



November 21, 2000

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

RE: Food Labeling: Health Claims; Plant Sterol/Stanol Esters and Coronary Heart Disease  
Docket Nos. 00P-1275 and 00P-1276  
65 Fed. Reg. 54686 (September 8, 2000)

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This letter is submitted by the Altus Food Company ("Altus") in response to the Food and Drug Administration's ("FDA") request for comments on its interim final rule permitting health claims stating that plant sterol/stanol esters may reduce the risk of coronary heart disease.

Altus is a joint venture of The Quaker Oats Company and Novartis Consumer Health Inc. ("Novartis"). Altus is developing processed foods and beverages for the retail market. The Altus mission is to become the functional food and beverage leader with trusted brands that exceed consumer expectations by delivering the peak of health and taste, with valuable information backed by proven science, and with innovative solutions to unmet needs. Altus will soon be introducing products which specifically support cardiovascular health. Altus strongly believes that food labeling should ensure that consumers are fully informed and not misled about the nutritional content and health benefits of their food, while still encouraging companies to innovate and develop safe and health promoting products. Altus appreciates the opportunity to comment on this important topic.

The interim final rule on plant sterol/stanol esters and the risk of coronary health disease derives from the petitions submitted separately by two companies with products containing plant sterol esters and plant stanol esters, respectively. There are other, very similar, related substances which are equally safe and efficacious.

Altus is preparing to market products with a combination of free (not esterified) plant sterols and stanols. The Jones, et al., study (FDA ref. 74 in the September 8, 2000 interim final rule) demonstrates the efficacy of the active ingredient in the Altus products. This study was reviewed and cited with approval by the FDA as part of the evidence demonstrating that plant sterols and stanols are efficacious. Altus comments request that the final rule reflect that free sterols and stanols are as effective as plant sterol esters and plant stanol esters, that free plant sterols and stanols from kraft paper pulping by-products are safe, and that plant sterols and plant stanols are approximately equal in their effects

00P-1275

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on blood cholesterol levels. Additional detail on these issues will be found in the Novartis Consumer Health comments on the interim final rule which we support.

Our comments also request that food products eligible to bear the claim not be specified in the final rule. The methods for quantifying the amount of plant sterol and plant stanol in food products are fundamentally similar and the sample preparation methods are comparable to existing methods for lipids in general and cholesterol specifically. Altus recommends that the FDA require that companies have on file validated analytical methods for the products that carry the claim.

Altus also requests an exception from §101.14(e)(6) for fruit drinks and smoothies because such products offer a significant, alternative, low fat delivery system for plant sterols and stanols.

**I. Free sterols/stanols are functionally equivalent to esterified sterols/stanols**

The FDA specified in §101.83(c)(2)(ii)(A) and (B) that substances eligible to bear the claim are plant sterol esters and plant stanol esters. Altus requests that this section be modified to include sterols and stanols which are unesterified or free. The FDA determined in its review of the relevant data that sterol and stanol esters are converted to free sterols and stanols before exerting their effect in the body (65 FR 54690). Further, the FDA found that blood total cholesterol and LDL-cholesterol levels were significantly reduced in studies in which the sterols and stanols were consumed in the free, unesterified form (65 FR 54704). Additional detail may be found in the Novartis comments on the interim final rule.

**II. By-products of the kraft paper pulping process should be included as an additional source of plant sterols**

The interim final rule specifies at §101.83(c)(2)(ii)(A)(1) that plant sterol esters from edible oils are eligible to bear the claim. At §101.83(c)(2)(ii)(B)(1) plant stanols from edible oils or from the by-products of the kraft paper pulping process are made eligible to bear the claim. The language implies that plant sterols from the by-products of the kraft paper pulping process are not eligible for the claim. Altus requests that the by-products of the kraft paper pulping process be included in §101.83(c)(2)(ii)(A)(1) as an additional approved source of plant sterols. Novartis has previously notified the FDA that it has determined that free plant sterols are GRAS for use in spreads and no safety concerns were raised by the FDA. The Novartis interim final rule comments and the attached "Notification of GRAS Determination for Tall Oil Phytosterols (Phytrol™) Use in Vegetable Oil Spreads" note these details.

**III. Free plant sterols and stanols have comparable efficacy**

In §101.83(c)(2)(i)(G) the FDA specifies that the intake levels associated with reduced CHD risk are 1.3 g or more per day of plant sterol esters and 3.4 g or

more per day of plant stanol esters. As discussed in the preamble, these levels are based on the lowest levels which consistently caused significant blood LDL-cholesterol reductions in clinical studies. Altus believes that if all studies are considered as a whole, plant sterols and plant stanols have approximately equal ability to reduce blood cholesterol levels. Further, we believe that differentiating the effectiveness of sterols and stanols will lead to unnecessary consumer confusion making it less likely that consumers will actually use beneficial products with these ingredients and will therefore, fail to derive any benefit.

Only a few studies compared both a primarily plant sterol intervention and a primarily plant stanol intervention. Weststrate JA, 1998 (FR ref. 67) reported separate sterol ester and stanol ester groups with similar cholesterol responses. Miettinen, TA, 1994 (FR ref. 63) reported similar responses with sitosterol and sitostanol ester, although they did report that sitostanol did not perform as well. The Vanhanen, HT, 1992 (FR ref. 64) version of the study did report that sitosterol and sitostanol were approximately equal in effect. Jones, PJH, 1999, (FR ref. 74) reported that a plant sterol mixture containing about 20% stanols gave results similar to comparable amounts of sterol alone which is consistent with sterols and stanols having comparable efficacy. Note that Jones, PJ, 2000 (FR ref. 58) tested equal amounts of sterol esters and stanol esters and found the stanol esters less effective. This appears to be an anomalous finding. The dose response studies of Hendriks, HFJ, 1999 (FR ref. 57) and Hallikainen, MA, 2000 (FR ref. 88) for plant sterol esters and plant stanol esters, respectively, suggest that efficacy is approximately comparable. The apparent difference in efficacy between low and high doses is more likely to be an artifact of the small sample size than actual differences between sterols and stanols.

Furthermore, we note that the ranges of doses tested and the blood cholesterol changes observed for sterols and the corresponding ranges for stanols overlap very closely. This is consistent with the dose response effects of sterols and stanols being more equivalent than different. In the Table 1 of the Federal Register notice the range of sterols tested was 0.7 to 3.24 grams per day excluding a single test of 8.6 g/d. In Table 2 the range of stanols tested was 0.63 to 4.0 g/d. The total cholesterol reductions were -3.8 to -12.3 % and -2.8 to -12 % for sterols and stanols, respectively. The values for LDL-cholesterol reductions were -6 to -15 % and -1.03 to -15.2 %, excluding the Denke, et al. value (FR ref. 97). Taken as a whole the results are consistent with substantial equivalence in the effectiveness between sterols and stanols on a weight basis. We strongly urge the FDA to reconsider the differences in effective amounts for sterols and stanols that were incorporated into the interim final rule, and make proper amendments in finalizing the rule.

We believe that the FDA has used an appropriate standard, i.e., the lowest daily amount that consistently, significantly lowers blood LDL-cholesterol, to set the daily effective amount. This is consistent with the belief that even small changes in blood cholesterol levels will have a useful public health benefit. It is also

consistent with the approach taken in developing the soluble fiber and soy protein health claims. The effective amount identified, 0.8 grams per day of free plant sterols is also a correct interpretation of the available science.

#### IV. **Acceptable food formats should not be specified in the health claim final rule**

In §101.83(c)(2)(iii)(A)(1) the FDA specifies that the food products eligible to bear the health claims are limited specifically to spreads and dressings for salad for sterol esters and in §101.83(c)(2)(iii)(A)(2) that only spreads, dressings for salad, snack bars and dietary supplements in softgel form containing stanol esters are eligible to bear the claim. Altus requests that the FDA not specify the foods eligible to bear the claim in the final rule. Instead, in order to satisfy FDA's concern for a means of determining the content of the active component in the product, the FDA can specify that companies producing a product bearing the claim have on file a validated method of analysis for the identified components. Companies uncertain of the validity of their analytical method could consult with the FDA regarding validated methods. This approach would be consistent with §101.13(j)(ii)(A), which requires that companies possess substantiation for certain nutrient content claims and make the information available to appropriate officials on request. It is also consistent with the §101.82(c)(2)(ii)(B) in the soy health claim final rule, which requires manufacturers to maintain records confirming that their products contain the required amount of soy protein, but does not otherwise limit or specify the food forms to which soy protein may be added.

The same fundamental procedures are used to determine both the sterol and stanol contents of foods. Samples are saponified, if needed, to release free sterols. It should be noted that the methods do not measure the amount of esterified sterols or esterified stanols, only the free sterols and stanols. The free sterols are extracted with solvents; then concentrated, derivatized, separated by gas-liquid chromatography and quantified by flame ionization detection or mass spectroscopy. Sample preparation is generally similar to the methods used in cholesterol analysis. Appropriate standards and chromatographic systems are available with the resolution and sensitivity to readily quantify the plant sterols and stanols in any food matrix. Altus is prepared to work with other parties to develop universal, validated methods for measuring plant sterols and stanols in food matrices.

In the event that the agency decides to continue to specify the products eligible to bear the claim Altus requests that cereals, food bars, fruit drinks and smoothies be included for both plant sterols and plant stanols. Novartis, and its external panel of experts, has determined that these additional food forms are GRAS. This determination was based on: 1) the fact that plant sterols and stanols use in vegetable oil-based spreads is GRAS; 2) an evaluation of consumer exposure which concluded that intake would not be substantially increased by the additional food forms; and 3) on the FDA evaluation of safety in the interim final rule. The complete report entitled "Report for Expert GRAS Evaluation of

Phytrol™ Phytosterol Enriched Cereals, Food Bars, Fruit Drinks, and Smoothie Beverages” is included with this submission.

Altus has developed a working method for analysis of the plant sterol and stanol content in these food forms and is currently validating those methods. A copy of the working method is included with these comments headed “Phytrol Content in Smoothie Drinks-Modified to Include Internal Standard.” When validated, these methods will be submitted to the agency.

As indicated by the FDA in the preamble to the interim final rule plant sterols and stanols, both free and esterified, can be incorporated into a variety of food forms and retain the capacity to significantly reduce blood cholesterol levels (65 FR 54701).

**V. Claim statements should not limit the number of servings to two per day**

The FDA in the interim final rule at §101.83(c)(2)(i)(H) requires the claim specify that the daily intake of plant sterol or stanol esters should be consumed in two servings eaten at different times of the day with other foods. The requirement that it be consumed with other foods was based on the proposed mechanism of action, interference with intestinal absorption of dietary and biliary cholesterol. Maximum interference is hypothesized to occur when plant sterols or stanols and cholesterol are present simultaneously in the intestine, i.e., after meals. The designation of two servings was based on the assumption that the number of foods which could contain sufficient plant sterols or stanols to be effective is small, hence the opportunities to consume the necessary amount of plant sterol or stanol may be limited. For plant sterol esters, the FDA also identified a minimal effective amount as opposed to an optimum or maximum effective amount.

In reality, consumers are likely to benefit by consuming plant sterols or stanols on more occasions during the day. Altus requests that the requirement in §101.83(c)(2)(i)(H) be modified to permit “at least two” or “two or more” or “two or three” in the claim. It is noted that a group in Holland (Plat, J., et al, European Journal of Clinical Nutrition 54: 671-677, 2000, copy attached) has published study results which suggest that plant sterols and stanols may be effective even when consumed once per day. In lieu of more extensive evidence that less frequent consumption is as effective, this change will allow claimants to more accurately communicate the most effective manner for consumers to incorporate the product into their diet.

**VI. Request for exemption from the minimum nutrient content requirement for fruit drinks and smoothies**

In §101.83(c)(2)(iii)(D) dressings for salad were exempted from the minimum nutrient contribution requirement in §101.14(e)(6). Even though dressings for salad do not meet the usual minimum nutrient requirements for health claims,

they do provide low levels of some essential nutrients and are not typical of foods that provide only calories but no meaningful levels of nutrients. Further, they provide a useful alternate means of consuming the active substances.

Altus requests that the FDA exempt fruit drinks and smoothies from the minimum nutrient contribution requirements of §101.14(e)(6). Juice-based beverages contain small amounts of essential vitamins and minerals, and in some cases, fiber. In addition, juice-based beverages could provide a very useful low fat alternative to spreads and dressings for delivering plant sterols and stanols.

**VII. Urgency of rulemaking**

The Altus Food Company is rapidly developing food products containing plant sterols and stanols to provide consumers with effective alternate means of reducing coronary heart disease risk by reducing blood LDL-cholesterol. Timely resolution of the issues cited above will increase consumer benefit by allowing more accurate communication of the benefits of the products.

Sincerely,



Marcus Chambers  
Director of Research &  
Development  
Altus Food Company  
Chicago, Illinois



Fred Shinnick, Ph.D.  
Science & Discovery Manager  
Altus Food Company  
Chicago, Illinois

Attachments:

Notification of GRAS Determination for Tall Oil Phytosterols (Phytrol™) Use in Vegetable Oil Spreads.

Report for Expert GRAS Evaluation of Phytrol™ Phytosterol Enriched Cereals, Food Bars, Fruit Drinks, and Smoothie Beverages.

Phytrol Content in Smoothie Drinks-Modified to Include Internal Standard.

Effects on serum lipids, lipoproteins and fat soluble antioxidant concentrations of consumption frequency of margarines and shortenings enriched with plant stanol esters. J. Plat, ENM van Onselen, MMA van Heugten and RP Mensink, European Journal of Clinical Nutrition, 54:671-677, 2000.

**NOTIFICATION OF GRAS  
DETERMINATION FOR TALL OIL  
PHYTOSTEROLS (PHYTROL™)  
USE IN VEGETABLE OIL SPREADS**

**NOVARTIS CONSUMER HEALTH, INC.**

**DECEMBER 13, 1999**



Judith A. Weinstein  
Associate General Counsel

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Tel 908 598 7048  
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December 13, 1999

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

Re: Notification of GRAS Determination for Tall Oil Phytosterols  
(Phytrol™) Use in Vegetable Oil Spreads

Dear Sir or Madam:

Novartis Consumer Health, Inc. hereby submits a notification to the Food and Drug Administration that tall oil phytosterols are generally recognized as safe (GRAS) for use in vegetable oil spreads. The notification consists of a GRAS exemption claim, attached to this letter, and a detailed summary of the basis for the GRAS determination, pursuant to proposed 21 C.F.R. §170.36 (62 Fed. Reg. 18960, April 17, 1997). Three copies of these materials are enclosed.

As discussed with FDA, this GRAS determination is based on scientific procedures and substantial equivalence to existing GRAS phytosterols. Phytosterol safety is well supported by numerous published clinical trials and by substantial marketing experience of the Benecol™ product in Finland. A substantial body of additional unpublished clinical and animal studies corroborates this extensive published database. Moreover, the composition of Novartis Consumer Health's tall oil phytosterol product (tradename Phytrol™) is substantially equivalent to that found in the vegetable oil phytosterol based product Take Control™ and the hydrogenated vegetable oil/ tall oil phytosterols in Benecol™. The submission provides a summary of the clinical basis of this determination. In addition, it contains detailed information about the structure and composition of the notified substance, its intended use, the expected consumer exposure, and details of the absorption, distribution, metabolism and excretion of the substance.

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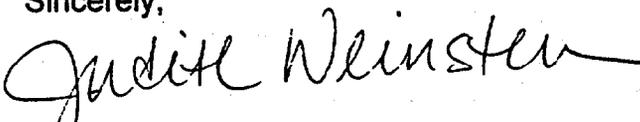
The GRAS notification and data and information contained herein are being submitted voluntarily by Novartis Consumer Health as part of the company's efforts to work cooperatively with FDA. The submission contains information (discussions, summaries, analyses, panel statement etc.) not previously disclosed to the public on the safety, efficacy, and functionality of tall oil phytosterols for use in a Phytrol™ product. We believe that the agency's disclosure of this information is precluded by 21 C.F.R. §20.111(d), which exempts disclosure of such voluntarily submitted information.

The notification also contains information relating to manufacturing methods and processes, quantitative and semi-quantitative formulae, and Novartis Consumer Health's plans for marketing and distribution. This information comprises trade secrets of Novartis Consumer Health and is thus also exempt under FDA's regulations implementing the Freedom of Information Act (FOIA) in 21 C.F.R. Part 20.

Should FDA conclude that any of the confidential commercial information described herein is subject to public disclosure, we would appreciate the opportunity to meet with the agency as part of the notification process described in 21 C.F.R. §20.61(e), prior to the public release of this information.

We appreciate the agency's guidance in the preparation of this submission and look forward to discussing it following the preliminary review.

Sincerely,



Judith A. Weinstein, Esq.



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December 13, 1999

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

Re: Notification of GRAS Determination for Tall Oil Phytosterols  
(Phytrol™) Use in Vegetable Oil Spreads:  
GRAS Exemption Claim

Dear Sir or Madam:

Pursuant to FDA's policy described at 62 Fed. Reg. 18938, 18960 (April 17, 1997), Novartis Consumer Health, Inc. hereby notifies the Food and Drug Administration (FDA) that it has determined that the use of tall oil phytosterols (Phytrol™) in vegetable oil spreads is "generally recognized as safe" (GRAS) and is therefore exempt from the premarket approval requirements of the Federal Food, Drug and Cosmetic Act. The following information is provided under proposed 21 C.F.R. §170.36(c)(1):

Notifier: Novartis Consumer Health, Inc.  
560 Morris Avenue  
Summit, New Jersey 07901  
Attn: Judith A. Weinstein, Esq.  
Associate General Counsel

GRAS Substance: Tall Oil Phytosterols

Intended Use: The substance will be used in vegetable oil spreads at a level up to 12% free phytosterols, an amount intended to provide approximately 1.5 g of the phytosterol compound per day to the average consumer of vegetable oil spreads. The phytosterols are intended for use as nutrients in food to reduce the absorption of cholesterol from the gastrointestinal tract.

Basis for GRAS  
Determination: Scientific Procedures

The data and information that are the basis for Novartis Consumer Health's GRAS determination are available for FDA's review and copying at reasonable times at the offices of Sidley & Austin, 1722 Eye Street, Washington D.C. 20006, Attn: I. Scott Bass (202-736-8684). In addition, Novartis Consumer Health agrees to send the materials to FDA at the agency's request.

Respectfully submitted,



Judith A. Weinstein, Esq.  
Associate General Counsel



**Notification of GRAS Determination**  
**for Tall Oil Phytosterols (Phytrol™) Use**  
**in Vegetable Oil Spreads**

**Novartis Consumer Health, Inc.**

**December 13, 1999**

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## 1.0 INTRODUCTION

Phytosterols or plant sterols occur naturally as a mixture in the non-saponifiable material of plant oils. The most abundant phytosterol in nature is  $\beta$ -sitosterol, hereafter referred to simply as sitosterol. The phytosterols campesterol, stigmasterol and dihydrobrassicasterol occur at lower concentrations. Sitostanol, the saturated derivative of sitosterol and stigmasterol, is found in minor concentrations in food plant sources but occurs at significant levels in the unsaponifiable matter of oil derived from trees, particularly conifers. Tree oil is commonly referred to as tall oil. The phytosterols therein are tall oil phytosterols. This product, as manufactured by Forbes Medi-Tech Inc., shall be referred to in this document as Phytrol™, and is composed of phytosterols derived from tall oil.

Phytosterols or plant sterols as found in beans, were originally considered by Hesse [1878] as isomers of cholesterol. With the development of nuclear magnetic resonance (NMR), infra-red, ultraviolet and mass spectrometry (MS), the structure and empirical formula of many phytosterols have been identified. Nonetheless, the formula and structure of a large number of minor phytosterols are still not well characterized [Pollak and Kritchevsky, 1981]. Most phytosterols are similar to cholesterol in their basic skeletal structure except that they contain methyl, ethyl, di-methyl, di-ethyl or other groups next to their C<sub>24</sub> position on the aliphatic side chain of the compound [Pollak and Kritchevsky, 1981]. When phytosterols are saturated at the 5- $\alpha$  position, such as through the use of a commercial process, compounds such as sitostanol are formed. The difference in the chemical structure between cholesterol and phytosterols has significant physiological implications. For instance, phytosterols are not synthesized in humans [Salen et al., 1970]; they are absorbed in the intestine at a rate of about 0 to 5% [Grundy et al., 1969]. In contrast, cholesterol which is produced in humans and exhibits an absorption rate of 40 to 50 % in normal subjects [Grundy et al., 1969].

Regardless of differences in the absorption rates of the various phytosterols when compared with cholesterol, there is substantial evidence that increased phytosterol consumption impedes cholesterol absorption and provides a beneficial effect with regards to maintaining a healthy cholesterol level in the human blood stream. Currently, there are two self-affirmed GRAS vegetable oil spread products containing phytosterols on the market which contain up to 20% by weight of added fatty acid esterified phytosterols and which make this claim. Take Control™ is manufactured by Lipton. The

phytosterols therein are predominantly sterols derived from vegetable oil. Benecol™ is manufactured by McNeil Consumer Healthcare and contains primarily hydrogenated tall oil and vegetable oil sterols (stanols). Both products include phytosterols in their matrices in order to promote a healthy level of cholesterol. With this in mind, Novartis Consumer Health Inc. has formulated a similar vegetable oil based spread product. This product, called Phytrol™, is based on a non-hydrogenated tall oil phytosterol product manufactured by Forbes Medi-Tech Inc. It is intended to be consumed in a manner identical to Benecol™ and Take Control™ and provide consumers with an additional product choice in order to promote a healthy cholesterol level. The Phytrol™ tall oil phytosterol product merely revises the ratio of major sterols to stanols present in the vegetable oil spread to an intermediate composition when compared to the other two currently marketed products. GRAS status has already been established for the constituent sterol and stanol mixtures in the other phytosterol product formulations. Furthermore, the levels of the individual component phytosterols in Phytrol™ are at or lower than those levels in the other products considered GRAS. Therefore, this GRAS submission is based upon the principle of substantial equivalence: that differences between Phytrol™ and the products Benecol™ and Take Control™ are inconsequential and that all data and considerations of safety and use which apply to Benecol™ and Take Control™ apply equally to Phytrol™. The FDA has full knowledge of and does not disagree that the use of sterols and stanols in the other products, at the same level and manner of use as Phytrol™, is considered by their manufacturers to be Generally Recognized as Safe (GRAS). Similarly, Novartis Consumer Health Inc. has determined that the Phytrol™ tall oil phytosterol product is also GRAS.

### **1.1 Historical Background**

The presence and distribution of phytosterols across plant species have been extensively described by Pollak and Kritchevsky [1981]. Phytosterols are found in plants that include ornamental, edible types as well as herbs, shrubs and trees [Pollak and Kritchevsky, 1981]. At least 44 sterols from seven different plant classes have been identified [Bean, 1973]. The list of phytosterols, their sources and botanical functions is growing steadily. Crombie [1961], Shoppee [1964] and Bean [1973] listed a large number of sterols and their sources in plants. The greatest number of phytosterols, naturally present in pure or esterified form, or conjugated as glycosides, were found in the angiosperms and the most dominant were sitosterol, campesterol and stigmasterol.

There are several factors that affect the distribution of phytosterols in plants. Among other factors, the phytosterol content of any given plant depends on the length of daylight, degree of soil alkalinity, and time of plant harvest. For example, light exposure or photoperiod has been shown to lower sitosterol in leaves of *Solanum audigena* [Bae and Mercer, 1970]. Soil alkalinity, seasonal changes, and leaf shedding have also been reported to alter the concentrations of sitosterol and campesterol in plants [Misra et al., 1961; Davis, 1971]. Such natural variation in phytosterol occurrence affects their level of intake by those ingesting plant derived foods.

Dietary phytosterol intake levels among different populations vary greatly depending primarily on the type and amount of plant foods consumed. Western diets, for example, typically contain lower levels of phytosterols than diets of many other parts of the world. In 1991, the British consumed 104, 49, 10, and 4 mg per day of sitosterol, campesterol, stigmasterol and stigmastanol, respectively, representing a total phytosterol intake of 167 mg per day [Morton et al., 1995]. The primary sources of phytosterols in the British diet are fats and oils, although breads and other cereals were also important sources [Morton et al., 1995]. A trend was observed toward increased phytosterol intakes between 1987 and 1991 in Britain, possibly due to increased utilization of vegetable oils for cooking.

The only source of phytosterols in humans is the diet. However, within the same population, dietary intake of phytosterols can vary substantially. A comparison was made of phytosterol intakes of vegetarian and non-vegetarian Seventh Day Adventists (SDA) and non-vegetarians from the general population in the United States [Nair et al, 1984]. Pure SDA vegetarians, lacto-ovo SDA vegetarians, and non-vegetarians who were SDA or from the general population ingested sitosterol + stigmasterol / cholesterol in ratios of 16.0, 3.3, 1.0, and 0.5, respectively. Plant sterol intake for pure vegetarians was approximately 89.1 mg/day, 343.6 mg/day for lacto-ovo vegetarians, 230.7 mg/day for non-vegetarian SDA's, and 77.9 mg/day for members of the general population. The Tarahumara Indians of Mexico consume a diet containing unusually high amounts of beans and corn reportedly ingest over 400 mg of phytosterols per day [Cerqueira et al, 1979]. In Japan, phytosterol intake remained at approximately 373 mg per day from 1957 to 1982, while cholesterol consumption simultaneously increased over twofold [Hirai et al., 1986]. The most commonly ingested phytosterol is sitosterol (54%), while significant levels of campesterol (14%), brassicasterol (10%), and

stigmasterol (7.5%) are also consumed [Hirai et al, 1986]. The quantity and quality of phytosterol consumption has not changed in Japan over the last few decades.

## 1.2 Regulatory Basis for GRAS Status

As described at 62 Fed. Reg. 18938, 18960 (April 17, 1997) (proposed 21 C.F.R. §170.36), Novartis Consumer Health Inc. (NCH) notifies the Food and Drug Administration (FDA) that it has determined that the use of Phytrol™ tall oil phytosterols, as manufactured by Forbes Medi-Tech Inc., in a vegetable oil-based spread at a level up to 12% free phytosterols is Generally Recognized as Safe (GRAS). This is based on review by a panel of Experts qualified by scientific training and experience to evaluate the safety of food and food ingredients using scientific procedures and is therefore exempt from the pre-market approval requirements of the Federal Food, Drug and Cosmetic Act. The Phytrol™ tall oil phytosterols are derived from tall oil soap. In aggregate, the composition of Phytrol™ is substantially equivalent to that found in the vegetable oil phytosterol based product Take Control™ and the hydrogenated vegetable oil / tall oil phytosterols in Benecol™. Both products are currently GRAS and are available in the form of a vegetable oil based spread in the marketplace.

This document provides information required by proposed 21 CFR. §170.36(c)(2), (3), and (4). The requirements of the proposed regulation with the sections containing the relevant information is described below.

### Requirements of the proposed rule

### Section No(s).

- §170.36(c)(2): Detailed information about the identity of the notified substance; composition; method of manufacture; characteristic properties; and specifications 1, 3, 4
- §170.36(c)(3): Information on any self-limiting levels of use 1,5,6
- §170.36(c)(4)(I)(a): Comprehensive discussion of, and citations to, generally available and accepted scientific data and information, including consideration of probable consumption 1, 2, 3, 4, 5, 6, 7, 8, 9, 10
- §170.36(c)(4)(I)(c): The basis for concluding that there is a consensus of qualified experts that there is reasonable certainty that the substance is not harmful under the intended conditions of use 11

The determination that Phytrol™ is GRAS for use in vegetable oil-based spreads meets the applicable requirements for the technical element and common knowledge element of a GRAS determination based on scientific procedures. The scientific data and information summarized in this notification reflect a thorough review of the relevant literature and the results of studies of tall oil phytosterols conducted in accordance with generally accepted scientific procedures. Furthermore, this information has been supplemented by the use of all available scientific and statistical reference sources and compendia; new, relevant books and reviews; and those relevant regulatory documents available from the Food and Drug Administration. Of particular importance was the information, data, and considerations contained within the recently accepted GRAS notification dossiers for the esterified vegetable oil phytosterol product Take Control™ and the esterified hydrogenated vegetable oil / tall oil phytosterol product Benecol™.

Novartis Consumer Health Inc.'s GRAS determination is based on the weight of all of the available scientific information and grounded upon generally available scientific data. It is further based on the principle of substantial equivalence to other phytosterol based GRAS products currently available for consumer use on a daily basis. The consensus among a variety of qualified experts is that there is reasonable certainty that these substances will not be harmful under the intended conditions of use.

This GRAS determination therefore meets the requirements of §201(s) of the Federal Food, Drug, and Cosmetic Act; 21 C.F.R. §170.3 and §170.30; and the amendments to these rules proposed at 62 Fed. Reg. 18960.

### 1.3 Substantial Equivalence to Current GRAS Products

The phytosterols found in Phytrol™ are substantially equivalent to those comprising the vegetable and hydrogenated vegetable / tall oil phytosterol GRAS products Take Control™ and Benecol™. Substantial equivalence was established through examination of potential differences between Phytrol™ and the other two products. No differences exist which materially affect considerations of safety and effectiveness. Phytrol™ is equivalent to these GRAS products on the basis of aggregate composition, intended conditions of use, intended level of intake, physiologic properties and by extension, safety. In essence, the tall oil phytosterols in Phytrol™, as manufactured by Forbes Medi-Tech Inc., are a product whose total sterol and stanol profile corresponds approximately to a 60:40 combination of the other two substantially equivalent GRAS products.

#### 1.3.1 Composition

The composition of Phytrol™ exhibits a ratio of major sterol to stanol fractions intermediate to that of Take Control™ and Benecol™. Table 1-1 compares the approximate compositions of the three products. While in each product significant natural variation may occur in specific component content, the data in Table 1-1 indicates that levels of the individual component phytosterols in Phytrol™ are comparable or below the aggregate levels in the other GRAS phytosterol products.

The variation in constituent phytosterol profile among the three products arises from two main factors: phytosterol source and use of hydrogenation processing. A third variation arises from the use of fatty acid esterification of the current GRAS products to modify solubility properties for product application purposes.

##### 1.3.1.1 Source and Hydrogenation

Phytrol™ contains significant levels of sitosterol and campesterol, similar to those occurring in Take Control™. Unlike Take Control™, it contains only minor quantities of stigmasterol and other sterols but significant levels of the naturally occurring saturated (stanol) compounds sitostanol and campestanol as found in Benecol™. However, Benecol™ employs a hydrogenation process to

saturate most double bonds present in the sterol components, converting them to stanols, predominantly sitostanol and campestanol.

Since many of the minor components in these products are variously unsaturated congeners of the same saturated structures, hydrogenation may also reduce, somewhat, the diversity of minor components. However, Benecol™ still contains a range of minor phytosterols of up to 6% [ref. Benecol™ submission]. The phytosterols in Take Control™ are not hydrogenated and contain up to 8% by weight of minor sterol and non-sterol components (see Table 1-1). Similarly, Phytrol™ contains a number of the same minor components, primarily representing variation in the position and / or number of double bonds within sitosterol (C<sub>29</sub>) and campesterol (C<sub>28</sub>) structures (see Section 3 for compositional details). Also present are trace quantities of C<sub>15</sub>-C<sub>25</sub> saturated aliphatic alcohols. All minor components represent substances commonly found in the diet and in one or both of the current products. Thus Phytrol™ has compositional elements which are common to one or both of the other GRAS products and which, in aggregate, supports its substantial equivalence.

**Table 1-1: Comparison of Phytosterol Compositions**

Sterol	Take Control™ (Sterols from ADM and Cargill)	Phytrol™	Benecol™
Sitosterol	42	47	4
Campesterol	25	14	3
Stigmasterol	18		
Brassicasterol	5		
Sitostanol	2	26	64
Campestanol		5	23
Minor Sterols	8	8	6
Total Phytosterols	98	69	13
Total Phytostanols	2	31	87

Take Control™ uses vegetable sterols esterified with fatty acids. Data are averages of batches from Archer Daniels Midland (ADM) and Cargill. Benecol™ is a mixture of vegetable and tall oil phytosterols that have been hydrogenated and then esterified with fatty acids. The Phytrol™ values are typical of most batches and fit within current specifications. Percentages refer only to sterol content and are approximations. The estimated sterol proportions will vary depending on the methodology used for measurement. The response factors vary between different sterols when compared within the same detection system, e.g. flame ion detection (FID). The response factors also vary between detection systems, i.e. FID vs. GC/MS or LC/MS. The Phytrol™ phytosterols were quantitated by the use of GC-FID using in-house standards. The figures for Take Control™ and Benecol™ are area under the curve estimates by GC/MS.

### 1.3.1.2 Esterification

The phytosterols in Phytrol™ are in a free non-esterified form while those in the two GRAS products have been esterified to common vegetable oil fatty acids to enhance their solubility in a vegetable oil product matrix. Esterification does not materially affect the substantial equivalence of Phytrol™ to the other products. As discussed in the sections on physiologic equivalence (1.3.3.1) and safety (1.3.4), the ester forms are rapidly de-esterified *in vivo* through the action of lipase enzymes yielding the active free phytosterols. Esterification does affect quantitative parameters of equivalence. The two GRAS products are approximately 60% by weight phytosterols; the remainder being fatty acids. Accordingly, 0.6 grams of Phytrol™ are equivalent to the phytosterol content of 1.0 grams of the esterified products.

### 1.3.2 *Intended Use and Intake*

The intended use of Phytrol™ is to incorporate it into a vegetable oil based spread product at a concentration of up to 12% by weight. This represents an application and phytosterol content which is identical to that of the current GRAS products whose incorporation rate is up to 20% by weight of esterified phytosterols which is in turn 60% by weight free phytosterol.

The intended consumer daily consumption of a spread containing Phytrol™ is that which will contain 1.5 grams of phytosterols. This intake rate is equivalent to, or less than, that for the two existing GRAS product spreads, based on free phytosterol content. This is further summarized in Table 1-2.

**Table 1-2: Intended Daily Intake of Phytosterol Products**

		Take Control™	Phytrol™	Benecol™
Per Serving:		1.12 g (esters)	0.5 g	1.7 g (esters)
Servings Per Day		1 - 2	3	Up to 3
Daily Intake:	Esters	1.12 - 2.24	-	1.7 - 5.1
	Phytosterols	0.7 - 1.3	1.5	1.0 - 3.0

### 1.3.3 *Physiologic Properties*

The phytosterols in Phytrol™ are substantially equivalent in physiologic properties to those in Take Control™ and Benecol™ in regards to their active form and their effects on blood cholesterol parameters, blood phytosterol levels and absorption of vitamins and nutrients.

#### 1.3.3.1 Active Form

Esterified phytosterols and stanols are converted *in vivo* to, and are physiologically equivalent to, proportional amounts of free phytosterols and stanols. Evidence indicates that the active form of the sterol esters is the free sterol. Pancreatic cholesterol esterase hydrolyzes both cholesterol esters and phytosterol esters to their free forms [Swell et al, 1954]. Cholesterol is not absorbed in the esterified form but must first be cleaved before it can be absorbed into the body. Hellman et al [1953] fed labeled cholesterol to rats and observed that the labeled sterol appeared in the lymph in the free fraction before it appeared in the ester fraction. Although the above experiment has not been performed with phytosterol esters, it can be inferred that only phytosterols in the free form are absorbed. Direct comparisons between free phytosterols and esterified phytosterols in the rat found that the esterified forms were less effective as inhibitors of cholesterol absorption [Pollak & Kritchevsky, 1981]. Similarly, Mattson et al [1982] reported lower efficacy of sitosterol oleate than free sitosterol in inhibiting absorption of cholesterol in the human and stigmasterol oleate was less effective than free stigmasterol in decreasing cholesterol absorption in the human [Matson et al, 1977]. These results indicate that cleavage of the sterol esters controls the availability of phytosterols for interaction with the cholesterol absorption mechanism. It can also be inferred that it is primarily the free phytosterols which interact with the cholesterol absorption mechanism.

