

November 21, 2000

Dockets Management Branch  
Food and Drug Administration  
5630 Fishers Lane  
Room 1061  
HFA-305  
Rockville, Maryland 20852

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**Re: Docket No. 00P-1276  
Comments in Response to Interim Final Rule on Health Claim for Plant  
Sterol/Stanol Esters and Coronary Heart Disease**

Dear Madam/Sir:

Arent Fox submits the following comments to the Food and Drug Administration ("FDA") on behalf of Raisio Benecol Ltd., Raisio, Finland ("Raisio"), in response to the Agency's interim final health claim rule for plant sterol/stanol esters and coronary heart disease ("CHD"). 65 Fed Reg. 54686 (Sept. 8, 2000). The purpose of these comments is three-fold: (1) to demonstrate that scientific studies support a health claim for plant stanol esters at a level of 1.4 g/d;<sup>1/</sup> (2) to identify marked discrepancies in certain studies comparing the cholesterol-lowering effects of sterol esters and stanol esters; and (3) to explain errors in FDA's proposed factor(s) for the conversion of intake levels of sterols and stanols to the corresponding esters, thereby avoiding the anomaly that less sterols than stanols would be required to produce purportedly equivalent amounts of the esters.

## **I. BACKGROUND**

On January 6, 1993, FDA issued a final rule implementing the health claim provisions of the Nutrition Labeling and Education Act of 1990 ("NLEA"), which amended the Federal Food, Drug, and Cosmetic Act ("the Act"), to provide procedures for FDA's regulation of health claims on food labels and in food labeling. 58 Fed. Reg. 2478 (Jan. 6, 1993). In that final rule, FDA set forth the procedure for petitioning FDA to authorize a health claim for a substance-disease relationship, and identified the types of information that must be included in such a petition. 21 C.F.R. §101.70. In order for FDA to authorize a health claim, the petitioner must be able to establish that "based on the totality of publicly available scientific evidence," there is significant scientific agreement, among experts qualified to evaluate such claims, that the claim is supported by such evidence. *Id.*

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<sup>1/</sup> For purposes of convenience, Raisio has rounded 1.36 g/d stanol esters to 1.4 g/d throughout this Comment. Further, for purposes of convenience, we have omitted the word "plant" from the terms "plant sterols," "plant stanols," "plant sterol esters," and "plant stanol esters" throughout this Comment.

As part of its statutory mandate under the NLEA, FDA also evaluated several substance-disease relationships to determine whether there was sufficient scientific support to justify the Agency's authorization of health claims for them. One such substance-disease relationship focused on the relationship between dietary saturated fat and cholesterol and reduced risk of coronary heart disease ("CHD"). FDA determined that the publicly available data supported an association between diets low in saturated fat and cholesterol and reduced risk of CHD. 21 C.F.R. § 101.75.

The Agency had previously set forth the criteria for evaluating evidence on the relationship between diet and CHD, and the relationship of diet to other cardiovascular disease generally, in its proposed rule entitled "Health Claims and Label Statements; Lipids and Cardiovascular Disease." 56 Fed. Reg. 60727 (Nov. 27, 1991). In that proposed rule, FDA acknowledged that there is general agreement that elevated serum cholesterol levels are one of the major modifiable risk factors in developing CHD. FDA also recognized that there is voluminous epidemiologic and clinical evidence establishing that high levels of serum total and low density lipoprotein ("LDL") cholesterol are major risk factors for CHD. Therefore, as serum total and LDL cholesterol levels are decreased, so is the risk of CHD.

Over the years, FDA has authorized numerous health claims establishing a relationship between reducing the risk of CHD by lowering levels of total and LDL cholesterol. See, e.g., 21 C.F.R. § 101.77 (authorizing health claim for fruits, vegetables, and grain products and reduced risk of CHD); 21 C.F.R. § 101.81 (authorizing health claim for beta-glucan from oat sources and psyllium seed husk and reduced risk of CHD); and 21 C.F.R. § 101.82 (authorizing health claim for soy protein and reduced risk of CHD).

Last February, FDA accepted for filing two separate health claims petitions -- one for plant sterol esters and one for plant stanol esters -- and reduced risk of CHD. Specifically, on February 1, 2000, Lipton submitted a health claim petition for sterol esters.<sup>2</sup> In its petition, Lipton requested a health claim for sterol esters and reduced risk of CHD, and proposed 1.6 g/d sterol esters (1 g/d sterols) as the daily dietary intake level. On February 15, 2000, McNeil Consumer Healthcare ("McNeil") submitted its health claim petition for stanol esters.<sup>3</sup> In its petition, McNeil requested a health claim for stanol esters and reduced risk of CHD, and proposed 3.4 g/d stanol esters (2 g/d stanols) as the daily dietary intake level.

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<sup>2</sup> Lipton, "Petition for Health Claim - Vegetable Oil Sterol Esters and Coronary Heart Disease," Item CP1, Docket 00P-1275, Dockets Management Branch, Feb. 1, 2000 ("Lipton's Health Claim Petition").

<sup>3</sup> McNeil Consumer Healthcare, "Petition for Health Claims - Plant Stanol Esters and Coronary Heart Disease," Item CP1, Docket 00P-1276, Dockets Management Branch, Feb. 15, 2000 ("McNeil's Health Claim Petition").

McNeil's request for 3.4 g/d stanol esters as the daily dietary intake level corresponds to its currently labeled product. Unlike McNeil's request, however, the daily dose requested by Lipton is much lower than Lipton's marketed product. Its product is currently labeled for 1650 mg of sterol esters per serving, two to three times a day, or a total of 3.3 to 5.0 g/d sterol esters. Yet, Lipton requested a health claim for the same daily dose of sterol esters as that currently in just one serving of its product (i.e., 1.6 g sterol esters, or 1 g/d sterols). Lipton purported to have requested a lower daily dose than its current product labeling because it wanted "to assure that consumers who consume a small quantity of these foods will still obtain the benefit of the plant sterol esters." Lipton's Health Claim Petition, p. 70. As set forth in Section III below, Raisio believes that there is also significant scientific agreement in support of a health claim for stanol esters at a daily dose of 1.4 g/d (0.8 g/d stanols).

