other candies and snack foods. The FDA has indicated that these are not the types of foods that consumers should be encouraged to eat on the basis of their being labeled with a health claim (even if they technically might be eligible for the claim). Salad dressings, however; are significantly different; they provide various nutrients, including Vitamins E and K, although not at the 10% level, Further, salad dressings are never eaten alone, but are normally consumed with other nutritious foods. (Salads, for example, are usually rich in vegetables that provide important nutrients at significant levels, e.g. tomatoes-vitamins A & C; carrots-vitamin A; spinach- vitamin A & calcium.) Dressings provide flavor, making the salad more appealing and so more likely to be eaten. Most importantly, of course, VOSE-containing dressings are entirely different than "jellybeans" because they contain VOSE, which provides significant nutritive value.

Salad dressings represent an important source of VOSE, and it would assist consumers in maintaining healthy dietary practices if dressings could be labeled with the proposed health claim. Lipton believes that the information outlined above provides a reasonable basis upon which to grant an exemption.

C. Expert Opinion in Support of the Requested Exemptions

Lipton asked several experts, qualified by expert training, to evaluate the appropriateness of the above-requested exemptions. Sachiko Jeor, PhD, RD states in Appendix J, "VOSE are necessarily added to spreads and salad dressings because of their fat solubility and should be exempt from certain regulatory requirements that would otherwise not permit a health claim as Lipton has proposed." Barbara Howard, PhD states in Appendix H, "In my experience, the most difficult problem in reducing CHD risk is achieving meaningful long-term dietary modification. If vegetable oil sterol esters can be formulated in convenient and palatable foods such as spreads and salad dressings, this will increase the likelihood that dietary modification will be successful. Therefore, I believe it is appropriate that Lipton's request for exemption be granted and that a health claim be permitted on the labeling of these foods." Fergus Clydesdale, PhD also supports of the exemptions by stating in Appendix I that "products containing phytosterols are being marketed as replacements for existing products that consumers use. That is, Lipton is not attempting to market these products so that overall uptake will increase, but rather is offering them as a healthy alternative to present usage."

For reasons outlined above, and in the opinions qualified experts (as provided in Appendices H-M), Lipton requests an exemption from the 13 g fat disqualifying level for health claims (21 C.F.R. §101.14(a)(4)) and, for dressings for salads, from the requirement that a serving contain at least 10% of the Reference Daily Intake or the Daily Reference Value of dietary fiber, protein, vitamin A, vitamin C, calcium or iron (21 C.F.R. §101.14(e)(6)).

VI. Proposed Model Health Claims

(1) 1.6 grams of vegetable oil sterol esters a day, as part of a diet low in saturated fat and cholesterol, may reduce the risk of heart disease. A serving of [name of the food] supplies ____ grams of vegetable oil sterol esters.

(2) Diets low in saturated fat and cholesterol that include 1.6 grams of vegetable oil sterol esters may reduce the risk of heart disease. One serving of [name of food] provides ____ grams of vegetable oil sterol esters.

(3) [Name of food], containing vegetable oil sterol esters, may lower LDL-cholesterol levels and reduce the risk of heart disease. The minimum recommended level of vegetable oil sterol esters in the diet is 1.6 grams; this food contains ____ grams.

VII. Environmental Impact

Lipton claims a categorical exclusion from the requirement to prepare an Environmental Assessment and Environmental Impact Statement under 21 C.F.R. §25.32(p).

VIII. Proposed Rule and Certification

PROPOSED RULE

§101. __ Health Claims: Vegetable oil sterol esters and risk of coronary heart disease (CHD).

(a) Relationship between diets that are low in saturated fat and cholesterol and that include vegetable oil sterol esters and the risk of CHD.

(1) [same as §101.81(a)(1)]

(2) [same as §101.81(a)(2)]

(3) Scientific evidence demonstrates that diets low in saturated fat and cholesterol may reduce the risk of CHD. Other evidence demonstrates that the addition of vegetable oil sterol esters from certain foods to a diet that is low in saturated fat and cholesterol may also help to reduce the risk of CHD.

(b) Significance of the relationship between diets that are low in saturated fat and cholesterol and that include vegetable oil sterol esters and the risk of CHD.

(1) [same as §101.81(b)(1)]

(2) Intakes of saturated fat exceed recommended levels in the diets of many people in the United States. One of the major public health recommendations relative to CHD risk is to consume less than 10 percent of calories from saturated fat and an

average of 30 percent or less of total calories from all fat. Recommended daily cholesterol intakes are 300 mg or less per day. Scientific evidence demonstrates that diets low in saturated fat and cholesterol are associated with lower blood total- and LDL-cholesterol levels. Vegetable oil sterol esters, when included in a low saturated fat and cholesterol diet, also help to lower blood total- and LDL-cholesterol levels.

(c) Requirements.

(1) All requirements set forth in §101.14 shall be met, except §101.14(e)(6) with respect to dressings for salads.