#### 1.3.3.2 Effectiveness in Reducing Blood Cholesterol Levels

It is well established that phytosterols are effective in lowering blood cholesterol levels when administered orally in animals and humans. A summary of published clinical studies is provided in Section 10.2. The maximum lowering of LDL cholesterol observed in human studies with plant derived sterols is in the range of 13% - 15%. One study [Westrate et al, 1998] directly compared the

Take Control™ product with that of the Benecol™ product in hypercholesterolemics over a 25 day treatment interval. The data from this study is shown in Table 1-3. Data from a study (CLF9701) by Jones et al [1999] in which Phytrol™ was administered in a margarine matrix to hypercholesterolemics over an interval of 30 days is also presented in Table 1-3 for purposes of comparison.

**Table 1-3: Comparative Effectiveness of Sterol Products in a Margarine Matrix**

Product:	Take Control™	Benecol™	Phytrol™ in a Margarine Matrix
Dosage	3 g per day <sup>1</sup>	2.7 g per day <sup>1</sup>	1.5 g/70kg/day <sup>2</sup>
Δ Total Cholesterol <sup>3</sup>	-8.3%	-7.3%	-9.1%
Δ LDL Cholesterol <sup>3</sup>	-13.0%	-13.0%	-15.5%
Δ HDL Cholesterol <sup>3</sup>	+0.6%	+0.1%	-4.4%

<sup>1</sup> These data are from the Westrate [1998] study, which indicates that the average body weight of the men was 82.5 kg and for women was 66.8 kg. Converting the dose to an equivalent body weight (bw) basis, the dose of Take Control™ would have been 2.5 g /70 kg bw in men and 3.0 g / 70 kg bw in women. The same conversion to an equivalent body weight yields a Benecol™ dose of 2.3 g / 70 kg bw in men and 2.9 g / 70 kg bw in women.

<sup>2</sup> These data are from the Jones et al [1999] study conducted in males, only.

<sup>3</sup> Values are corrected for the change that occurred in the control group.

These data demonstrate that the tall oil phytosterols in Phytrol™ are at least as effective as the other two products in decreasing total and LDL serum cholesterol values.

#### 1.3.3.3 Effects on Circulating HDL-Cholesterol

There was no evidence of any significant effect on plasma HDL levels for any of the three products. In the Phytrol™ study reported in Table 1-3, decreases in mean HDL values of 6.3% and 10.7 % were reported in the control and treated groups, respectively. The 4.4 % difference between the groups attributable to Phytrol™, per se, was well within the 6.5% coefficient of variation for this assay procedure in the reported study and was not clinically significant.

As with the previous clinical studies involving Take Control™ and Benecol™, no adverse effects were observed in any of the subjects in the study by Jones et al [1999], including those consuming Phytrol™.

1.3.3.4 Effects on Circulating Levels of Phytosterols

Phytosterols are a normal circulating constituent in the blood. Phytosterols are not synthesized by the body but are absorbed from plant material in the diet. The major circulating phytosterols are sitosterol and campesterol, reflecting the higher content of these sterols in food sources. Ingestion of Take Control™, which has a high content of these phytosterols, results in an increase in their circulating blood levels. Ingestion of Benecol™ has an opposite effect, which can be attributed to its elevated stanol content. Sitostanol, which is particularly poorly absorbed, appears to inhibit not only the absorption of cholesterol but also that of other sterols. The phytosterols in Phytrol™ fall in between these two extremes in its effect on blood phytosterol levels, as illustrated below in Tables 1-4 and 1-5. In the three studies where data on blood phytosterol levels are available, no consistent effects on blood levels were obtained. This result is consistent with the intermediate composition of the Phytrol™ product in which sufficient sitosterol and campesterol are present to increase blood levels of these sterols, but in which the coincident presence of sitostanol impedes their absorption.

**Table 1-4: Effect of Phytosterol Products on Blood Phytosterols Levels**

	Take Control™	Phytrol™	Benecol™	Benecol™
References	Westrate et al 1998	See Table 4 Below	Westrate et al 1998	Gylling et al 1995
Sitosterol	+38.8%	No Consistent Change	-36.2%	-36.1%
Campesterol	+72.6%	No Consistent Change	-17.1%	-48.2%

**Table 1-5: Plasma Concentrations of Phytosterols in Humans after Treatment with Phytrol™ at a Dose of 1.5 g/70kg/day**

Study Number	Treatment Time (days)	Sitosterol (μmol/L)		Campesterol (μmol/L)	
		Control	Treated	Control	Treated
CLF9601	10	1.58 ± 0.30	3.04 ± 1.1	19.6 ± 3.7	13.4 ± 4.0
CLF9602	10	9.6 ± 3.0	9.2 ± 3.3	12.3 ± 2.8	18.1 ± 6.0
CLF9701	30	6.1 ± 0.5	4.4 ± 1.7	26.4 ± 12.0	27.5 ± 11.7

Note: CLF9701 is the same study as that of Jones et al [1999].

#### 1.3.3.5 Effects Upon Vitamin and Nutrient Absorption

The activity of ingested phytosterols with regard to absorption of fat soluble nutrients including vitamins A, E, D and K, has been thoroughly reviewed and discussed in the process of establishing the GRAS status of Take Control™ and Benecol™ [see Benecol™ and Take Control™ GRAS Notification filings]. While some decrease in the absorption of carotenoids such as beta-carotene and lycopene has been reported, a significant impairment of the availability of fat-soluble vitamins has not been noted. This included data from the intake of 30 g / day of Cytellin™ for extended periods of use. Expert and FDA reviews of the issue have resulted in findings of no expected significant risk and that these phytosterol products may be regarded as GRAS. While the effect of Phytrol™ has not been specifically tested in this regard, its substantial compositional equivalence, equivalent product matrix, and intended use level assures that it presents no significant risk of adversely altering vitamin and nutrient absorption to any degree materially different from Benecol™ and Take Control™.

#### 1.3.4 *Safety*

The safety of ingested phytosterols has been thoroughly reviewed and discussed in the process of establishing the respective GRAS status of the Take Control™ and Benecol™ vegetable oil spreads. The development of the Lipton product, Take Control™, has yielded substantial research into the safety of phytosterols, particularly sitosterol, campesterol, and stigmasterol [Baker et al, 1999; Hepburn et al, 1999; Jones et al, 1999; Wallkens-Berendsen et al, 1999]. Similarly, the development of the McNeil product, Benecol™, has also yielded substantial research into the safety of

phytosterols, particularly sitostanol, campestanol and stigmastanol [Slesinski et al, 1999; Turnbull et al, 1999; Turnbull et al, 1999; Whittaker et al, 1999].

The information reviewed included extensive clinical and non-clinical data. Clinical data included a history of safe intake of these phytosterols as components of everyday foodstuffs. Additionally, safe exposure to phytosterols at levels of up to 30 grams per day for extended periods during the use of the pharmaceutical agent Cytellin™, as well as results of direct clinical and nutritional investigations with these products in over 2000 subjects. Non-clinical studies included those designed to assess acute, short term and sub-chronic safety, genetic toxicity, estrogenic activity, developmental and reproductive safety, AME (absorption, metabolism and excretion) and nutrient effects.

Expert and FDA reviews have resulted in findings of a reasonable certainty of no harmful effects and that phytosterols may be regarded as GRAS when used in a vegetable oil spread. The safety of Phytrol™ is assured by the substantial compositional equivalence to other GRAS products and by their use in an equivalent product matrix and at an equivalent intended level of intake.

In support of this, direct testing of Phytrol™ has confirmed an absence of genotoxicity or estrogenic activity (see sections 9.1 and 9.2). Analyses for trace contaminants such as dioxins, pesticides, and heavy metals confirmed their absence or presence at levels within specifications for food grade fats and oils. Furthermore, a total of 55 human subjects were exposed to doses of 1.5 g / 70 kg body weight / day for up to 8 weeks during various clinical nutrition studies without reported adverse events (see section 10.1).

## **2.0 SAFE HISTORY OF USE**

Phytosterols are abundant in nature and naturally present in many varieties of fruits and vegetables ingested by humans. The average intake of phytosterols in the United States is approximately 250 mg/day, with vegetarians consuming almost twice this amount. Three of the most important and common major sterols are sitosterol, campesterol, and stigmasterol. Often, they are esterified with C<sub>12</sub> - C<sub>18</sub> fatty acids. Some crude vegetable oils contain 100 - 500 mg of phytosterols per 100 g of vegetable oil. Certain vegetable oils from sources rich in phytosterols like rice bran, wheat germ, and

oats may contain up to 4% phytosterols. Some reduced fat and low fat spreads currently available in the market place may contain phytosterol levels between 0.3% and 0.4%. Two previously mentioned examples of vegetable oil based spreads containing phytosterols available to consumers of vegetable oil base spreads are Lipton's Take Control™ and McNeil's Benecol™. The Novartis Consumer Health Inc. tall oil phytosterol product Phytrol™ is another example of the use of phytosterols employed in a vegetable oil base spread. All three of these products seek to provide consumers of vegetable oil base spreads with a choice in products while seeking to maintain healthy blood cholesterol levels.

Over the past fifty years, research has indicated that increased phytosterol intake can have an effect on lowering blood cholesterol levels in humans. Human studies with dose levels of up to 25,000 mg per day (i.e., 100 times the average dietary intake) and lasting up to several years, have been performed [Pollak and Kritchevsky, 1981; Pollak, 1985]. Some of these studies, which have been conducted as early as the 1950s, have involved over 1800 men, women, adolescents, and even children. Within the context of these studies, repeated observation of no adverse side effects has occurred [Lees et al., 1977; Oster et al., 1977]. Attempts to capitalize upon the perceived benefits of increased phytosterol intake are not new to the market place. A preparation marketed by Eli Lilly between the 1950s into the 1980s, called Cytellin™, was available in the United States to treat hypercholesterolemia. The same product was also available in Canada and sold under the brand name Positol™. The phytosterol composition of Cytellin™ was also derived from tall oil phytosterols. It was composed of approximately 80% sitosterol and 10% campesterol with another 9% of the product composed of stanols. Therapeutic levels ranged from 9000 to 30,000 mg/day. Repeated clinical investigation of Cytellin™ reported no contraindications or side-effects [Lesesne et al., 1955; Joyner and Kuo, 1955; Kuo, 1956; Best et al., 1955; Duncan and Best, 1963; Farquhar et al., 1956].

With the recent marketing of Lipton's Take Control™ and McNeil's Benecol™, phytosterols are available in the United States for every day consumer use with the aim of achieving a healthy cholesterol level. The phytosterols employed in McNeil's Benecol™ product are sourced from a blend of vegetable oil and tall oil. The resultant phytosterol blend is then hydrogenated to convert the plant sterols to stanols; approximately 62% sitostanol and 32% campestanol, respectively. The remaining 6% by weight are comprised of unsaturated sterols. The phytosterols employed in Take Control™ by Lipton are obtained from vegetable oil (e.g., soybean, canola, corn) distillate. The major sterol components show some variation from batch to batch depending upon which form of vegetable oil is employed in the production of the phytosterol product. However, the predominant phytosterols in the product, by weight, are sitosterol (40% - 55%), campesterol (20% - 28%), and stigmasterol (14% - 23%). Up to 8% of this product may be comprised of 20 to 30 different minor sterol components. The phytosterols in each of these products are esterified to vegetable oil fatty acids.

The same phytosterols found in all three of these products are commonly consumed through the diet. Furthermore, the constituent phytosterols present in Phytrol™ are already present in GRAS vegetable oil spread products employing phytosterols in order to maintain healthy cholesterol levels in humans.

### **3.0 CHEMICAL IDENTITY AND COMPOSITION**

#### **3.1 Chemical Identity**

The Novartis Consumer Health Inc. Phytrol™ product under consideration is predominantly a mixture of four phytosterols. These are: sitosterol, sitostanol, campesterol, and campestanol. A small percentage of minor phytosterols such as stigmasterol is also present as well as a fraction of a percentage of long chain aliphatic alcohols. The properties of the major phytosterol components are described below.

Sitosterol: (3 $\beta$ )-Stigmast-5-en-3-ol; C<sub>29</sub>H<sub>50</sub>O;

Approximate percentage in Phytrol™: 38% to 60%

Mol. wt. 414.72. Plates from alcohol.

Melting point 140 (138-142) degrees Celsius.

Soluble in acetone and ethyl acetate.

Sitostanol: (3 $\beta$ , 5 $\alpha$ )-Stigmastan-3-ol; C<sub>29</sub>H<sub>52</sub>O;

Approximate percentage in Phytrol™: 14% to 34%

Mol. wt. 416.73. Monohydrate, crystals, melting point 138-139 degrees Celsius.

Melting point 144-145 degrees Celsius when dry.

Soluble in acetone and ethyl acetate.

Campesterol: (3 $\beta$ ,24R)-Ergost-5-en-3-ol; C<sub>28</sub>H<sub>48</sub>O;

Approximate percentage in Phytrol™: 9% to 18%

Mol. wt. 400.69

Melting point 157-158 degrees Celsius.

Soluble in acetone and ethyl acetate.

Campestanol: (3 $\beta$ , 5 $\alpha$ , 24R)-Ergostan-3-ol; C<sub>28</sub>H<sub>50</sub>O;

Approximate percentage in Phytrol™: 2% to 14%

Mol. wt. 402.70

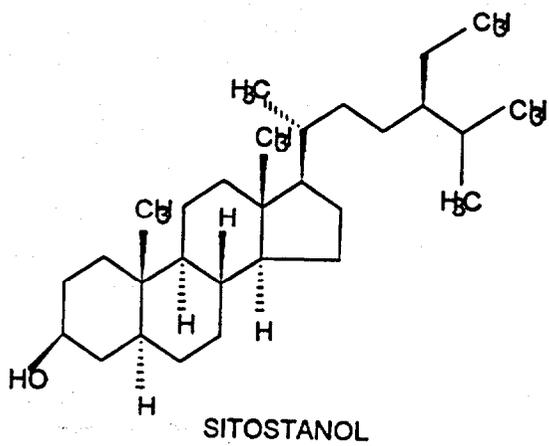
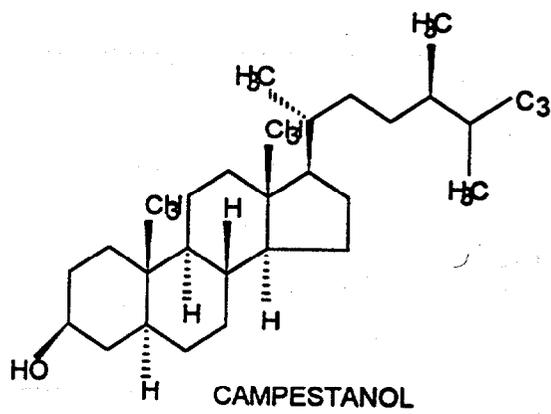
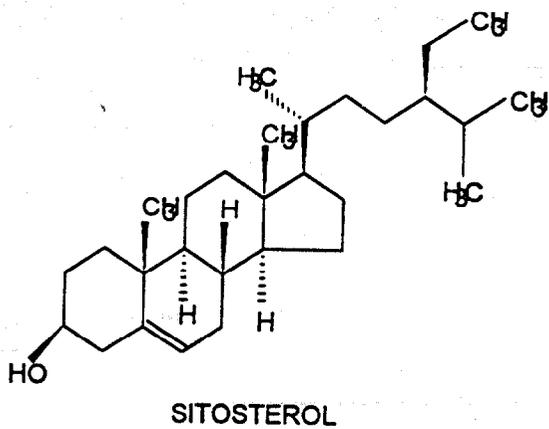
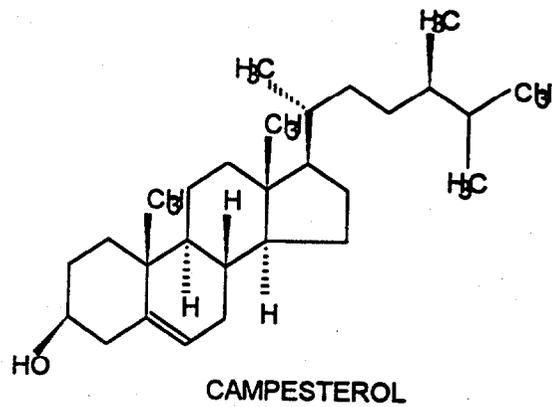
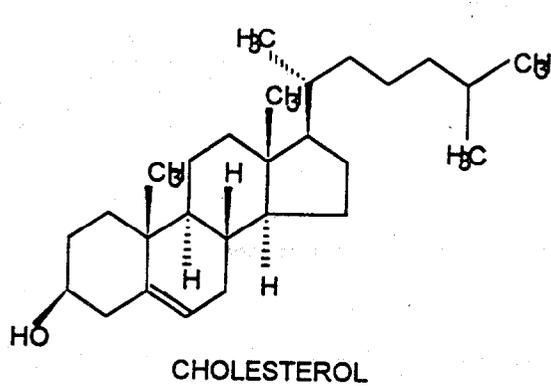
Melting point 146.5-147.8 degrees Celsius.

Soluble in acetone and ethyl acetate.

### 3.2 Chemical Structure of Component Phytosterols

The chemical structures of the four major phytosterol constituents of Phytrol™ are displayed below. For the purposes of comparison, the structure of the cholesterol molecule has also been provided.

**Figure 1: Cholesterol and Major Phytol™ Constituent Phytosterols**



### 3.3

### Phytosterol Components of Phytrol™

The only potential, safety related difference in composition among the three products lie with their minor phytosterol-like components. Table 3-1 below, lists minor sterol components that have been observed in batches of Phytrol™. The minor sterol components primarily represent variation in the position and / or number of double bonds within sitosterol (C<sub>29</sub>) and campesterol (C<sub>28</sub>) structures. Also present in trace amounts are saturated long chain (C<sub>15</sub> – C<sub>25</sub>) aliphatic alcohols. These minor, long chain alcohol components are substances commonly found in the diet and the Expert Panel concluded they were not toxic contaminants and their presence does not adversely affect general recognition of safety of the intended use of Phytrol™.

Comparative analysis of Take Control™, Benecol™, vegetable sterols produced by Archer Daniels Midland and Phytrol™ revealed a total of 45 major and minor sterol components. Twenty-two were present in Phytrol™. None were unique to Phytrol™ as all 22 components were found either in Take Control™, Benecol™, or vegetable sterols or reported present in vegetable oils in the scientific literature. Of the 22 phytosterol-like components in Phytrol™, 15 were found in Take Control, 11 in Benecol™ and 12 in vegetable sterols. Three phytosterol-like components were found in Phytrol™ which were not identified in Take Control™, Benecol™, or vegetable sterols. All three have been previously reported present in vegetable oils by Mennie et al [1994]; Goad [1966]; Grob et al [1994]; Bortolomeazzi et al [1996]; and Anderson et al [1926]. The Expert Panel concluded that the presence of Phytrol™'s phytosterol-like minor components in either GRAS products or vegetable oils allays any safety concern about these components under the intended conditions of use of Phytrol™. These data also support the applicability to Phytrol™ of safety study results obtained during testing of Benecol™ and Take Control™.

**Table 3-1: Phytrol™ Minor Components**

• α-Sitosterol	• 24-Methyl diene isomers (C <sub>28</sub> compounds)
• Stigmasterol	• 24-Ethyl di- and tri- ene isomers (C <sub>29</sub> compounds)
• Ergosterol	• Trace phytosterols and phytostanols
• C <sub>15</sub> - C <sub>25</sub> Aliphatic Alcohols (<0.5%)	

### 3.4

### Proposed Food-Grade Specifications

As a food additive, Phytrol™ can be directly incorporated into various food products. For optimal effect however, formulations appropriate to individual food products will be necessary. Thus, a variety of methods for incorporation of Phytrol™ into various food products have been developed. Additional formulation technologies are under development and U.S. patents will be applied for at the appropriate time. The proposed food grade specifications for Phytrol™ are as shown below:

Phytosterol content	> 95%
Sitosterol	38% to 60%
Sitostanol	14% to 34%
Campesterol	9% to 18%
Campestanol	2% to 14%
Total major sterols	> 86%
Loss on drying (water)	< 5%
Solvents	< 0.5%
Residue on ignition	< 0.1%
Heavy metals	< 10 ppm
Arsenic	< 5 ppm
Lead	< 0.25 ppm
Total aerobic count	< 10,000 CFU/g
Combined molds & yeasts	< 100 CFU/g
Coliformes	negative
E. Coli	negative
Salmonella	negative

In order to detect the presence of heavy metal contaminants, the following analytical methodologies are routinely employed as described below in Table 3-2.

**Table 3-2: Tests Employed to Detect the Presence of Heavy Metals in Phytrol™**

Test Item	Test Method	Limits
Total heavy metals, including Pb, Hg and Cd	ICP scan and CVUV scan	NMT 10 ppm
Arsenic	ICP scan	NMT 5 ppm

The results of the heavy metal testing for batches FM-PH-15 and FM-PH-52 and the range of values over the last six batches of the tall oil phytosterol product are shown below in Table 3-3.

**Table 3-3: Test Results for Heavy Metals in Batches of Phytrol™**

Test Item	Limit	Results		
		FM-PH-15	FM-PH-52	Range Previous 6 Batches
Total heavy metals, including Pb, Hg and Cd	NMT 10 ppm	Not Detected	< 1.07 ppm	0 - <1.06 ppm
Arsenic	NMT 5 ppm	Not Detected	< 2 ppm	0 - <2 ppm

Analysis of one batch of the Phytrol™ tall oil phytosterol product (Batch E7-04-017) was conducted to confirm the absence of pentachlorophenols, dioxins, and furans. The results indicated no detectable levels of these compounds. These structures would not be expected to survive the alkaline digestion used to free wood fibers in the pulping process.

### **3.5 Physical Properties of the Vegetable Oil Based Spread Containing Phytrol™**

The physical properties of the vegetable oil spread, in texture, taste, and consistency, will be the same as other vegetable oil spread products currently available in the marketplace.

### **3.6 Nutrition Information of the Vegetable Oil Based Spread Containing Phytrol™**

The nutritional profile of the vegetable oil spread will be similar in character to other vegetable oil spread products currently available in the marketplace.

## 4.0 PRODUCTION METHODS

Tall oil phytosterols in Phytrol™ are extracted from tall oil soap, a by-product of the pulping process used for coniferous trees in North America and Europe. The trees are reduced to fine wood chips and then digested at pH 14 for about 18 hours at 50 degrees to free the wood fibers. The lipid layer that forms at the top of the digestion is tall oil soap. It is skimmed off and used as a source of phytosterols. The phytosterols are then extracted directly from the tall oil soap using the Forbes Medi-Tech Inc. proprietary and patented extraction processes (patent WO 96/10033). The sterols are extracted and purified in a three-step process.

### 4.1 Extraction

Starting Material: TOS with >2% sterols

Product: Extract with 15 to 25% sterols

The first step is a solvent extraction of the tall oil soap. Organic solvents, water and tall oil soap are mixed while heating in stainless steel reactors. The mixture is allowed to separate into distinct aqueous and organic phases.

The aqueous phase contains residual solvents, residual tall oil soap, and water. The residual solvents are recovered, the water is removed from the tall oil soap and the extracted tall oil soap is then sent to an acidulation plant for further processing.

The organic phase contains extracted organic materials, phytosterols and approximately 70% of the solvents. The organic solvents are recovered. The extract, largely free of solvents, contains 15 to 25% sterols and is then used in the next step of the process.

**4.2                   Complexation**

Starting Material:     Extract from Step 1 containing 15 – 25% sterols.  
Product:                Crude sterols with 60 –75% purity

The second step consists of a complexation-washing process that removes the bulk of the organic material. The extract from Step 1 is mixed while heating with solvent, and complexing agent in a stainless steel reactor. The sterols rapidly bind to the agent. The complexed sterols are separated from the solvent phase by centrifugation. Next, the complexing agent is dissolved from the crude complex by heating in water. The water is removed and the resulting material, which contains 60-75% sterols, is called crude sterols and is used in the next step of the process.

**4.3                   Crystallization**

Starting Material:     Crude sterols from step 2 with 60-75% purity  
Product:                Purified sterols with >95% purity

The crude sterols are dissolved in alcohol at elevated temperature. The temperature of the mixture is reduced to allow crystallization of the sterols. The crystals are recovered and then dried. The mixture is tested for content of sterols. If the desired purity is not achieved, the mixture is re-crystallized a second time.

**4.4                   Statement of Good Manufacturing Practices (GMP)**

The Novartis Consumer Health Inc. tall oil phytosterol product Phytrol™ is manufactured by Forbes Medi-Tech Inc. under conditions that are in accord with the principles of Food Good Manufacturing Practices according to 21 CFR Part 110.

## 5.0

### INTENDED USE IN FOOD

Phytosterols are a group of plant compounds naturally occurring in a variety of foods in the human diet, such as minor components of vegetable oils. Novartis Consumer Health Inc. is interested in using the phytosterols found in the tall oil phytosterol blend Phytrol™, as manufactured by Forbes Medi-Tech Inc., in a new vegetable oil-based spread at levels formulated to provide approximately 1.5 g of Phytrol™ per day to the average consumer of vegetable oil spreads. The use of Phytrol™ in this spread base is intended to help maintain healthy cholesterol levels as part of a diet low in saturated fat and cholesterol. The phytosterols present in Phytrol™ have already been very well characterized by both Novartis Consumer Health Inc. and by their competitors in the marketplace. The use of the phytosterols sitosterol, sitostanol, campesterol and campestanol represent a variant of similar products such as Lipton's Take Control™ and McNeil's Benecol™.

## 6.0

### CONSUMER EXPOSURE

Novartis Consumer Health Inc. plans to market Phytrol™ in a vegetable oil based spread. The non-sterol composition of this spread will not differ from other vegetable base spreads currently available in the market place. Phytrol™ will be included in the formulation in an amount that will provide the consumer with the recommended amount of approximately 1.5 g of phytosterols per day over the course of three product servings.

No significant change in individual consumer intake of phytosterols is anticipated. The major constituent Phytrol™ phytosterols sitosterol, sitostanol, campesterol, and campestanol are all already established for use in other vegetable oil based spreads such as McNeil's Benecol™ product or the Lipton Take Control™ product. Phytrol™ merely represents an additional product choice for consumers seeking to maintain a healthy cholesterol level through the consumption of vegetable oil spread.

Analysis of structure activity relationships is a useful approach to correlating the molecular structure of a chemical with its biological activity [Food and Drug Administration, 1982]. The phytosterols contained within the tall oil phytosterol product Phytrol™ must therefore be placed into Structure Category B as the FDA has classified mixtures as belonging to this group. However, the constituent phytosterols of Phytrol™ belong in Structure Category A, as having low toxic potential. Phytosterols also bear a close structural resemblance to the intermediate products of lipid metabolism in humans, namely cholesterol. In conclusion, the constituent phytosterol contained in Phytrol™, based upon this type of structure activity relationship, would indicate that this product would not cause any adverse effects in humans.

Phytosterols are plant sterols structurally related to cholesterol, but differ in their side chain configuration. There are a wide variety of phytosterol structures, but the most abundant in nature are sitosterol, campesterol, and stigmasterol. Less common are saturated phytosterols or stanols, such as sitostanol and campestanol. Sitostanol and campestanol are formed by hydrogenation of the 5-alpha position on sitosterol and campesterol, respectively.

Phytosterols are not endogenously synthesized in the body with the possible exception of phytosterolemia, a rare condition where stanols are thought to be formed from unsaturated phytosterols in the liver which enter the body from the diet [Ling & Jones, 1995]. Phytosterols are not converted to cholesterol or vice versa in humans or other mammals [Subbiah & Kuksis, 1973; Kritchevsky *et al.*, 1981; Boberg *et al.*, 1990a]. Sources of phytosterols usually come from corn, bean, and plant oils, which are common components of diet. Vegetarian diets contain higher amounts of phytosterols compared to western diets. In the United States, about 250 mg/day of phytosterols are consumed through diet. In contrast, a vegetarian diet would provide twice this amount. Saturated phytosterols are present only in trace amounts in conventional diets [Connor, 1968; Cerqueira *et al.*, 1979].

## 8.1 Absorption

Phytosterols are poorly absorbed from the intestinal tract. Absorption of tritium labeled sitosterol has been studied in man and other mammals, see Table 8-1 below. Most of the studies indicate an absorption rate of approximately 5%.

**Table 8-1 Tritiated Sitosterol Absorption in Humans and Rats**

Species	Mean absorption (% of dose)	Reference
Man	5	Gould <i>et al.</i> , 1969
Man	10	Borgstrom, 1968
Rat	5	Gould, 1955
Rat	5	Syiven & Borgstrom, 1969

For healthy humans, the absorption rate of phytosterols is usually less than 5% of dietary levels. This is considerably lower than the absorption rate of cholesterol, which is over 40% [Salen *et al.*, 1989; Miettinen *et al.*, 1990]. Thus, approximately 95% of dietary phytosterols enter the colon and are eliminated from the body. Intestinal absorption of phytosterols is selective. In a study with 10 healthy subjects, absorption of phytosterols was compared during perfusion of the upper jejunum. The percentage absorption of campestanol, campesterol, stigmasterol, and sitosterol was 12.5%, 9.6%, 4.8%, and 4.2%, respectively. Sitostanol (the 5 $\alpha$ -saturated derivative of sitosterol) is not absorbed to any significant extent [Heinemann *et al.*, 1993].

### 8.1.1 Basal Phytosterol Levels in Plasma

Salen *et al.* [1970] presented basal plasma level data to show that sitosterol is not synthesized endogenously in the body. All the sitosterol and campesterol present had been absorbed from dietary sources. Miettinen *et al.* [1990] reported mean basal levels of sitosterol and campesterol to be 6.19  $\mu$ moles/L (1.62-12.72) and 9.31  $\mu$ moles/L (3.47-21.36) respectively in 63 Finnish male subjects. Estimates of plasma levels vary somewhat from one laboratory to another.

Plant sterols are significantly higher in the plasma of patients with hypercholesterolemia than in normocholesterolemic controls. This is illustrated in Table 8-2. However, the ratios of phytosterols to cholesterol are not different [von Bergmann & Lütjohann, 1998]. This is probably a result of plant sterols and cholesterol being carried in the same fractions.

**Table 8-2 Plasma Sterol Concentration in Normocholesterolemic Subjects and Patients with Hypercholesterolemia**

Group	Cholesterol	Sitosterol	Campesterol
Normal	169 (mg/dL)	0.3 mg/dL (7.2 $\mu$ mol/L)	0.42 mg/dL (105 $\mu$ mol/L)
Hypercholesterolemic	361 (mg/dL)	0.55 mg/dL (13.3 $\mu$ mol/L)	0.78 mg/dL (19.5 $\mu$ mol/L)

### 8.1.2 Mechanism of Phytosterol and Cholesterol Absorption

Plant sterols are absorbed to a much lesser extent than cholesterol. This suggests that the intestinal mucosa is discriminative toward sterols containing an extra methyl or ethyl group in the side chain.

On the basis of the events involved in sterol absorption, the specificity determining the rate of phytosterol absorption could lie in any one of four steps: (1) partition of the phytosterol between an oil and a micellar phase of intestinal contents (2) uptake of the sterols by mucosal cell membrane, (3) esterification of the sterol in the mucosal cell, and (4) transport to chylomicrons.

A difference in the partition coefficient between cholesterol and sitosterol in the intestinal contents is not an adequate explanation of the difference in absorption rates. Borgstrom [1967] determined the partition coefficient of sitosterol between the micellar and oil phases and found that it was nearly identical to that of cholesterol. Esterification of phytosterols and cholesterol is not obligatory for absorption. Hellman *et al.* [1953] fed labeled cholesterol to rats and observed that the labeled sterol appeared in the lymph as the free fraction before it appeared in the ester fraction. The most likely explanation is that selectivity occurs at the uptake mechanism for cholesterol and phytosterols in the mucosal membrane. Glover and Green [1953] postulated that lipoproteins in the mucosal membrane are specific toward cholesterol and might not readily take up the foreign plant sterols. The demonstration that the uptake of sitosterol by the intestinal wall was considerably less than that of

cholesterol suggests that the rate of absorption of these sterols might be determined at this step [see review by Subbiah, 1971].

Furthermore, increased absorption of all dietary sterols with resultant high phytosterols and serum cholesterol may be a heritable, atherogenic trait separate from the rare, recessive familial sitosterolemia. Von Bergmann & Lütjohann, [1998] reported that although the phytosterol levels were higher in the plasma of hypercholesteroleemics, the ratio of phytosterols to cholesterol did not change. In addition, these authors demonstrated that feeding cholesterol to animals elevates blood levels of both cholesterol and phytosterols.

## **8.2            Distribution**

### **8.2.1           *Distribution in Animals***

Rabbits were fed a low cholesterol diet containing 2% plant sterols for 10 weeks. The plasma levels of sitosterol and campesterol were 0.76 and 8.9 mg/100 ml respectively after 10 weeks. The blood levels of sitosterol and campesterol plateaued after about 5 weeks. The initial blood levels of phytosterols were below 0.01mg/100 ml [Bhattacharyya and Lopez, 1979]. Although the source of the phytosterols was not identified, the high content of campesterol (34%) suggests that the source was soy.

The effect of administration of the Novartis Consumer Health Inc. tall oil Phytrol™ product on plasma phytosterols levels is shown in Table 8-3. In these studies, Phytrol™ was included in the diet and the concentration of both sitosterol and campesterol increased.

**Table 8-3 Plasma Concentrations of Phytosterols in Animals Administered Phytrol™**

Study	Species	Test Article Dose in mg/kg/d	Sitosterol ( $\mu\text{mol/L}$ )		Campesterol ( $\mu\text{mol/L}$ )	
			Control	Treated	Control	Treated
PHF9502	Hamster	680	19	37	15	18
PHF9401	Rat	1000	$4.87 \pm 0.65$	$20.30 \pm 2.50$	$3.75 \pm 0.50$	$13.91 \pm 1.92$
PHF9401	Rat	1000 (Soy Phytosterols)	$5.78 \pm 0.89$	$23.29 \pm 2.93$	$7.07 \pm 1.88$	$14.59 \pm 1.47$

In non-primates, plant sterols have been shown to have a tissue distribution similar to cholesterol. Six hours after injection of labeled sitosterol into rats, about 50% of the injected radioactivity is located in the liver, which decreases to about 8% by the end of the ninth day. At that time, minor but significant amounts of radioactivity were observed in the adrenal and testis. Minor amounts of radioactivity were also present in adipose tissue and skeletal muscle. In a separate study in rats, Subbiah and Kuksis [1973] reported that sitosterol was initially taken up by the liver and to a lesser extent by other tissues. On the basis of tissue weight, adrenals displayed the highest uptake. In the monkey, phytosterols are not present in sperm, indicating that phytosterols are absent from testes tissue.

High intakes of dietary phytosterol (3% sitosterol + 2% campesterol in diet) for 21 days in rats leads to an increased phytosterol incorporation into liver microsomes. There was no change in either the phospholipid or total sterol content of liver cell membranes with high phytosterol intake, however the phytosterol / cholesterol ratio was increased [Leikin & Brenner, 1989]. Dogs fed soy phytosterols for over 19 months at a dose of 1000 mg/kg/day showed no evidence of accumulation of phytosterols in the liver. Rabbits fed soy phytosterols at a dose of 4g per day for over 2 years showed no evidence of accumulation of phytosterols in liver or aortic tissue [Shipley *et al.*, 1958].