On September 8, 2000, in response to the two health claim petitions, FDA published its interim final health claims rule for sterol/stanol esters and reduced risk of CHD. The Agency's determinations are set forth below in Section II.

## **II. PROPOSED HEALTH CLAIM RULE FOR STEROL/STANOL ESTERS**

### **A. Established Criteria for FDA's Review of Scientific Studies**

FDA may authorize a health claim where there is significant scientific agreement among qualified experts that the totality of the publicly available scientific evidence supports the claimed health benefit. 21 U.S.C. § 343(r)(3)(B); 21 C.F.R. § 101.70(f). As both Congress and FDA have explained, the standard does not require a consensus among scientists, but rather only significant agreement.<sup>4/</sup> FDA has recognized that requiring full consensus would cause many valid health claims to be denied because of the difficulty in achieving unanimous agreement. 58 Fed. Reg. 2478, 2505 (Jan. 6, 1993).

In the proposed rule for sterol/stanol esters and CHD, FDA stated that in selecting the most pertinent studies to evaluate both health claim petitions, the Agency relied on the same criteria that it has used to evaluate the relationship between other substances and CHD. 65 Fed. Reg. 54686, 54691. Specifically, FDA required that the studies (1) present data and adequate descriptions of the study designs and methods; (2) be available in English; (3) include estimates of, or enough information to estimate, intakes of sterols or stanols and their esters; (4) include direct measurement of blood total cholesterol and other blood lipids related to CHD; and (5) be

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<sup>4/</sup> House of Representatives, House Report 101-538, "Nutrition Labeling and Education Action of 1990," June 13, 1990; 58 Fed. Reg. 2478, 2505 (Jan. 6, 1993).

conducted in persons who represent the general U.S. population (i.e., adults with blood total cholesterol levels less than 300 mg/dL). *Id.*

#### **B. FDA's Review of Health Claim Petition for Sterol Esters**

With regard to its review of Lipton's Health Claim Petition for sterol esters, FDA concurred with Lipton that the data supported a health claim for sterol esters at a daily dose of 1.6 g/d sterol esters (1 g/d sterols). However, FDA also reviewed the studies to determine whether there was sufficient support for a health claim at an even lower daily dose. Citing to the Hendriks et al. study, (Ref. 57)<sup>5/</sup> and the Sierksma et al. study (Ref. 75) as adequate support, FDA authorized a health claim for sterols at a dose of 1.3 g/d plant sterol esters (0.8 g/d sterols).

#### **C. FDA's Review of Health Claim Petition for Stanol Esters**

With regard to McNeil's Health Claim Petition for stanol esters, FDA concurred that there was significant scientific agreement in support of a health claim for stanol esters at a daily dose of 3.4 g/d stanol esters (2 g/d stanols). The Agency then reviewed the studies submitted by McNeil to determine whether there was sufficient support for a health claim at a lower daily dose than the amount McNeil originally requested. FDA concluded that the Miettinen and Vanhanen study (Refs. 63 & 64) supported a dose of 1.4 g/d stanol esters (0.8 g/d stanols).<sup>6/</sup> (See discussion in Section III.C below about problems with FDA's conversion factors.) FDA also noted that the Hallikainen et al. study (Ref. 88) found a significant reduction in both total and LDL cholesterol levels at 2.7 g/d stanol esters (1.6 g/d stanols). In reviewing the Hallikainen study, however, FDA concluded that the data did not support a lower daily intake because the study did not demonstrate a statistically significant reduction for both serum total and LDL cholesterol levels at 1.4 g/d stanol esters (0.8 g/d stanols). FDA also rejected that portion of the Hallikainen study that supported a dose of 2.7 g/d stanol esters (1.6 g/d stanols) because the study by Jones et al. (Ref. 58), as discussed below, did not replicate that finding.

FDA also cited the findings from the Jones study as inconsistent with a daily dose lower than 3.4 g/d stanol esters (2 g/d stanols). Although the Jones study reported a statistically significant

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<sup>5/</sup> Unless otherwise indicated, the reference numbers cited to in these comments correspond to the reference numbers used by FDA in its Interim Final Rule on Health Claims for Plant Sterol/Stanol Esters and Coronary Heart Disease.

<sup>6/</sup> As discussed in Section III, the weight that FDA accorded to the Miettinen and Vanhanen study is not entirely clear. The Agency did, however, cite this study as support for a health claim at a dose of 1.4 g/d of stanol esters (0.8 g/d stanols). Elsewhere in the interim final rule, FDA identified what it perceived to be inconsistencies in the study. Those perceived inconsistencies are fully explained and reconciled in Section III.A.1. below.

reduction in serum LDL cholesterol at 3.3 g/d stanol esters (1.9 g/d stanols), it did not find a statistically significant decrease in total cholesterol versus control at this dose. Therefore, FDA approved McNeil's request for a health claim for 3.4 g/d stanol esters (2 g/d stanols).

Raisio believes that the data discussed below clearly demonstrate that stanol esters are effective at significantly reducing serum total and LDL cholesterol levels when taken in two separate servings at a dose as low as 1.4 g/d stanol esters (0.8 g/d stanols).

### III. DISCUSSION

This discussion has three primary objectives:

- to demonstrate that data from scientific studies cited by FDA support a health claim for stanol esters at levels of 1.4 g/d, equivalent to 0.8 g/d stanols, divided into two daily doses;
- to point out discrepancies in certain studies on the cholesterol-lowering effects of sterols and sterol esters; and
- to correct errors in the proposed factor(s) used to convert weights of sterols and stanols to weights of the corresponding esters, since such errors falsely mandate that less sterol than stanol is required to produce equivalent amounts of the ester.