(2) Specific Requirements -

(i) Nature of the claim. A health claim associating diets that are low in saturated fat and cholesterol and include vegetable oil sterol esters with reduced risk of heart disease may be made on the label or labeling of a food described in paragraph (c)(2)(iii) of this section, provided that:

(A) The claim states that diets that are low in saturated fat and cholesterol and that include vegetable oil sterol esters "may" or "might" reduce the risk of heart disease;

(B) In specifying the disease, the claim uses the following terms: "heart disease" or "coronary heart disease";

(C) In specifying the substance the claim uses the term "vegetable oil sterol esters";

(D) In specifying the fat component, the claim uses the terms "saturated fat" and "cholesterol";

(E) The claim does not attribute any degree of risk reduction for CHD to diets that are low in saturated fat and cholesterol and that include vegetable oil sterol esters;

(F) The claim does not imply that consumption of diets that are low in saturated fat and cholesterol and that include vegetable oil sterol esters is the only recognized means of achieving a reduced risk of CHD; and

(G) The claim specifies the daily dietary intake of vegetable oil sterol esters that is necessary to reduce the risk of CHD and the contribution one serving of the product makes to the specified daily dietary intake level. The daily dietary intake level of vegetable oil sterol esters that has been associated with reduced risk of CHD is 1.6 gram (g) or more per day of vegetable oil sterol esters.

(ii) Nature of the substance.

(A) Vegetable oil sterol esters are a mixture, prepared by esterifying plant sterols from edible oils such as soy, canola, and corn, with mainly unsaturated fatty acids from sunflower oil. The plant sterols consist principally of β-sitosterol. The substance has a purity of no less than 94% sterol esters and free sterols combined. The unsaturated fatty acids comprise over 75% of the fatty acids esterified to the vegetable oil sterol. Saturated fatty acids comprise less than 10% of the fatty acids esterified to the vegetable oil sterol.

Analyses of VOSE is based on standard triglyceride or cholesterol determination using sample saponification, followed by heptane extraction and gas

chromatography. Contact FDA for details of the method.

(iii) Nature of the Food Eligible to Bear the Claim.

(A) The food product is a vegetable oil spread or dressing for salad that shall contain at least 1.6 g vegetable oil sterol esters per reference amount customarily consumed;

(B) Pursuant to section 101.14(e)(3), FDA has made a finding that, despite the fact that a disqualifying level of fat may be present in the foods described in paragraph (c)(iii)(A) of this section, a health claim for vegetable oil sterol esters in such foods will assist consumers in maintaining healthy dietary practices.

(C) The food shall meet the nutrient content requirements in §101.62 for a "low saturated fat" and "low cholesterol" food.

(d) Optional information.

(1) [same as §101.81(d)(1)]

(2) The claim may state that the relationship between intake of diets that are low in saturated fat and cholesterol and that include vegetable oil sterol esters and reduced risk of heart disease is through the intermediate link of blood cholesterol levels, including blood total- and/or blood LDL-cholesterol levels;

(3) The claim may include information from paragraphs (a) and (b) of this section, which summarize the relationship between diets that are low in saturated fat and

cholesterol and that include vegetable oil sterol esters and CHD and the significance of the relationship;

(4) The claim may state that a diet low in saturated fat and cholesterol that includes vegetable oil sterol esters is consistent with "Nutrition and Your Health: Dietary guidelines for Americans," U. S. Department of Agriculture (USDA) and Department of Health and Human Services (DHHS), Government Printing Office (GPO);

(5) [same as §101.81(d)(6)]

(6) [same as §101.81(d)(7)]

(e) Model health claim. The following model health claims may be used in food labeling to describe the relationship between diets that are low in saturated fat and cholesterol and that include vegetable oil sterol esters and reduced risk of heart disease:

(1) 1.6 grams of vegetable oil sterol esters a day, as part of a diet low in saturated fat and cholesterol, may reduce the risk of heart disease. A serving of [name of the food] supplies ____ grams of vegetable oil sterol esters.

(2) Diets low in saturated fat and cholesterol that include 1.6 grams of vegetable oil sterol esters may reduce the risk of heart disease. One serving of [name of food] provides ____ grams of vegetable oil sterol esters.

(3) [Name of food], containing vegetable oil sterol esters, may lower LDL-cholesterol levels and reduce the risk of heart disease. The minimum recommended level of vegetable oil sterol esters in the diet is 1.6 grams; this food contains ____ grams.

CERTIFICATION

Attached herewith are copies of the scientific studies, literature searches, and other information referenced in, and constituting the basis for, this petition. To the best of our knowledge, all nonclinical studies relied upon were conducted in compliance with the good laboratory practice regulations set forth in 21 C.F.R. Part 58, and all clinical or other human investigations relied upon were either conducted in compliance with the requirements for institutional review set forth in 21 C.F.R. Part 56 or were not subject to such requirements in accordance with 21 C.F. R. § 56.104 or 56.105, and were conducted in compliance with the requirements for informed consent set forth at 21 C.F.R. Part 50.

Any questions regarding this petition may be directed to Nancy Schnell, Esq. (212-906-4573 at Lipton) or Daniel R. Dwyer, Esq. (202-223-5120 at Kleinfeld, Kaplan and Becker).

To the best of the undersigned's knowledge, this petition is a representative and balanced submission that includes unfavorable information as well as favorable information, known by Lipton to be pertinent to the evaluation of the proposed health claim.

Sincerely,

Lipton

By: William C. Franke

William Franke, PhD.
Vice President, Scientific and Regulatory Affairs

Appendices