The effect of tall oil phytosterol treatment on phytosterol content in the liver tissue of hamsters is shown in Table 8-4. Tall oil phytosterol intake increased the liver content of both sitosterol and campesterol. Soy phytosterols also increased the liver content of these sterols.

**Table 8-4 Tissue Concentrations of Phytosterols in Hamster after Treatment with Phytosterols**

Study	Species (Treatment Time)	Phytosterol Dose and Source (mg/kg/d)	Tissue	Sitosterol (mg/g)		Campesterol (mg/g)	
				Control	Treated	Control	Treated
PHF9502	Hamster (45 days)	680 Tall Oil	Liver	0.001	0.031	0.001	0.035
PHF9402	Hamster (34 days)	680 Soy	Liver	0.05	0.02	0.30	0.74
PHF9501	Hamster (90 days)	680 Tall Oil	Liver	0.09	0.19	0.11	0.30
PHF9501	Hamster (90 days)	680 Soy	Liver	0.09	0.84	0.11	0.36

### 8.2.2 *Distribution in Humans*

Only 1 to 42  $\mu\text{mol/L}$  of phytosterols are found in human serum under normal conditions with dietary intakes of 160-360 mg/day, however plasma levels have been shown to increase up to two-fold by dietary supplementation [Connor, 1968; Cerquira *et al.*, 1979; Salen *et al.*, 1970]. The effect of orally administered phytosterols is dependent on the sterol composition administered. Where the sterols have a high sitosterol content, plasma levels of campesterol are depressed and plasma levels of sitosterol are raised. Refer to Tables 8-5 and 8-6 for studies in adults and children. Phytosterol esters of phytosterols from soy, which have a high content of sitosterol and campesterol, raised the concentration of both phytosterols in the plasma [Westrate & Meijer, 1998]. Hydrogenated phytosterols, which contain primarily sitostanol, when administered orally, consistently depress plasma levels of both campesterol and sitosterol. For example, 3 g per day of sitostanol ester depresses campesterol and sitosterol levels by 44% and 43%, respectively [Gylling and Mietinen, 1994]. It was concluded that phytostanols not only interfere with cholesterol uptake but also interfere with the uptake of other phytosterols. Administration of sitosterol, while reducing uptake of campesterol, increases the blood levels of sitosterol because of the excess sitosterol available for uptake from the gut. A preparation with a high content of sitosterol and campesterol raises plasma levels of both components. Administered sitostanol blocks the uptake of sitosterol and campesterol in addition to blocking cholesterol uptake. The Novartis Consumer Health Inc. tall oil phytosterol

blend Phytrol™ contains enough sitostanol to partially block the uptake of both sitosterol and campesterol and thus offsets the increased load of phytosterols presented to the uptake mechanism in the intestine.

**Table 8-5 Effect of Oral Phytosterols on Plasma Levels of Sitosterol and Campesterol in Adult Humans**

Dose of Plant Sterols g/day	Plasma Levels		Reference
	Sitosterol (μmol/L)	Campesterol (μmol/L)	
0.22 g/d sitosterol	13.7	No data	Salen <i>et al.</i> , 1970
5.65 g/d sitosterol	26.5	No data	
Control	8.0	17.5	Weststrate and Meijer, 1998
3 g/d soy phytosterol esters	11.1	30.2	
3 g/d stanol esters	5.1	14.5	
Control	No data	18.6	Miettinen <i>et al.</i> , 1995.
2.6 g/d stanol esters	No data	10.7	

**Table 8-6 Effect of Oral Phytosterols on Plasma Levels of Sitosterol and Campesterol in Children**

Dose of Plant Sterols (g/day)	Plasma Levels		Reference
	Sitosterol (μmol/L)	Campesterol (μmol/L)	
Control	37.8 ± 26.2	36.7 ± 26.8	Becker, 1993
1.5 (Stanol)	18.6 ± 7.5	18.9 ± 8.4	
6 (Sterol)	43.0 ± 19.4	26.5 ± 12.0	
Control	1.56 ± 1.12	1.45 ± 1.26	Becker, 1992
6.0 (Sterols)	1.90 ± 0.98	1.11 ± 0.59	
Control	0.88 ± 0.24	No data	Schlierf <i>et al.</i> , 1978
12.0 (Sterols)	1.48 ± 0.62	No data	
Control	13.3 ± 1.0	30.1 ± 2.4	Gylling <i>et al.</i> , 1995.
3.0 (Stanol esters)	8.5 ± 0.6	15.6 ± 1.4	

Note: Becker 1992, 1993: appears to be a phytosterol preparation with a high content of sitosterol similar to Cytellin™.

Human tissue levels of phytosterols have been measured at autopsy. Mellies *et al.*, [1976] examined aortic tissue from adults and infants, as shown in Table 8-7.

**Table 8-7 Cholesterol and Phytosterol Content of Human Aorta at Autopsy [Mellies *et al.*, 1976]**

Age	Number	Cholesterol (mg/g)	Campesterol (µg/g)	Sitosterol (µg/g)	Stigmasterol (µg/g)
Abortus and Neonates	4	0.55 ± 0.24	1.12 ± 0.4	1.8 ± 0.6	0.51 ± 0.2
Infants	11	0.66 ± 0.11	3.57 ± 1.3	8.93 ± 1.3	9.22 ± 2.7
Adult	11	3.4 ± 0.7	14 ± 4	16 ± 4	12.8 ± 4
Atheromatous Plaque	4	54 ± 23	112 ± 48	236 ± 147	167 ± 60

The effects of Phytrol™ intake on plasma levels of sitosterol and campesterol in human studies are shown in Table 8-8. At a dosage of 1.5 g/70 kg per day, there was no consistent effect on plasma levels of sitosterol or campesterol.

**Table 8-8 Phytosterol Plasma Concentration in Humans after Treatment with 1.5 g/70kg/day of Phytrol™**

Study	Treatment Time (days)	Sitosterol (µmol/L)		Campesterol (µmol/L)	
		Control	Treated	Control	Treated
CLF9601	10	1.58 ± 0.30	3.04 ± 1.1	19.6 ± 3.7	13.4 ± 4.0
CLF9602	10	9.6 ± 3.0	9.2 ± 3.3	12.3 ± 2.8	18.1 ± 6.0
CLF9701	30	6.1 ± 0.5	4.4 ± 1.7	26.4 ± 12.0	27.5 ± 11.7

#### 8.2.2.1 Phytosterolemia in Humans

Phytosterolemia (Sitosterolemia), a very rare lipid storage disease characterized chemically by increased plant sterol levels and 5α-saturated stanols in plasma and tissue, is associated with premature atherosclerosis. As of 1992, 27 individuals with phytosterolemia had been detected [Bhattacharyya *et al.*, 1991; Salen *et al.*, 1992]. Table 8-9 lists plasma levels of phytosterols that occur in this disease. Phytosterols account for an average of 13% of the total sterols present in plasma in phytosteroleemics, compared to about 0.4% in normal subjects.

**Table 8-9 Plasma Levels of Phytosterols in Phytosterolemia**

Sterol	Average Plasma Level ( $\mu\text{mol/L}$ )	Range ( $\mu\text{mol/L}$ )	References
Sitosterol	850	340 - 1570	Salen <i>et al.</i> , 1985
Campesterol	425	186 - 596	
Sitostanol	100	46 - 144	Bhattacharyya <i>et al.</i> , 1974
Campestanol	70	20 - 99	
Cholesterol	6300	3300 - 12000	Miettinen, 1980

Phytosterolemia is inherited as a recessive trait. Heterozygotes are clinically and biochemically normal although plasma phytosterol levels of some heterozygotes may be slightly increased over control levels [Salen *et al.*, 1992]. The absorption rate of phytosterols is very high in phytosterolemics. The sterol uptake mechanism in the intestine does not distinguish between cholesterol and phytosterols, thus approximately equal proportions of sitosterol and cholesterol are absorbed [Salen *et al.*, 1992]. As diet contains only trace amounts of  $5\alpha$ -saturated stanols, it is thought that the stanols are produced endogenously in large amounts. In normal subjects, the liver secretes sitosterol into the bile so there is a three-fold enrichment of sitosterol to cholesterol as compared to blood [Salen *et al.*, 1970]. In phytosterolemic subjects, sitosterol appears in the same or lower proportions relative to cholesterol in the bile as compared to blood. In addition, less cholesterol is secreted into the bile [Bhattacharyya & Connor, 1974]. The large quantities of sitosterol and cholestanol in the liver of phytosterolemic subjects competitively inhibits cholesterol  $7\alpha$ -hydroxylase mediated bile acid synthesis [Shefer *et al.*, 1994].

### 8.3 Metabolism

In the same manner as cholesterol, sitosterol is esterified with fatty acids, by a reaction mediated by cholesterol-lecithin acyltransferase. A study in the rat wherein orally administered tritiated sitosterol was compared with  $^{14}\text{C}$ -labelled cholesterol, the extent of esterification of sitosterol in the plasma was 5-10% lower than that of cholesterol. About 65% of the plasma sitosterol esters were tetraenes and 25% were dienes, proportions similar to that of cholesterol [Subbiah & Kuksis, 1973]. In contrast to cholesterol, from which  $\text{C}_{24}$  bile acids are formed,  $\text{C}_{21}$  bile acids are formed from sitosterol in the

rat. Two bile acids have been tentatively identified as having hydroxyl groups at C<sub>3</sub> and C<sub>15</sub>. One bile acid has a keto group and the other an additional hydroxyl group, positions unknown for both acids [Boberg *et al.*, 1990b]. Compared with cholesterol, sitosterol is excreted to a larger extent as free sterol [Boberg & Skrede, 1988].

#### 8.4 Elimination

Phytosterol elimination takes place primarily *via* the biliary route and appears to be more rapid than that of cholesterol [Lin *et al.*, 1984]. Correspondingly, endogenous phytosterol pool size is low compared to cholesterol due to poor absorption in the intestine and faster excretion via the bile.

A fraction of absorbed phytosterols is excreted through the skin. Phytosterols which were absorbed into the plasma through the diet were excreted into skin surface lipids after being transferred from the plasma to the skin [Bhattacharyya *et al.*, 1983]. The excretion of phytosterols and cholesterol from skin and feces was studied over 24-h in a hyperlipoproteinemic (type IIa) patient fed formula diets providing varying quantities of phytosterols (0-30 g/day) and cholesterol (0-1000 mg/day). Sitosterol excretion decreased progressively upon feeding a sterol-free diet from about 6 mg/day to 0.08 mg/day by 83 days and then completely disappeared. When dietary phytosterols (about 30 g/day) were added to the formula diet, sitosterol reappeared in the skin surface lipids and rose to nearly 5 mg/day/ by 6 weeks. Fecal excretion of phytosterols responded similarly to skin surface lipids which demonstrate that dietary phytosterols could be excreted through the surface of the skin as well as feces [Bhattacharyya *et al.*, 1983].

## 9.0 PRECLINICAL TOXICOLOGY

### 9.1 Genotoxicity Assays

#### 9.1.1 *In Vitro* Genotoxicity Assays

A genotoxic evaluation of Phytrol™ was conducted in the *Salmonella typhimurium* / *Escherichia coli* plate incorporation / pre-incubation mutation assay in the presence and absence of induced rat liver S-9 microsomal fraction. Phytrol™ was tested in *Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537 (104, 208, 417, 834 and 1667 µg/plate) and *Escherichia coli* strain WP2UvrA (104, 208, 417, 834 and 1667 µg/plate), for the potential to cause mutation both in the presence and absence of metabolic activation. The plate incorporation method was employed in the definitive assay, as well as, the confirmatory assay. Results of both mutation assays indicated that the test article did not induce a significant increase in the number of revertant colonies for any of the strains tested in the presence or absence of the S-9 fraction. Therefore, under the conditions of this study, Phytrol™ was reported to be negative for mutagenic potential in *Salmonella typhimurium* and *Escherichia coli*.

An *in vitro* evaluation of Phytrol™ in the L5178Y TK +/- mouse lymphoma mutagenesis assay with colony size evaluation in the presence and absence of induced rat liver S-9 microsomal fraction was conducted along with a confirmatory study. This is an *in vitro* mammalian cell mutation assay based on the detection and quantitation of forward mutation in a sub-line of mouse lymphoma L5178Y cells at the thymidine kinase locus. It was used to test the mutagenic potential of Phytrol™ at levels of 5.0, 10, 20, 40, 60, 80, 100 and 167 µg/ml. Following a 4-hour treatment period, all responses were negative, both in the presence and absence of metabolic activation. Relative total growth (RTG) for the non-activated cultures was greater than 100%, and the RTG for S-9 activated cultures ranged from 54-110%. A confirmatory assay was subsequently performed without S-9 activation. Following a 24-hour treatment period, all responses were also negative in this assay. The RTG for treated cultures ranged from 71% to 133%. The solvent controls (DMSO and acetone) and positive controls (hycanthone methane sulfonate without activation, and 7,12-dimethylbenz(α)anthracene with

activation) all produced acceptable colony size distributions. Based on these results, it was concluded that Phytrol™ was not considered mutagenic under the conditions tested.

In an *in vitro* test for chemical induction of chromosome aberrations in cultured human peripheral lymphocytes, with and without metabolic activation, the mutagenic potential of Phytrol™ was investigated. Using the chromosome aberration assay in cultured human peripheral blood lymphocytes, the mutagenic potential of Phytrol™ was investigated at 100, 150, 300, 600, 750, 900 and 1200 µg/ml, with and without rat liver S-9 fraction. The test article was prepared in acetone, and duplicate cultures of each dose were established. In addition, solvent and positive controls (mitomycin at 0.1 and 0.2 µg/ml, and cyclophosphamide at 10 and 20 µg/ml, in non-activated and activated systems, respectively) were used to verify testing conditions. Cells were harvested 21 hours after treatment initiation in both systems, with 0.1 µg/ml colcemid present during the final two hours. Toxicity was measured by determining the Relative Mitotic Index (RMI), and the percentage of polyploid and endoreduplicated cells was determined at each concentration level. Data showed that Phytrol™ did not induce a statistically significant increase in the percentage of cells with aberrations, as compared to solvent controls, at any of the concentrations tested with and without metabolic activation. Results were subsequently confirmed by a confirmatory assay performed without S-9 activation. Given the results of the definitive and confirmatory assays, Phytrol™ was reported to have no effect on the frequency of chromosome aberration in peripheral blood lymphocytes, both in the presence and absence of S-9 metabolic activation.

### 9.1.2 *In Vivo Genotoxicity Assays*

Phytrol™ was evaluated at levels of 500, 1000 and 2000 mg/kg for the potential to induce micronucleated polychromatic erythrocytes (MPCE) in the bone marrow cells of male and female CD-1 mice. A single dose of the test article was administered *via* oral gavage, and the percentage of polychromatic erythrocytes (PCE) and micronucleated polychromatic erythrocytes (MPCE) frequency was determined at approximately 24, 48 and 72 hours after dose administration. Two thousand PCEs per animal were analyzed for the frequency of micronuclei, and cytotoxicity was assessed by scoring the number of PCEs and normochromatic erythrocytes (NCEs) in the first 200 erythrocytes for each animal. Results indicated there was no statistically significant increase in the number of MPCE in the

Phytrol™ treated groups relative to control. In addition, there were no reductions (more than 20% of vehicle) in the percentage of PCE in other test groups receiving Phytrol™. Based on the results summarized above, it was concluded that under the current test conditions, Phytrol™ did not cause chromosome damage *in vivo*, nor was it a clastogenic agent.

## 9.2 Toxicological Studies with Phytrol™

Forbes Medi-Tech Inc. sponsored a number of studies for the purpose of examining the safety and potential toxicological effects of Phytrol™ in four species of animals. All species tolerated intake of Phytrol™ well and with no apparent effects on body weight gain or food intake even at very high dosages. See Tables 9-1 and 9-2 for a summary of studies where weight gain and food intake were monitored.

**Table 9-1 Effect of Oral Phytrol™ Administration on Food Intake & Body Weight Gain in Animals**

Study Number	Species, Number, Gender	% Chol. added to Diet (w/w)	% Tall Oil Phytosterols in Diet (w/w)	Approx. Dose <sup>2</sup> mg/kg/day	Treatment Time (days)	% Change in Food Intake <sup>1</sup>	% Change in Body Weight <sup>1</sup>
<b>Subchronic Studies</b>							
PHF9501	Hamster <sup>3</sup> 20 M & F	0.25%	0.5% 1.0%	340 680	90	+10.2% NS	-3.25%
PHF9601	Mouse <sup>4</sup> 10 M	0.15%	2.0%	3340	126	NS	+6.13% p<0.05
PHF9602	Mouse <sup>4</sup> 8 M	0%	2.0%	3340	140	NS	NS
PHF9703	Mouse <sup>4</sup> 8 M	0%	2.0%	3340	42 91 140	NS	NS

1. Percentage change with respect to appropriate dietary control.

2. The calculated daily dosage is based on estimated food consumption or percent of body weight per day as follows: Mouse, 16.7%; Rat, 10%; Hamster, 6.8%; Rabbit, 3.7%.

3. Syrian Golden Hamster

4. ApoE-Deficient Mouse

**Table 9-2 Effect of Oral Phytrol™ Administration on Food Intake & Body Weight Gain in Animals**

Study Number	Species, Number, Gender	% Chol. added to Diet (w/w)	% Tall Oil Phytosterols in Diet (w/w)	Approx. Dose <sup>2</sup> mg/kg/day	Treatment Time (days)	% Change in Food Intake <sup>1</sup>	% Change in Body Weight <sup>1</sup>
<b>Subacute Studies</b>							
PHF9401	Rat <sup>4</sup> 6 M	1.0%	1.0%	1000	10	-2.56% NS	-1.72% NS
PHF9402	Hamster <sup>3</sup> 8 M	0.25%	0.25% 0.50% 1.00%	170 340 680	34	-1.5% p<0.05	-13.07% NS
PHF9502	Hamster <sup>3</sup> 10 M	0.25%	1.0%	680	45	+0.38% NS	+0.61% NS
PHF9503	Rabbit <sup>5</sup> 6 M	0.5%	1.0%	370	50	No Data	-3.8% NS

1. Percentage change with respect to appropriate dietary control.
2. The calculated daily dosage is based on estimated food consumption or percent of body weight per day as follows: Mouse, 16.7%; Rat, 10%; Hamster, 6.8%; Rabbit, 3.7%.
3. Syrian Golden Hamster
4. Wistar Rat
5. New Zealand White Rabbit

In addition to the aforementioned studies, there are three other Novartis Consumer Health Inc. sponsored studies, which include toxicological data. Refer to Table 9-3 for tabulated summaries of these studies. Additionally, data from a uterotrophic assay in the rat is included.

In study TXF9501, Syrian Golden Hamsters were treated with 0.5% or 1.0% Phytrol™ incorporated into their diet for 90 days. The approximate doses were 0.34 and 0.68g/kg/day. No histopathological changes were noted in any of the intestinal sections submitted, usually taken from the duodenal level. No other tissues were examined.

In study TXF9605, Syrian Golden Hamsters were treated with 1.0% Phytrol™ incorporated into their diet for 60 days. The approximate dose was 0.68g/kg/day. The hamsters were also injected with Phytrol™ at a dose of 0.5mg / 100g body weight. Histopathological tissue examination from the duodenum, epididymis, liver, ovaries, testes, and uterus revealed no changes between treated and experimental groups, irrespective of the route of administration. No other tissues were examined.

In study TXF9503, New Zealand White Rabbits were treated with 1% Phytrol™ incorporated into

their diet for 50 days. The approximate dose in this case was 0.370 g/kg/day. No significant differences in the histology of liver and small intestine tissue were observed between the experimental group and the control group. No other tissues were examined.

In study TXF9904, a uterotrophic assay in immature female rats was conducted to evaluate the estrogenic potential of Phytrol™ (FCP-3P1). Beginning on day 19 postpartum, 50 immature female rats were administered *via* oral gavage, either 0 (vehicle control), 1000, 2500 or 5000 mg/kg/day of Phytrol™ for 4 consecutive days. Body weights and body weight gains were slightly reduced in both the 2500 and 5000 mg/kg/day dosage groups compared to the control group. Uterine weights and the ratios of the uterine weight to the terminal body weight were unaffected by dosages of the test article. All values in the test groups were comparable to control group values. A fifth group received a positive control benchmark substance, ethinyl oestradiol, which increased uterine weight and relative uterine weights to over 500% that of the control group.

**Table 9-3 Summary of Phytrol™ Toxicological Studies**

Study Number	Species, Number, Gender	Duration	Diet	Route of Administration Dosage	Approx. Dose per Day	Tissues Examined	Findings
<b>Subchronic Studies</b>							
TXF9501	Hamster 10M and 10F per group	90 days	Control diet or diet with 1% cholesterol	Oral: % of diet Control 0.5% FCP 1.0% FCP	0.34 g/kg/d 0.68 g/kg/d	Liver Intestine	The atherogenic diet resulted in periportal to diffuse microvesicular vacuolation of the hepatocytes. It was interpreted to be a hepatocellular fatty change graded as mild to moderate in severity and tangibly more severe in females than males. The administration of FCP was associated with a dose related decrease in the incidence and/or severity of the hepatocellular vacuolation in both sexes. No histopathological changes were noted in any of the intestinal sections submitted, usually taken from the duodenal level.
<b>Subacute Studies</b>							
TXF9605	Hamster Control 4M,3F Treated 5M, 9F	60 days	Standard diet or diet with 0.25% cholesterol	Subcutaneous Oil:ethanol (6:1) vehicle; volume injected 0.06 ml Controls rec'd vehicle Treated 35 mg/kg administered weekly	5 mg/kg/d	duodenum liver epididymis ovary testis uterus	No histological findings that could be related to treatment
TXF9503	New Zealand White Rabbit Control 2 Treated 6	50 days	Diet with 0.5% cholesterol	Oral: % of diet 1% FCP in diet	0.37 g/kg/d	liver and small intestine	No significant differences were observed between experimental groups.
<b>Reproductive Studies</b>							
TXF9904	Immature Rats (female)	4 days	NA	Oral gavage	1000, 2500 and 5000 mg/kg/day	Uterus	Dosages as high as 5000 mg/kg/day administered for 4 days to immature female rats did not affect uterine weights and thus had no uterotrophic potential.

FCP = Forbes Medi-Tech Inc. phytosterols

### 9.3 Toxicology Studies with Cytellin™ (Positol™)

Between 1954 and 1982, Eli Lilly Research Laboratories marketed a mixture of phytosterols extracted from tall oil in the United States (Cytellin™) and in Canada (Positol™). Cytellin™ / Positol™, marketed as an anti-hypercholesterolemic agent, was available either as a powder or liquid suspension, and the reported composition was sitosterol, sitostanol, campesterol, campestanol; 80:10:7:2. The Novartis Consumer Health Inc. product Phytrol™ is also extracted from tall oil and is composed of the same four major constituent sterols. However, it differs in that the proportions of sitostanol and campestanol, are higher and lower, respectively. Although Cytellin™ / Positol™ was eventually withdrawn from the market due to business considerations, several toxicology studies had been conducted with the product. Refer to Table 9-4 for a tabulated summary of these studies.

**Table 9-4 Toxicology Studies with Cytellin™**

Species and Number	Dosage	Duration	Results
<b>Acute Studies</b>			
Albino mice 565	5g/kg sitosterol triturated in sesame oil by stomach tube	Single dose	Sitosterol from tall oil show little or no toxicity following administration of large single oral doses to mice.
<b>Subchronic Studies</b>			
Rats 30 female	1% and 5% sitosterol in diet	18 months	Rats fed doses containing 5% sitosterol from tall oil for 18 months survived, gained weight comparable to controls, and upon sacrifice showed no visceral or hematopoetic damage and no alterations in serum cholesterol, lipid phosphorus or blood protein fractions.
Rats 20 female	Diet containing 5%Formula 226	8 months	Rats fed diets containing 5% formula 226 for 8 months responded in similar manner
Dogs 8 female mongrel dogs	Capsules: 4 dogs 500mg/kg/d 4 dogs 1000mg/kg/d	18 months	Dogs that received daily doses of 1000 mg/kg for 18 months survived, gained weight and had no hematological or visceral damage. Serum cholesterol, calcium and phosphorus, total lipids, lipid phosphorus, vitamin A and blood protein fractions were unaltered. The ultracentrifugal pattern was similar for treated and control dogs. Total lipid and free and total cholesterol values of the livers were also unchanged.
Dogs 3 dogs	1000mg/kg/d of Formula 226*	8 months	Dogs that received daily doses of 1000mg/kg of Formula 226 for 8 months were also normal.

\* Formula 226, Each 100cc. Contains:

- Tall oil sterols 20g
- Benzoic acid 0.1g
- Sodium Carboxymethylcellulose 3.0g
- Saccharin Soluble 10mg, Raspberry Flavor 0.0015 cc.
- Sodium Lauryl Sulfate Purified 50mg

The above information was obtained under "Freedom of Information" from the FDA in the United States.

## 9.4 Published Toxicology Studies with Phytosterols

Phytosterols have been extensively documented in many readily available scientific publications. This section seeks to document the general safety of phytosterols by reviewing scientific publications which discuss the safety of phytosterols in general. The results of this review are documented below and summarized in Table 9-5.

### 9.4.1 Genotoxicity

The results of a panel of genotoxicity tests with vegetable and tall oil stanol esters was reported by Turbull *et al.*, [1999]. The study was in compliance with OECD Guideline 473. All tests gave negative results.

### 9.4.2 Subchronic Toxicity

Shipley *et al.*, [1958] reported that no evidence of toxicity was observed in rabbits and dogs given large daily oral dietary supplements of sitosterol (mostly of tall oil origin), for periods of up to 2 years. Gross or microscopic alterations were not observed in any tissue, and there was no histological evidence of disposition of the plant sterols. In addition, chemical analysis of the aorta and liver showed no increase in sterol content.

An abstract by Robinson *et al.*, [1998] describes a 90 day subchronic feeding study conducted in 160 Sprague-Dawley rats (80 male/80 female) to investigate the safety of phytosterols. Stanols (61, 305 and 915 mg stanol/kg bw/day) were administered *via* oral gavage in a cottonseed/soybean oil mixture, consisting of 65% sitostanol, 30% campestanol, 2.5% campesterol and 2.5% other sterols. Following the 13-week treatment period, no significant toxicological effects were reported.

A second study investigated the safety of stanol esters in male and female Wistar rats. Animals received either a wood-derived stanol ester preparation or a vegetable oil-derived stanol ester preparation, at dietary concentrations of 0, 0.2, 1 and 5% total stanols (174-5509 mg stanol esters/kg bw/day). Approximately 0.5 g total stanols/kg bw/day was provided at this dietary level. Following

a 13-week treatment period, slightly decreased levels of plasma cholesterol and phospholipids were reported in stanol-treated males. Decreased levels of plant sterols and increased levels of stanols were observed in both males and females. A marked increase in the fecal excretion of sterols, including cholesterol and stanols, was reported in the stanol ester groups. Animals treated with the high-dose diets experienced a decrease in plasma levels of vitamin E, vitamin K, and to a lesser extent, vitamin D. Similar changes were also observed in hepatic levels of vitamins E and D. Based on these results, and the absence of any significant adverse clinical, pathological or histopathological effects, both preparations were considered well tolerated. The no observable adverse effect level (NOAEL) was reported to be the mid-dose level of 1% total dietary stanols. [Turnbull *et al.*, 1999].

Malini and Vanithakumari [1990] described a study in which rats were administered sitosterol by subcutaneous injection at doses of 2.5, 5.0 and 10.0 mg/kg/day for 60 days. The sitosterol was well tolerated and no evidence of gross microscopic lesions either in the liver or kidney was observed. Furthermore, liver and kidney function tests were assessed by determining blood/serum parameters such as hemoglobin, blood glucose, serum protein, serum bilirubin, serum GPT and GOT. All clinical biochemical parameters were in the normal range with the exception of serum cholesterol, which was reduced at all doses of sitosterol.

The effect of tall oil phytosterols administered in the diet was investigated in the apo-E-KO-deficient mouse. Histological, hematological, and biochemical characteristics were examined. No toxicity was observed in the phytosterol treated group. Both treated and untreated mice exhibited arrested spermatogenesis and atrophy of the seminiferous tubules to a variable extent. This effect may be related to the difficulty of breeding this particular strain. The apo-E-KO-deficient mouse exhibits a number of abnormalities related to the genetic defect including xanthomatous skin lesions and oil red O-negative vacuolation in the liver and kidney parenchymal cells. The phytosterol treatment prevented these lesions [Moghadasian *et al.*, 1999].

Daily injections of soy phytosterols for three weeks resulted in a progressive accumulation in the serum, liver, and bile of exposed neonatal piglets. Serum bile acid levels were significantly higher in the sterol-treated piglets. In addition, a significant inhibition of secretory function in isolated rat hepatocyte couplets was observed [Clayton *et al.*, 1998]. Furthermore, neonatal piglets receiving

daily injections of phytosterols in the absence of other parenteral nutrition components, experienced reduced bile flow [Iyer *et al.*, 1998].

#### 9.4.3 *Reproductive Toxicity*

Two tests of potential estrogenic activity were reported for plant stanols (soy or tall oil) and plant stanol esters by Turnbull *et al.*, [1999]. These were the E-screen test, which measures the ability of a substance to induce proliferation of estrogen-responsive human breast adenocarcinoma (MCF-7) cells in culture, and an *in vivo* test, which measures uterotrophic activity in immature female rats fed the test substance. In the E-screen test, none of the stanol preparations produced any increase in cell proliferation when tested at 1, 10, and 100  $\mu\text{M}$ . In the *in vivo* test, neither stanol ester preparation caused any significant change in uterine weight when fed at a concentration of 8.3% in the diet for 4 days.

Whittaker *et al.*, [1999] reported the results of a two-generation reproductive toxicity study performed according to OECD Guideline 414, and in compliance with the OECD principles of GLP. The test article was vegetable oil stanol esters at doses of up to 5% stanols in the diet. No adverse treatment related effects were noted on reproductive performance of male or female rats in any dose group. In addition, no adverse developmental effects were noted in F<sub>1</sub> or F<sub>2</sub> pups of the low and mid-dose groups. A treatment related effect on body weight and body weight change was observed in both the F<sub>1</sub> and F<sub>2</sub> male and female pups of the high-dose group, particularly during the later stages of lactation. However, the lower body weight in the high-dose group pups was attributed to a reduction in the caloric intake of the test diet compared to the control.

Another two generation reproductive study investigated the effects of soy phytosterol esters in the rat was reported in the form of an abstract [Waalekns-Berendsen *et al.*, 1999]. Soy phytosterol esters of up to 5000 mg/kg/day of phytosterols were tested. No effect on the reproduction of parental F<sub>0</sub>- and F<sub>1</sub>-generation Wistar rats or the development of F<sub>1</sub> and F<sub>2</sub> pups was reported.

A developmental toxicity study in rats was performed according to OECD Guideline 414 and was in compliance with OECD Principles of GLP. The test article was vegetable oil stanol esters administered in doses up to 5% stanols in the diet from days 0 to 21 of gestation. No adverse effects

on reproduction or development were observed [Slesinski *et al.*, 1999].

Malini *et al.* [1991; 1993] investigated effects on male and female rat reproductive tissues. The investigators, using nonpurified sitosterol plant extracts reported various effects in both males and females which are at variance with findings reported by other investigators using purified sitosterol.

Burck *et al.*, [1982] reported that introduction of 0.5 mg sitosterol sulfate into the vagina of female belted rabbits reduced the number of pregnancies. The number of embryos per pregnant rabbit was not affected. Sitosterol sulfate, but not sitosterol, has an acrosin inhibitory activity, which would reduce the efficiency of sperm in fertilizing the ova. Implantation of silicone rods containing sitosterol sulfate into uterine horns of rabbits for 16 days, significantly reduced the number of embryos present in those horns. No birth defects were reported. The release rate of sitosterol sulfate from the silicone rods was 1-2  $\mu\text{g}$  per day. Neither treatment affected the number of corpora lutea.

In conclusion, the only evidence of toxicity to animals reported in the literature is for injected phytosterols. The blood levels of phytosterols achieved by this route of administration would be much higher than could be obtained by oral administration, where absorption is quite low.

**Table 9-5 Published Studies with Toxicology Findings**

Reference	Species/Strain, Sex, No/Group	Phytosterol	Source	Route of Administration	Dosage mg/kg/day	Duration	Tissues / Parameters Examined	Findings
Robison <i>et al.</i> , 1998.	Sprague-Dawley Rat 20M+20F per groups; 4 groups	Hydrogenated soy phytosterols	Soy	Oral , cotton-seed/soy oil mixture by gavage	Control 61 305 915	90 days	Standard tissue screen for GLP study	No toxicological effects
Turnbull <i>et al.</i> , 1999.	Wistar rats (M&F) 20 rats/sex/group	Plant stanol esters	Tall oil (3 groups)  Vegetable oil (3 groups)	Oral in diet	0.2%; 1%; 5% stanols in diet  (0.34%; 1.68%; 8.39% stanol esters from tall oil 0.2%, 1%, 5% stanol esters from vegetable)	13 weeks	GLP study standard for US and EU requirements	<p>No toxicity was associated with the subchronic ingestion of wood or vegetable oil derived stanol esters at dietary concentrations up to 1% (as free stanol; equivalent to about 0.5g total stanols/kg bwt/d). At dietary levels of 5% (as free stanol), subchronic ingestion of these substances resulted in decreased plasma levels of the fat soluble vitamins E and K1 (~50%), and, to a lesser extent, vitamin D (-15%). Hepatic levels of vitamins E and D showed similar changes.</p> <p>Both wood and vegetable oil derived stanol esters were well tolerated, as evidenced by the absence of clinical changes or major abnormalities in growth, food and water consumption, ophthalmoscopic findings, routine hematological and clinical chemistry values, renal concentrating ability, composition of the urine, appearance of the feces, estrus cycle length, organ weights, gross necropsy findings, and histopathological findings.</p> <p>Females of the wood-derived stanol 5% dose group showed a statistically significant increase in thrombocyte count, and females of the vegetable derived stanol 5% dose group had an increased percentage of neutrophils and decreased percentage of lymphocytes (not ascribed to treatment because there was no clear dose- response relationship and no significant changes in absolute numbers of these cell types).</p> <p>Plasma sitostanol was increased in males of the 1 and 5% dose groups and in females in all treatment groups. Campestanol was increased in all groups fed vegetable oil-derived stanols.</p> <p>Uterine luminal dilatation was observed more frequently in females fed vegetable oil-derived stanols (5%) than in controls (not significant) and it was not accompanied by any histopathological urine changes, nor by treatment related changes in estrous cycle length or other reproductive organs.</p>

**Table 9-5 Published Studies with Toxicology Findings (continued)**

Reference	Species/Strain, Sex, No/Group	Phytosterol	Source	Route of Administration	Dosage mg/kg/day	Duration	Tissues / Parameters Examined	Findings
Turnbull <i>et al.</i> , 1999.	E-screen test human breast carcinoma (MCF-7) cells in culture	Stanols (88-99% stanols)	Four samples of vegetable oil-derived stanols (88-99% stanols)	Cell culture	0,1,10, and 100µM stanols	6 days	GLP study standard for US and EU requirements	None of the stanol preparations produced any increase in cell proliferation when tested at 1,10, and 100µM. The highest dose of each stanol sample was associated with microscopic evidence of cytotoxicity and crystalline precipitation in the culture dishes. Slight to moderate cytotoxicity was seen with all four stanol samples at the highest dose tested. This was accompanied by crystals at the bottom of the culture wells at this dose level.
	In vivo test (immature 15 day old female Wistar rats)	Stanol fatty acid esters	One sample of tall-oil and one of vegetable oil-derived stanol fatty acid esters	Oral in diet	8.3% stanol esters(w/w) diet	4 days		In <i>in vivo</i> test, neither of two stanol ester preparations caused any significant change in absolute or relative uterus weight when fed at a concentration of 8.3% in the diet for 4 days. Thus, under the conditions of testing used, neither the free stanols nor the stanol fatty acid ester preparations showed evidence of estrogenic or uterotrophic activity. Animals fed stanol esters showed a slightly reduced body weight gain over the 4-day treatment period significant in the wood stanol ester group only). This was associated with a slightly reduced food consumption in these animals.
Whittaker <i>et al.</i> , 1999.	Wistar rats (M&F) 28rats/group/ generation	Plant stanol esters	Tall oil and vegetable	Oral in diet	1%; 2.5% and 5% stanols in diet (1.75%; 4.38%; 8.76% total stanol esters)	10-13 weeks	GLP study standard for US and EU requirements	No effects on reproduction of parental F0- and F-1 generation Wistar rats. Consumption of plant stanol esters at dietary percentages up to 4.76% (equivalent to 2.5% total stanols) was not associated with adverse effects upon the reproduction or development of male or female rats over two generations. At dietary concentrations of 8.76% stanol esters (equivalent to 5.0% total stanols), ingestion of plant stanol esters was associated with increases in food consumption in male and female F0 and F1 generation rats, as well as decreases in body weight in male and female F1 and F2 pups (attributable to consumption of test substance, which is not absorbed and reduces the caloric value of the test diet compared to control).  In the F1 generation both absolute and relative weights of the testes were increased in the 4.38% dose only. Furthermore, the relative weight of the epididymides of the F1 males of the 4.38% dose group was statistically significantly increased. These statistically significant effects on organ weights were not observed in the high-dose group and were not considered treatment related.