#### A. Data Support a Daily Dose of 1.4 g Stanol Esters (0.8 g Stanols)

FDA has already recognized that a daily intake of 1.4 g stanol esters (0.8 g stanols) produced a statistically significant reduction in serum total and LDL cholesterol in one study by Miettinen and Vanhanen (1994), reported in 2 papers cited by FDA as Refs. 63 and 64 (65 Fed. Reg. 54698, 54700, 54704). As explained below, that study is critical because it establishes the efficacy of stanol esters (but not unesterified sterols) even when used as part of a diet with high intake levels of cholesterol-lowering vegetable oil. Raisio believes that a second study considered by FDA, Hallikainen et al. (2000) (Ref. 88), also supports the efficacy of a daily intake of 1.4 g stanol esters. 65 Fed. Reg. 54698, 54704. Finally, discussed below are several other studies that support a health claim for stanol esters at daily doses lower than 3.4 g sterol esters (2 g stanols).

##### 1. Miettinen and Vanhanen, 1994; Vanhanen and Miettinen, 1992.

These two papers, taken together, describe the results of a single study on the efficacy of small quantities of sterols, stanols or stanol esters in reducing serum total and LDL cholesterol. Stanol

esters were administered at a daily rate of 1.4 g/d stanol esters (0.8 g/d stanols), and were found to have a significant cholesterol-lowering effect over and above that of the oil-based spread into which they were mixed. Although FDA appears to accept this finding (Id.), Raisio is concerned that FDA noted that the results of these papers were "inconclusive because of inconsistencies in the descriptions of methods and results" (65 Fed. Reg. 54698, 54700). Raisio believes that one of the two apparent inconsistencies cited by FDA arises from what is clearly a recording error.<sup>7/</sup> The other, relating to the degree of cholesterol reduction, derives from confusing presentation of results in the two papers.<sup>8/</sup> Raisio therefore believes that these papers do provide conclusive support for the efficacy of a daily intake of 1.4 g stanol esters (0.8 g stanols).

It is important to note that in this study, the sterols, stanols, and stanol esters were administered in a mayonnaise-type spread made from rapeseed oil (canola oil) and water. Rapeseed oil mayonnaise alone was used in a 6-week run-in prior to the test period, and served as the control. Targeted daily consumption of rapeseed oil in the run-in period was 50 grams, considerably higher than in later stanol ester studies using a margarine. Not unexpectedly, this high daily intake of rapeseed oil alone caused a significant reduction in serum total and LDL cholesterol compared with home-diet baseline. (Miettinen and Vanhanen, 1994, Figure 1 and Table 3 (Ref. 63)). The average reduction in serum total cholesterol in 24 subjects after a 6-week run-in period with 50 g/day rapeseed oil alone was 9.2%<sup>9/</sup> (Ref. 64). (The paper does not provide a numerical value for the percentage reduction in serum LDL cholesterol levels by rapeseed oil alone.)

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<sup>7/</sup> FDA notes that it was difficult to "decipher" from the two papers the amount of stanol esters that was consumed, because the experimental design section of the 1994 paper (Ref. 63) shows the daily intake of sitostanol ester to be equivalent to 800 mg of *sitostanol* (i.e., 1360 mg sitostanol ester), but Table 1 shows it to be 830 mg "*sitostanol ester*". However, the data presented in the paper make it clear that the table heading should be "*sitostanol*" and not "*sitostanol ester*." Thus, the real discrepancy between the two stanol values -- a targeted intake of 800 mg/d and a measured intake of 830 mg/d -- is of no practical significance.

<sup>8/</sup> FDA also notes that it was difficult to decipher the amount of cholesterol-lowering that was observed. The agency stated that, by using information in the results section of the "Miettinen reference", it calculated the total cholesterol reduction resulting from ingesting sitostanol esters to be 18%, while the abstract reported the value as 7%. It is not apparent how the Agency calculated a value of 18%. Significant reductions of 5.6% and 8.6% in total and LDL cholesterol levels, respectively, compared with control can be readily calculated from Table 2 in the results section. Although it is not clear which of these is referred to in the abstract, they are both close to 7%.

<sup>9/</sup> The abstract erroneously states 8.5% for the effect of rapeseed oil alone.

Notably, there were already significant reductions in serum total and LDL cholesterol as a consequence of rapeseed oil intake. Thus, the effect of the treatment ingredients could have been masked were such effects marginal. In fact, 1.4 g/d stanol esters (0.8 g/d stanols) resulted in significant additional reduction of 5.6% in serum total and 8.2% in LDL cholesterol levels compared with control. The fact that 1.4 g/d stanol esters produced further statistically significant reductions in serum total and LDL cholesterol compared to pre-treatment diet and control (Table 2 of Miettinen & Vanhanen (Ref. 63)) demonstrates that the effects of stanol esters were robust.

These substantial results obtained with stanol esters stand in marked contrast to the results obtained with similar quantities of unesterified sterols and stanols. Administration of unesterified sterols and stanols after the run-in period produced no additional cholesterol-lowering effects (Vanhanen and Miettinen, 1992) (Ref. 64). Those results are consistent with other studies that demonstrate that once dietary modifications have been made with a run-in of corn oil, there are no further decreases in serum total and LDL cholesterol from unesterified sterols even at higher daily intakes up to 12 g/d. (Pollak and Kritchevsky, 1981, § II.E.6(g), citing Tobian and Tuna, 1958; Engelberg, 1957.<sup>10/</sup>)

The findings in these older studies are echoed by a recent study by Ostlund et al.,<sup>11/</sup> which showed that the effect of unesterified sitostanol on cholesterol absorption is highly dependent on the physical form in which it is administered. FDA concludes that the cholesterol lowering effects of unesterified sterols and sterol esters are equivalent. Consequently, the Agency allowed the use of data from a study Sierksma et al. (Ref. 75) with unesterified sterols to evaluate the efficacy of soy sterols. Raisio disagrees with that conclusion, and believes that studies using unesterified sterols and stanols are not comparable to those using the esters. The findings from the Sierksma study are specific to the product prepared for that study (a margarine), and are not relevant to the commercial products available. This is because free sterols must be intimately mixed with fat to be effective. Thus, free (unesterified) sterols would not be effective in products in which the fat dispersion is different from Sierksma's formulation.