**Table 9-5 Published Studies with Toxicology Findings (continued)**

Reference	Species/Strain, Sex, No/Group	Phytosterol	Source	Route of Administration	Dosage mg/kg/day	Duration	Tissues / Parameters Examined	Findings
Waalkens-Berendsen <i>et al.</i> , 1999.	Wistar Rats, 28 rats/group/ generation	Phytosterol esters	Soy	Oral	Max 8.1% PE in diet 5000 mg/kg/day sterols	NA	GLP study standard for US and EU requirements	No effects on reproduction of parental F0- and F1-generation Wistar rats and the development of F1- and F2 pups.
Slesinski <i>et al.</i> , 1999.	28 Wistar rats per dose group	Stanol esters	Vegetable oil (Sito - 70) (68% sitostanol, 30% campestanol, 2% unsaturated sterol)	Oral in diet	0, 1, 2.5, 5% total stanols (equivalent to 0, 1.75, 4.38, 8.76% plant stanol esters)	21 days	GLP study standard for US and EU requirements	No adverse treatment-related maternal or fetal developmental effects were produced following ingestion of a diet containing up to 8.76% plant stanol fatty acid esters. This diet provided up to 5% of total dietary stanols equivalent to 2.4-3.5g stanols/kg bw/d. No significant differences were seen in reproductive performance, maternal and fetal body weights, sex distribution, or visceral or skeletal malformations, anomalies, and variations. Vegetable oil-derived stanol fatty acid esters are concluded not to be developmental toxicants and did not produce any embryotoxic, fetotoxic, or teratogenic effects in Wistar rats under the conditions of this study.  Statistically significant differences were noted in mean body weight relative to controls at the 0-7-day and 7- to 14-day period and in body weight gains during 0-7 days for the high dose group (attributable to decrease in caloric content of the diet from the levels of unabsorbable stanols at the highest dose). These changes were relatively small, transient in nature, and were not considered biologically meaningful as they were not seen in the 14- to 21-day terminal portion of the study.
Turnbull <i>et al.</i> , 1999.	Ames assay (s. typhimurium) bacterial cell genotoxicity test  LS178Y assay (mammalian cell) gene mutation assays  Mammalian cell chromosome aberration assay (CHO cells)	Plant stanol fatty acid esters	Tall Oil and vegetable-derived plant stanol fatty acid esters  Tall Oil  Vegetable  Tall Oil  Vegetable	Cell culture  Cell culture  Cell culture	0, 62, 185, 556, 1667, 5000 µg/plate  20-500µg/ml  250-3000µg/ml  125-500µg/ml  500-2000µg/ml	4hrs  18 or 32 h without S9 rat liver microsomal metabolic) and 3h with S9	GLP study standard for US and EU requirements	All tests gave negative results for both wood and vegetable oil stanol ester formulations. Thus, plant stanol esters are not genotoxic under the conditions of exposure tested.

**Table 9-5 Published Studies with Toxicology Findings (continued)**

Reference	Species/Strain Sex, No/Group	Phytosterol	Source	Route of Administration	Dosage mg/kg/day	Duration	Tissues / Parameters Examined	Findings
Shipley <i>et al.</i> , 1958.	Dogs, 13	Sitosterol in diet	Cytellin™, derived from Tall Oil, study from Eli Lilly Laboratories	Oral in diet	1000 mg/kg/day	8 to 22 months	Blood hematology, biochemistry, aorta, heart, lungs, liver, spleen, kidneys, stomach, intestine, thymus, thyroid, adrenal glands, bone marrow	No gross or microscopic pathological changes; biochemistry and hematology normal. No evidence of phytosterol accumulation in any tissues. Vitamin A levels unchanged in blood.
	New Zealand White Rabbits, 6 M, 6F	Sitosterol in diet	Derived from either tall oil or cottonseed oil	Oral in diet	4000 mg/per rabbit per day	348 to 842 days	Heart, blood vessels, thyroid spleen, liver, intestine	No gross or microscopic pathological changes; biochemistry and hematology normal. No evidence of phytosterol accumulation in any tissues.
Malini <i>et al.</i> , 1990.	Wistar albino rats 10 M & 10 F	Sitosterol	Anacardium occidentale	Subcutaneously	2.5 mg/kg/D 5.0 10.0	60 days	liver kidney	There was no clear cut evidence of any gross or microscopic lesions in the liver or kidney. A marked fall in serum protein level only at dose of 1000µg of sitosterol. All parameters (blood/serum) were in normal range.
Moghadasian <i>et al.</i> , 1999.	Apo-E-KO mice 6M Control 6M Treated	Phytosterols	Tall Oil	Oral in diet	3.34g/kg/d	18 weeks	Hematology, urinalysis, heart, lung, brain, kidney, skeletal muscle, skin, esophagus, stomach, small & large intestine, liver, adrenal gland, spleen, pancreas, bladder	Hematology: Hemoglobin concentration, red cell counts, and hematocrit were comparable between groups; but there was a statistically significant reduction in platelet counts. Leukocyte counts showed a large but not significant variation between the two groups. Urinalysis: No significant differences were observed in the urine parameters. Macroscopic Organ Examination: No abnormalities except for skin lesions (thickened, red, alopecia) in two control mice. Histological Examination: The affected skin revealed numerous cholesterol crystals, cholesterol granulomas along with cellular reaction with eosinophils and histocytes. Routine histochemical staining revealed no histological abnormalities in the tissues examined except for slight histological changes in liver and kidney which were reduced in extent in the FCP treated group Arrested spermatogenesis and atrophy in the seminiferous tubules was observed to a variable extent in both treated and untreated groups.
Iver <i>et al.</i> , 1998.	Neonatal Piglets	Soy phytosterols	Soy	Intravenous	18 nM per kg/ per day	14 days	Bile, liver, serum	Serum bile acid levels increased. Reduction in bile acid-stimulated bile flow. Normal liver function tests, liver histology remained normal.

**Table 9-5 Published Studies with Toxicology Findings (continued)**

Reference	Species/Strain Sex, No/Group	Phytosterol	Source	Route of Administration	Dosage mg/kg/day	Duration	Tissues / Parameter Examined	Findings
Malini <i>et al.</i> , 1993.	Wistar albino rats 10 F	Sitosterol	Anacardiu m occidental c	Subcutaneously	0.5 mg/kg/D 2.5 5.0	10 days	Uterus RNA, DNA, protein concentrations	Uterine weight and RNA concentrations increased in a dose dependent manner indicating that sitosterol has some intrinsic estrogenic property.
Burck <i>et al.</i> , 1982.	Dutch-belted rabbits 20 F	Sitosterol Sulphate	Not identified	Intravaginal  Intrauterine	0.5  1-2µg	16 days	pregnancy rate  corpora lutea  number of embryos	Introduction of 0.5 mg sitosterol sulphate into the vagina of rabbits before coitus lowered the pregnancy rate, but did not significantly reduce the number of embryos produced per pregnant animal. Sitosterol sulfate but not sitosterol is a potent acrosin inhibitor which would reduce the efficiency of fertilization. Implantation of silicone rods containing sitosterol sulfate into the uterine horns of rabbits significantly reduced the number of embryos present in those horns. Neither treatment affected the number of corpora lutea.
Malini <i>et al.</i> , 1991.	Wistar albino rats 10 M	Sitosterol	Anacardiu m occidental c	Subcutaneously	0.5 mg/kg/D  5.0	16 days 32 days 48 days	testes	A significant decrease in testicular weight and sperm concentrations after long-term treatment with low dose of sitosterol. The weights of all accessory sex tissues except the epididymis increased following low dose sitosterol treatment. High dose treatment reduced the sperm concentrations as well as the weights of testis and accessory sex tissues to near normal conditions.

## 10.0 CLINICAL TOXICOLOGY

### 10.1 Clinical Studies Employing Phytrol™

Table 10-1 summarizes the clinical studies conducted to date with Phytrol™ (FCP-3P1) in human subjects. A total of 55 subjects were exposed to Phytrol™ in their diet at a dose of 1.5g per 70 kg body weight per day. No clinically significant adverse events were observed in these studies.

**Table 10-1 Clinical Studies on Dietary Administration of Phytrol™**

Study Number	Cholesterol Levels	Number & Sex	Food Matrix	Dosage g/70kg/day	Duration (days)
CLF9601	Normal	6M 5F	Vegetable Oil	1.5	10
CLF9602	Elevated	12M	Vegetable Oil	1.5	10
CLF9701*	Elevated	32M	Margarine	1.5	30

\* Jones et al [1999].

In study CLF9601, Phytrol™ (FCP-3P1) was incorporated into the standard diet of 11 healthy male and female volunteer test subjects at a dose level of 1.5 g phytosterol per 70 kg body weight. This was conducted over the course of 10 days, followed by a 14-day washout period, followed again by a second 10-day administration. When compared to the control group, results indicate that at relatively low doses, the phytosterol mixture effectively impeded cholesterol absorption, thus improving the plasma lipid profile through decreasing total and LDL-cholesterol levels as well as increasing the HDL/LDL ratio. No adverse effects were reported.

In study CLF9602, Phytrol™ (FCP-3P1) was incorporated into the standard diet of 12 healthy male volunteer test subjects at a dose level of 1.5 g phytosterol per 70 kg body weight. This study was also conducted over the course of 10 days, followed by a 14-day washout period, followed again by a second 10-day administration period. Post treatment plasma LDL cholesterol level ( $4.1 \pm 0.2$  mmol/l) was lower ( $p < 0.05$ ) than that of post placebo treatment ( $4.3 \pm 0.1$  mmol/l). The treatment had no effect on plasma HDL and triglycerides versus placebo. No adverse effects were reported.

In study CLF9701, published by Jones et al [1999], Phytrol™ (FCP-3P1) was incorporated into a double-blind, randomized, placebo controlled diet. A standard test diet consisting of 15% protein, 50% carbohydrates, and 35% fat was administered to 32 healthy volunteer test subjects for a period of 30 days. Treated subjects received a dose level of 1.5 g Phytrol™ per 70 kg body weight per day, incorporated into margarine at a ratio of 1:20 (w/w). Another 16 volunteers received a placebo. Both the placebo and Phytrol™ containing diets were well tolerated with no reported discomfort and no significant adverse events. No change in body weight was noted for each of the study groups. The most significant dietary effect noted was the mean decline in total and LDL cholesterol. The difference between placebo and treated groups at day 30 for total and LDL cholesterol was 9.1% and 15.5% respectively. A small decrease in HDL occurred in both the control and treated groups. The mean decrease in the treated group was slightly greater than that of the control group but the difference was not clinically significant, was well within the variability of measurement, and was not statistically significant, as indicated in Section 1.3.3.3.

## 10.2 Literature Review

The safety of tall oil phytosterols in general, is further supported by the extensive history of human exposure to the constituent phytosterols, as documented in the published literature cited below.

Humans are continually exposed to phytosterols in the diet. The average dietary phytosterol intake is about 250 mg per day, with perhaps double that amount consumed by vegetarians. The scientific literature on the effects of human exposure to elevated intakes of phytosterols is extensive and dates from the early 1950's. Pollak and Kritchevsky [1981] reviewed published studies on the clinical use of phytosterols up to 1981. The authors estimate that clinical data on the cholesterol-lowering action of phytosterols in about 1800 subjects was available at the time of their review.

Table 10-2 summarizes clinical studies of phytosterols published since the review by Pollak & Kritchevsky [1981], as well as some earlier studies. Most of the recent studies have been conducted using sitostanol ester. As reflected in Table 10-2, the occurrence of adverse effects associated with the use of phytosterols is rare. Prior to 1981, reports of adverse events consisted primarily of gastrointestinal disturbances. In more recent studies, reported adverse effects were mild and

presented no consistent pattern that might suggest a relation to the use of phytosterols. Furthermore, to our knowledge, there has not been a single report of a serious adverse event associated with the use of phytosterols.

**Table 10-2 Summary of Safety of Orally Administered Phytosterols in Human Subjects from Published Sources**

Reference	Population			Study Drug			Exposure			Safety
	Disease State	Number & Sex	Age Range or Mean (yr)	Phytosterol	Plant Source	Dosage Form	Dosage g/day <sup>2</sup>	Duration (days)	Extent g/d x days x subjects	Adverse Events Reported
Weststrate JA and Meijer GW, 1998.	NC and mildly HC	95 M&F	48±12.8	Sitostanol ester (Benecol™)	Tall oil	Margarine suspension	2.74	24-25	6377	None except effects on vitamin and nutrient levels in plasma (See Table 10-1).
				Soy PS ester	Soy		3.24		7541	
Plat J and Mensink R, 1998.	Healthy volunteers	112		SITO 70	Vegetable	Margarine suspension	3.8	56	23833.6	Hematology and blood chemistry parameters remain within normal range.
				SITO 90	Tall oil		4.9		25088	
Kris-Etherton PM <i>et al.</i> , 1998.	HC	35M & 23F		Sitostanol mixture	Vegetable	Margarine suspension	3	28	4872	none
Cobb MM <i>et al.</i> , 1997.	Sitosterolemic homozygote	1F	9	Sitosterol	Soybean oil Sesame oil	Oil suspension	0.06	56	34-67	none
							0.09			
							0.122			
Gylling <i>et al.</i> , 1997.	Woman with angiographically documented CAD	22F		Sitostanol ester (Benecol™)	Tall oil	Margarine suspension	3	49	3234	None
	Women treated with simvastatin for more than 1 year	10F					3	90	2700	
Gylling <i>et al.</i> , 1996	NIDDM with HC	8 M	60.2±1.6	Sitostanol Ester	Tall Oil	Margarine suspension	3.0	42	1008	None

1 NC = Normocholesterolemic; FH = Familial Hypercholesterolemia; NIDDM = Non-insulin dependent diabetes mellitus; HC = Hypercholesterolemia

2 Total combined dose phytosterols where phytosterols are a mixture

**Table 10-2 Summary of Safety of Orally Administered Phytosterols in Human Subjects from Published Sources (continued)**

Reference	Population			Study Drug			Exposure			Safety
	Disease State	Number & Sex	Age Range or Mean (yr)	Phytosterol	Plant Source	Dosage Form	Dosage g/day <sup>2</sup>	Duration (days)	Extent g/d x days x subjects	Adverse Events Reported
Gylling <i>et al</i> , 1995	FH	7 M 7 F	9.1±1.1	Sitostanol ester	Tall Oil	Margarine suspension	3.0	42	1764	none
Gylling <i>et al</i> , 1995	NIDDM with HC	6 M	63.2±1.2	Sitostanol ester	Tall Oil	Margarine suspension	3.0	28	504	none
Pelletier <i>et al</i> , 1995	Healthy volunteers	12M	22.7±2.6	Sitostanol ester	Soybean Phytosterol	Margarine suspension	0.740	28	249	none
Miettinen <i>et al</i> , 1995	HC	64 M 89 F	25-64	Sitostanol Ester	Tall Oil	Margarine suspension	2.6 (n=51) 2.6 (n=51) 1.8 (n=51)	365 180 180	48399 23868 16254	none
Denke <i>et al</i> , 1995	HC	33 M	31-70	Sitostanol	Tall Oil	Margarine suspension	3.0	30	2970	none
Gylling <i>et al</i> , 1994	NIDDM with HC	11 M	57.8±1.9	Sitostanol Ester	Tall Oil	Margarine suspension	3.0	42	1386	none
Miettinen & Vanhanen, 1994	HC	22 M 9F	45±3	Sitosterol Sitostanol Sitostanol ester	Tall Oil	Margarine suspension	0.7 (n=9) 0.7 (n=7) 0.8 (n=7)	63 63 63	1367	none
Vanhanen <i>et al</i> , 1994	HC	11M 4F	33-60 M 37-55 F	Sitostanol ester Sitosterol	No data	Margarine suspension	0.8 (n=7) 2.0 (n=7)	63 42	352 588	none
Vanhanen <i>et al</i> , 1993	HC	47M 20F	25-60	Sitostanol Ester	No data	Margarine suspension	3.4 (n=34)	42	9568	none
Becker <i>et al</i> , 1993	FH	6 M 3 F	10-14	Sitosterol Sitostanol	No data	Pastil	6.0 1.5	84 196	4536 2646	none

1 NC = Normocholesterolemic; FH = Familial Hypercholesterolemia; NIDDM = Non-insulin dependent diabetes mellitus; HC = Hypercholesterolemia

2 Total combined dose phytosterols where phytosterols are a mixture

**Table 10-2 Summary of Safety of Orally Administered Phytosterols in Human Subjects from Published Sources (continued)**

Reference	Population			Study Drug			Exposure			Safety
	Disease State	Number & Sex	Age Range or Mean (yr)	Phytosterol	Plant Source	Dosage Form	Dosage g/day <sup>2</sup>	Duration (days)	Extent g/d x days x subjects	Adverse Events Reported
Becker <i>et al</i> , 1992	FH	7 M&F	5-10	Sitosterol	No data	Pastil	6.0	84	3528	Slight, but significant decrease in hemoglobin concentration (-5%), decrease alkaline phosphatase activity (-19%), decrease in appetite in 2 children for about 2 weeks.
Vanhanen & Mietinen, 1992	HC	24 M&F	25-45	Sitosterol	No data	Margarine suspension	0.625 (n=8)	54	270	none
				Sitostanol			0.630 (n=8)	54	272	
Heinemann <i>et al</i> , 1986	HC and FH	3 M 3 F	27-59	Sitostanol	No data	Capsule	1.5	28	252	none
Weisweiler <i>et al</i> , 1984	FH (type IIa)	6M 4F	29-67	Sitosterol	No data	Capsule ?	6.0	56	3360	none
Mattson <i>et al</i> , 1982	Unknown cholesterol status	9 M&F	adults	Sitosterol	No data	Aqueous Suspension (Cytellin™)	1.0	30	270	none
Schlierf <i>et al</i> , 1978	FH (type II)	12 M&F	8-20	Sitosterol	No data	Granule	12.0	56	8064	none
Lees <i>et al</i> , 1977	FH (type II)	9M 3F	Adults	Sitosterol Campesterol	Soybean	Capsule	18.0	280 (average) (364-728)	60480	none
Lees <i>et al</i> , 1977	FH (type II)	6 M	Adults	Sitosterol Campesterol	Soybean	Capsule	18.0	Ave 280	30240	none

1 NC = Normocholesterolemic; FH = Familial Hypercholesterolemia; NIDDM = Non-insulin dependent diabetes mellitus; HC = Hypercholesterolemia

2 Total combined dose phytosterols where phytosterols are a mixture

**Table 10-2 Summary of Safety of Orally Administered Phytosterols in Human Subjects from Published Sources (continued)**

Reference	Population			Study Drug			Exposure			Safety
	Disease State	Number & Sex	Age Range or Mean (yr)	Phytosterol	Plant Source	Dosage Form	Dosage g/day <sup>2</sup>	Duration (days)	Extent g/d x days x subjects	Adverse Events Reported
Lees <i>et al</i> , 1977	FH (type II)	9 M	adults	Campesterol	Tall Oil	Capsule	3.0	Ave 196	5292	none
Lees <i>et al</i> , 1977	FH (type II)	14 M 17 F	adults and children	Phytosterol mixture	Tall Oil	Capsule	3.0	Ave 168	15624	mild constipation in a few patients
Lees <i>et al</i> , 1977	FH (type II)	5 M 13 F	adults and children	Phytosterol mixture	Tall Oil	Capsule	6.0	Ave 140	15120	
Duncan <i>et al.</i> , 1963	HC	1M 1F	58 69	Sitosterol	Unknown	unknown	18-20	2190 240	43800 4800	none
Reeves, 1959	Healthy volunteers	7M 1F	31-61	20% Sitosterol suspension	Tall Oil	Cytellin™	6-18	30 (5 patients) 60 (3 patients)	2700-3240	The only side effect was a slight to moderate increase in the number of daily bowel movements but no actual diarrhea occurred.
Cooper, 1958	Atherosclerotic patients	25	unknown	Sitosterol	Tall Oil	Cytellin™	12	140	42000	Three patients reported constipation, the rest thought their stools were bulkier and looser.
Lehmann, 1957.	MI (6) Angina (6) Familial tuberous xanthomatosis (1) HC (1)	9M 6F	adults	Sitosterol	Tall Oil	Cytellin™	20	30-150	9000-45000	none
Farquhar <i>et al</i> , 1956	Patients with myocardial infarction	15 M	26-45	Sitosterol	No data	Capsule (Cytellin™)	12.0-18.0	84-168	15120-45360	none

1 NC = Normocholesterolemic; FH = Familial Hypercholesterolemia; NIDDM = Non-insulin dependent diabetes mellitus; HC = Hypercholesterolemia

2 Total combined dose phytosterols where phytosterols are a mixture

**Table 10-2 Summary of Safety of Orally Administered Phytosterols in Human Subjects from Published Sources (continued)**

Reference	Population			Study Drug			Exposure			Safety
	Disease State	Number & Sex	Age Range or Mean (yr)	Phytosterol	Plant Source	Dosage Form	Dosage g/day <sup>2</sup>	Duration (days)	Extent g/d x days x subjects	Adverse Events Reported
Sachs and Weston, 1956	5 healthy subjects; 1 FH	6	Unknown	Sitosterol and sitostanol	Tall Oil	Cytellin™	9-12	56	3024-4032	none
	4 healthy subjects; 2 CAD; 3HC	9					9-45	90-180	7290-72900	none
	1 biliary cirrhosis	1	73				18	28	504	none
Lesesne <i>et al.</i> , 1955	6 with HC 5 with atherosclerotic and/or hypertensive heart disease	4M 3F	33-55	Mixtures of phytosterols, primarily Sitosterol	Soybean (n=3) Tall Oil (n=4)	Powder	9 plus extra 3 g with extra meals	84-224 Ave 192	14112 Ave 12096	1 Subject: Fatigue and unexplained weight loss of 10 lb.; 1 Subject: on weight reduction diet for 2 months prior to treatment, continued to lose weight. No other events reported.
Best <i>et al.</i> , 1955	12 HC 2 volunteers	10M 4F	33-77	Sitosterol	Tall Oil	Cytellin™	20-25 on occasion 50	91-448 Ave 280	98000	none
Barber <i>et al.</i> , 1955	Coronary artery disease	18M 8F	unknown	Sitosterol	Unknown	palatable biscuit	9	147	34398	none
Joyner <i>et al.</i> , 1955	4 hypertension, the other angina pectoris, 1 HC	I part: 4F&3M II part: 2 HC	39-50 F 34-62 M	13% Sitosterol 85% Sitosterol	Tall-Oil	Cytellin™	6-15	28	3780	none
Best <i>et al.</i> , 1954	2 Volunteers 7 HC	9	unknown	Sitosterol	Tall Oil	Cytellin™	5-6	91-203 Ave 154	8316	none

1 NC = Normocholesterolemic; FH = Familial Hypercholesterolemia; NIDDM = Non-insulin dependent diabetes mellitus; HC = Hypercholesterolemia

2 Total combined dose phytosterols where phytosterols are a mixture.

## 10.3

## Tall Oil Phytosterol Effects on Vitamin and Nutrient Levels

Table 10-3 summarizes the effects of phytosterols on plasma levels of vitamins and nutrients in humans. There have been a number of reports which indicate that phytosterols esterified with fatty acids may interfere with the uptake of fat soluble vitamins and nutrients, primarily carotenoids, from the intestine. All of these reports were for esters dissolved in margarine. The effect may depend on incomplete hydrolysis of the fatty acid esters with the ester remaining in the intestine and acting as a reservoir to hold fat-soluble vitamins. The impact on human safety is not clear. These changes are small enough that they could be offset by supplementation of the diet with these vitamins or nutrients. The impact of free phytosterols on the absorption of drugs and hormones has not been studied to the same extent. As reported by Shipley *et al.*, [1958], Cytellin™ phytosterols had no effect on vitamin D absorption in rats and dogs. In addition, Gylling and Miettinen [1998] reported that stanol ester had no effect on serum estradiol levels in postmenopausal women with coronary artery disease. Phytrol™ is not expected to exhibit any difference in activity in this regard compared to the phytosterols in Take Control™ and Benecol™.

**Table 10-3 Effect of Phytosterols on Plasma Levels of Vitamins in the Human**

Reference	Phytosterols Administered	Vitamin E Levels	Carotene levels $\alpha+\beta$ carotene	Vitamin D or A	Lycopene
Weststrate & Meijer, 1998	Esters of soy sterols	-	-23%	-	-20%
	Sheanut esterified sterols	-	-43%	-	-40%
	Rice bran esterified sterols	-	-8.3%	-	-5.1%
	Stanol esters	-	-22%	-	-22%
Uusitupa, 1998	Stanol esters	No change	No change	-	No change
Mensink & Plat, 1998.	Stanol esters	-10%	-19%	-	-
Simell <i>et al.</i> , 1998.	Stanol esters	$\alpha$ tocopherol / LDL ratio unchanged	$\beta$ -carotene/LDL ratio - 17.6%	No change in Vit. A or D	-
Gylling <i>et al.</i> , 1996	Stanol esters	-9.8% ( $p<0.001$ )	-30% ( $p<0.001$ )	-	-

Data summarized above is from phytosterols administered in margarine.

11.0

**DETERMINATION OF THE GRAS STATUS OF TALL OIL DERIVED  
PHYTOSTEROLS USED AS AN INGREDIENT OF VEGETABLE OIL-  
BASED SPREADS**

An independent panel of recognized experts, qualified by their scientific training and relevant national and international experience to evaluate the safety of food and food ingredients, was requested by Novartis Consumer Health Inc., to determine the Generally Recognized as Safe (GRAS) status of a natural product from tall oil (wood pulp derived). The Expert Panel statement follows:

## EXPERT PANEL STATEMENT

### DETERMINATION OF THE GRAS STATUS OF TALL OIL DERIVED PHYTOSTEROLS USED AS AN INGREDIENT OF VEGETABLE OIL-BASED SPREADS

The undersigned, an independent panel of recognized experts (hereinafter referred to as Expert Panel), qualified by their scientific training and relevant national and international experience to evaluate the safety of food and food ingredients, was requested by Novartis Consumer Health Inc. to determine the Generally Recognized as Safe (GRAS) status of a natural product from tall oil (wood pulp derived). This product, which shall be referred to in this document as Phytrol™, contains phytosterols and stanols for addition to a vegetable oil-based spread at a level up to 12%. Phytrol™ is manufactured by Forbes Medi-Tech, Inc. and supplied to Novartis Consumer Health Inc. for manufacture of the spread product. The intended purpose of Phytrol™ is to help maintain normal cholesterol levels in blood. Because the phytosterol/stanol ingredients in two similar products, Benecol™ and Take Control™, are in many ways the same as Phytrol™ and are currently being marketed for the same intended use with FDA's knowledge and consent, the principal focus of this review and evaluation is on the nature and relevance of any differences between these marketed products and Phytrol™. A comprehensive search of the scientific literature for safety and toxicity information on phytosterols or stanols and their presence in food was conducted through October 1999 and made available to the Expert Panel. A report by CanTox U.S. Inc. based on this comprehensive literature review and analysis of safety and nutritional studies of phytosterols and stanols aided and facilitated the work of the Expert Panel. The Expert Panel independently evaluated materials submitted by Novartis Consumer Health Inc. and its agent, CanTox U.S. Inc., as well as other materials deemed appropriate or necessary. Following independent, critical evaluation, the Expert Panel conferred and unanimously agreed to the decision described herein.

The composition of Phytrol™ is intermediate between that of the phytosterol/stanols ingredients of Take Control™ and Benecol™. Henceforth, for the purpose of this document, these ingredients will be simply referred to as Take Control™ and Benecol™. Table 1 compares approximate compositions of the three products. While significant natural variation may occur in specific component content of each product, the data in Table 1 indicate that on average, levels of the individual component phytosterols in Phytrol™ do not significantly exceed the highest level present in either Take Control™ or Benecol™. Both of these products are marketed in the US with FDA's knowledge and consent based solely on independent self-GRAS determinations. Thus, in terms of the main phytosterol and stanol components, the Expert Panel concludes that Phytrol™ is substantially the compositional equivalent to a mixture of Take Control™ and Benecol™ and that the main components are considered GRAS for their intended use in vegetable oil-based spreads at a level not to exceed 12% for any given product or portion size.

**Table 1 Comparison of Phytosterol Compositions (% by weight)**

<b>Sterol</b>	<b>Take Control™ (phytosterols from Soybean Oil)</b>	<b>PHYTROL™ (Forbes Medi-Tech natural Tall Oil Phytosterols)</b>	<b>Benecol™ (Hydrogenated Tall and Soybean Oil Phytosterols)</b>
Sitosterol	42	47	4
Campesterol	25	14	3
Stigmasterol	18		
Brassicasterol	5		
Sitostanol	2	26	64
Campestanol		5	23
Minor Sterols	8	8	6
Total Phytosterols	98	69	13
Total Phytostanols	2	31	87

*Take Control™ uses vegetable sterols esterified with fatty acids. Data are averages of batches from ADM and Cargill. Benecol™ is a mixture of vegetable and tall oil phytosterols that have been hydrogenated and then esterified with fatty acids. The Phytrol™ values are typical of most batches and fit within current specifications. Percentages refer only to sterol content and are approximations. The estimated sterol proportions will vary depending on the methodology used for measurement. The response factors vary between different sterols when compared within the same detection system, e.g. flame ion detection (FID). Further, the response factors vary between detection systems, i.e. FID versus GC/MS or LC/MS. The Phytrol™ phytosterols were quantitated by the use of GC-FID using in-house standards. The figures for Take Control™ and Benecol™ are area under the curve estimates by GC/MS.*

The difference in constituent phytosterol profiles among the three products arises from two main factors: (1) phytosterol source with respect to Take Control™ and (2) use of hydrogenation processing for Benecol™. A third difference arises from the use of fatty acid esterification of the Take Control™ and Benecol™ products to modify their solubility properties for product application purposes.

Phytrol™, which is derived from tall oil, contains significant levels of sitosterol and campesterol, similar to those occurring in Take Control™ which is derived predominantly from soybean oil. The specifications for Phytrol™ are given in Table 2. Unlike Take Control™, Phytrol™ contains only minor quantities of stigmasterol and brassicasterol but significant levels of the saturated (stanol) compounds, sitostanol and campestanol, as occurs in Benecol™. Benecol™, which is also derived from tall oil, utilizes hydrogenation to saturate double bonds present in the sterol components, thus converting most

phytosterols to stanols, predominantly sitostanol and campestanol. However, a minor portion of the phytosterols remain unhydrogenated following hydrogenation processing of Benecol™ as indicated by the data in Table 1. Many of the minor components in each of the three products are unsaturated congeners of the same saturated structures. Consequently, hydrogenation tends to reduce, somewhat, the level and diversity of minor components. However, Benecol™ still contains a low percentage of minor component phytosterols that remain unsaturated, in the range of 2% to 3% (ref. Benecol™ submission).

**Table 2**

**Proposed food-grade specifications for Phytrol™**

Phytosterol content	> 95%
Sitosterol	38% to 60%
Sitostanol	14% to 34%
Campesterol	9% to 18%
Campestanol	2% to 14%
Total major sterols	> 86%
Loss on drying (water)	< 5%
Solvents	< 0.5%
Residue on ignition	< 0.1%
Heavy metals	< 10 ppm
Lead	< 0.25 ppm
Total aerobic count	< 10,000 CFU/g
Combined molds & yeasts	< 100 CFU/g
Coliformes	negative
E. Coli	negative
Salmonella	negative

The phytosterols in Take Control™ are not hydrogenated and contain up to 8% by weight of minor sterol and non-sterol components (see Table 1). Similarly, Phytrol™ contains a number of minor components primarily representing variations in the position and/or number of double bonds within sitosterol (C29) and campesterol (C28) structures. Also present are trace quantities of C<sub>15</sub>-C<sub>25</sub> saturated aliphatic alcohols. These minor, long chain alcohol components are substances commonly found in the diet and the Expert Panel concluded they were not toxic contaminants and their presence does not adversely affect

general recognition of safety of the intended use of Phytrol™.

The only potential, safety related difference in composition among the three products lies with their minor phytosterol-like components. Comparative analysis of these substances in Take Control™, Benecol™, vegetable sterols (produced by ADM) and Phytrol™ revealed a total of 45 major and minor sterol components. Twenty-two were present in Phytrol™.

None were unique to Phytrol™ as all 22 components were found either in Take Control™, Benecol™, or vegetable sterols or reported present in vegetable oils in the scientific literature. Of the 22 phytosterol-like components in Phytrol™, 15 were found in Take Control™, 11 in Benecol™ and 12 in vegetable sterols. Three phytosterol-like components were found in Phytrol™ which were not identified in Take Control™, Benecol™, or vegetable sterols. These were sitosta-4, 6, 22-triene (C<sub>29</sub>H<sub>46</sub>), 24-methylene lophenol (C<sub>29</sub>H<sub>48</sub>O), and alpha-1-sitosterol. All three have been previously reported present in vegetable oils by Mennie et al [1994]; Goad [1966]; Grob et al [1994]; and Bortolomeazzi et al [1996]. The Expert Panel concludes that the presence of Phytrol™'s phytosterol-like minor components in either GRAS products or vegetable oils allays any safety concern about these components under the intended conditions of use of Phytrol™.

While Take Control™ and Benecol™ have been esterified and Phytrol™ has not, the Expert Panel, based on the following data and discussion, concludes that their esterification does not affect either the safety or effectiveness of these products.

**Table 3: Comparative Effectiveness of Sterol Products in a Margarine Matrix**

<b>Product:</b>	<b>Take Control™</b>	<b>Benecol™</b>	<b>Phytrol™ in a Margarine Matrix</b>
Dosage	3 g per day <sup>1</sup>	2.7 g per day <sup>1</sup>	1.5 g/70kg/day <sup>2</sup>
Δ Total Cholesterol <sup>3</sup>	-8.3%	-7.3%	-9.1%
Δ LDL Cholesterol <sup>3</sup>	-13.0%	-13.0%	-15.5%
Δ HDL Cholesterol <sup>3</sup>	+0.6%	+0.1%	-4.4%

<sup>1</sup> These data are from the Westrate [1998] study, which indicates that the average body weight of the men was 82.5 kg and for women was 66.8 kg. Converting the dose to an equivalent body weight (bw) basis, the dose of Take Control™ would have been 2.5 g /70 kg bw in men and 3.0 g / 70 kg bw in women. The same conversion to an equivalent body weight yields a Benecol™ dose of 2.3 g / 70 kg bw in men and 2.9 g / 70 kg bw in women.

<sup>2</sup> These data are from the Jones et al [1999] study conducted in males, only.

<sup>3</sup> Values are corrected for the change that occurred in the control group.

Take Control™ and Benecol™ products have been esterified with common vegetable oil fatty acids to enhance their solubility in a vegetable oil product matrix. Lack of esterification does not detract from the observed equivalence of Phytrol™ phytosterols compared to Take Control™ and Benecol™. In fact, the ester forms are rapidly de-esterified *in vivo* through the action of lipase enzymes in order to yield the active free phytosterols. Only the free phytosterol or stanol affect blood cholesterol levels. Thus, equivalence between gut concentrations of the active free phytosterol plus stanols in esterified products (Take Control™ and Benecol™) compared to non-esterified (Phytrol™) is established by clinical studies showing closely similar effects on cholesterol lowering for the time and amount consumed (Table 3). The somewhat lower effectiveness of Take Control™ and Benecol™ on a gram/day basis is probably a reflection of a less than complete de-esterification of the phytosterol and stanols esters in Take Control™ and Benecol™ following their ingestion. These data demonstrate that the tall oil phytosterols in Phytrol™ are substantially equivalent to the other two products in decreasing total and LDL serum cholesterol values. Furthermore, there is no evidence of any significant effect on plasma HDL levels for any of the three products. In the Phytrol™ study reported in Table 3, decreases in mean HDL values of 6.3 and 10.7 % were reported in the control and treated groups, respectively. The 4.4 % difference between the groups attributable to Phytrol *per se* was well within the 6.5% coefficient of variation for this assay procedure in the reported study and is not clinically significant.

As with the previous clinical studies involving Take Control™ and Benecol™, no adverse effects were observed in any of the subjects in the study by Jones *et al* [1999] including those consuming Phytrol™.

As Phytrol™ is intended for use as an ingredient in vegetable oil-based spreads at levels of free phytosterols and stanols similar to that of Take Control™ and Benecol™, Phytrol™'s use and purpose in food are identical to that of the two currently marketed products, Take Control™ and Benecol™. The Expert Panel, based on a critical review of the information assembled and discussed by CanTox U.S. Inc., concludes that plant phytosterols and stanols as described and used by Lipton (Take Control™) and McNeil (Benecol™) in their submissions to FDA of January 11, 1999 and February 18, 1999, respectively, are GRAS by scientific procedures for their intended use in vegetable oil-spreads. The published studies relied upon for this conclusion are listed in Attachment I. In view of these facts and given the compositional equivalency of Phytrol™ to Take Control™ and Benecol™, the Expert Panel concludes that the intended use of Phytrol™ does not raise questions concerning safety, including those related to potential, adverse nutritional effects. Such nutritional matters have been addressed and adequately resolved in the course of establishing the self-determined GRAS status of Take Control™ and Benecol™ based on studies included in Attachment I.

Based on the critical evaluation discussed above, the Expert Panel has determined that Phytrol™, meeting the specifications cited above, is generally recognized as safe (GRAS) by scientific procedures when used in vegetable oil-based spreads for the purpose of helping to maintain a healthy blood cholesterol level, providing it is used in accordance with current good manufacturing practice (21 CFR § 182.1(b)) in an amount not to exceed 12% phytosterol plus stanol in the finished spread.



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This concludes the Expert Panel statement. Attachment 1, as cited within the Expert Panel's discussion of Phytrol™ is redundant with the reference section (Section 12.0) of this notification and has not been included.

- Anderson et al. 1926. J. Am. Chem. Soc. 48, 2987. As cited in: The Merck Index: An Encyclopedia of Chemicals, Drugs, and Biologicals. 12th Edition. Eds: Budavari S, O'Neil MJ, Smith A, Heckelman PE, Kinneary JF. Merck & Co., Inc. Whitehouse Station NJ. p8697.
- Bae M, Mercer EI. 1970. The effect on long and short day photoperiods on the sterol levels in the leaves of *Solanum andigena*. *Phytochemistry*. 9:63-68.
- Baker VA, Hepburn PA, Kennedy SJ, Jones PA, Lea LJ, Sumpter JP, and Ashby J. 1999. Safety evaluation of phytosterol esters: Part 1. Assessment of oestrogenicity using a combination of *in vivo* and *in vitro* assays. *Food Chem Toxicol* 37(1):13-22.
- Barber JM, Grant AP. 1955. The serum cholesterol and other lipids after administration of sitosterol. *Br. Heart J.* 17: 296-298.
- Bean GA. 1973. Phytosterols. *Adv. Lipid Res.* 11:193-218.
- Becker M, Staab D, von Bergmann K. 1992. Long-term treatment of severe familial hypercholesterolemia in children: effect of sitosterol and bezafibrate. *Pediatr* 89:138-42.
- Becker M, Staab D, von Bergmann K. 1993. Treatment of severe familial hypercholesterolemia in childhood with sitosterol and sitostanol. *J Pediatr* 122:292-6.
- Best MM, Duncan CH, Van Loon EJ, Wathen JD. 1954. Lowering of serum cholesterol by the administration of a plant sterol. *Circulation* 10: 201-206.
- Best MM, Duncan CH, Van Loon EJ, Wathen JD. 1955. The effects of sitosterol on serum lipids. *Am. J. Med.* 19: 61-70.
- Bhattacharyya A, Connor WE, Lin DS. 1991. Sluggish sitosterol turnover and hepatic failure to excrete sitosterol into bile cause expansion of body pool of sitosterol in patients with sitosterolemia and xanthomatosis. *Arterioscler. Thromb.* Vol 11. 5:1287-1294
- Bhattacharyya A, Connor WE. 1974.  $\beta$ -sitosterolemia and xanthomatosis: A newly described lipid storage disease in two sisters. *J Clin Invest.* 53: 1033-1043.
- Bhattacharyya A, Connor WE. Familial diseases with storage of sterols other than cholesterol. The metabolic basis of inherited disease, 4<sup>th</sup> ed., New York, McGraw-Hill, Chapter 31, pp656-669.
- Bhattacharyya A and Lopez. 1979. Absorbability of plant sterols and their distribution in rabbit tissues. *Biochim. Biophys. Acta* 574:146 - 153.
- Bhattacharyya, A.K., Connor, W.E., and Lin, D.S. 1983. The origin of plant sterols in the skin surface lipids in humans: from diet to plasma to skin. *J. Invest. Dermat.* 80, 294-296.
- Boberg KM, Einarsson K, Bjorkhem I. 1990a. Apparent lack of conversion of sitosterol into C<sub>24</sub>-bile

- acids in humans. *J Lipid Res.* 31: 1083-1088.
- Boberg KM, Lund E, Olund J, Bjorkhem I. 1990b. Formation of C<sub>21</sub> bile acids from plant sterols in the rat. *J Bio Chem.* (263) 14: 7967-7975.
- Boberg KM, Skrede S. 1988. Content of sitosterol, cholestanol, and cholesterol in very low density lipoproteins of rat liver perfusate. *Scand J gastroenterol.* 23: 442-448.
- Borgstrom B. 1968. Quantitative aspects of the intestinal absorption and metabolism of cholesterol and  $\beta$ -sitosterol in the rat. *J. Lipid. Res.* 9:473-481.
- Borgstrom B. 1967. Absorption of fats. *Proc. Nutr. Soc.* 26:34-46.
- Bortolomeazzi R, Pizzale L, Novelli A, Conte L. 1996. *La Rivista Italiana delle Sostanze Grasse* 73: 457.
- Burck PJ, Thakkar AL, Zimmerman RE. 1982. Antifertility action of a sterol sulphate in the rabbit. *J Reprod Fert* 66:109-12.
- Cerqueira MT, McMarry Fry M, Connor WE. 1979. The food and nutrient intakes of the Tarahumara Indians of Mexico. *Am J Clin Nutr* 32:905-15.
- Clayton PT, Whitfield P, and Iyer, K. 1998. The role of phytosterols in the pathogenesis of liver complications of pediatric parenteral nutrition. *Nutrition.* 14, 158-164.
- Cobb MM, Salen G, Tint GS. 1997. Comparative effect of dietary sitosterol on plasma sterols and cholesterol and bile acid synthesis in a sitosterolemic homozygote and heterozygote subjects. *J Am College Nutr.* 16 (6): 605-613.
- Cooper EE. 1958. Dietary and pharmaceutical approaches to atherosclerosis; special reference to beta-sitosterol. *Texas St. J. Med.* 54:29-36.
- Crombie WML. 1961. Chemical composition of plant tissues and related data. In *Long Biochemist handbook.* Spon, ed. pp. 937-1053.
- Davis DL. 1971. Sterol distribution within green and air cured tobacco. *Phytochemistry*:11: 489-494.
- Denke MA. 1995. Lack of efficacy of low-dose sitostanol therapy as an adjunct to a cholesterol-lowering diet in men with moderate hypercholesterolemia. *Am J Clin Nutr* 61:392-6.
- Duncan CH, Best MM. 1963. Long-term use of sitosterol as a hypocholesterolemic agent. *J Kentucky med. Ass.* 61: 45-47.
- Farquhar JW, Smith RE, Dempsey ME. 1956. The effect of beta sitosterol on the serum lipids of young men with arteriosclerotic heart disease. *Circulation* 14:77-82.
- Glover J, Green C. 1953. Sterol metabolism 3. The distribution and transport of sterols across the

- intestinal mucosa of the guinea pig. *Biochem. J.* 67:308-316.
- Goad LJ. 1967. Aspects of phytosterol biosynthesis. In: *Terpenoids in Plants*. Ed. Pridham JB. Academic Press Inc. London. pp159-190.
- Gould RGI, et al. 1969. Absorbability of  $\beta$ -sitosterol in humans. *Metabolism* 18:652-662, 1969.
- Gould RGI. 1955. Absorbability of beta sitosterol. *Trans.N.Y.Acad.Sci.* 18, 129-134.
- Grob K, Biedermann M, Artho A, Schmid J. 1994. *La Rivista Italiana delle Sostanze Grasse* 71: 533.
- Grundy S, et al. 1969. The interaction of cholesterol absorption and cholesterol synthesis in man. *J Lipid Res.* 10: 304-315.
- Gylling H, Miettinen TA. 1994. Serum cholesterol lipoprotein metabolism in hypercholesterolemic NIDDM patients before and during sitostanol ester-margarine treatment. *Diabetologia* 37:773-80.
- Gylling H, Miettinen TA. 1995. The effect of cholesterol absorption inhibition on low density lipoprotein cholesterol level. *Atherosclerosis* 117:305-8.
- Gylling H, Miettinen TA. 1996. Effects of inhibiting cholesterol absorption and synthesis on cholesterol and lipoprotein metabolism in hypercholesterolemic non-insulin-dependent diabetic men. *J. Lipid Research* 37:1776-85.
- Gylling H, Radhakrishnan R, Miettinen TA. 1997. Reduction of serum cholesterol in postmenopausal women with previous myocardial infarction and cholesterol malabsorption induced by dietary sitostanol ester margarine. *Circulation*. No 12. 96: 42264231.
- Gylling H, Siimes MA, Miettinen TA. 1995. Sitostanol ester margarine in dietary treatment of children with familial hypercholesterolemia. *J. Lipid Research* 36:1807-12.
- Gylling HK, Puska P, Vartiainen E, et al. Serum retinol, beta-tocopherol, carotens, and lipid peroxide production during serum cholesterol lowering by sitostanol ester margarine in a mildly hypercholesterolemic population (abstract 3379). *Circulation* 1996; 94 (Suppl 1): 1-578.
- Gylling HK, and Miettinen TA. Efficacy of plant stanol ester in lowering cholesterol in postmenopausal women and patients with diabetes. *Postgraduate Medicine -A Special Report: New developments in the dietary management of high cholesterol*, November 1998, p.39.
- Heinemann T, Axtmann G, von Bergmann K. 1993. Comparison of intestinal absorption of cholesterol with different plant sterols in man. *Eur J Clin Investig* 23:827-31.
- Heinemann T, Leiss O, von Bergmann K. 1986. Effect of low-dose sitostanol on serum cholesterol in patients with hypercholesterolemia. *Atheroscler* 61:219-23.
- Hellman L, et al. 1953. Metabolism of cholesterol-4-C14 in hypercholesterolemia. *Circulation* 8:434-435.

- Hesse D. 1878. Uber phytosterin und cholesterin. *Ann.* 192, 175-179.
- Hepburn PA, Homer SA, and Smith M. 1999. Safety evaluation of phytosterol esters: Part 2. Subchronic 90-day oral toxicity study on phytosterol esters: A novel functional food. *Food Chem Toxicol* 37(5):521-532.
- Hirai K, et al. 1986. Cholesterol, phytosterol and polyunsaturated fatty acid levels in 1982 and 1957 Japanese diets. *J. Nutr. Sci. Vitaminol.* 32: 363-372.
- Iyer KR, Spitz L, Clayton P. 1998. New insight into mechanisms of parenteral nutrition-associated cholestasis: role of plant sterols. *J. Ped. Surg.* 33 (1) 1-6.
- Jones PJH, Ntanos FY, Raeini-Sarjaz M, Vanstone CA. 1999. Cholesterol-lowering efficacy of a sitosterol-containing phytosterol mixture with a prudent diet in hyperlipidemic men. *Am J. Clin Nutr* 69: 1140-50.
- Joyner C, Kuo PT. 1955. The effect of sitosterol administration upon the serum cholesterol level and lipoprotein pattern. *Am. J. med. Sci.* 230: 636-647.
- Kris-Etherton PM, Etherton TD, Pearson TA, Phyllips K, Reed R, Windhouser M, Champagne C, Lefevre M. 1998. Stanol supplemented margarine (SM) lowers LDL-C in moderately hypercholesterolemic subjects fed an average American diet (AAD). *Experimental Biology Meeting, San Francisco.*
- Kritchevsky DL, Davidson LM, Mosbach EH, Cohen BI. 1981 Identification of acid steroids in feces of monkeys fed  $\beta$ -sitosterol. *Lipids* 16: 77-78.
- Lees AM, Mok HY, Lees RS, McCluskey MA, Grundy SM. 1977. Plant sterols as cholesterol-lowering agents: clinical trials in patients with hypercholesterolemia and studies of sterol balance. *Atheroscler* 28:325-38.
- Lehmann JH. 1957. Clinical experiences with beta-sitosterol, a new anti-cholesterolemic agent. *NW. Med., Seattle* 56: 43-46.
- Leikin AI, Brenner RR. 1989. Fatty acid desaturase activities are modulated by phytosterol incorporation in microsomes. *Bichim et Biophys Acta* 1005:187-91.
- Lesesne JM, Castor CW, Hoobler SW. 1955. Prolonged reduction in human blood cholesterol levels induced by plant sterols. *Univ. Mich. Med. Bull.* 21: 13-17.
- Lin DS, Connor WE, Phillipson BE. 1984. Sterol composition of normal human bile. Effects of feeding shellfish (marine) sterols. *Gastroenterology* 86, 611-617.
- Ling WH, Jones PJH. 1995. Dietary phytosterols: a review of metabolism, benefits and and side effects. *Life Sciences* 57:195-206.

- Malini T, Vanithakumari G. 1990. Rat toxicity studies with beta-sitosterol. *J. Ethnopharma* 28:221-34.
- Malini T, Vanithakumari G. 1991. Antifertility effects of beta-sitosterol in male albino rats. *J. Ethnopharm* 35:149-53.
- Malini T, Vanithakumari G. 1993. Effect of beta-sitosterol on uterine biochemistry: a comparative study with estradiol and progesterone. *Biochem Molecular Biol Int* 31:659-68.
- Mattson FH, Grundy SM, Crouse JR. 1982. Optimizing the effect of plant sterols on cholesterol absorption in man. *Am J. Clin Nutr* 35:697-700.
- Mellies MJ, Ishikawa TT, Glueck CJ, Bove K, Morrison J. 1976. Phytosterols in aortic tissue in adults and infants. *J. Lab Clin Med. Vol* 88. 6: 914-921.
- Mennie D, Moffat C, McGill A. 1994. *Journal of High Resolution Chromatography* 17: 831.
- Mensink PR, Plat J. October 1998. Studies of effects of plant stanol ester on lipid parameters, vitamin levels, and thrombogenic factors in a normocholesterolemic population. Dallas conference: Plant sterol ester: a new tool in dietary management of cholesterol.
- Miettinen TA, Vanhanen H. 1994. Dietary sitostanol related to absorption, synthesis and serum level of cholesterol in different apolipoprotein E phenotypes. *Atheroscler* 105:217-26.
- Miettinen TA, Puska P, Gylling H, Vanhanen H, Vartiainen E. 1995. Reduction of serum cholesterol with sitostanol-ester margarine in a mildly hypercholesterolemic population. *New Engl J Med* 333:1308-12.
- Miettinen TA, Tilvis RS, Kesaniemi YA. 1990. Serum plant sterol and cholesterol precursors reflect cholesterol absorption and synthesis in volunteers of a randomly selected male population. *Am J Epidemiol* 131:20-31.
- Miettinen TA. 1980. Phytosterolemia, xanthomatosis and premature atherosclerotic arterial disease: a case with high plant sterol absorption, impaired sterol elimination and low cholesterol synthesis. *Eur J Clin Invest.* 10: 27-35.
- Moghadasian MH, et al. 1999. Histologic, hematologic, and biochemical characteristics of Apo-E-KO mice E-deficient mice: Effects of dietary cholesterol and phytosterols. *Laboratory Investigation* 79:355-364.
- Morton GM, Lee SM, Buss DH, Lawrence P. 1995. Intakes and major dietary sources of cholesterol and phytosterols in the British diet. *J. Hum. Nutr. Diet.* 8: 429-440.
- Nair P, et al. 1984. Diet, nutrition intake, and metabolism in populations at high and low risk for colon cancer: dietary cholesterol,  $\beta$ -sitosterol, and stigmasterol. *Am J. Clin Nutr.* 40: 927-930.

- Plat J and Mensink R. 1998. Safety aspects of dietary plant sterols and stanols. Postgraduate Medicine -A Special Report: New developments in the dietary management of high cholesterol, November 1998.
- Pollak OJ, Kritchevsky D. 1981. Monographs on atherosclerosis. ISBN 3-8055-0568-X.
- Pollak OJ. 1985. Effect of plant sterols on serum lipids and atherosclerosis. *Pharmac Ther* 31:177-208.
- Reeves JE. 1959. Hypercholesterolemia: treatment with sitosterol and a low cholesterol diet. *Am. Practit.* 10: 1193-1197.
- Robinson M, Wnorowski G, Dreher M. 1998. Dietary stanols as anti-hypercholesterolemic agents: a 90-day sub-chronic feeding trial as a safety assessment in the rat. #1202 Abstract from F.A.S.B. meeting in San Francisco. (Nabisco Inc, East Hanover, NJ 07936 and Product Safety Labs, East Brunswick, NJ 08816).
- Sachs BA and Weston RE. 1956. Sitosterol administration in normal and hypercholesterolemic subjects; the effect in man of sitosterol therapy on serum lipids and lipoproteins. *Archs intern. Med.* 97: 738-752.
- Salen G, Ahrens EH, Grundy SM. 1970. Metabolism of beta-sitosterol in man. *J. Clin Invest.* 49:952-967.
- Salen G, Kwiterovich PO, Shefer S, Tint GS, Horak I, Shore V, Dayal B, Horak E. 1985. Increased plasma cholestanol and 5 $\alpha$ -saturated plant sterol derivatives in subjects with sitosterolemia and xanthomatosis. *J. Lipid Res* 26: 203-209.
- Salen G, Shefer L, Nguyen L, Ness GC, Tint GS, Shore V. 1992. *J. Lipid Res* 33:945-55.
- Salen G, Shore V, Tint GS, Forte T, Shefer S, Horak I, Horak E, Dayal B, Nguyen L, Batta AK, Lindgren T, Kwiterovich PO. 1989. *J Lipid Res* 30:1319-30.
- Schlierf G, Oster P, Heuck CC, Raetzer H, Schellenberg B. 1978. Sitosterol in juvenile type II hyperlipoproteinemia. *Atheroscler* 30:245-48.
- Shefer S, Salen G, Bullock J. 1994. *Hepatology* 20:213-19.
- Shibley RE, Pfeiffer RR, Marsh MM, Anderson RC. 1958. Sitosterol feeding: Chronic animal and clinical toxicology and tissue analysis. *Circulation Research*, 6: 373-382.
- Shoppee CW. 1964. *Chemistry of steroids* (London: Butterworth).
- Simell O, Tammi A, Gylling H, Pulkki K, Ronnema T, and the STRIP Study Group. October 1998. Studies with plant stanol ester in children. Dallas conference: Plant sterol ester: a new tool in dietary management of cholesterol.

- Slesinski RS, Turnbull D, Frankos VH, Wolterbeek APM and Waalkens-Berendsen DH. 1999. Developmental toxicity study in vegetable-oil derived stanol fatty acid esters. *Regulatory Toxicology and Pharmacology* 29: 227-233.
- Subbiah MTR. 1971. Subject review: Significance of dietary plant sterols in man and experimental animals. *Mayo Clin Proc.* 46: 549-559.
- Subbiah MTR and Kuksis A. 1973. Differences in metabolism of cholesterol and sitosterol following intravenous injection in rats. *Biochim et Biophys Acta* 306:95-105.
- Swell, et al. 1954. Sterol specificity of pancreatic cholesterol esterase. *Proc. Soc. Exp Biol. Med.* 87:21-218.
- Sylvén C and Borgström B. 1969. Absorption and lymphatic transport of cholesterol and sitosterol in the rat. *J. Lipid Res.* 10: 179-182.
- Turnbull D, Frankos VH, Leeman WR, and Jonker D. 1999. Short-term tests of estrogenic potential of plant stanols and plant stanol esters. *Regulatory Toxicology and Pharmacology* 29:211-215.
- Turnbull D, Frankos VH, van Delft JHM, and deVogel N. 1999. Genotoxicity evaluation of wood-derived and vegetable-oil derived stanol esters. *Regulatory Toxicology and Pharmacology* 29: 205-210.
- Turnbull D, Whittaker MH, Frankos VH, and Jonker D. 1999. 13-week oral toxicity study with stanol esters in rats. *Regulatory Toxicology and Pharmacology* 29:216-226.
- Uusitupa M. October 1998. Study of efficacy of plant stanol ester in subjects consuming NCEP diets. Dallas conference: Plant sterol ester: a new tool in dietary management of cholesterol.
- Vanhanen HT, Blomqvist S, Ehnholm C, Hyvonen M, Jauhiainen M, Torstila I, and Miettinen TA. 1993. Serum cholesterol, cholesterol precursors, and plant sterols in hypercholesterolemic subjects with different apoE phenotypes during dietary sitostanol ester treatment. *J. Lipid Res* 34:1535-44.
- Vanhanen HT, Kajander J, Lehtovirta H, and Miettinen TA. 1994. Serum levels, absorption efficiency, fecal elimination and synthesis of cholesterol during increasing doses of dietary sitostanol esters in hypercholesterolemic subjects. *Clin Sci* 87:61-7.
- Vanhanen HT and Miettinen TA. 1992. Effects of unsaturated and saturated dietary plant sterols on their serum contents. *Clin Chim Acta* 205:97-107.
- von Bergman K and Lutjohann D. November 1998. Review of the absorption and safety of plant sterols. Postgraduate medicine: A Special Report: New developments in the dietary management of high cholesterol.
- Waalkens-Berendsen DH, Wolterbeek APM, Wijnands MVW, Richold M, Hepburn PA. 1999. Safety evaluation of phytosterol esters. Part 3. Two-generation reproduction study of phytosterol esters in rats. A novel functional food. *Food Chem Toxicol* 37(7):683-696.

Weisweiler P, Heinemann V, Schwandt P. 1984. Serum lipoproteins and lecithin: cholesterol Acyltransferase (LCAT) activity in hypercholesterolemic subjects given  $\beta$ -sitosterol. *Int J. Clin Pharmacol Therapy Tox* 22:204-6.

Weststrate JA and Meijer GW. 1998. Plant sterol-enriched margarines and reduction of plasma total- and LDL cholesterol concentrations in normocholesterolemic and mildly hypercholesterolemic subjects. *Eur J. Clin Nutr* 52: 334-343.

Whittaker MH, Frankos VH, Wolterbeek APM, and Waalkens-Berendsen DH. 1999. Two-generation reproductive toxicity study of plant stanol esters in rats. *Regulatory Toxicology and Pharmacology* 29:196-204.

# **CANTOX**

**HEALTH SCIENCES INTERNATIONAL**

## **REPORT FOR EXPERT GRAS EVALUATION OF PHYTROL™ PHYTOSTEROL ENRICHED CEREALS, FOOD BARS, FRUIT DRINKS, AND SMOOTHIE BEVERAGES**

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**APPENDICES**

- 1 **Report to the Expert Panel Regarding the Evaluation of the GRAS Status of a Novartis Consumer Health Product Containing Phytrol™ in a Vegetable Oil Spread**
- 2 **Report Amendment to the GRAS Expert Panel Regarding the Revised Manufacturing Process and Specifications of Phytrol™ for Use in a Novartis Consumer Health Vegetable Oil Spread**
- 3 **Supplemental Estimates for the Daily Intake of Phytrol™ from the Consumption of Cereal(s), Food Bar(s), Fruit Drink(s) and Smoothie Beverage(s) In the United States**
- 4 **Signed Expert Panel Statement Regarding the Evaluation of the GRAS Status of a Novartis Consumer Health Product Containing Phytrol™ in a Vegetable Oil Spread**
- 5 **Signed Expert Panel Statement Regarding the Report Amendment to the GRAS Expert Panel Regarding the Revised Manufacturing Process and Specifications of Phytrol™ for Use in a Novartis Consumer Health Vegetable Oil Spread**
- 6 **References**

## 1.0 INTRODUCTION

Plant sterols, or phytosterols, are similar to cholesterol in their chemical structure but have a significantly lower absorption rate and are gaining popularity in consumer products. Phytosterols, which are found in a variety of plant sources, cannot be manufactured by the human body and are obtained exclusively through the diet. Because phytosterols can compete with and reduce cholesterol absorption in the gastrointestinal tract, they provide health benefits by helping to maintain healthy blood cholesterol levels. The Department of Health and Human Services of the FDA has recently published an Interim Final Rule [21 CFR Part 101] in the Federal Register entitled "Food Labeling: Health Claims; Plant Sterol / Stanol Esters and Coronary Heart Disease" which permits such claims.

Recently marketed vegetable oil spreads such as Take Control™, Benecol™, and Reducol™ are three examples of phytosterol-containing products intended to promote healthy cholesterol levels. The Take Control™ and Benecol™ vegetable oil spreads contain up to 20% by weight added fatty acid esterified phytosterols. Reducol™ incorporates Phytrol™ phytosterols, in an un-esterified form, into vegetable oil spread at a concentration of up to 12% by weight. This represents an application and phytosterol content which is identical to that of the other two products whose incorporation rate is 20% by weight of esterified phytosterols which is in turn 60% by weight free phytosterol.

Take Control™ is manufactured by Lipton. The esterified phytosterols therein are predominantly sterols derived from vegetable oil. Benecol™ is manufactured by McNeil Consumer Healthcare and contains esterified hydrogenated tall oil and vegetable oil sterols (stanols). The third product, Reducol™, is produced by Novartis Consumer Health Inc. and incorporates Phytrol™ in a vegetable oil spread product. Phytrol™ consists of hydrogenated and non-hydrogenated tall oil phytosterols manufactured by Forbes Medi-Tech Inc. at the Quest facility in Houston, Texas. Reducol™ is intended to be consumed in a manner identical to Benecol™ and Take Control™ as all three products are intended to provide consumers with an additional product choice in order to promote a healthy cholesterol level.

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GRAS status has already been established for Reducol™ vegetable oil spread and its constituent Phytrol™ sterol and stanol mixture. Reducol™, Benecol™, and Take Control™ have all been self-affirmed by their respective manufacturers as Generally Recognized as Safe (GRAS). The U.S. Food and Drug Administration (FDA) has been notified of and after review is not in disagreement with these opinions. Establishment of the GRAS status of Reducol™ was based in part upon the principle of substantial equivalence, such that any differences between Phytrol™ in a vegetable oil spread and the products Benecol™ and Take Control™ are inconsequential and that all data and considerations of safety and use which apply to Benecol™ and Take Control™ apply equally to Phytrol™. Furthermore, the concentrations of the major component phytosterols and stanols in Phytrol™ are comparable to, or lower than, the aggregate levels in the other products considered GRAS.

The manufacturers of Benecol™ and Take Control™ have each marketed additional self-determined GRAS products under their respective brand names. The FDA is aware of these additional phytosterol-containing products and has considered them in the recently published Interim Final Rule for phytosterol health claims regarding coronary heart disease. Similarly, the Altus Foods Company, a joint venture between Novartis Consumer Health Inc. and Quakerfoods Company, has incorporated the Phytrol™ phytosterol product into a variety of food products such as cereal(s), food bar(s), fruit drink(s) and smoothie beverage(s); all of which are intended for consumption by those individuals seeking to promote a healthy cholesterol level. These may be viewed as additional dietary sources of phytosterols, however, their intended use is in amounts which would provide an intake of Phytrol™ comparable to the intake of Phytrol™ when consumed in the Reducol™ vegetable oil spread. This is discussed in greater detail in Section 5.0 of this document and in the supplemental intake assessment found in Appendix 3.

An independent recognized expert, qualified by scientific training and relevant national and international experience to evaluate the safety of food and food ingredients, has been commissioned by Altus Foods to determine the Generally Recognized as Safe (GRAS) status of the proposed Phytrol™ phytosterol enriched food products. It is proposed that the Altus Foods phytosterol

enriched cereal(s), food bar(s), fruit drink(s), and smoothie beverage(s) be considered GRAS based upon the fact that they contain the same phytosterol product, Phytrol™, as contained in Reducol™. Furthermore, their consumption would be in an amount equal to or less than the amount from Reducol™. These phytosterol-enriched products are intended to provide additional product choices for consumers. In summary, the phytosterols in the food products:

- 1) Are of compositional equivalence to the phytosterol constituents found in the Reducol™ vegetable oil spread and are of similar constituent nature to the phytosterol esters found in other currently marketed products, particularly Take Control™;
- 2) Have an expected safety and physiologic activity profile equivalent to the current application of Phytrol™ in Reducol™;
- 3) Are to be consumed in an amount similar to that from the currently marketed Reducol™ product and based upon intake assessments of the expected additional intake of Phytrol™ phytosterols, from use in phytosterol enriched cereal(s), food bar(s), fruit drink(s), and smoothie beverage(s), would not present any additional risk to consumers of such products;
- 4) Are to be consumed in an amount which is at least as great as that identified by FDA as the minimum efficacious amount for which a coronary heart disease health claim may be permitted.

This report provides a summary of necessary technical, safety and product information and considerations to support an evaluation by a qualified expert as to whether the use of Phytrol™ in phytosterol enriched cereal(s), food bar(s), fruit drink(s), and smoothie beverage(s) may be also considered to be generally recognized as safe based on scientific procedures. The assessment of safety is based upon the Report to the Expert Panel used to determine the GRAS status of Phytrol™ as it was employed in the Reducol™ vegetable oil spread, provided herein as Appendix 1. Certain sections of this document, particularly those regarding the safe history of use, structure-activity relationships, ADME, preclinical and clinical toxicology directly reference the previous Report to the Expert Panel. Further information regarding the phytosterol source, Phytrol™ product specifications, constituent chemical identities, and the method of Phytrol™ production at the Quest facility is provided in the Report Amendment to the GRAS Expert Panel Regarding the Revised Manufacturing process and Specifications of Phytrol™ for use in a Novartis Consumer Health

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Vegetable Oil Spread, as found in Appendix 2.

**1.1 Regulatory Basis for GRAS Status**

As described in 62 Fed. Reg. 18938, 18960 (April 17, 1997) (proposed 21 C.F.R. §170.36), Altus Foods Company wishes to make the determination that the use of Phytrol™ tall oil phytosterols, as manufactured by Forbes Medi-Tech Inc. at the Quest facility, in phytosterol enriched cereal(s), food bar(s), fruit drink(s), and smoothie beverage(s) at a level of 0.6 grams free phytosterols in a single product serving is Generally Recognized as Safe (GRAS). The concentration of Phytrol™ found in these products will result in a total daily intake of 1.8 grams of phytosterols per day if three servings per day of the Altus Foods products are consumed as recommend by the manufacturer. The determination of GRAS status would be supported by a review by an expert qualified by scientific training and experience to evaluate the safety of food and food ingredients using scientific procedures and would assert exemption from the pre-market approval requirements of the Federal Food, Drug and Cosmetic Act.

Phytrol™ tall oil phytosterols are derived from coniferous trees at the Quest facility in Houston, Texas. The composition of Phytrol™ in the proposed products adheres to the same product specifications as that found in the vegetable oil spread Reducol™ and is described in Appendix 2.

The constituent phytosterols within Phytrol™ are also substantially equivalent to the phytosterols found in Take Control™ and the hydrogenated vegetable oil / tall oil phytosterols in Benecol™. All three vegetable oil spreads are currently self-determined to be GRAS by their manufacturers and are available in the marketplace with the full knowledge of the FDA.

This report provides information required by proposed 21 CFR. §170.36(c)(2), (3), and (4) to support an evaluation by a qualified expert in fulfillment of the requirements of 21 CFR. §170.36(c)(4)(I)(c). The requirements of the proposed regulation are described below in Table 1-1.

**Table 1-1: Subset of Requirements for GRAS Determination by Scientific Procedures**

Proposed Rule 21 CFR Section	Specific Requirements
Identity and Specifications: 170.36(c)(2)	Notice must include detailed information about the identity of the notified substance, including chemical name, structural formula, quantitative composition, method of manufacture, characteristic properties, specifications, etc.
Self-Limiting Levels of Use: 170.36(c)(3)	Notice must include any self-limiting levels of use of the substance.
Technical Evidence of Safety: 170.36(c)(4)(i)(A)	Notice must include a detailed summary of the basis for determination that use of the substance is GRAS by scientific procedures. Summary should include a comprehensive discussion of, and citations to, generally available and accepted scientific data, information, methods, or principles used to establish safety, as well as consideration of probable consumption and cumulative effect of the substance in the diet.
Basis for Concluding Expert Consensus: 170.36(c)(4)(i)(C)	Notice summary of a scientific procedure GRAS determination must include the basis for concluding that there is a consensus among qualified experts that there is reasonable certainty that the substance is not harmful under the intended conditions of use.