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<sup>10/</sup> Pollak, O.J.; Kritchevsky, "Sitosterol," In *Monographs on Atherosclerosis*, Vol. 10. Clarkson, T.B.; Kritchevsky, D.; Pollak, O.J., Eds.; Karger: New York, 1981, pp. 1-219. Engelberg, H., "Studies of Serum Cholesterol and Low-Density Lipoprotein Levels, Previously Lowered by a Reduced Fat Intake, After the Addition of Corn Oil to the Diet," *Journal of Chronic Diseases*, Vol. 6, pp. 229-233, 1957. Tobian, L.; N. Tuna, "The Efficacy of Corn Oil in Lowering the Serum Cholesterol of Patients with Coronary Atherosclerosis", *American Journal of Medical Sciences*, Vol. 235, pp. 133-137, 1958.

<sup>11/</sup> Ostlund, R.E., C.A. Spilburg, W.F. Stenson, "Sitostanol Administered in Lecithin Micelles Potently Reduces Cholesterol Absorption in Humans", *American Journal of Clinical Nutrition*, Vol. 70, pp. 826-831, 1999.

Ostlund et al. showed that sitostanol in a crystalline powder form (which should have very low solubility in bile) caused only a marginal reduction in cholesterol absorption, whereas sitostanol incorporated into lipid micelles (which should be readily soluble in bile) reduced cholesterol absorption significantly at much lower dose levels. As discussed by Ostlund et al., the negative results obtained by Vanhanen and Miettinen (Ref. 64) with unesterified sitostanol are consistent with its low solubility in vegetable oil. The greater solubility of stanol esters in oil is responsible for their greater efficacy.

Thus, as FDA has acknowledged, the study by Miettinen and Vanhanen (Ref. 63) clearly demonstrates the efficacy of a daily intake of 1.4 g stanol esters (0.8 g stanols) in significantly reducing serum total and LDL cholesterol levels. On the other hand, FDA has noted that the dose-response study by Hallikainen et al. (2000, Ref. 88) discussed below, did not find a significant reduction in serum total or LDL cholesterol levels at a daily intake of 1.4 g/d stanol esters (0.8 g/d stanols). As set forth below, Raisio disagrees with the Agency's conclusions concerning the Hallikainen study.

## 2. Hallikainen et al., 2000.

Raisio believes that the Hallikainen study supports the conclusion that 1.4 g/d stanol esters are effective in reducing the risk of CHD. A detailed examination of the study, and of additional unpublished findings obtained from the authors, shows that 1.4 g/d stanol esters (0.8 g/d stanols) produced not only a significant reduction in serum total and LDL cholesterol levels, but also a significant reduction in Apolipoprotein B ("Apo B") levels. Four important observations may be made based on the full set of data:

- Apo B (a marker of LDL cholesterol metabolism) was significantly reduced at 4 weeks versus control;
- Daily intakes of 1.4 g stanol esters (0.8 g stanols) significantly reduced serum total and LDL cholesterol levels at 2 weeks, compared with the control period;
- Daily intakes of 1.4 g stanol esters (0.8 g stanols) significantly reduced serum total and LDL cholesterol levels at 4 weeks, compared with home diet baseline; and
- A temporary diet change during the control period may well account for the apparent failure of 1.4 g/d stanol esters (0.8 g/d stanols) to produce a significant reduction in serum total and LDL cholesterol at 4 weeks, compared with control.

**a. Significant Reduction of Apo B at 4 Weeks**

Apo B is the major apolipoprotein of low density lipoproteins ("LDLs"). In addition to being essential for the synthesis and secretion of very low-density lipoprotein ("VLDL"), the precursor of LDL, it also is crucial for the clearance of LDL by its receptor.

Lipoproteins transport cholesterol through the bloodstream and lymphatic fluid. There are two major types of lipoprotein involved in binding and transporting cholesterol: LDLs and high-density lipoproteins ("HDLs"). About 70 percent of all cholesterol in the blood is carried by LDL particles; most of the remainder is carried by HDLs. LDLs transport cholesterol from its site of synthesis in the liver to the body's cells, where the cholesterol is separated from the LDL and is then used by the cells for various purposes. Body cells extract cholesterol from the blood by means of receptors on their surfaces; these receptors bind with the LDL particles (and their attached cholesterol) and draw them from the blood into the cell. Apo B-carrying lipoprotein is primarily responsible for the atherosclerotic buildup of fatty deposits on the blood vessel walls. Thus, the number of LDL particles circulating in the blood, rather than the concentration of serum LDL cholesterol, represents the true atherogenic factor.

More than 90% of serum Apo B resides in the LDL fraction of lipoproteins. There are, however, several different forms of LDL cholesterol, which contain varying proportions of cholesterol. Measurement of Apo B levels provides, therefore, a far more accurate reflection of the number of LDL particles than does calculation of LDL cholesterol levels.

Highly standardized, automated, and precise methods for the direct measurement of plasma Apo B have become available in most clinical laboratories. In fact, the measurement of Apo B is significantly more precise than the measurement of LDL cholesterol. The biological (day-to-day) variability of the direct measurement of Apo B ranges from 5% to 8%. Assessment of LDL cholesterol levels is more variable because LDL cholesterol levels are not measured directly, but are calculated, based on total cholesterol, triglycerides and HDL.<sup>12/</sup> The biological variability for these three parameters is 6.1%, 22.6%, and 9.5%, respectively. The calculation error involved in working with these highly variable parameters produces a much wider variability in the assessment of LDL cholesterol, compared to the direct measurement of Apo B.

The variation in the analytical measurement of Apo B is now less than 5%, equivalent to that of both total and LDL cholesterol. Yet while it is necessary to take fasting samples for measurement of serum cholesterol, blood samples for measurement of Apo B can be taken at any time. Given the potential calculation errors for LDL cholesterol and ease of measuring Apo B, it is clear why Apo B has become the preferred measurement.

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<sup>12/</sup> LDL cholesterol is generally calculated using the Friedwald equation: LDL cholesterol = total cholesterol - HDL cholesterol - triglycerides/5.

Much evidence implicates Apo B-containing lipoproteins in the pathogenesis of atherosclerosis. In controlled clinical studies in patients with CHD, concentrations of plasma Apo B have been found to be more discriminating than those of other plasma lipids and lipoproteins. (See references in Attachment A.) Also, prospective studies have confirmed the utility of plasma Apo B levels in determining risk for CHD. (See references in Attachment A). Since Apo B enjoys superiority with respect to biological variability compared to LDL cholesterol, the significant reduction of 8.7% in Apo B demonstrated in the Hallikainen et al. study should also be accepted to show that ingestion of stanol esters at a dose of 1.4 g/d would result in a significant reduction in cardiovascular events.