The scientific and technical data presented herein, in the original Report to the Expert Panel [Appendix 1], and in the Amendment Report to the Expert Panel [Appendix 2] are in support of a GRAS determination by a qualified expert on behalf of Altus Foods Company for an additional food use of the Forbes Medi-Tech Phytosterol product Phytrol™ to be consumed in phytosterol enriched cereal(s), food bar(s), fruit drink(s), and smoothie beverage(s). These supportive materials were obtained from the following sources and are available for further review by the qualified expert:

- 1) The Report to the Expert Panel as prepared for Novartis Consumer Health Inc. This document is the basis for the GRAS Notification supplied to the FDA by Novartis Consumer Health Inc. for the vegetable oil spread later marketed as Reducol™. A complete copy of the Report to the Expert Panel has been provided in Appendix 1 and is intended to serve as a reference to the health and safety of the Phytrol™ tall oil phytosterol product;
- 2) Further information regarding the phytosterol source, Phytrol™ product specifications, constituent chemical identities, and the method of Phytrol™ production at the Quest facility is provided in the Report Amendment to the GRAS Expert Panel Regarding the Revised Manufacturing Process and Specifications of Phytrol™ for use in a Novartis Consumer Health Vegetable Oil Spread, as found in Appendix 2;

- 3) An intake assessment based on the 1989 - 1991 USDA CSFII for the consumption of a Phytrol™ phytosterol enriched cereal(s), food bar(s), fruit drink(s), and smoothie beverage(s) is incorporated into this document. A supplemental intake assessment based upon more recent but less reliable data (1994 - 1996 USDA CSFII) is included for completeness in Appendix 3;
- 4) A copy of the original signed statement issued by the Expert Panel following their review of the information provided in the original Report to the Expert Panel regarding the safety of Phytrol™ when employed in a vegetable oil spread (Reduacol™) has been provided in Appendix 4;
- 5) A copy of the original signed statement issued by the Expert Panel following their review of the information provided in the Amendment Report to the Expert Panel regarding the phytosterol source, Phytrol™ product specifications, constituent chemical identities, and the method of production at the Quest facility for Phytrol™ when employed in a vegetable oil spread (Reduacol™) has been provided in Appendix 5;
- 6) A compilation of the scientific literature for data on the safety of sterols, particularly phytosterols, conducted in the preparation of the Report to the Expert Panel concerning Phytrol™ and information from recent GRAS notifications for substantially equivalent products (e.g., Take Control™ and Benecol™) is supplied in Appendix 6;

The determination of GRAS status is based upon affirmation by a qualified expert that the substance is not harmful under the intended conditions of use and that it is equivalent to other GRAS Phytrol™ phytosterol-containing products currently marketed in the United States. By meeting the requirements outlined in the Proposed Rule for substances Generally Recognized as Safe (21 CFR Parts 170 *et al.*) in Volume 62, Number 74 of the April 17, 1997 Federal Register, Pages 18937-18964, it is assumed that the requirements outlined in Parts 201 *et al.* of the Federal Food, Drug, and Cosmetic Act for this product would have been met.

## 1.2 Equivalence to Current GRAS Products

The Phytrol™ phytosterols contained within the Altus Foods phytosterol enriched cereal(s), food bar(s), fruit drink(s), and smoothie beverage(s) are also available in the Novartis Consumer Health product Reduacol™. Phytrol™, which is based upon tall oil phytosterols, is manufactured by Forbes Medi-Tech Inc. at the Quest facility. Phytrol™ phytosterols in a vegetable oil spread have been previously determined as having GRAS status. A chemical analyses of the Phytrol™ phytosterols

in Reducoil™, which are employed in the proposed phytosterol enriched Altus products, is available in the Amendment Report to the Expert Panel found in Appendix 2. Inclusion of these phytosterols in the proposed products is not expected to materially affect their physiologic properties. Therefore, Phytrol™ found in Reducoil™ and in the proposed Altus Foods phytosterol enriched cereal(s), food bar(s), fruit drink(s), and smoothie beverage(s) may be considered equivalent with respect to safety, physiologic properties, product specifications, constituent chemical identities, and the method of Phytrol™ production at the Quest facility. Accordingly, the previous Expert Panel Report regarding the application of Phytrol™ in a vegetable oil spread (Reducoil™) has been provided as Appendix 1. The Amendment Report to the Expert Panel regarding the revised manufacturing process and specifications of Phytrol™ for use in the Novartis Consumer Health vegetable oil spread product (Reducoil™) has been provided as Appendix 2. The Expert Panel statements of conclusion regarding the GRAS status of the original and the Quest produced Phytrol™ have been provided in Appendices 4 and 5.

### *1.2.1 Composition*

The composition of Phytrol™ exhibits a ratio of major sterol to stanol fractions intermediate to that of the phytosterols in Take Control™ and Benecol™. Table 1-2 compares the approximate phytosterol composition of each product. Three batches of Phytrol™, manufactured using the Quest process, were analyzed by GC-FID. The results demonstrate that the major phytosterols in the final product fall within the revised product specifications. GC-FID data presented in the original Report to the Expert Panel are also provided for comparison. While significant natural variation may occur in specific component content, the data in Table 1-2 indicate that concentration of the major component phytosterols and stanols in Phytrol™ are comparable to or below the aggregate levels in the other GRAS phytosterol products.

Table 1-2: Analysis of Phytosterol Products by GC-FID

	Sitosterol (%w/w)	Sitostanol (%w/w)	Campesterol (%w/w)	Campestanol (%w/w)
<b>Phytrol™ Batch Number (Quest)</b>				
272	57.8	23	6.5	2.9
273	61.4	19.8	7	2.6
274	60.9	21.2	6.4	3.1
<b>Original Phytrol™</b>	47	26	14	5
<b>Take Control™</b>	42	2	25	
<b>Benecol™</b>	4	64	3	23

The variation in constituent phytosterol profile among the three products arises from two main factors: phytosterol source and use of hydrogenation processing. A third variation arises from fatty acid esterification of the phytosterols in the GRAS products Take Control™ and Benecol™ in order to modify solubility properties for product application purposes. The safety of ingested phytosterols has been thoroughly reviewed and discussed in the process of establishing GRAS status for Phytrol™ when employed in Reducol™ vegetable oil spread [see Appendix 1].

#### 1.2.1.1 Source and Hydrogenation

Phytrol™ contains significant levels of sitosterol and campesterol, similar to those occurring in Take Control™. Unlike Take Control™, Phytrol™ contains only minor quantities of stigmasterol and other sterols but significant levels of the naturally occurring saturated (stanol) compound sitostanol and, to a lesser extent, campestanol. Both of these compounds are found in high concentration in Benecol™. This is due to the extensive hydrogenation process used in Benecol™ production which saturates most of the double bonds present in the sterol components, converting them to stanols, predominantly sitostanol and campestanol. This is in contrast to the hydrogenation process component used to restore Phytrol™ stanol levels to product specifications. The hydrogenation process component is necessary due to the relocation of the Phytrol™ production site to the Quest

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manufacturing facility in Houston, Texas. This relocation resulted in the inclusion of southern conifers as the primary source of tall oil phytosterols. This source is naturally lower in stanols and in order to remain within product specifications, a standard food industry compensatory hydrogenation process component was added. Further information regarding product specifications and the manufacturing process is available in the Amendment Report to the Expert Panel in Appendix 2.

Since many of the minor components in these products are variously unsaturated congeners of the same saturated structures, hydrogenation may reduce, somewhat, the diversity of minor components. However, Benecol™ still contains a range of minor phytosterols of up to 6% [ref. Benecol™ GRAS notification in Appendix 6]. The phytosterols in Take Control™ are not hydrogenated and contain up to 8% by weight of minor sterol and non-sterol components. Similarly, Phytrol™ contains a number of the same minor components, primarily representing variation in the position and / or number of double bonds within sitosterol (C<sub>29</sub>) and campesterol (C<sub>28</sub>) structures (see Appendix 2 for compositional details). Also present are trace quantities of C<sub>15</sub>-C<sub>25</sub> saturated aliphatic alcohols.

All minor components in Phytrol™ are substances commonly found in the diet and in one or both of the other GRAS products. A single minor component phytosterol was present in the Quest manufactured Phytrol™, sitosta-6-ene [CAS RN 152914-67-5], which was not present in Phytrol™ as manufactured originally by Forbes, or in Take Control™ or Benecol™. This compound has been found in other products employing hydrogenated oil. The following references for this compound are available:

1. Softly BJ, Huang AS, Finley JW, Petersheim M, Yarger RG, Chrysam MM, Wieczorek RL, Otterburn MS, Manz A, Templeman GJ. Composition of Representative SALATRIM Preparations. Nabisco Foods Group, East Hanover, NJ 07936, USA
2. J. Agric. Food Chem., 42(2), 461-467. 1994 CODEN:JAFCAU.ISSN:0021-8561.

**Confidential GRAS Report for Expert Review****1.2.1.2      Esterification**

The Phytrol™ phytosterols in Reducol™ are in a free non-esterified form while those in Take Control™ and Benecol™ have been esterified to common vegetable oil fatty acids to enhance their solubility in a vegetable oil product matrix. Esterification does not materially affect the substantial equivalence of Phytrol™ to the other products. As discussed in the sections on physiologic equivalence (1.3.3.1) and safety (1.3.4) as found in the Report to the Expert Panel in Appendix 1, the ester forms are rapidly de-esterified *in vivo* through the action of lipase enzymes, yielding the active free phytosterols. Esterification does affect quantitative parameters of equivalence. The Take Control™ and Benecol™ products contain fatty acid esterified phytosterols which are approximately 60% by weight phytosterol, the remainder being fatty acids. Accordingly, 0.6 grams of Phytrol™ are equivalent to the phytosterol content of 1.0 grams of the esterified products. Phytrol™, when employed in the proposed Altus Foods products will not be esterified to any vegetable oil fatty acids.

**1.2.2      Intended Use and Intake**

The intended application of Phytrol™ in this instance is to incorporate it into phytosterol enriched cereal(s), food bar(s), fruit drink(s), and smoothie beverage(s) in an amount of 0.6 grams per product serving. The proposed products are intended to be consumed three times per day, resulting in a daily Phytrol™ intake of 1.8 grams, based upon product labeling. This represents a recommended daily Phytrol™ intake which is similar to that of the GRAS vegetable oil spread product Reducol™ and is comparable to that of the other GRAS vegetable oil spread products, based on free phytosterol content. This is further summarized in Table 1-3. The proposed products are intended to provide consumers with additional product choices with the goal of maintaining a healthy cholesterol level. The 0.6 grams of Phytrol™ per serving have a sterol content of at least 65% or 0.39 grams of plant sterols, the remainder being stanols. This meets or exceeds the per-serving amount designated by FDA in the Interim Final Rule to permit a labeling health claim regarding coronary heart disease.

**Table 1-3: Intended Daily Intake of Existing GRAS Phytosterol Products**

		Take Control™	Reduocol™	Benecol™
Per Serving:		1.9 g (esters)	0.75 g	1.7 g (esters)
Servings Per Day		1 - 2	2	Up to 3
Daily Intake:	Esters	1.9 - 3.8	-	1.7 - 5.1
	Phytosterols	1.12 - 2.24	1.5	1.0 - 3.0

### 1.2.3 *Physiologic Properties*

The phytosterols in Phytrol™ are substantially equivalent in physiologic properties to those in Take Control™ and Benecol™ products in regards to their active form and their effects on blood cholesterol parameters, blood phytosterol levels and absorption of vitamins and nutrients. These factors were all taken into account in the determination of GRAS status of Phytrol™ when incorporated in the Reduocol™ vegetable oil spread. The incorporation of Phytrol™ into phytosterol enriched cereal(s), food bar(s), fruit drink(s), and smoothie beverage(s) to provide an aggregate intake of 1.8 grams per day does not pose any new or differential physiological properties when compared to the recommended serving of 1.5 grams per day of Phytrol™ found in Reduocol™. Furthermore, 0.6 grams per serving of Phytrol™ meets or exceeds the FDA's Interim Final Rule regarding labeling of health claims and coronary heart disease as Phytrol™ contains at least 65% sterols or 0.39 grams of sterol. In order to review the physiological properties of Phytrol™, please refer to Section 1.3.3 of Appendix 1.

### 1.2.4 *Safety*

The safety of ingested phytosterols has been thoroughly reviewed and discussed in the process of establishing the GRAS status of Phytrol™ when employed in Reduocol™ vegetable oil spreads as well as Take Control™ and Benecol™. The development of the Lipton product, Take Control™, has yielded substantial research into the safety of phytosterols, particularly sitosterol, campesterol, and stigmasterol. Similarly, the development of the McNeil product, Benecol™, has also yielded

substantial research into the safety of phytosterols, particularly sitostanol, campestanol and stigmasterol. The information used to establish the safety of the Phytrol™ product was based upon the principle of substantial equivalence between the constituent phytosterols found in all three of these products and is discussed in greater detail in Section 1.3.4 of Appendix 1.

## **2.0 CHEMICAL IDENTITY AND COMPOSITION**

The constituent phytosterols of which Phytrol™ is composed have been very well characterized. The composition of Phytrol™ in the proposed Altus Foods products has the same product specifications as the Phytrol™ employed in the manufacture of the Novartis vegetable oil spread Reducol™. Phytrol™ is manufactured by the Forbes Medi-Tech Company at the Quest facility and has been added to these products in order to help promote a healthy cholesterol level in the respective consumers of phytosterol enriched vegetable oil spread (Reducol™), cereal(s), food bar(s), fruit drink(s), and smoothie beverage(s). For further detail and information regarding the chemical identity and composition of Phytrol™, please refer to Section 3.0 of the Report to the Expert Panel, as contained in Appendix 1 and the Amendment Report to the Expert panel, as contained in Appendix 2.

## **3.0 PRODUCTION METHODS**

The method by which Phytrol™ is manufactured by Forbes Medi-Tech at the Quest facility is well established and is within compliance of Good Manufacturing Practices (GMPs). The relocation of the production of Phytrol™ to the Quest manufacturing facility in Houston, Texas has resulted in the inclusion of southern conifers as the primary source of tall oil phytosterols. This source is naturally lower in stanols and has resulted in the inclusion of a compensatory standard food industry hydrogenation process component to restore the stanol concentrations. The Quest production method of Phytrol is provided in Section 3.0 in Appendix 2.

**Confidential GRAS Report for Expert Review****4.0 INTENDED USE IN FOOD**

Phytosterols are a group of plant compounds naturally occurring in a variety of foods in the human diet, such as minor components in vegetable oils. The Altus Foods Company is interested in using the phytosterols found in the tall oil phytosterol blend Phytrol™, as manufactured by Forbes Meditech Inc., in phytosterol enriched cereal(s), food bar(s), fruit drink(s), and smoothie beverage(s). The projected types of foods, serving size, and the amount of Phytrol™ proposed for use in these food products has been provided below in Table 4-1. Phytrol™ will be incorporated into these proposed products in an amount intended to provide a consumer with approximately 1.8 grams of Phytrol™ per day from the consumption of three labeled servings of the various Altus Foods products. The use of Phytrol™ in these products is intended to provide additional choices to consumers of phytosterol products in order to help maintain healthy cholesterol levels as part of a diet low in saturated fat and cholesterol.

**Table 4-1: Proposed Altus Foods Company Products, Serving Sizes and Projected Phytrol™ Content**

<b>Proposed Food Product</b>	<b>Product Serving Size</b>	<b>Proposed Phytrol™ Content</b>
Breakfast Cereal(s)		
Cereal: Extruded	27 grams per serving	0.6 grams
Cereal: Flake	49 grams per serving	0.6 grams
Food Bar(s)	48 grams per serving	0.6 grams
Fruit Drink Beverage(s)	9.5 fluid ounces	0.6 grams
Smoothie Beverage(s)	9.5 fluid ounces	0.6 grams

**5.0 CONSUMER EXPOSURE****5.1 Introduction**

The intake of the proposed Altus Foods phytosterol enriched cereal(s), food bar(s), fruit drink(s), and

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smoothie beverage(s) in the United States was estimated in order to quantify the expected levels of phytosterol intake which may arise through use of these products. The calculations for intake were based upon the results of the U.S. Department of Agriculture (USDA) 1989 - 1991 Continuing Survey of Food Intakes by Individuals (CSFII). Calculations of the mean all-person intake, and 90<sup>th</sup> percentile per-user intake and percent consuming were made for the intake of cereal(s), food bar(s), fruit drink(s), and smoothie beverage(s). The intake of the Altus Foods phytosterol enriched cereal(s), food bar(s), fruit drink(s), and smoothie beverage(s) per person and per kilogram body weight was calculated for the following population groups based upon available information:

- Infants, ages 0 to 2;
- Children, ages 3 to 11;
- Female teenagers, ages 12 to 19;
- Male teenagers, ages 12 to 19;
- Female adults, ages 20 and up;
- Male adults, ages 20 and up; and,
- Total population (all population and gender groups combined).

A supplemental intake assessment, found in Appendix 3 [CanTox, 2000], provides estimates for the daily intake of Phytrol™ from the consumption of phytosterol enriched cereal(s), food bar(s), fruit drink(s), and smoothie beverage(s) developed from data contained within the more recent USDA 1994 - 1996 Continuing Survey of Food Intakes by Individuals (USDA CSFII 1994 - 1996) and the 1998 Supplemental Children's Survey (USDA CSFII 1998). These data indicate an increase in the number of users compared to the CSFII 1989 - 1991 database. However, the data in the 1994 - 1996 survey were gathered over a 2-day period, whereas the data in the 1989 - 1991 survey were gathered over a 3-day period. Therefore, the 1989 - 1991 data are generally considered to be more statistically reliable and scientifically rigorous than the 1994 - 1996 food intake survey and forms the basis for the following discussion of Phytrol™ phytosterol intake from the proposed food products. The supplemental intake assessment provided in Appendix 3 is based upon the 1994 - 1996 food intake survey has been provided as an additional reference for completeness and due diligence.

**5.2 Estimated Total Daily Intake of the Altus Foods Phytrol™ Enriched Cereal(s), Food Bar(s), Fruit Drink(s), and Smoothie Beverage(s)**

Food codes representative of all food products proposed to contain Phytrol™ were identified within the USDA CSFII 1989 - 1991 consumption survey. Ready-to-eat cereals were grouped into two separate categories, extruded (0.6 grams of Phytrol™ per 27 gram serving) and flake (0.6 grams of Phytrol™ per 49 gram serving), according to density and serving size (USDA, 1999). Food codes for bars (0.6 grams of Phytrol™ per 48 gram serving), fruit drinks (0.6 grams of Phytrol™ per 9.5 fl. oz. serving), and smoothies (0.6 grams Phytrol™ per 9.5 fl. oz. serving) were grouped to allow for separate determinations of Phytrol™ intake of from these food sources. The list of all food codes and the calculated percentages of Phytrol™ by weight used in estimating the intake of Phytrol™ from all assessed food groups remained unchanged between the 1989 - 1991 and 1994 - 1996 CSFII surveys. This information has been provided in the supplemental intake assessment [CanTox, 2000] found in Section A of Appendix 3.

Calculated estimates for the daily intake of Phytrol™ from each of the proposed individual food groups represent 3-day projected averages. Intake data for individuals within the USDA CSFII 1989 - 1991 survey were collated by computer and the resulting distributions analyzed statistically. All-person intake refers to the intake of Phytrol™ averaged over all people surveyed regardless of whether they consumed food products containing Phytrol™, hence the 'all-person' designation. Per-user intake refers to the intake of Phytrol™ by individuals who only consumed foods containing Phytrol™, hence the 'per-user' designation. Individuals within the survey were defined as users if they consumed one or more of the food products containing Phytrol™ on any 3 days of the survey.

Estimates for the mean and 90<sup>th</sup> percentile daily intake of Phytrol™ from each of the individual food products by population group have been summarized in Tables 5-1 to 5-7 and 5-8 to 5-14, on a milligram and mg/ kg body weight per day basis, respectively. Tables 5-7 and 5-14 summarize the estimates for the mean per person Phytrol™ intake by the total population (all ages) from each of the individual food products in milligram and milligram per kg body weight per day basis, respectively. Consumption of ready to eat cereals by the total population made the most significant

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contribution to the mean all-person intake of Phytrol™, 262 mg/person/day (5.85 mg/kg body weight/day). The heavy consumer (90<sup>th</sup> percentile) all-person intake of Phytrol™ from ready to eat cereals was determined to be 799 mg/person/day (17.4 mg/kg body weight/day). Approximately 46% of individuals within the total population were determined to be consumers of ready to eat cereals. All other food products made less significant (< 50 mg/person/day) contributions to the all person intake of Phytrol™ by the total population. This is expected due to the smaller number of individuals within the total population consuming these food products (*i.e.*, bars, fruit drinks, smoothies).

As with the total population, consumption of ready to eat cereals made the most significant contribution to the all-person intake of Phytrol™ when assessed on an individual population group basis (Tables 5-1 to 5-6 and Tables 5-8 to 5-13). The highest all-person mean intake of Phytrol™ was reported in male teenagers (aged 12 to 19) consuming ready to eat cereals, 454 mg/person/day (7.82 mg/kg body weight/day). However, when assessed on a per kilogram body weight basis, children (aged 3 to 11) consuming ready to eat cereals, experienced a higher mean all-person intake of 16.7 mg/kg body weight/day. The highest heavy consumer (90<sup>th</sup> percentile) all-person intake of Phytrol™ was also reported in male teenagers (1270 mg/person/day), but on a per kilogram body weight basis, in children (38.2 mg/kg body weight/day). This was expected, since children consume the largest amounts of food and energy on a body weight basis.

Tables 5-7 and 5-14 summarize the estimates for the mean per-user Phytrol™ intake by the total population (all ages) from each of the individual food products in milligram and mg/kg body weight/day, respectively. The consumption of ready to eat cereals and ready to drink fruit-drink mixtures made the most significant contributions to the mean per-user intake of Phytrol™ by the total population. Estimates for the mean per-user intake of Phytrol™ from ready to eat cereals and fruit drinks were 573 mg/person/day (12.8 mg/kg body weight/day) and 350 mg/person/day (7.52 mg/kg body weight/day), respectively. The heavy consumer (90<sup>th</sup> percentile) per-user intake of Phytrol™ for the total population from the consumption of ready to eat cereals was 1110 mg/person/day (27.2 mg/kg body weight/day), and 700 mg/person/day (14.88 mg/kg body

weight/day) for fruit drinks. All other food products (*i.e.*, food bars and smoothies) were determined to make less significant (< 550 mg/person/day) contributions to the per user intake of Phytrol™ by the total population.

As with the total population, ready to eat cereals and fruit drinks also made the most significant contributions to the mean per-user intake of Phytrol™ when assessed on an individual population group basis (Tables 5-1 to 5-6 and Tables 5-8 to 5-13). The highest per-user Phytrol™ intake was reported in male teenagers (aged 12 to 19) consuming ready to eat cereals, 784 mg/person/day (13.5 mg/kg body weight/day). However, when assessed on a per kilogram body weight basis, infants (age 0 to 2) experienced the highest mean per-user intake of 24.9 mg/kg body weight/day from the consumption of ready to eat cereals. The highest heavy consumer (90<sup>th</sup> percentile) per-user intakes were reported in male teenagers and infants consuming ready to eat cereals, 1510 mg/person/day and 47 mg/kg body weight/day, on a per-person and per kilogram body weight basis, respectively.

**Table 5-1 Estimated Daily Phytrol Intake From The Consumption of Various Food Products By Infants Aged 0 to 2 Years Within The United States**

Food Category	Food Product	% Users	Actual Number of Total Users	All-Person Consumption		Per-User Consumption	
				Mean (mg)	90th Percentile (mg)	Mean (mg)	90th Percentile (mg)
Baked goods and baking mixes	Bars	0.2	1	0.13	n/a	89.6	89.6
Beverages and beverage bases	Ready to Drink Fruit Drink Mixtures and Blends	13.2	100	34.4	88.0	260.8	524.0
	Smoothies	0.1	2	0.84	n/a	752.0	776.0
Breakfast cereals	Ready to Eat Cereals (extruded and flaked)	48.7	407	162.0	456.0	332.0	622.0

**Table 5-2 Estimated Daily Phytrol Intake From The Consumption of Various Food Products By Children Aged 3 to 11 Years Within the United States**

Food Category	Food Product	% Users	Actual Number of Total Users	All-Person Consumption		Per-User Consumption	
				Mean (mg)	90th Percentile (mg)	Mean (mg)	90th Percentile (mg)
Baked goods and baking mixes	Bars	2.1	32	4.53	n/a	221.0	358.0
Beverages and beverage bases	Ready to Drink Fruit Drink Mixtures and Blends	19.2	441	59.2	219.2	308.0	672.0
	Smoothies	0.9	8	2.64	n/a	282.4	338.0
Breakfast cereals	Ready to Eat Cereals (extruded and flaked)	76.8	1652	414.0	925.0	540.0	1010.0

**Table 5-3 Estimated Daily Phytol Intake From The Consumption of Various Food Products By Female Teenagers Aged 12 to 19 Within the United States**

Food Category	Food Product	% Users	Actual Number of Total Users	All-Person Consumption		Per-User Consumption	
				Mean (mg)	90th Percentile (mg)	Mean (mg)	90th Percentile (mg)
Baked goods and baking mixes	Bars	2.1	15	7.09	n/a	341.0	538.0
Beverages and beverage bases	Ready to Drink Fruit Drink Mixtures and Blends	19.9	165	74.4	262.0	372.4	700.0
	Smoothies	0.0	0	n/a	n/a	n/a	n/a
Breakfast cereals	Ready to Eat Cereals (extruded and flaked)	52.2	395	314.0	855.0	602.0	1040.0

**Table 5-4 Estimated Daily Phytol Intake From The Consumption of Various Food Products By Male Teenagers Aged 12 to 19 Years Within the United States**

Food Category	Food Product	% Users	Actual Number of Total Users	All-Person Consumption		Per-User Consumption	
				Mean (mg)	90th Percentile (mg)	Mean (mg)	90th Percentile (mg)
Baked goods and baking mixes	Bars	3.0	16	11.3	n/a	373.0	896.0
Beverages and beverage bases	Ready to Drink Fruit Drink Mixtures and Blends	17.5	129	60.0	185.2	341.6	788.0
	Smoothies	0.6	3	2.24	n/a	396.4	740.0
Breakfast cereals	Ready to Eat Cereals (extruded and flaked)	57.9	423	454.0	1270.0	784.0	1510.0

**Table 5-5 Estimated Daily Phytol Intake From The Consumption of Various Food Products By Female Adults Aged 20 years and Up Within the United States**

Food Category	Food Product	% Users	Actual Number of Total Users	All-Person Consumption		Per-User Consumption	
				Mean (mg)	90th Percentile (mg)	Mean (mg)	90th Percentile (mg)
Baked goods and baking mixes	Bars	1.2	53	3.18	n/a	258.0	467.0
Beverages and beverage bases	Ready to Drink Fruit Drink Mixtures and Blends	12.4	637	41.6	175.2	334.0	668.0
	Smoothies	0.2	11	0.48	n/a	241.2	370.4
Breakfast cereals	Ready to Eat Cereals (extruded and flaked)	39.2	2174	194.0	633.0	495.0	965.0

**Table 5-6 Estimated Daily Phytol Intake From The Consumption of Various Food Products By Male Adults Aged 20 Years And Up Within the United States**

Food Category	Food Product	% Users	Actual Number of Total Users	All-Person Consumption		Per-User Consumption	
				Mean (mg)	90th Percentile (mg)	Mean (mg)	90th Percentile (mg)
Baked goods and baking mixes	Bars	1.0	33	3.39	n/a	347.0	717.0
Beverages and beverage bases	Ready to Drink Fruit Drink Mixtures and Blends	9.6	379	40.4	n/a	420.0	760.0
	Smoothies	< 0.01	2	0.02	n/a	260.0	262.8
Breakfast cereals	Ready to Eat Cereals (extruded and flaked)	36.4	1400	250.0	869.0	687.0	1310.0

**Table 5-7 Estimated Daily Phytol Intake From The Consumption of Various Food Products For The Total U.S. Population (All Ages) Within the United States**

Food Category	Food Product	% Users	Actual Number of Total Users	All-Person Consumption		Per-User Consumption	
				Mean (mg)	90th Percentile (mg)	Mean (mg)	90th Percentile (mg)
Baked goods and baking mixes	Bars	1.4	150	3.93	n/a	291.0	538.0
Beverages and beverage bases	Ready to Drink Fruit Drink Mixtures and Blends	13.2	1851	46.0	175.2	350.0	700.0
	Smoothies	0.2	26	0.72	n/a	293.6	388.0
Breakfast cereals	Ready to Eat Cereals (extruded and flaked)	45.8	6451	262.0	799.0	573.0	1110.0

**Table 5-8 Estimated Daily Per Kilogram Body Weight Phytrol Consumption By Infants Aged 0 To 2 Years From Individual Proposed Food-Uses**

Food Category	Food Product	% Users	Actual Number of Total Users	All-Person Consumption		Per-User Consumption	
				Mean (mg/kg)	90th Percentile (mg/kg)	Mean (mg/kg)	90th Percentile (mg/kg)
Baked goods and baking mixes	Bars	0.2	1	0.01	n/a	6.89	6.89
Beverages and beverage bases	Ready to Drink Fruit Drink Mixtures and Blends	13.2	100	2.39	7.32	18.08	44.0
	Smoothies	0.1	2	0.05	n/a	45.6	45.6
Breakfast cereals	Ready to Eat Cereals (extruded and flaked)	48.7	407	12.1	36.1	24.9	47.0

**Table 5-9 Estimated Daily Per Kilogram Body Weight Phytrol Consumption By Children Aged 3 To 11 From Individual Proposed Food-Uses**

Food Category	Food Product	% Users	Actual Number of Total Users	All-Person Consumption		Per-User Consumption	
				Mean (mg/kg)	90th Percentile (mg/kg)	Mean (mg/kg)	90th Percentile (mg/kg)
Baked goods and baking mixes	Bars	2.1	32	0.21	n/a	10.2	20.6
Beverages and beverage bases	Ready to Drink Fruit Drink Mixtures and Blends	19.2	441	2.32	8.08	12.04	23.56
	Smoothies	0.9	8	0.13	n/a	13.84	20.4
Breakfast cereals	Ready to Eat Cereals (extruded and flaked)	76.8	1652	16.7	38.2	21.8	42.2

**Table 5-10 Estimated Daily Per Kilogram Body Weight Phytrol Consumption By Female Teenagers Aged 12 To 19 From Individual Proposed Food-Uses**

Food Category	Food Product	% Users	Actual Number of Total Users	All-Person Consumption		Per-User Consumption	
				Mean (mg/kg)	90th Percentile (mg/kg)	Mean (mg/kg)	90th Percentile (mg/kg)
Baked goods and baking mixes	Bars	2.1	15	0.13	n/a	6.41	8.82
Beverages and beverage bases	Ready to Drink Fruit Drink Mixtures and Blends	19.7	165	1.42	3.66	7.12	12.24
	Smoothies	0.0	0	n/a	n/a	n/a	n/a
Breakfast cereals	Ready to Eat Cereals (extruded and flaked)	52.2	395	5.98	15.4	11.5	21.8

**Table 5-11 Estimated Daily Per Kilogram Body Weight Phytrol Consumption By Male Teenagers Aged 12 To 19 From Individual Proposed Food-Uses**

Food Category	Food Product	% Users	Actual Number of Total Users	All-Person Consumption		Per-User Consumption	
				Mean (mg/kg)	90th Percentile (mg/kg)	Mean (mg/kg)	90th Percentile (mg/kg)
Baked goods and baking mixes	Bars	3.0	16	0.19	n/a	6.18	12.8
Beverages and beverage bases	Ready to Drink Fruit Drink Mixtures and Blends	17.5	129	1.02	3.8	5.8	12.36
	Smoothies	0.6	3	0.04	n/a	7.32	11.76
Breakfast cereals	Ready to Eat Cereals (extruded and flaked)	57.9	423	7.82	21.1	13.5	26.7

**Table 5-12 Estimated Daily Per Kilogram Body Weight Phytrol Consumption By Female Adults Aged 20 Years And Up From Individual Proposed Food-Uses**

Food Category	Food Product	% Users	Actual Number of Total Users	All-Person Consumption		Per-User Consumption	
				Mean (mg/kg)	90th Percentile (mg/kg)	Mean (mg/kg)	90th Percentile (mg/kg)
Baked goods and baking mixes	Bars	1.2	53	0.05	n/a	4.29	7.47
Beverages and beverage bases	Ready to Drink Fruit Drink Mixtures and Blends	12.4	637	0.66	2.3	5.28	10.48
	Smoothies	0.2	11	0.01	n/a	3.34	3.9
Breakfast cereals	Ready to Eat Cereals (extruded and flaked)	39.2	2174	3.04	9.89	7.76	15.5

**Table 5-13 Estimated Daily Per Kilogram Body Weight Phytrol Consumption By Male Adults Aged 20 Years And Up From Individual Proposed Food-Uses**

Food Category	Food Product	% Users	Actual Number of Total Users	All-Person Consumption		Per-User Consumption	
				Mean (mg/kg)	90th Percentile (mg/kg)	Mean (mg/kg)	90th Percentile (mg/kg)
Baked goods and baking mixes	Bars	1.0	33	0.04	n/a	3.94	9.33
Beverages and beverage bases	Ready to Drink Fruit Drink Mixtures and Blends	9.6	379	0.52	n/a	5.44	10.48
	Smoothies	< 0.001	2	< 0.01	n/a	4.36	4.52
Breakfast cereals	Ready to Eat Cereals (extruded and flaked)	36.4	1400	3.14	10.8	8.63	16.8

**Table 5-14 Estimated Daily Per Kilogram Body Weight Phytrol Consumption For The Total U.S. Population (All Ages) From Individual Proposed Food-Uses**

Food Category	Food Product	% Users	Actual Number of Total Users	All-Person Consumption		Per-User Consumption	
				Mean (mg/kg)	90th Percentile (mg/kg)	Mean (mg/kg)	90th Percentile (mg/kg)
Baked goods and baking mixes	Bars	1.4	150	0.08	n/a	5.88	11.2
Beverages and beverage bases	Ready to Drink Fruit Drink Mixtures and Blends	13.2	1851	0.99	2.84	7.52	14.88
	Smoothies	0.2	26	0.02	n/a	10.52	20.4
Breakfast cereals	Ready to Eat Cereals (extruded and flaked)	45.8	6451	5.85	17.4	12.8	27.2

### 5.3 Conclusions

Estimates of means and 90th percentile intakes based on sample sizes of less than 30 and 80, respectively, or perhaps higher depending on the coefficient of variation may not necessarily be considered statistically reliable due to limited sampling size. As such, estimates of the intake of Phytrol™ based on the consumption of smoothies and bars by some individual population groups may be unreliable. This type of methodology is generally considered to be 'worst case' in terms of potential intake as a result of several conservative assumptions made in estimating consumption. For example, it is often assumed that all food products within a food category contain the ingredient at the maximum level of use. In addition, it is well established that the length of a dietary survey affects the estimated consumption of individual users. Short term surveys, such as the typical 3-day dietary surveys, overestimate consumption of food products which are consumed relatively infrequently. Nevertheless, this intake assessment specific to Phytrol™, demonstrates a usage pattern in the total population for the proposed products below the label recommended amount in the all-person and per user consumption categories for both the mean and heavy (90<sup>th</sup> percentile) consumers.

### 6.0 STRUCTURE-ACTIVITY RELATIONSHIPS

Analysis of structure activity relationships is a useful approach to correlating the molecular structure of a chemical with its biological activity [Food and Drug Administration, 1982]. The phytosterols contained within the tall oil phytosterol product Phytrol™ must therefore be placed into Structure Category B as the FDA has classified mixtures as belonging to this group. However, the constituent phytosterols of Phytrol™ belong in Structure Category A, as having low toxic potential. Phytosterols also bear a close structural resemblance to the intermediate products of lipid metabolism in humans, namely cholesterol. In conclusion, the constituent phytosterol contained in Phytrol™, based upon this type of structure activity relationship, would indicate that this product would not cause any adverse effects in humans.

**7.0 ABSORPTION, DISTRIBUTION, METABOLISM AND EXCRETION**

The absorption, distribution, metabolism, and excretion of the phytosterols constituent to the Phytrol™ product have been very well characterized in the Report to the Expert Panel provided in Appendix 1. The Expert Panel Report was written in support of the use of Phytrol™ in a Novartis vegetable oil spread (Reduacol™). In the case of the Altus Foods phytosterol enriched cereal(s), food bar(s), fruit drink(s), and smoothie beverage(s), Phytrol™ is provided as a unique ingredient intended to help the consumer maintain a healthy cholesterol level. The recommended daily intake of Phytrol™ from the various Altus Foods products is 1.8 grams in total. The Phytrol™ in the proposed products is expected to have exactly the same ADME profile as the Phytrol™ provided in a vegetable oil spread (Reduacol™). In order to prevent a duplication of effort, please refer to Section 8.0 in Appendix 1 for further information on the ADME profile of Phytrol™.

**8.0 PRECLINICAL TOXICOLOGY**

The preclinical toxicological profile of Phytrol™ and the constituent phytosterols has been very well characterized in the Report to the Expert Panel provided in Appendix 1. The Expert Panel Report was written in support of the use of Phytrol™ in a Novartis vegetable oil spread (Reduacol™). In the case of the proposed Altus Foods phytosterol enriched cereal(s), food bar(s), fruit drink(s), and smoothie beverage(s), Phytrol™ is provided as an ingredient intended to help the consumer maintain a healthy cholesterol level. The recommended daily intake of Phytrol™ from the various Altus Foods products is 1.8 grams in total. The Phytrol™ in the phytosterol enriched cereal(s), food bar(s), fruit drink(s), and smoothie beverage(s) is expected to have the same preclinical toxicological profile as the Phytrol™ provided in a vegetable oil spread (Reduacol™). Please refer to Section 9.0 in Appendix 1 for further information on the preclinical toxicological profile of Phytrol™.

9.0 **CLINICAL TOXICOLOGY**

The clinical toxicological profile of Phytrol™ and the constituent phytosterols has been very well characterized in the Report to the Expert Panel provided in Appendix 1. The Expert Panel Report was written in support of the use of Phytrol™ in a Novartis vegetable oil spread (Reducol™). In the case of the proposed Altus Foods products, Phytrol™ is provided as an ingredient intended to help the consumer maintain a healthy cholesterol level. The recommended daily intake of Phytrol™ from the various Altus Foods products is 1.8 grams in total. The Phytrol™ in the phytosterol enriched cereal(s), food bar(s), fruit drink(s), and smoothie beverage(s) is expected to have the same clinical toxicological profile as the Phytrol™ provided in a vegetable oil spread (Reducol™). Please refer to Section 10.0 in Appendix 1 for further information on the clinical toxicological profile of Phytrol™.



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**SUPPLEMENTAL ESTIMATED DAILY INTAKE OF  
PHYTROL™ FROM THE CONSUMPTION OF READY TO  
EAT CEREALS, BARS, FRUIT DRINKS, AND SMOOTHIE  
BEVERAGES IN THE UNITED STATES**

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**ESTIMATED DAILY INTAKE OF PHYTROL™ FROM THE CONSUMPTION OF  
READY TO EAT CEREALS, BARS, FRUIT DRINKS, AND SMOOTHIE BEVERAGES  
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**ESTIMATED DAILY INTAKE OF PHYTROL™ FROM THE CONSUMPTION OF  
READY TO EAT CEREALS, BARS, FRUIT DRINKS, AND SMOOTHIE BEVERAGES  
IN THE UNITED STATES**

**OVERVIEW**

Estimates for the daily intake of Phytrol™ from ready-to-eat cereals, bars, fruit drinks, and smoothie beverages were developed based on data contained within the United States Department of Agriculture (USDA) 1994-1996 Continuing Survey of Food Intakes by Individuals (USDA CSFII 1994-1996) and the 1998 Supplemental Children's Survey (USDA CSFII 1998) (USDA, 2000). USDA CSFII (1994-1996) provides data on persons of all ages; whereas, USDA CSFII (1998) is limited to children birth through 9 years of age. Combined, these surveys provide the most up-to-date data for evaluating food use and food-consumption patterns in the United States, containing 4 years of data on individuals selected *via* stratified, multistage area probability sampling of American households within all 50 states.

USDA CSFII (1994-1996, 1998) survey data were collected from individuals and households *via* 24-hour dietary recalls administered on two non-consecutive days (Day 1 and Day 2) throughout all 4 seasons of the year. Data was collected in-person, a minimum of 3 days apart, on different days of the week, to achieve the desired degree of statistical independence. USDA CSFII (1994-1996) contains 2-day dietary food consumption data for more than 15,000 individuals of all ages, and 1-day data for 16,103 individuals. USDA CSFII (1998) contributes consumption data from an additional 5,559 children birth through 9 years of age to data reported for 4,253 children of the same ages within USDA CSFII (1994-1996). The overall USDA CSFII (1994-1996, 1998) response rate for individuals selected for participation in surveys was 81.5 and 77.5% for Day 1 and Day 2, respectively.

In addition to collecting information on the types and quantities of foods being consumed, USDA CSFII (1994-1996, 1998) collected physiological and demographic information from individual participants in the survey, such as sex, age, self-reported height and weight, and other variables useful in characterizing consumption. The inclusion of this information allows for further assessment of food intake based on consumption by specific population groups of interest within the total population. USDA sample weights were developed and incorporated with USDA CSFII (1994-1996, 1998) data to correct for potential under-representation of intake, that results from variability in samples due to survey design, non-response, or other factors.

Calculations of the mean all-person intake, mean per-user intake, 90<sup>th</sup> percentile intake, and percent consuming were performed for each of the following population groups:

- infants, ages 0 to 2;
- children, ages 3 to 11;
- female teenagers, ages 12 to 19;
- male teenagers, ages 12 to 19;
- female adults, ages 20 and up;
- male adults, ages 20 and up; and,
- total population (all population and gender groups combined).

Foods reported as being consumed during USDA CSFII (1994-1996, 1998) survey were coded according to a system developed by the USDA. The USDA database of food codes contains descriptions and portion size weights for greater than 7,500 food products and food mixtures as consumed by individuals. Ready-to-eat cereals were grouped into two separate categories, extruded (0.6 g Phytrol<sup>TM</sup> per 27 g serving) and flake (0.6 g Phytrol<sup>TM</sup> per 49 g serving), according to density and NLEA serving size (USDA, 1999). Food codes for bars (0.6 g Phytrol<sup>TM</sup> per 48 g serving), fruit drinks (0.6 g Phytrol<sup>TM</sup> per 9.5 fl. oz. serving), and smoothies (0.6 g Phytrol<sup>TM</sup> per 9.5 fl. oz. serving) were grouped to allow for separate determinations of the intake of Phytrol<sup>TM</sup> from these food sources. A summary of all food codes, use-levels, and adjustment factors included in our current intake assessment is provided in Section 1.

Estimates for the daily intake of Phytrol<sup>TM</sup> represent projected averages over 2 days (Day 1 and Day 2) of USDA CSF II (1994-96, 1998) data. Individual consumption data was collated by computer and the resulting distributions were analyzed statistically. All-person intake refers to the intake of Phytrol<sup>TM</sup> averaged over all people surveyed regardless of whether they consumed food products formulated with Phytrol<sup>TM</sup>, hence the 'all-person' designation. Per-user intake refers only to the intake of Phytrol<sup>TM</sup> by individuals consuming food products formulated with Phytrol<sup>TM</sup>, hence the 'per-user' designation. Individuals were considered users if they consumed one or more food products formulated with Phytrol<sup>TM</sup> on either Day 1 or Day 2 of the survey.

Estimates for the mean and 90<sup>th</sup> percentile intake of Phytrol<sup>TM</sup> from each of the individual food products by population group are summarized in Tables 2-1 to 2-7 and 3-1 to 3-7 of Section 2 and C, on a mg and mg per kg body weight per day basis, respectively. Tables 2-7 and 3-7 summarize the intake of Phytrol<sup>TM</sup> by the total population (all ages) from each of the individually assessed food products on a mg and mg/kg body weight/day basis, respectively.

Consumption of ready to eat cereals by the total population made the most significant contribution to the mean all-person intake of Phytrol™, 299.00 mg/person/day (6.65 mg/kg body weight/day). Heavy consumer (90<sup>th</sup> percentile) all-person intake of Phytrol™ by the total population from ready to eat cereals was 966.00 mg/person/day (20.80 mg/kg body weight/day). Approximately 40% of individuals within the total population were determined to be consumers of ready to eat cereals. Ready to drink fruit drink mixtures (105.00 mg/person/day) and bars (12.40 mg/person/day) proved to be less significant sources of Phytrol™ for the total population. Smoothies made the smallest (< 1.4 mg/person/day) contribution to the all person intake of Phytrol™, with less than 1% of individuals within the total population considered to be consumers of smoothies.

As with the total population, consumption of ready to eat cereals made the most significant contribution to the all-person intake of Phytrol™ on an individual population group basis (Tables 2-1 to 2-6 and Tables 3-1 to 3-6). The highest mean all-person intake of Phytrol™ was reported in male teenagers (aged 12 to 19) consuming ready to eat cereals, 503.00 mg/person/day (8.55 mg/kg body weight/day). However, on a per kilogram body weight basis, in children (aged 3 to 11) consuming ready to eat cereals, 20.20 mg/kg body weight/day. Heavy consumer (90<sup>th</sup> percentile) all-person intake of Phytrol™ was also the highest in male teenagers consuming ready to eat cereals (1490.00 mg/person/day), but on a per kilogram body weight basis, in children (48.60 mg/kg body weight/day). This is expected, since children consume the largest amounts of food and energy on a body weight basis.

Tables 2-7 and 3-7 summarize the estimates for the mean per user Phytrol™ intake by the total population (all ages) from each of the individual food products in mg and mg/kg body weight/day, respectively. Consumption of ready to eat cereals and ready to drink fruit drink mixtures made the most significant contributions to the mean per-user intake of Phytrol™ by the total population. Estimates for the mean per-user intake of Phytrol™ from ready to eat cereals and ready to drink fruit drink mixtures were 751.0 mg/person/day (16.70 mg/kg body weight/day) and 555.00 mg/person/day (12.50 mg/kg body weight/day), respectively. Heavy consumer (90<sup>th</sup> percentile) per-user intake of Phytrol™ by the total population was 1390.00 mg/person/day (35.00 g/kg body weight/day) from ready to eat cereals and 1060.00 mg/person/day (26.70 mg/kg body weight/day) from ready to drink fruit drink mixtures. Estimates of the mean user and heavy consumer intake of Phytrol™ by the total population from the consumption of bars and smoothies were less significant (< 783 mg/person/day) but similar.

As with the total population, ready to eat cereals and ready to drink fruit drink mixtures made the most significant contributions to the mean per-user intake of Phytrol™ in most individual population

groups (Tables 2-1 to 2-6 and Tables 3-1 to 3-6). Male teenagers (aged 12 to 19) consuming ready to eat cereals experienced the highest per-user intake of Phytrol™, 1120.00 mg/person/day (19.00 mg/kg body weight/day). However, on a per kilogram body weight basis, infants (age 0 to 2) consuming ready to eat cereals experienced the highest mean per-user Phytrol™ intake of 31.50 mg/kg body weight/day. Similarly, the highest heavy consumer (90<sup>th</sup> percentile) per-user intakes of 2090.00 mg/person/day and 61.40 mg/kg body weight/day were reported in male teenagers and infants consuming ready to eat cereals, respectively.

Only 73 individuals within the total population were considered to be consumers of smoothies formulated with Phytrol™. Of which, 43 consumers were determined to be children between the ages of 3 to 11. Mean and 90<sup>th</sup> percentile intake estimates based on sample sizes of less than 30 and 80, respectively, or perhaps higher depending on the coefficient of variation may not necessarily be considered statistically reliable due to limited sampling size (LSRO, 1995). As such, estimates of the intake of Phytrol™ based on the consumption of smoothies by some individual population groups are likely unreliable.

This type of methodology is generally considered to be 'worst-case' in terms of potential intake as a result of several conservative assumptions made in estimating consumption. For example, it is assumed all food products within a food category contain Phytrol™ at the maximum specified level of use. In addition, the length of a dietary survey can affect the accuracy of estimates of consumption for individual users. Short term surveys, *e.g.*, 1 or 2-day surveys, are well known to overestimate consumption of food products that are consumed on a relatively infrequent-basis.

**REFERENCES**

FASEB, 1995. Third Report on Nutrition Monitoring in the United States, Volume 1. Interagency Board for Nutrition Monitoring and Related Research. Prepared by the Life Sciences Research Office, Federation of American Societies for Experimental Biology. U.S. Government Printing Office, Washington.

U.S. Department of Agriculture (USDA). 1999. USDA Nutrient Database for Standard Reference, Release 13. Nutrient Data Laboratory Home Page, <http://www.nal.usda.gov/fnic/foodcomp>

U.S. Department of Agriculture (USDA). 2000. 1994-1996, 1998 Continuing Survey of Food Intakes by Individuals (CSFII) and Diet and Health Knowledge Survey (DHKS) (On CD-ROM) U.S. Department of Agriculture (USDA), Riverdale, MD, (Apr.) Supercedes PB98-500457 ; PB2000-500027

**SECTION 1**

**Representative USDA CSF II 1994-1996, 1998 Food Codes for  
Ready to Eat Cereals, Bars, Fruit Drinks, and Smoothie Beverages**

Representative USDA CSF II 1994-1996, 1998 Food Codes for  
Ready to Eat Cereals, Bars, Fruit Drinks, and Smoothie Beverages

Ready-To-Eat Cereals

*Extruded* (0.6 g Phytrol™ per 27 g serving size)

[Phytrol™] = 2.22 %

57000000	Cereal, NFS
57000050	Kashi Cereal, Not Specified as to Ready-to-eat or Cooked
57000100	Oat Cereal, NFS
57100100	Cereal, Ready-to-eat, NFS
57101000	All-bran Cereal
57101020	All Bran Cereal with Extra Fibre
57101500	Almond Delight Cereal
57103000	Alpha-bits Cereal
57103020	Alpha-bits with Marshmallows Cereal
57103050	Amaranth Flakes Cereal
57103100	Apple Cinnamon Cheerios
57103400	Apple Cinnamon Oh's Cereal
57103450	Apple Cinnamon Rice Krispies Cereal
57104000	Apple Jacks Cereal
57106250	Berry Berry Kix
57107000	Booberry Cereal
57109000	Body Buddies Cereal, Natural Fruit Flavor
57110000	Bran Buds Cereal
57117000	Cap'n Crunch Cereal
57117500	Christmas Crunch
57119000	Cap'n Crunch's Crunch Berries Cereal
57119500	Cap'n Crunch's Deep Sea Crunch Cereal
57120000	Cap'n Crunch's Peanut Butter Crunch Cereal
57123000	Cheerios
57124000	Chex Cereal, NFS
57124200	Chocolate flavored Frosted puffed corn Cereal
57124500	Cinnamon Grahams Cereal, General Mills
57125000	Cinnamon Toast Crunch Cereal
57125900	Clusters Cereal
57126000	Cocoa Krispies Cereal
57126500	Cocoa Blasts Cereal, Quaker
57127000	Cocoa Pebbles Cereal
57128000	Cocoa Puffs Cereal
57128880	Common Sense Oat Bran Cereal, Plain

57128900 Common Sense Oat Bran Cereal, with Raisins  
57130000 Cookie-crisp Cereal (Includes All Flavors)  
57131000 Crunchy Bran Cereal  
57132000 Corn Chex Cereal  
57134000 Corn Flakes, NFS (Includes Store Brands)  
57134090 Corn Flakes, Low Sodium  
57135000 Corn Flakes, Kellogg  
57137000 Corn Puffs Cereal  
57138000 Corn Total Cereal  
57139000 Count Chocula Cereal  
57144000 Crisp Crunch Cereal  
57148000 Crispix Cereal  
57148500 Crispy Brown Rice Cereal  
57151000 Crispy Rice Cereal  
57205260 Double Dip Crunch, Kellogg's  
57206700 Fibre One Cereal  
57211000 Frankenberry Cereal  
57212100 French Toast Crunch Cereal, General Mills  
57213000 Froot Loops Cereal  
57213800 Frosted Bran, Kellogg's  
57213850 Frosted Cheerios Cereal  
57214100 Frosted Wheat Bites  
57215000 Frosty O's Cereal  
57218000 Frosted Rice Krispies Cereal  
57219000 Fruit 'N Fibre Cereal, NFS  
57220000 Fruit 'N Fibre Cereal, with Apples and Cinnamon  
57221000 Fruit 'N Fibre Cereal, with Dates, Raisins, and Walnuts  
57221600 Fruit and fibre Cereal with Peach, Raisin, Almond and Oat Clusters  
57221700 Fruit Rings, NFS (Includes Store Brands)  
57221800 Fruit Whirls Cereal  
57223000 Fruity Pebbles Cereal  
57223200 Fruity Yummy Mummy Cereal  
57224000 Golden Grahams Cereal  
57231000 Grape-nut Flakes  
57232120 Healthy Choice Multi-grain Flakes Cereal, Kellogg's  
57235600 Heartwise with Fruit Nuggets Cereal  
57237000 Honey Bran Cereal  
57237100 Honey Bunches of Oats Cereal  
57237300 Honey Bunches of Oats with Almonds, Post  
57238000 Honeycomb Cereal, Plain  
57239000 Honeycomb Cereal, Strawberry  
57239100 Honey Crunch Corn Flakes Cereal, Kellogg's  
57240100 Honey Nut Chex Cereal  
57241000 Honey Nut Cheerios

57241200 Honey Nut Shredded Wheat Cereal, Post  
57243000 Honey Smacks Cereal  
57243870 Jenny O's  
57301100 Kabbom Cereal  
57301500 Kashi, Puffed  
57302100 King Vitamin Cereal  
57303100 Kix Cereal  
57305100 Lucky Charms Cereal  
57305150 Frosted Oat Cereal with Marshmallows  
57305170 Malt-o-meal Coco-Roos Cereal  
57305180 Malt-meal Corn Bursts Cereal  
57305200 Malt-o-meal Crisp Rice Cereal  
57305500 Malt-o-meal Honey and Nut Toasty O's Cereal  
57305600 Malt-o-meal marshmallow mateys Cereal  
57306100 Malt-o-meal puffed rice Cereal  
57306120 Malt-o-meal puffed wheat Cereal  
57306500 Malt-o-meal Sugar Puffs Cereal  
57306700 Malt-o-meal Toasted Oat Cereal  
57306800 Malt-o-meal Tootie Fruities (Rte Cereal)  
57307100 Fruity Marshmallow Krispies Cereal  
57307150 Marshmallow Safari Cereal, Quaker  
57307500 Millet, Puffed (Cereal)  
57307550 Mini Buns Cereal (Cinnamon)  
57308220 Strawberry Muesli w/pecans & raisins Ralston  
57308400 Multi-Grain Cherrios  
57308410 Multi-Grain Cherrios Plus Cereal  
57312100 Nutri-grain Biscuits, Shredded Wheat Cereal  
57315000 Nutri-grain Wheat Cereal  
57316100 Nutri-grain Almond Raisin Cereal  
57316200 Nutty Nuggets (Ralston)  
57316300 Oat Bran Flakes, Health Valley  
57316700 Oh's, Crunchy Nut Cereal  
57316710 Oh's, Honey Graham Cereal  
57316750 Oh's Fruitangy Cereal  
57317200 Oat Flakes Cereal, Post  
57322500 Oreo's Cereal, Post  
57323000 Popeye Cereal  
57323050 Sweet Puffs Cereal, Quaker  
57323200 Pop Tarts Crunch Cereal  
57325000 Product 19 Cereal  
57327450 Quaker Oat Bran Cereal  
57328000 Quisp Cereal  
57335530 Razzle Dazzle Rice Krispies Cereal  
57335550 Reese's Peanut Butter Puffs Cereal

57336000	Rice Chex Cereal
57337000	Rice Flakes, NFS
57339000	Rice Krispies Cereal
57339500	Rice Krispies Treats Cereal (Kellogg's)
57340000	Puffed Rice Cereal
57340200	Ripple Crisp Golden Corn
57340210	Ripple Crisp Honey Bran Cereal, General Mills
57342500	S'mores Crunch Cereal
57344000	Special K Cereal
57344050	Spider-man Cereal, Ralston
57344100	Sprinkle Sprangle Cereal
57346200	Sun Crunchers Cereal, General Mills
57346500	Toasted Oatmeal, Honey Nut (Quaker)
57347000	Corn Pops Cereal
57348000	Frosted Corn Flakes, NFS
57349000	Frosted Flakes, Kellogg
57349010	Cocoa Frosted Flakes Cereal, Kellogg's
57350000	Frosted Flakes, Ralston Purina
57352000	Sugar-sparkled Flakes
57353000	Sugar-sparkled Rice Krinkles Cereal
57354000	Sun Flakes Cereal
57355000	Super Golden Crisp Cereal
57401100	Tasteeos Cereal
57402000	Team Cereal
57402600	Temptations Cereal, French Vanilla Almond, Kellogg's
57402610	Temptations Cereal, Honey Roasted Pecan, Kellogg's
57403100	Toasties, Post
57404100	Toasty O's Cereal
57406100	Total Cereal
57406200	Triples (Rte Cereal)
57407100	Trix Cereal
57409100	Waffle Crisp Cereal, Post
57410000	Weetabix Whole Wheat Cereal
57411000	Wheat Chex Cereal
57416000	Puffed Wheat Cereal, Plain
57416010	Wheat, Puffed, presweetened w/sugar
57417000	Shredded Wheat, 100%
57417500	Shredded Wheat with Oat Bran (Rte Cereal)
57418000	Wheaties Cereal
57418200	Wheaties Cereal, Honey Frosted (Formerly Wheaties Honey Gold)

Flake (0.6 g Phytrol™ per 49 g serving size)

[Phytrol™] = 1.22 %

57102000	Alpen Cereal
57103500	Apple Cinnamon Squares Cereal
57105000	Apple Raisin Crisp Cereal
57106050	Banana Nut Crunch Cereal, Post
57106100	Basic 4 (Rte Cereal)
57106530	Blueberry Morning, Post
57111000	Bran Chex Cereal
57112000	Branola Cereal
57143000	Cracklin' Oat Bran Cereal
57152000	Crispy Wheats'n Raisins Cereal
57205250	Double Chex Cereal
57206000	Familia Cereal
57206800	Fiber 7 Flakes Cereal, Health Valley
57207000	40% Bran Flakes, NFS
57208000	40% Bran Flakes, Kellogg
57209000	Natural Bran Flakes Cereal, Post
57210100	40+ Bran Flakes Cereal
57214000	Frosted Mini-wheats Cereal (Includes All Flavors)
57216000	Frosted Rice Cereal, NFS
57217000	Frosted Rice Krinkles Cereal
57222500	Fruit Wheats Cereal
57225000	Golden Harvest Proteinola Cereal
57227000	Granola, NFS
57228000	Granola, Homemade
57229000	Granola, Lowfat, Kellogg's
57229500	Granola W/Raisins, Lowfat, Kellogg's
57230000	Grape-nuts Cereal
57231200	Great Grains, Raisins, Date & Pecan, Whole Grain Cereal, Post
57231250	Great Grains Double Pecan Whole Grain Cereal, Post
57232100	Healthy Choice Almond Crunch Cereal W/Raisins
57232110	Healthy Choice Multi-Grain Squares, Kellogg's
57233000	Heartland Natural Cereal, Plain
57234000	Heartland Natural Cereal, with Raisins
57235000	Heartland Natural Cereal, with Coconut
57240000	Honey Graham Chex Cereal
57244000	Just Right Cereal
57245000	Just Right with Raisins, Dates, and Nuts Cereal
57304100	Life Cereal (Plain and Cinnamon)
57308150	Mueslix Bran Muesli Cereal(includes Mueslix, NFS)
57308160	Muesli with Raisins, Walnuts, and Cranberries
57308170	Muesli with Raisins, Peaches and Pecans
57308180	Mueslix Five Grain Muesli Cereal
57308190	Muesli with Raisins, Dates, and Almonds

57308200 Mueslix Golden Crunch Cereal  
57308210 Muesli, with Apples and Almonds, Ralston Purina  
57308300 Multi Bran Chex  
57308900 Natural Muesli, Jenny's Cuisine  
57309100 Nature Valley Granola, with Fruit and Nuts  
57310000 Nature Valley Granola, with Cinnamon and Raisins  
57311000 Nature Valley Granola, Toasted Oat Mixture  
57311700 Nu System Cuisine Toasted Grain Circles Cereal  
57311800 Nut and Honey Crunch Flakes Cereal  
57316400 Oatmeal Crisp (Rte Cereal)  
57316410 Apple Cinnamon Oatmeal Crisp Cereal (Oatmeal Crisp w/Apples)  
57316450 Oatmeal Crisp w/Almonds Cereal  
57316500 Oatmeal Raisin Crisp Cereal  
57317000 Oat Flakes, Fortified  
57318000 100% Bran Cereal  
57319000 100% Natural Cereal, Plain  
57319500 Sun Country 100% Natural Granola, with Almonds  
57320500 100% Natural Cereal, w/Oats, Honey & Raisins, Quaker  
57321000 100% Natural Cereal, with Raisins and Dates  
57321500 100% Natural Wholegrain Cereal w/Raisins, Lowfat, Quaker  
57327500 Quaker Oat Squares Cereal  
57329000 Raisin Bran Cereal, NFS  
57330000 Raisin Bran Cereal, Kellogg  
57330500 Raisin Bran Cereal, Nutri/system  
57331000 Raisin Bran Cereal, Post  
57332000 Raisin Bran Cereal, Ralston Purina  
57332050 Raisin Bran, Total  
57332100 Raisin Nut Bran Cereal  
57332300 Super Raisin Bran, New Morning  
57333000 Raisin Grape-nuts Cereal  
57334000 Raisin Life Cereal  
57335500 Raisin Squares Mini-Wheats Cereal (formerly Raisin Squares)  
57341000 Shredded Wheat N' Bran Cereal  
57347500 Strawberry Squares Cereal  
57408100 Uncle Sam's Hi Fibre Cereal  
57412000 Wheat Germ Cereal, Plain  
57413000 Wheat Germ Cereal, with Sugar and Honey

**Bars**

(0.6 g Phytrol™ per 48 g bar)

[Phytrol™] = 1.25 %

- 41435010 High Protein Bar, Soy Base
- 41435110 High Protein Bar, Candy-like, Soy and Milk Base
- 41435200 High Protein Bar, Cookie Type, Soy and Milk Base
- 41460010 High-protein Wafers
- 53540000 Breakfast Bar, NFS
- 53540100 Breakfast Bar, Cake-like
- 53540200 Breakfast Bar, Cereal Crust, with Fruit Filling
- 53540500 Breakfast Bar, Date, with Yogurt Coating
- 53542100 Granola Bar with Oats, Sugar, Raisins, Coconut
- 53543100 Granola Bar with Peanuts, Oats, Sugar, Wheat Germ
- 53544100 Granola Bar, with Nougat
- 53544200 Granola Bar, Chocolate-coated
- 53544220 Granola Bar with Nuts, Chocolate-coated
- 53544250 Granola Bar, Coated with Nonchocolate Coating
- 53544300 Granola Bar, High Fibre, Yogurt Coating, Not Choc
- 53544400 Granola Bars, with Rice Cereal

**Fruit Drinks**

(0.6 g Phytrol™ per 9.5 Fl Oz)

*Phytrol™* = 0.214 %

92510110	Apple Drink
92510120	Apple-Cherry Drink
92510150	Apple Juice Drink
92510200	Apple-Orange-Pineapple Juice Drink
92510220	Apricot-Pineapple Juice Drink
92510310	Banana-Orange Drink
92510410	Black Cherry Drink
92510610	Fruit Drink (Includes Fruit Punch and Fruit Ade)
92510630	Fruit Juice Drink, NFS
92510650	Tamarind Drink, P.R. (Refresco De Tamarindo)
92510720	Fruit Punch, Made with Fruit Juice and Soda
92510730	Fruit Punch, Made with Soda, Fruit Juice and Sherbet
92510810	Grapeade and Grape Drink
92510820	Grape Juice Drink
92510910	Grapefruit Juice Drink
92510950	Guava Drink
92511010	Lemonade
92511020	Lemon-Limeade
92511110	Limeade
92511200	Orange-Mango Juice Drink
92511220	Orange Drink
92511230	Orange-Apricot Juice Drink
92511240	Orange-Lemon Drink
92511250	Citrus Fruit Juice Drink (60% fruit juice)
92511260	Orange-Cranberry Juice Drink
92511270	Orange-Peach Juice Drink
92511280	Orange-Grape-Banana Juice Drink
92511290	Papaya Juice Drink
92511310	Pineapple-Grapefruit Juice Drink
92511340	Pineapple-Orange Juice Drink
92511400	Raspberry-Flavored Drink
92511510	Strawberry-Flavored Drink
92530110	Apple Drink With Vitamin C Added
92530210	Black Cherry Drink With Vitamin C Added
92530310	Cherry Drink With Vitamin C Added
92530410	Citrus Drink With Vitamin C Added
92530510	Cranberry Juice Drink With Vitamin C Added

92530520	Cranberry-Apple Juice Drink With Vitamin C Added
92530610	Fruit Punch, Fruit Drinks, or Fruitades With Vitamin C Added
92530710	Grape Drink With Vitamin C Added
92530810	Grapefruit Juice Drink With Vitamin C Added
92530840	Guava Juice Drink With Vitamin C Added
92530910	Lemonade With Vitamin C Added
92531010	Orange Drink and Orangeade With Vitamin C Added
92531020	Orange Breakfast Drink, From Frozen Concentrate
92531110	Pineapple-Grapefruit Juice Drink With Vitamin C Added
92531120	Pineapple-Orange Juice Drink With Vitamin C Added
92531150	Pineapple-Orange-Grapefruit Juice Drink with Vitamin C Added
92531210	Strawberry-Flavored Drink With Vitamin C Added

**Smoothie Beverages**

(0.6 g Phytrol™ per 9.5 fl oz)

*Phytrol™* = 0.214 %

11551050	Milk Fruit Drink (Includes Licuado)
11551100	Milk Fruit Drink, Hispanic Style
11552200	Milk-based Fruit Drink (Includes Orange Julius)
11553000	Fruit Smoothie drink, w/fruit and dairy products
11553100	Fruit Smoothie drink, NFS
11560000	Choc-flavored Drink, Whey-&milk-based (includes Yoo-hoo)
11560020	Milk Drink, Whey&milk-base, Not Chocolate(Includes Yoo-hoo)
11560100	Flav Milk Drink,skim Milk&cream-based,not Choc
11560110	Chocolate Flav Milk Drink, Skim Milk and Cream-based

**SECTION 2**

**Estimated Daily Phytrol™ Intake Resulting From The Consumption of Ready to Eat Cereals, Bars, Fruit Drinks, and Smoothie Beverages by Different Population Groups Within The United States**

**Table 2-1 Estimated Daily Phytol Intake From The Consumption of Various Food Products By Infants Aged 0 to 2 Years Within The United States**

Food Category	Food Product	% Users	Actual # of Total Users	All-Person Consumption		Per-User Consumption	
				Mean (mg)	90th Percentile (mg)	Mean (mg)	90th Percentile (mg)
<u>Baked goods and baking mixes</u>							
	Bars	3.9	116	10.00	n/a	258.00	463.00
<u>Beverages and beverage bases</u>							
	Fruit Drink Mixtures and Blends (Ready to Drink)	20.1	632	81.60	268.00	405.00	803.00
	Smoothies	0.1	7	0.14	n/a	125.00	261.00
<u>Breakfast cereals</u>							
	Cereals (Ready to Eat) (extruded and flaked)	48.6	1543	196.00	622.00	404.00	799.00

**Table 2-2 Estimated Daily Phytrol Intake From The Consumption of Various Food Products By Children Aged 3 To 11 Within The United States**

Food Category	Food Product	% Users	Actual # of Total Users	All-Person Consumption		Per-User Consumption	
				Mean (mg)	90th Percentile (mg)	Mean (mg)	90th Percentile (mg)
<u>Baked goods and baking mixes</u>							
	Bars	6.4	356	18.20	n/a	284.00	500.00
<u>Beverages and beverage bases</u>							
	Fruit Drink Mixtures and Blends (Ready to Drink)	37.2	2293	172.00	541.00	464.00	862.00
	Smoothies	0.8	48	3.20	n/a	412.00	1040.00
<u>Breakfast cereals</u>							
	Cereals (Ready to Eat) (extruded and flaked)	69.2	4367	494.00	1150.00	715.00	1330.00

**Table 2-3 Estimated Daily Phytol Intake From The Consumption of Various Food Products By Female Teenagers Aged 12 To 19 Within The United States**

Food Category	Food Product	% Users	Actual # of Total Users	All-Person Consumption		Per-User Consumption	
				Mean (mg)	90th Percentile (mg)	Mean (mg)	90th Percentile (mg)
<u>Baked goods and baking mixes</u>							
	Bars	6.2	37	20.20	n/a	327.00	463.00
<u>Beverages and beverage bases</u>							
	Fruit Drink Mixtures and Blends (Ready to Drink)	30.4	213	168.00	531.00	551.00	1060.00
	Smoothies	0.5	3	2.83	n/a	604.00	769.00
<u>Breakfast cereals</u>							
	Cereals (Ready to Eat) (extruded and flaked)	46.7	320	342.00	999.00	732.00	1330.00

**Table 2-4 Estimated Daily Phytol Intake From The Consumption of Various Food Products By Male Teenagers Aged 12 To 19 Within The United States**

Food Category	Food Product	% Users	Actual # of Total Users	All-Person Consumption		Per-User Consumption	
				Mean (mg)	90th Percentile (mg)	Mean (mg)	90th Percentile (mg)
<u>Baked goods and baking mixes</u>							
	Bars	4.3	31	14.70	n/a	340.00	538.00
<u>Beverages and beverage bases</u>							
	Fruit Drink Mixtures and Blends (Ready to Drink)	24.2	175	185.00	751.00	765.00	1590.00
	Smoothies	0.3	2	1.89	n/a	561.00	561.00
<u>Breakfast cereals</u>							
	Cereals (Ready to Eat) (extruded and flaked)	45.1	325	503.00	1490.00	1120.00	2090.00

**Table 2-5 Estimated Daily Phytrol Intake From The Consumption of Various Food Products By Female Adults Aged 20 Years And Up Within The United States**

Food Category	Food Product	% Users	Actual # of Total Users	All-Person Consumption		Per-User Consumption	
				Mean (mg)	90th Percentile (mg)	Mean (mg)	90th Percentile (mg)
<u>Baked goods and baking mixes</u>							
	Bars	3.0	120	9.97	n/a	329.00	538.00
<u>Beverages and beverage bases</u>							
	Fruit Drink Mixtures and Blends (Ready to Drink)	13.7	653	68.10	265.00	497.00	929.00
	Smoothies	0.2	9	1.03	n/a	501.00	770.00
<u>Breakfast cereals</u>							
	Cereals (Ready to Eat) (extruded and flaked)	34.9	1627	223.00	733.00	638.00	1180.00

**Table 2-6 Estimated Daily Phytrol Intake From The Consumption of Various Food Products By Male Adults Aged 20 Years And Up Within The United States**