**b. Significant Reduction in Serum Total and LDL Cholesterol at Two Weeks Compared With Controls**

Unpublished data from the Hallikainen et al. study (Attachment B) demonstrates that 1.4 g/d stanol esters (0.8 g/d stanols) produced a significant reduction in serum total and LDL cholesterol levels at 2 weeks compared with 0 g/d control values and with the home diet baseline. The cholesterol reduction was also statistically significant in all other dose groups. (See Attachment B, Tables 3 and 6). Because these data, provided to Raisio by the study's authors, are derived from a large published study, Hallikainen et al. (Ref. 88), they are valid and should be recognized. These important data are not suspect for any of the reasons that ordinarily cause FDA to discount unpublished findings. 65 Fed. Reg. 54686, 54691. Specifically, the design and methodologies of the study are described in detail in Hallikainen et al. (Ref. 88); the necessary primary data are clearly identified; and the published study from which the data derived was subject to peer-review. The time-related data, which would have shown the anomalies in the data of the control period, were considered redundant and were eliminated from the published paper during the review process when the manuscript was submitted for publication. In light of the fact that these data, albeit unpublished, are valid and well-supported, and are derived from a peer-reviewed published study, they should be accorded a weight similar to their published counterpart.

Other studies confirm that a significant cholesterol-lowering effect can be seen at 2 weeks. Nguyen et al. (1999, Ref. 90) found that a daily intake of 3.4 or 5 g stanol esters (2 or 3 g stanols) significantly reduces serum total (Figure 1) and LDL cholesterol (Figure 2) after just 2 weeks of dietary use. Similarly, Weststrate and Meijer (1998, Ref. 67) reported the cholesterol-lowering effects of stanol esters at 2.5 weeks. The 2-week measurement in the Nguyen study and the 2.5 week measurement in the Weststrate and Meijer study represent the earliest measurement after the start of these studies.

Recent studies have shown significant reductions in serum total and LDL cholesterol after as short a time as 7 or 8 days. First, a study by Miettinen et al. (2000)<sup>13/</sup> found that the daily intake of 4.3 g stanol esters (2.5 g stanols) by colectomized subjects reduced serum total and LDL cholesterol levels by 16.6% and 13.8%, respectively, after only 7 days.

Second, in an unpublished sub-trial by Hallikainen et al., 3.3 g/d stanol ester (2 g/d plant stanols) reduced total cholesterol by 8.8% and LDL cholesterol levels by 10% after only 8 days (protocol and data at Attachment C).

Third, Mensink et al. studied the effect of plant stanol ester delivered in a virtually fat-free yogurt. The study design comprised a 3 week run-in period with control yogurt, a 4 week experimental period and a 2 week wash-out period. Sixty subjects consumed 3 cups/day of the control yogurt during the run-in period after which they were randomized to either a control group continuing with the control yogurt, or a stanol ester group receiving 3 cups per day of stanol ester yogurt containing 1.7 g stanol esters (1 g stanol) per cup. Fasting blood was sampled at 0, 2, 3, 4, 5, 6, 7, 8, 9 weeks, with the 3-week point representing the start of the stanol ester test period. At week 4, after only 1 week on the stanol ester yogurt, serum total and LDL cholesterol were significantly reduced by 9.4% and 15% in test subjects relative to controls, and these were the maximum reductions observed throughout the 4-week experimental period. (Attachment D.)

Finally, a recent study by Prange et al. showed that a single week's ingestion of 2.6 g/d stanol esters (1.5 g/d stanols) significantly reduced serum total and LDL cholesterol levels by 7.3 and 12.4%, respectively.<sup>14/</sup>

Although the studies discussed above employed higher daily intakes than the two lowest used in the Hallikainen et al. study (Ref. 88), these studies, taken together, clearly support Raisio's position that the 2-week data of Hallikainen et al. are valid for evaluating the cholesterol-lowering effect of stanol esters.

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<sup>13/</sup> Miettinen, T.A., et al., "Serum, Biliary, and Fecal Cholesterol and Plant Sterols in Colectomized Patients Before and During Consumption of Stanol Ester Margarine", *American Journal of Clinical Nutrition*, Vol. 71, pp. 1095-1102, 2000.

<sup>14/</sup> See Attachment E, poster presented at the XIIth International Symposium on Atherosclerosis in Stockholm, Sweden, June 25-29, 2000. Prange, W., D. Lütjohann, T. Sudhop, K. von Bergmann, "Increased Serum Concentrations of Sitostanol in Volunteers after Feeding of Sitostanol-Oleate enriched Margarine," *Atherosclerosis*, vol. 151, TuP14:W16, 2000.

**c. Significant Reduction in Serum Total and LDL Cholesterol at Four Weeks Compared With Baseline**

FDA states that stanol esters have to reduce cholesterol compared to baseline in order for the risk of CHD to decrease, yet not all of the studies reviewed by FDA reported whether reductions in cholesterol were compared to baseline (65 Fed. Reg. 54686, 54702). Although it is unclear what is meant by "baseline," it would be reasonable to assume that "baseline" in the Hallikainen study means home diet baseline. Both the supplementary and the published data demonstrate that all intake levels used in the study, from 1.4 to 5.4 g/d stanol esters (0.8 g/d to 3.2 g/d stanols), caused a significant reduction in total and LDL-cholesterol at 4 weeks when compared with home diet baseline.<sup>15/</sup>

Only if FDA interprets "baseline" to mean the 0 g/d stanol control is there no significant reduction in serum total and LDL cholesterol levels at 4 weeks with a daily intake of 1.4 g/d stanol esters (0.8 g/d stanols). Since 1.4 g/d stanol esters (0.8 g/d stanols) produced such a significant reduction in both parameters at 2 weeks, compared to control, the apparent lack of a significant effect at 4 weeks is surprising and probably aberrant. The cholesterol-lowering effect of stanol esters, once achieved, is known to be persistent. Moreover, the significant reduction in Apo B levels observed at 4 weeks indicates that there should have been an accompanying effect on cholesterol levels. It is very possible that the dietary changes discussed below accounted for this discrepancy.

**d. Temporary Diet Change in Control Period Group**

One possible explanation for the dichotomy between the 2-week and 4-week measurements is that the 4-week control serum samples (but not the 4-week test serum samples) were taken during a period of increased alcohol intake and other dietary disruptions coinciding with the Finnish national holiday, "First May."<sup>16/</sup> These dietary disruptions may have had a confounding effect on the 4-week control measurements. In particular, alcohol consumption may have affected serum

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<sup>15/</sup> Attachment C also presents a tabulation of mean values of serum total and LDL cholesterol at 2 and 4 weeks (equivalent to 3-week data), and these show significant reductions in serum total and LDL cholesterol levels compared with both 0 g control values and home diet baseline, in all intake groups, including the 1.4 g and 2.7 g stanol ester groups (Table 4).

<sup>16/</sup> Because of the timing of the various intake trials, alcohol consumption was significantly higher in the 0 g/d (control) group (April 6 - May 8) than in the 1.6 g/d, 2.4 g/d, and 3.2 g/d stanol groups (January 12 - April 9) (Table 3 of Ref. 88, and Table 1 of Attachment B). The effect of alcohol on the control group would have been apparent at 4 weeks, but not at 2 weeks, because the 4-week blood sampling coincided with a Finnish national holiday -- a week of festivities, around May 1, during which high levels of alcohol consumption are prevalent.

total and LDL cholesterol levels in the 0 g/d (control) group and thus skewed the 4-week results for this group. This would have inadvertently reduced or obscured the apparent cholesterol-reducing effect of stanols at 4 weeks compared to 0 g/d controls, but not compared to home diet baseline values since these differences were significant for all intake groups. Studies suggest that high acute alcohol intake may actually reduce LDL-cholesterol levels.<sup>17/</sup> This explanation for the odd 4-week control value is supported by the fact that 1.4 g/d stanol esters (0.8 g/d stanols) significantly reduced Apo B levels (which are not affected by alcohol intake<sup>18/</sup>), compared to controls. (Table 4 of Ref. 88.)

### 3. Other Supporting Low-Dose Studies of Stanol Esters

The totality of the published and unpublished data from Hallikainen et al. (Ref. 88) clearly demonstrates that intake of 1.4 or 2.7 g/d stanol esters (0.8 g/d or 1.6 g/d stanols) significantly reduces serum total and LDL cholesterol. These data are consistent with the findings of Miettinen and Vanhanen (1994), discussed above, and with those of Miettinen et al. (1995) and Prange et al. (2000), discussed below.

Although FDA (65 Fed. Reg. 54704) recognized that the Hallikainen study demonstrates that 2.7 g/d stanol esters significantly reduces serum total and LDL cholesterol levels, the Agency rejected that finding as support for a qualifying level for the CHD health claim because it was not replicated by Jones et al. (Ref. 58), which failed to find significant reduction in serum total cholesterol at higher daily intake of 3.3 g/d stanol esters. The flaws in the Jones study, which account for its anomalous findings, are discussed in the next section. Moreover, the Hallikainen data are fully consistent with data from two other studies discussed below, which used comparable intakes of 3.1 and 2.6 g/d stanol esters (1.8 and 1.5 g/d stanols).

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<sup>17/</sup> See: Chenecky, C.C., B.J. Berger, In *Laboratory Tests and Diagnostic Procedures*, 2nd ed., Saunders, 1997, p. 688.

Mishra, L., N.A. Le, W.V. Brown, E. Mezey, "Effect of Acute Intravenous Alcohol on Plasma Lipoproteins in Man," *Metabolism*, Vol. 40(11), pp. 1128-30, 1991.

Clevidence, B.A., et al., "Effects of Alcohol Consumption on Lipoproteins of Premenopausal Women. A Controlled Diet Study," *Arteriosclerosis, Thrombosis and Vascular Biology*, Vol. 15(2), pp. 179-84, 1995.

Koyama, H., H. Hosokai, S. Tamura, H. Satoh, "Positive Association Between Serum Zinc and Apolipoprotein A-II Concentrations in Middle-aged Males Who Regularly Consume Alcohol," *American Journal of Clinical Nutrition*, Vol. 57(5), pp. 657-61, 1993.

(These four references will be submitted to FDA separately.)

<sup>18/</sup> Taskinen, M-R. et al., Alcohol-Induced Changes in Serum Lipoproteins and in their Metabolism", *American Heart Journal*, vol. 113, pp. 458-464, 1987.

All three studies demonstrate not only significant reductions in serum total and LDL cholesterol, but reductions by comparable percentages. The Hallikainen 2-week results, for example, show that 2.7 g/d stanol esters (1.6 g/d stanols) significantly reduced serum total and LDL cholesterol levels by 7.7% and 11.2%, respectively. Similarly, in a key study by Miettinen et al. (1995) (Ref. 89, Table 2), an intake of 3.1 g/d stanol esters (1.8 g/d stanols) consumed for 6 months resulted in sustained reductions of 9.7% and 12% in serum total and LDL cholesterol, respectively. Finally, as described above, a recent study by Prange et al. showed that a single week's ingestion of 2.6 g/d stanol esters (1.5 g/d stanols) significantly reduced serum total and LDL cholesterol levels by 7.3% and 12.4% respectively.<sup>19</sup>

The overall conclusions to be drawn from all the studies discussed above are:

- The maximum treatment effects of stanol esters in reducing serum total and LDL cholesterol may be seen after only 1 week. Stanol esters produce significant reductions in serum total and LDL cholesterol levels after both 1 and 2 weeks, so FDA should accept the 2-week data presented above rather than require a minimum of three weeks for the demonstration of efficacy.
- Daily intakes of 1.4 g/d stanol esters (0.8 g/d stanol) or 2.7 g/d stanol esters (1.6 g/d stanol) cause significant reductions in serum total and LDL cholesterol levels. FDA should therefore re-examine the data and reduce the qualifying level of stanol esters required daily from the proposed 3.4 g/d to 1.4 g/d.

**B. Discrepancies in Certain Studies on the Cholesterol-Lowering Effects of Sterols and Sterol Esters**

**1. Jones et al., 2000.**

In light of the studies described above, the data presented by Jones et al. (2000) (Ref. 58) appear anomalous and contradict the findings of several investigators that a daily intake of 1.4 to 2.7 g/d stanol esters (0.8 to 1.6 g/d stanols) is effective in reducing serum total and LDL cholesterol levels. The Jones study purports to demonstrate that 3.3 g/d stanol esters (1.8 g/d stanols) did not significantly reduce serum total cholesterol levels after 20 to 21 days, show little effect on serum total or LDL cholesterol levels at 7 days, and demonstrate lower efficacy than sterol esters in reducing serum total LDL cholesterol levels.

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<sup>19/</sup> See Attachment E, poster presented at the XIIth International Symposium on Atherosclerosis in Stockholm, Sweden, June 25-29,2000. Prange, W., D. Lütjohann, T. Sudhop, K. von Bergmann, "Increased Serum Concentrations of Sitostanol in Volunteers after Feeding of Sitostanol-Oleate Enriched Margarine," *Atherosclerosis*, Vol. 151, TuP14:W16, 2000.

FDA rejected the results of the Hallikainen study because of the anomalous findings of the Jones paper. A close examination of the data presented in Jones identifies several problems with study design and presentation that bias the findings in favor of sterols and that make the conclusions of the study questionable.

First, the tested products in the Jones, et al. study were described in the abstract as containing equal amounts of stanols and sterols (1.84 g/d), but Table 1 shows that the concentration of the sterol ester in the sterol ester spread (83.8 g/kg) was 11% higher than that of stanol ester in the stanol ester spread (76.7 g/kg), leading to an 11% higher daily intake of sterols compared to stanols. At the intake levels studied, small changes in daily intake could yield substantial differences in total and LDL cholesterol reduction. It is misleading and of great concern that different amounts of the sterols and stanols were tested, but that this fact was obscured both in the abstract and throughout the text of the paper.

Second, the criteria used to select subjects are questionable and not consistent with FDA criteria for inclusion. The subjects may not have been representative of hypercholesterolemic patients with serum total cholesterol levels at or below 300 mg/dl. Inclusion criteria were such that subjects with cholesterol values as high as 386 mg/dl could participate. Significantly, no mean cholesterol levels were disclosed in the paper. This is important because if, in fact, the mean cholesterol level of the subjects was greater than 300 mg/dl, FDA should have excluded the study from its review for failure to meet the criterion that subjects be representative of the U.S. population. 65 Fed. Reg. 54686, 54691.

Moreover, although subjects reporting a history of diabetes were excluded from the study, it is possible that some subjects could have had diabetes, since the paper does not report whether blood glucose values were fasting or post-prandial. Thus, again, if some subjects had either rather severe lipidemias or occult diabetes, the findings may not be representative of the hypercholesterolemic population identified by FDA as an appropriate representation of the U.S. population. *Id.*

Third, the cholesterol-lowering effects of daily stanol ester ingestion occurred much more slowly than those reported by other investigators. Although the investigators measured cholesterol levels after 8 days, they did not see cholesterol-lowering effects until 15 days after stanol intake began. This observation does not agree with the recent studies discussed above, which show significant reductions in serum total and LDL cholesterol levels after 7 or 8 days of stanol-ester ingestion. There are no explanations as to why the differences in cholesterol-lowering effect between stanols and sterols appeared only after 15 days, and why the effect of stanols was smaller than reported in other studies above. The Jones paper reported that the cholesterol-lowering effects of stanol esters plateaued after 15 days, while that of sterols continued to increase (Figures 2 and 3). As the cholesterol-lowering effect of stanol and sterol esters most

certainly is based on similar mechanisms, there are no scientific reasons for this difference in response over time. However, no explanation is offered in the paper.

Fourth, the study used very few subjects. There were only 18 subjects at the beginning of the study, and these were divided into 6 groups of three each. However, 3 of the subjects subsequently left or were terminated from the study, representing a 17% drop-out rate.

Finally, there are other discrepancies in the Jones paper that make the conclusions difficult to verify. For example, although Figures 2 and 3 show that both stanol and sterol esters significantly decreased total and LDL cholesterol at 15 days, the percentage reductions are not provided in Table 2, which shows only 0 and 21/22 day data. Taken together, the numerous flaws in design and data presentation make reliance on the Jones study results unfounded.

## 2. Weststrate and Meijer, 1998.

The Weststrate and Meijer study (1998) (Ref. 67), which also compares the cholesterol-lowering efficacy of stanol and sterol esters, is similarly flawed.

First, although the study was presented as a direct comparison between stanols and sterols and implied that sterol and stanol intake was equal, sterol intake was actually 19% higher than stanol intake (Table 1). This discrepancy is even higher than that in Jones et al. (Ref. 58). The difference in intake skews the study's findings in favor of sterols.

Second, the sterol ester test margarine had lower saturated and monounsaturated fatty acid content and more polyunsaturated fatty acid content than the stanol ester test margarine. These differences in fatty acid and sterol or stanol intake could, in themselves, account for at least 2% of the percentage reduction in serum cholesterol in the sterol group compared to the stanol group. For example, the authors reported 13% reductions in serum LDL cholesterol levels, in subjects taking the sterol ester product, and 13% in those taking the stanol ester product. Of the 13% reductions with sterol esters, 2% or more could be attributable to the sterol ester fatty acid composition compared to the stanol ester composition.<sup>20/</sup> Thus, the true decrease caused by sterol esters is more on the order of 11%, not 13%.

Third, because the sterol-ester product tested was only 65% esterified, compared to about 95% esterification in commercial products, and because the tested product had a considerably higher

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<sup>20/</sup> See Mensink, R.P. and M.B. Katan, "Effect of Dietary Fatty Acids on Serum Lipids and Lipoproteins", *Arteriosclerosis and Thrombosis*, vol. 12, pp. 911-919, 1992, for discussion of effects on LDL cholesterol levels of replacing saturated fatty acids with polyunsaturated fatty acids.

content of sterols than the commercial products, the value of this study in the FDA's health claim evaluation must be questioned.

Additionally, the test compounds were not equivalent, because vitamin E was added to sterols but not stanols. Cardiovascular effects of vitamin E addition are not clearly known. However, if future cardiovascular outcome studies are used to compare the long-term CHD-preventing effects of stanols and sterols, these results might be confounded by vitamin E addition.

Finally, in both Jones et al. (Ref. 58) and Weststrate and Meijer (Ref. 67), the test ingredients were not comparable in terms of degree of esterification and/or daily intake. Therefore, those studies are unacceptable as side-by-side efficacy trials of comparable sterol ester and stanol ester-containing products, and FDA should make no decisions as to the qualifying levels of such products based on the results of these flawed studies. By contrast, a recent, well-designed study by Hallikainen et al. (2000a)<sup>21/</sup> compared the cholesterol-lowering efficacy of sterol and stanol ester spreads prepared in the same way in the same laboratory using the same esterification process. The two test products had virtually identical fatty acid profiles, and virtually identical weights of total sterols and stanols: 10.3% in the sterol ester product and 10.1% in the stanol ester product. Subjects consumed 20 g/d of either control, sterol ester, or stanol ester spread. After four weeks with a daily intake of 2.0 g sterol or stanol esters, reductions in serum total and LDL cholesterol were 9.2% and 12.7%, respectively, with the stanol ester spread, and 7.3% and 10.4%, respectively, with the sterol ester spread, compared with control. These results provide conclusive evidence that when comparable products are used in efficacy trials, stanol ester spreads appear to be, if anything, more effective than the same weight of sterol ester spreads.

### C. Discrepancies in Proposed Conversion Factors for Plant Sterols and Stanols

In the interim final rule, FDA used an ester conversion factor of 1.6 for sterols and 1.7 for stanols. Raisio objects to FDA's use of different ester conversion factors for the conversion of weights of sterols and stanols to sterol and stanol esters. Sterol and stanol esters are virtually identical chemically and have virtually identical molecular weights. Thus, there is no scientific justification for using different conversion factors for the two compounds. The factor should be 1.7 in all cases.

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<sup>21/</sup> Hallikainen, M.A., et al., "Comparison of the Effects of Plant Sterol Ester and Plant Stanol Ester-Enriched Magazines in Lowering Serum Cholesterol Concentration, in Hypercholesterolemic Subjects on a Low-Fat Diet", *European Journal of Clinical Nutrition*, vol. 54, pp. 715-725, 2000(a).

In deriving its conversion factors, FDA inappropriately took into account the degree of esterification of the sterols or stanols present in the test product.<sup>22/</sup> Such a consideration would be appropriate only if the health claim were for the test product and not the ingredient (stanol or sterol esters). Because the health claim is for the esterified ingredient, however, the only relevant consideration in setting the conversion factors are the molecular weights of the unesterified and the esterified ingredients. Those molecular weights vary so little that there is no justification for any difference in the conversion factors.

FDA's improper selection of different conversion factors has serious repercussions. Using the FDA conversion factor means that an esterified sterol that purports to qualify for the health claim actually contains less sterol than would qualify for the health claim. In addition, the discrepancy means that stanol esters are unfairly disadvantaged, with no scientific justification for such a position.

## V. SUMMARY AND CONCLUSIONS

Clinical studies demonstrate that consumption of 1.4 g/d stanol esters (0.8 g/d stanols) produces a significant reduction in total serum and LDL cholesterol levels. As Miettinen and Vanhanen (Ref. 63) demonstrated, this low dose of stanol esters had a significant cholesterol-lowering effect over and above the reductions observed as a consequence ingesting 50 g/d rapeseed oil during the run-in period. The cholesterol-lowering effects of the treatment ingredients would have been easily masked had they been marginal. Instead, 1.4 g/d stanol esters (0.8 g/d stanols) resulted in a further significant reduction of 5.6% serum total and 8.25% LDL cholesterol levels compared with control.

The Hallikainen study (Ref. 88 and supplemental data) is in accord with the findings of Ref. 63, showing significant cholesterol-lowering effects of a daily dose of 1.4 g/d stanol esters (0.8 stanols). Not only did the Hallikainen study demonstrate statistical significance after two weeks in decreased levels of serum total and LDL cholesterol levels compared to control and home diet baseline, but also after four weeks in decreased levels of Apo B, a more accurate and thus preferred measurement of serum lipid status and thus CHD risk. Hallikainen also found significant reductions of serum total and LDL cholesterol levels, at four weeks compared to home diet baseline.

Moreover, contrary to FDA's contention, the Jones et al. study (Ref. 58) does not contradict the findings of Hallikainen and other supportive low-dose studies. For the reasons discussed in

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<sup>22/</sup> It should be noted that the conversion factors proposed by FDA are derived from studies as different product formulations by different toxicology laboratories and that apparent inter-laboratory differences in analytical methods might also have had some impact on the quantitative sterol analysis.

Food and Drug Administration

November 21, 2000

Page 19

Section III.B.1. above, the Jones study is flawed and should not be used as the basis for any regulatory decisions concerning the qualifying level of plant esters for a health claim.

Finally, Raisio objects to FDA's use of different conversion factors for sterols to sterol esters and stanols to stanol esters. Sterol esters and stanol esters share nearly identical chemical composition and molecular weights. Therefore, FDA should use the same conversion rates for them.

## VI. ACTION REQUESTED

For all of the reasons set forth in this document, Raisio respectfully requests that the Agency reconsider its proposed qualifying level of stanol esters required for a health claim for reduced risk of CHD. Specifically, Raisio requests that FDA approve a health claim for stanol esters at a level of 1.4 g/d stanol esters (0.8 g/d stanols). Raisio also requests that FDA base the ester conversion factors for sterols and stanols on their molecular weights, resulting in an ester conversion factor of 1.7 for both sterols and stanols.

Very truly yours,

 11/21/00

Marsha C. Wertzberger  
Counsel to Raisio Benecol Ltd.