Food Category	Food Product	% Users	Actual # of Total Users	All-Person Consumption		Per-User Consumption	
				Mean (mg)	90th Percentile (mg)	Mean (mg)	90th Percentile (mg)
<u>Baked goods and baking mixes</u>							
	Bars	2.6	109	11.30	n/a	426.00	806.00
<u>Beverages and beverage bases</u>							
	Fruit Drink Mixtures and Blends (Ready to Drink)	14.4	661	97.00	383.00	671.00	1200.00
	Smoothies	0.1	4	0.71	n/a	627.00	1040.00
<u>Breakfast cereals</u>							
	Cereals (Ready to Eat) (extruded and flaked)	30.8	1599	277.00	1010.00	900.00	1670.00

**Table 2-7 Estimated Daily Phytol Intake From The Consumption of Various Food Products For The Total U.S. Population (All Ages) Within The United States**

Food Category	Food Product	% Users	Actual # of Total Users	All-Person Consumption		Per-User Consumption	
				Mean (mg)	90th Percentile (mg)	Mean (mg)	90th Percentile (mg)
<u>Baked goods and baking mixes</u>							
	Bars	3.6	769	12.40	n/a	341.00	538.00
<u>Beverages and beverage bases</u>							
	Fruit Drink Mixtures and Blends (Ready to Drink)	18.8	4627	105.00	398.00	555.00	1060.00
	Smoothies	0.3	73	1.31	n/a	494.00	783.00
<u>Breakfast cereals</u>							
	Cereals (Ready to Eat) (extruded and flaked)	39.8	9781	299.00	966.00	751.00	1390.00

**SECTION 3**

**Estimated Daily per Kilogram Body Weight Phytrol™ Intake Resulting from the  
Consumption of Ready to Eat Cereals, Bars, Fruit Drinks, and Smoothie Beverages by  
Different Population Groups Within the United States**

**Table 3-1 Estimated Daily Per Kilogram Body Weight Phytrol Intake From The Consumption of Various Food Products By Infants Aged 0 to 2 Years Within The United States**

Food Category	Food Product	% Users	Actual # of Total Users	All-Person Consumption		Per-User Consumption	
				Mean (mg/kg)	90th Percentile (mg/kg)	Mean (mg/kg)	90th Percentile (mg/kg)
<u>Baked goods and baking mixes</u>							
	Bars	3.9	116	0.80	n/a	20.40	35.30
<u>Beverages and beverage bases</u>							
	Fruit Drink Mixtures and Blends (Ready to Drink)	20.1	632	6.40	22.80	31.80	68.40
	Smoothies	0.1	7	0.01	n/a	10.40	21.10
<u>Breakfast cereals</u>							
	Cereals (Ready to Eat) (extruded and flaked)	48.6	1543	15.30	45.80	31.50	61.40

**Table 3-2 Estimated Daily Per Kilogram Body Weight Phytrol Intake From The Consumption of Various Food Products By Children Aged 3 To 11 Within The United States**

Food Category	Food Product	% Users	Actual # of Total Users	All-Person Consumption		Per-User Consumption	
				Mean (mg/kg)	90th Percentile (mg/kg)	Mean (mg/kg)	90th Percentile (mg/kg)
<u>Baked goods and baking mixes</u>							
	Bars	6.4	356	0.75	n/a	11.60	20.50
<u>Beverages and beverage bases</u>							
	Fruit Drink Mixtures and Blends (Ready to Drink)	37.2	2293	7.14	23.40	19.20	37.40
	Smoothies	0.8	48	0.14	n/a	18.70	40.50
<u>Breakfast cereals</u>							
	Cereals (Ready to Eat) (extruded and flaked)	69.2	4367	20.20	48.60	29.30	55.40

**Table 3-3 Estimated Daily Per Kilogram Body Weight Phytol Intake From The Consumption of Various Food Products By Female Teenagers Aged 12 To 19 Within The United States**

Food Category	Food Product	% Users	Actual # of Total Users	All-Person Consumption		Per-User Consumption	
				Mean (mg/kg)	90th Percentile (mg/kg)	Mean (mg/kg)	90th Percentile (mg/kg)
<u>Baked goods and baking mixes</u>							
	Bars	6.2	37	0.38	n/a	6.11	10.90
<u>Beverages and beverage bases</u>							
	Fruit Drink Mixtures and Blends (Ready to Drink)	30.4	213	3.08	10.10	10.10	17.80
	Smoothies	0.5	3	0.05	n/a	11.20	14.40
<u>Breakfast cereals</u>							
	Cereals (Ready to Eat) (extruded and flaked)	46.7	320	6.42	18.20	13.70	24.00

**Table 3-4 Estimated Daily Per Kilogram Body Weight Phytol Intake From The Consumption of Various Food Products By Male Teenagers Aged 12 To 19 Within The United States**

Food Category	Food Product	% Users	Actual # of Total Users	All-Person Consumption		Per-User Consumption	
				Mean (mg/kg)	90th Percentile (mg/kg)	Mean (mg/kg)	90th Percentile (mg/kg)
<u>Baked goods and baking mixes</u>							
	Bars	4.3	31	0.28	n/a	6.37	12.70
<u>Beverages and beverage bases</u>							
	Fruit Drink Mixtures and Blends (Ready to Drink)	24.2	175	2.93	11.10	12.10	25.60
	Smoothies	0.3	2	0.04	n/a	10.50	10.80
<u>Breakfast cereals</u>							
	Cereals (Ready to Eat) (extruded and flaked)	45.1	325	8.55	26.00	19.00	34.80

**Table 3-5 Estimated Daily Per Kilogram Body Weight Phytrol Intake From The Consumption of Various Food Products By Female Adults Aged 20 Years And Up Within The United States**

Food Category	Food Product	% Users	Actual # of Total Users	All-Person Consumption		Per-User Consumption	
				Mean (mg/kg)	90th Percentile (mg/kg)	Mean (mg/kg)	90th Percentile (mg/kg)
<u>Baked goods and baking mixes</u>							
	Bars	3.0	120	0.16	n/a	5.25	9.68
<u>Beverages and beverage bases</u>							
	Fruit Drink Mixtures and Blends (Ready to Drink)	13.7	653	1.03	3.80	7.55	14.80
	Smoothies	0.2	9	0.02	n/a	8.31	12.20
<u>Breakfast cereals</u>							
	Cereals (Ready to Eat) (extruded and flaked)	34.9	1627	3.49	11.50	10.00	19.40

**Table 3-6 Estimated Daily Per Kilogram Body Weight Phytrol Intake From The Consumption of Various Food Products By Male Adults Aged 20 Years And Up Within The United States**

Food Category	Food Product	% Users	Actual # of Total Users	All-Person Consumption		Per-User Consumption	
				Mean (mg/kg)	90th Percentile (mg/kg)	Mean (mg/kg)	90th Percentile (mg/kg)
<u>Baked goods and baking mixes</u>							
	Bars	2.6	109	0.14	n/a	5.14	9.39
<u>Beverages and beverage bases</u>							
	Fruit Drink Mixtures and Blends (Ready to Drink)	14.4	661	1.22	4.19	8.44	15.80
	Smoothies	0.1	4	0.01	n/a	9.19	16.60
<u>Breakfast cereals</u>							
	Cereals (Ready to Eat) (extruded and flaked)	30.8	1599	3.45	12.40	11.20	21.30

**Table 3-7 Estimated Daily Per Kilogram Body Weight Phytrol Intake From The Consumption of Various Food Products For The Total U.S. Population (All Ages) Within The United States**

Food Category	Food Product	% Users	Actual # of Total Users	All-Person Consumption		Per-User Consumption	
				Mean (mg/kg)	90th Percentile (mg/kg)	Mean (mg/kg)	90th Percentile (mg/kg)
<u>Baked goods and baking mixes</u>							
	Bars	3.6	769	0.28	n/a	7.57	14.90
<u>Beverages and beverage bases</u>							
	Fruit Drink Mixtures and Blends (Ready to Drink)	18.8	4627	2.36	7.71	12.50	26.70
	Smoothies	0.3	73	0.03	n/a	12.90	18.80
<u>Breakfast cereals</u>							
	Cereals (Ready to Eat) (extruded and flaked)	39.8	9781	6.65	20.80	16.70	35.00

## **AN EXPERT OPINION STATEMENT**

### **GRAS Status of Reducol™ (Phytrol™) Phytosterols Used as an Ingredient of Cereals, Food Bars, Fruit Drinks and Smoothie Beverages**

The undersigned, an independent recognized expert (hereinafter referred to as Expert), qualified by scientific training and relevant national and international experience to evaluate the safety of food and food ingredients, was requested by Novartis Consumer Health, Inc. on behalf of Altus Foods Co. to determine the Generally Recognized as Safe (GRAS) status of the use of Reducol™ in Cereals, Food Bars, Fruit Drinks and Smoothie Beverages. These products are to be manufactured and marketed by Altus Foods Co., a joint venture between Novartis Consumer Health, Inc. and Quaker Oats Company.

Reducol™, originally named Phytrol™, is a tall-oil derived mixture of non-esterified phytosterols and stanols and would be incorporated as an ingredient in Cereals, Food Bars, Fruit Drinks and Smoothie Beverages at a concentration sufficient to provide a total of 1.8 grams phytosterols and stanols obtained daily through consumption of three servings from among the products (0.6 grams/serving), for the purpose of helping to maintain normal cholesterol blood levels.

Reducol™ is currently manufactured by Forbes Medi-Tech, Inc. at the Quest facility in Houston Texas. Its use in a vegetable oil-based spread product at a level up to 12% has been previously determined to be GRAS by Novartis Consumer Health, Inc. Novartis Consumer Health, Inc. subsequently submitted to FDA a notification (GRN39) that it had determined that Reducol™ (then termed Phytrol™) phytosterols are GRAS for use in vegetable oil spreads. The FDA completed a review of the Novartis notification and on April 24, 2000 replied that it had no questions at that time regarding Novartis' determination.

Subsequent to Novartis' GRAS determination and FDA review of their notification, the manufacture of Reducol™ was relocated to the Quest facility in Houston, Texas. This resulted in a change in Reducol™'s profile of constituent phytosterols and necessitated a change in product specifications to accommodate a somewhat higher range of sitosterol content and lower ranges of content for sitostanol, campesterol and campestanol. The Quest manufacturing process and

resultant Reducol™ composition were reassessed by the Expert Panel originally requested by Novartis to evaluate Phytrol™'s GRAS status for use in a vegetable oil-based spread. The Panel, of which this Expert was a member, concluded that the change in manufacture and component specifications were inconsequential with respect to safety and physiologic properties and that Reducol™, as manufactured at the Quest facility, continues to be GRAS when used in a vegetable oil-based spread at the level previously established.

In conducting the assessment of the GRAS status of the use of Reducol™ in the Altus Foods Co. products, this Expert had available and considered the information and data made available during the previous considerations of Phytrol™'s GRAS status for use in a vegetable oil-based spread. A report by CANTOX U.S. INC. providing detailed information regarding Cereals, Food Bars, Fruit Drinks and Smoothie Beverages product compositions, intended and estimated consumer exposures, as well as, summary safety information facilitated the work of this Expert. In this regard, FDA's recent publication of an Interim Final Rule which authorized, with certain conditions, the use of a coronary heart disease health claim for plant sterol esters and plant stanol esters was considered relevant to this review. The Interim Final Rule, which is currently undergoing a comment period, authorizes the health claim for several product forms wherein a single product serving contains at least 0.65 grams of plant sterol esters or 1.7 grams of plant stanol esters. FDA did not raise safety concerns regarding consumer exposure to plant sterols and stanols arising through possible use of multiple products in which they may be incorporated. FDA's position is considered consistent with and supporting the safety and effectiveness of consuming phytosterols and stanols for the purpose of maintaining healthy cholesterol blood levels. Attention is drawn to the consistency of the proposed use of Reducol™ in the Altus Foods Co. products with that authorized by FDA's health claim regulation.

With respect to critical evaluation of consumer exposure, this Expert considered the manufacturers' recommendation for daily product intake to represent best the intended conditions of use of the product. The recommended consumption of up to three servings from among the Cereals, Food Bars, Fruit Drinks and Smoothie Beverages products, providing a total of 1.8 grams of Reducol™ phytosterols and stanols, was determined to be similar with the intake associated with the recommended use of Reducol™ in a vegetable oil-based spread, as well as, similar in amount to other currently marketed products containing added phytosterols and stanols. While formal intake estimations based on data for Cereals, Food Bars, Fruit Drinks and Smoothie Beverage usage reported in the USDA CSFII surveys for 1989-1991 as well as more

recently for 1994-1996 were provided, the Expert found them to be of limited statistical reliability owing to the very small number (N) of users represented for certain product categories, particularly for Food Bars and Smoothie Beverages. Nevertheless, as tabulated below, values projected for mean and 90<sup>th</sup> percentile daily intake of Reducol™ among all users of the individual products, based on the CSFII surveys, were comparable to or less than the 1.8 grams derived from recommended product use.

**Table 1: Projected Daily Reducol™ Intake Among Cereals, Food Bars, Fruit Drinks and Smoothie Beverage Users**

Product and Reducol™ content per Serving size	Per Label	Intake Amount (gram Reducol™/day)					
		1989-1991 CSFII			1994-1996 CSFII		
		(N)	Mean	90 <sup>th</sup> %	(N)	Mean	90 <sup>th</sup> %
Food Bars 0.6g/48g	1.8  (3 servings from among product)	(150)	0.291	0.538	(769)	0.341	0.538
Fruit Drinks 0.6g/9.5 fluid ounces		(1851)	0.35	0.700	(4627)	0.555	1.060
Smoothies 0.6g/9.5 fluid ounces		(26)	0.294	0.388	(73)	0.494	0.783
Cereals, Flaked: 0.6g/49 grams Extruded: 0.6g/27 grams		(6451)	0.573	1.110	(9781)	0.751	1.390

The composition of Reducol™ phytosterols and stanols to be incorporated into the Cereals, Food Bars, Fruit Drinks and Smoothie Beverage products was determined to be the same as that incorporated into the vegetable oil-based spread and which has been determined by Novartis Consumer Health, Inc. to be GRAS. Following critical evaluation, no factors were identified which would suggest incorporation of Reducol™ into Cereals, Food Bars, Fruit Drinks and Smoothie Beverage products would materially alter its physiologic properties and effectiveness or create new or intensify previous safety considerations, including those regarding vitamin and nutrient availability.

Based on the critical evaluations discussed above and consistent with the authorized uses of phytosterols granted by FDA's Interim Final Rule (see above discussion), this Expert has concluded that Redurol™ is generally recognized a safe (GRAS) by scientific procedures when used in Cereals, Food Bars, Fruit Drinks and Smoothie Beverages for the purpose of helping to maintain a healthy cholesterol blood level, providing it is used in accordance with current good manufacturing practice (21 CFR § 182.1(b)) in an amount to provide 0.6 grams phytosterols and phytosterols per serving.



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W. Gary Flamm, Ph.D., F.A.C.T., F.A.T.S.  
President, Flamm Associates

## Phytrol Content in Smoothie Drinks – Modified to include Internal Standard:

**Principle** Extraction / Silylation / Capillary Gaschromatography

### Reference solution

Internal Standard Stock for Reference Solution (IS-R): Weigh 80 mg 5- $\alpha$ -cholestane (Internal Standard) into a 25 mL volumetric flask. Dissolve in pyridine. Dilute to volume with pyridine. Mix thoroughly.

Reference Solution Preparation: Accurately weigh 15 mg phytrol reference substance into a 10 ml reaction vial. Pipette 2 mL internal stock standard solution (IS-R) into this reaction vial. Add 600  $\mu$ l of BSTFA (1) and 1 ml pyridine. Close the reaction vial tightly and warm to 90 °C for 1 hour. Cool to room temperature and transfer the solution quantitatively with small portions of toluene into a 10 ml volumetric flask. Dilute to volume with toluene.

### Sample solution

Internal Standard Stock for Sample Solution (IS-S): Weigh 80 mg 5- $\alpha$ -cholestane (Internal Standard) into a 50 mL volumetric flask. Dissolve in toluene. Dilute to volume with toluene. Mix thoroughly.

Sample Solution Preparation: Accurately weight an amount of sample corresponding to 15 mg phytrol in a erlenmeyer flask. Pipette 4 mL of internal stock standard solution (IS-S) to the sample. Add 50 ml of toluene and 50 ml distilled water. Close the erlenmeyer flask with a stopper and stirr (magnetic stirrer) for 15 minutes. Transfer the mixture quantitatively into a separatory funnel and separate the layers. Extract the aqueous phase with 40 ml Toluene and unify the toluene layers in a second separatory funnel. Reject the aqueous layer. Wash the unified toluene layers twice with 20 ml distilled water and once with 20 ml sodium chloride solution (saturated). Add approx. 5 g of Na<sub>2</sub>SO<sub>4</sub> sicc. and filter (glass frit/vacuum) the toluene extract quantitatively into a 250 ml round bottomed flask. Rinse the separatory funnel and the Na<sub>2</sub>SO<sub>4</sub> sicc. with small quantities of toluene. Evaporate to dryness on rotary evaporator at 50 °C.

Dissolve the dry residue in 3 ml pyridine and transfer the solution with small quantities (3 x 1 ml) of toluene quantitatively into a 10 ml reaction vial. Add 600  $\mu$ l of BSTFA(1), close the reaction vial tightly and warm to 90 °C for 1 hour. Cool to room temperature and transfer the solution quantitatively with small portions of toluene into a 10 ml volumetric flask. Dilute to volume with toluene.

**Remark:** (1) N,O-Bis(trimethylsilyl)trifluoroacetamide

### Capillary Gaschromatography (Conditions)

<b>Column</b>	Fused Silica, 5 % phenyl- / 95 % methylpolysiloxane 0,25 $\mu$ m, length: 25 m, internal diameter: 0,25 mm (e.g. Optima 5, Machery Nagel, Oensingen, Switzerland)
<b>Autosampler Method</b>	Injected Volume: 1.0 $\mu$ l, split ratio = 100 : 1
<b>Carrier</b>	Helium, 40 cm/ s
<b>Detector Parameters</b>	Detector FID

Range: 1  
Time Constant: 200  
Auto Zero: ON

**Heated Zones**

Injector 275 °C  
Detector 340 °C

**Oven Program**

Initial Temp.: 230 °C  
Equilibration Time: 2.0 min  
Initial Hold: 1.00 min  
Equilibration Time: 2.0 min  
Ramp: 3.0 °C/min to 300 °C, hold for 6 min

**Calculation**

$$\text{g Phytol / 100 g} = (P_R \times \Sigma A_T) / (\Sigma A_R \times P_T \times 10)$$

$P_R$	=	weight of reference substance in mg
$\Sigma A_T$	=	sum of peak areas of the sterols in the sample solution
$\Sigma A_R$	=	sum of peak areas of the sterols in the reference solution
$P_T$	=	weight of sample in g
10	=	conversion factor to g/100 g



# Effects on serum lipids, lipoproteins and fat soluble antioxidant concentrations of consumption frequency of margarines and shortenings enriched with plant stanol esters

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**Objective:** To examine in humans the effects on serum lipids, lipoproteins and fat-soluble antioxidants of a daily consumption of 2.5 g plant stanols, consumed either once per day at lunch or divided over the three meals.

**Design:** A randomized, double-blind, placebo-controlled, cross-over design.

**Subjects:** Thirty-nine healthy normocholesterolemic or mildly hypercholesterolemic subjects participated.

**Interventions:** Each subject consumed in random order; no plant stanols; 2.5 g plant stanols at lunch; and 2.5 g plant stanols divided over the three meals (0.42 g at breakfast, 0.84 g at lunch and 1.25 g at dinner, which is proportional to dietary cholesterol intake). Each period lasted 4 weeks. Plant stanols were esterified with fatty acids from low erucic rapeseed oil (LEAR) and incorporated into margarines or shortenings.

**Results:** Consumption of 2.5 g plant stanols at lunch results in a similar low-density lipoprotein (LDL)-cholesterol-lowering efficacy compared to consumption of 2.5 g plant stanols divided over the three meals ( $-0.29$  mmol/l compared with the control period ( $P < 0.001$ ; 95% CI,  $-0.19$  to  $-0.39$  mmol/l) for the once per day diet and  $-0.31$  mmol/l ( $P < 0.001$ ; 95% CI,  $-0.20$  to  $-0.41$  mmol/l) for the three times per day period). High-density Lipoprotein (HDL) cholesterol and triacylglycerol concentrations did not change. After standardization for LDL cholesterol, the sum of the most lipophilic hydrocarbon carotenoids (ie  $\alpha$ -carotene,  $\beta$ -carotene and lycopene) in particular was slightly, though not significantly, lowered by  $-0.017 \pm 0.018$   $\mu$ mol/mmol LDL cholesterol ( $P = 0.307$ ) after the once per day period and by  $-0.032 \pm 0.016$   $\mu$ mol/mmol LDL cholesterol ( $P = 0.049$ ) after the three times per day period.

**Conclusions:** Our findings suggest that for lowering LDL cholesterol concentrations it is not necessary to consume products rich in plant stanol ester at each meal or simultaneously with dietary cholesterol.

**Sponsorship:** Raisio Group, Raisio, Finland.

**Descriptors:** plant stanols; consumption frequency; diet; serum lipids; serum lipoproteins; fat-soluble antioxidants

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## Introduction

Plant stanols are useful hypocholesterolemic agents since a daily intake of 2–3 g lowers LDL cholesterol concentrations by 10–15% as found in various populations (Wester, 1999; Law, 2000). The proposed mechanism is that plant stanols reduce the micellar solubility of cholesterol and consequently lower intestinal absorption of both exogenous and endogenous cholesterol (Heinemann *et al*, 1991). This suggests that plant stanol esters should be consumed at each meal to obtain a maximal cholesterol-lowering effect. However, consuming plant stanol esters at lunch and dinner only (Weststrate & Meijer, 1998) showed a decrease in LDL cholesterol comparable to that when consumed three times daily (Miettinen *et al*, 1995; Plat & Mensink, 2000). This suggests that plant stanols are active in the intestinal tract for at least a few hours. It has, however, never systematically been evaluated whether the efficacy of

plant stanols to lower serum LDL cholesterol depends on consumption frequency.

The main purpose of the present study therefore was to examine in a normocholesterolemic and mildly hypercholesterolemic population the effects on serum lipids and lipoproteins of a margarine and shortening enriched with plant stanol esters, consumed three times per day, vs an equal dose of plant stanol esters, consumed once per day. Also effects on plasma fat soluble antioxidant concentrations were evaluated, as these may be affected by consumption of plant sterol and stanol esters (Weststrate & Meijer, 1998; Gylling & Miettinen, 1999).

## Methods

### Subjects

Forty-three subjects from Maastricht and surrounding areas applied for the study. Twenty-six of these volunteers had participated in a previous study on the effects of plant stanol esters on serum lipids and lipoproteins (Plat & Mensink, 2000), while the others were recruited via posters in public buildings. Subjects were invited for a screening visit to see if they met our eligibility criteria: age 18–65 y, fasting serum total cholesterol concentration  $< 6.5$  mmol/l

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Contributors: This study was planned by RPM and JP. All authors have contributed to the execution, analysis, interpretation, and reporting of the study.

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(251 mg/dl), fasting serum triacylglycerol concentration < 3.0 mmol/l, body mass index < 30 kg/m<sup>2</sup>, diastolic blood pressure < 95 mmHg, systolic blood pressure < 160 mmHg, no presence of proteinuria or glucosuria, no use of medication or a diet known to affect serum lipids, and no history of coronary heart disease. Volunteers had not donated blood at least 4 weeks before or during this trial, and did not participate in another biomedical study. All subjects gave their written informed consent before the start of the study. A population of normocholesterolemic and mildly hypercholesterolemic subjects was used, since the serum cholesterol lowering efficacy of plant stanol esters—expressed as a percentage—does not depend on initial serum LDL cholesterol concentrations (Wester, 1999; Law, 2000). Hypercholesterolemic subjects were not included, as many of these patients have a history of cardiovascular disease, or use medication or a diet known to affect serum lipids, which were all exclusion criteria.

One subject was excluded for a serum total cholesterol concentration > 6.5 mmol/l and two subjects decided not to participate. Consequently, the study started with 40 volunteers. One subject dropped out during the first week, because she could not combine the study protocol with her lifestyle. The remaining 39 volunteers, 28 women and 11 men, completed the study successfully. These participants were 31 ± 14 y of age (mean ± s.d.) and had a body mass index of 22.7 ± 2.6 kg/m<sup>2</sup>. Before the study started, mean serum total cholesterol and triacylglycerol concentrations were 4.74 ± 0.85 mmol/l (range 2.83–6.28 mmol/l) and 0.99 ± 0.39 mmol/l (range 0.39–1.84 mmol/l) in women and 4.94 ± 0.89 mmol/l (range 3.37–6.15 mmol/l) and 0.97 ± 0.53 mmol/l (range 0.44–2.02 mmol/l) in men. Seventeen women had cholesterol concentrations below 5.0 mmol/l (normocholesterolemic) and 11 women had cholesterol concentrations between 5.0 and 6.5 mmol/l (mildly hypercholesterolemic). For men, these figures were seven and four, respectively. One man and three women smoked cigarettes, 19 women used oral contraceptives and one woman was postmenopausal.

**Design and diets**

The study, which was approved by the Medical Ethics Committee of Maastricht University, had a double-blind, placebo-controlled cross-over design (Figure 1). Each subject received three different diets for 4 weeks in one of the six possible treatment orders. There was no washout period between the three different dietary periods. Before the start

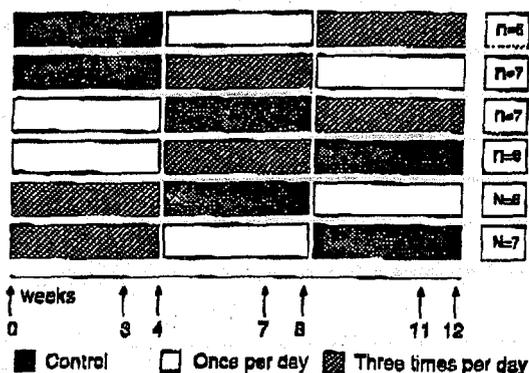


Figure 1 Experimental design of the study.

of the study, the subjects were randomly allocated to one of the six groups. The participants were instructed to maintain their customary lifestyles and home diets throughout the study. During the study, they recorded in diaries any symptoms, visits to physicians, medication used, menstrual phase, alcohol use and any deviation from the protocol. Body weight was recorded weekly.

During the study, the subjects were required to replace at breakfast and at lunch their habitual margarines for an experimental margarine of which, at breakfast 10 g and at lunch 20 g, had to be consumed. Within 1 h after dinner, each participant also had to eat a cake or cookie, which contained 10 g of an experimental shortening. These cakes and cookies were prepared every week by a local bakery especially for this study. To control fat and fatty acid intake as much as possible, each participant also received during each period a shortening without plant stanol esters that had to be used for baking and cooking.

One experimental margarine contained 4.2 g/100 g plant stanols as its fatty acid (low plant stanol ester margarine), and another margarine 12.5 g/100 g (high plant stanol ester margarine). The plant stanol concentration in the experimental shortening was 12.5 g/100 g. Products provided during the control period did not contain any plant stanol esters.

The mixture of vegetable oil and pinewood-derived plant stanols contained approximately 76% sitostanol and 24% campestanol. Sitostanol was prepared from β-sitosterol and stigmasterol, and campestanol from campesterol, both by hydrogenation. Free sitostanol and campestanol were transesterified with rapeseed oil fatty acids, forming fat-soluble sitostanol and campestanol esters. The plant stanol esters were then mixed with the experimental margarines and shortening. The plant stanol esters were added to the experimental margarines at the expense of water and to the experimental shortening at the expense of absorbable fats. All the margarines and shortenings were prepared from low erucic acid rapeseed oil (LEAR) and contained 68% (margarines), 99% (control shortening) or 86% (experimental shortening) absorbable fats. All margarines and the shortening were fortified with normal amounts of vitamin A and D. β-Carotene was used as a coloring agent, while vitamin E was present as a natural compound. The margarines and shortenings were produced and provided by the Raisio Group, Raisio, Finland.

At a daily intake of 10 g margarine at breakfast, 20 g margarine at lunch, and 10 g shortening incorporated into the cakes and cookies after dinner, the aimed plant stanol intake during the experimental periods was 2.5 g. The distribution of plant stanol intake over the day, however, was different (Figure 2). During the once per day period the 2.5 g of plant stanols were consumed once per day at lunch, while during the three times per day period the plant stanols were provided in amounts proportional to cholesterol intake (Ministeries van Welzijn, Volksgezondheid en Cultuur en van Landbouw, Natuurbeheer en Visserij, 1993). Thus, 0.42 g plant stanols were consumed at breakfast, 0.84 g at lunch and 1.25 g at dinner.

The volunteers had to come at least once a week to the Department to receive a new supply of products. The experimental margarines were given in color-labeled tubs, which contained 75 g margarine (breakfast) or 145 g margarine (lunch). The cookies or cakes were provided in similarly color-labeled bags. The tubs and the bags provided margarine, cakes and cookies for one week. Parts of

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	Breakfast	Lunch	Dinner	
Control	10 g control margarine	20 g control margarine	control shortening	control cake or cookie
Once per day	10 g control margarine	20 g stanol margarine (high)	control shortening	control cake or cookie
Three times per day	10 g stanol margarine (low)	20 g stanol margarine (low)	control shortening	cake or cookie with stanols
	low concentration stanol margarine: 4.2 g stanols /100 g high concentration stanol margarine: 12.5 g stanols /100 g cake or cookie with stanols: 1.25 g stanols /piece			

Figure 2 Distribution of plant stanol intake over the day.

all experimental products that were left over at the end of the week had to be returned and were weighed back to calculate the consumption of the experimental margarines and shortening for that week. The shortening without plant stanol esters was packed in a tub of 200 g, which could be used for more than one week.

During the last week of each period, the participants had to fill in a food frequency questionnaire about their eating habits of the previous 4 weeks, in order to estimate their energy and nutrient intakes. Details of the food frequency questionnaire have been published before (Plat & Mensink, 2000). A dietician immediately checked the questionnaires in presence of the subject, for completeness and inconsistencies. Food intake was divided over breakfast, between breakfast and lunch (morning snacks), lunch, afternoon snacks, dinner and evening snacks. Composition of the diets was calculated as described before (Plat & Mensink, 2000).

#### Blood sampling

Blood was sampled after an overnight fast and after abstinence from drinking alcohol the preceding day and smoking on the morning before blood sampling. All venipunctures were performed by the same person, at the same location and approximately at the same time of the day. No blood was sampled on Mondays. Blood was sampled once at the beginning of the study (day 1) and twice at the end of each dietary period (weeks 3 and 4, 7 and 8, 11 and 12).

A 10 ml clotting tube was always sampled (CORVAC, integrated serum separator tube, Sherwood Medical Company, St Louis, MO, USA). Serum was obtained by low-speed centrifugation at 2000 g for 15 min at 4°C, at least 1 h after venipuncture, and then immediately stored in small portions at -80°C. Serum was used for lipids and lipoprotein analysis. At weeks 0, 4, 8 and 12 blood was also sampled using a 10 ml EDTA tube (Sherwood Medical, Monoject). Plasma was prepared from EDTA blood by centrifuging at 2000 g for 30 min at 4°C. Aliquots were snap-frozen and stored directly at -80°C for analysis of antioxidants. Serum and EDTA blood were also used for analysis of parameters for liver and kidney function, C-reactive protein concentrations and hematological parameters. These parameters were not affected by the diets (Plat & Mensink, 1999).

#### Chemical analysis

All samples from one subject were analyzed in the same analytical run for total and HDL cholesterol and triacylglycerol concentrations as described before (Plat & Mensink, 2000). The coefficients of variation within runs were 1.9% for serum total cholesterol 2.0% for HDL cholesterol and 3.4% for triacylglycerol. LDL cholesterol concentrations were calculated using the Friedewald equation (Friedewald et al, 1972).

Plasma concentrations of tocopherols ( $\alpha$ -tocopherol,  $\delta$ -tocopherol,  $\beta + \gamma$ -tocopherol), several carotenoids ( $\alpha$ -carotene,  $\beta$ -carotene, lycopene, lutein/zeaxanthin,  $\beta$ -cryptoxanthin and phytofluene) and retinol were determined simultaneously, as described (Hess et al, 1991; Oostenbrug et al, 1997). Briefly, plasma samples were extracted twice with hexane, while retinylacetate was used as internal standard. Antioxidant concentrations were determined by reversed-phase high-pressure liquid chromatography (HPLC). Samples from one subject of weeks 3, 8 and 12 were analyzed in the same analytical run. The mean recovery of retinylacetate was  $96.0 \pm 7.9\%$ .

#### Statistical analysis

The data were analyzed with the General Linear Models (GLM) procedure of the SAS program (SAS Institute Inc., 1985). For each subject, lipid and lipoprotein concentrations of weeks 3 and 4, of 7 and 8, and of weeks 11 and 12 were first averaged. The model to examine diet effects included subject, diet, period, carry-over effect and diet  $\times$  sex as independent variables. Since the carry-over effect, period and the diet  $\times$  sex interaction term never reached statistical significance, these terms were subsequently omitted from the model. Thus the final model included subject and diet. When the analysis indicated a significant effect of diet ( $P < 0.05$ ), the Tukey method was used to compare the diets pairwise. All values are presented as their means  $\pm$  standard deviations (s.d.), except in Figure 3, in which values are presented as means  $\pm$  s.e.

#### Results

##### Dietary intakes and body weight

Table 1 shows the estimated daily plant sterol and stanol intakes, as derived from the experimental margarines and shortenings. As expected, total intakes of plant stanols during the once per day diet ( $2468 \pm 173$  mg) and during the three times per day diet ( $2456 \pm 121$  mg) were significantly higher than those during the control diet ( $P < 0.001$ ). Total plant stanol ( $P = 0.672$ ) and sitostanol ( $P = 0.578$ ) intake was similar during the once per day period and the three times per day period. The slightly higher campestanol intake of 23 mg or 4%, during the once per day period, compared to the three times per day period, was significant ( $P < 0.001$ ). This difference was due to a slight difference in the sitostanol/campestanol ratio of the plant stanol ester mixtures used for the preparation of the low and the high stanol ester margarines.

The daily energy intake and the proportion of energy from the macronutrients and alcohol, as well as cholesterol and fiber consumption, were essentially the same during the three periods of the study. Slight, statistically significant, differences existed in the intakes of fatty acids. This was mainly due to the slightly lower absorbable fat content of the stanol ester shortening compared with the control shortening.

Table 1 Estimated daily intake of plant sterols and plant stanols, energy and nutrients, during the three different diets<sup>a</sup>

	Control period	Once per day period	Three times per day period
Total plant sterols (mg) <sup>b</sup>	228 ± 44	2729 ± 199*	2682 ± 146*
Of which plant stanols (mg)	0 ± 0	2468 ± 173*	2456 ± 121*
Sitosterol (mg)	112 ± 22	132 ± 22*	123 ± 21**
Sitostanol (mg)	0 ± 0	1867 ± 131*	1879 ± 92*
Campesterol (mg)	81 ± 15	97 ± 15*	82 ± 15*
Campestanol (mg)	0 ± 0	601 ± 42*	578 ± 28*†
Energy (MJ)	11.0 ± 2.4	11.1 ± 2.5	11.0 ± 2.4
Fat (energy%)	38.8 ± 4.4	38.9 ± 4.1	38.2 ± 4.3
SAFA	13.3 ± 1.9	12.8 ± 1.9	13.5 ± 2.0†
MUFA	16.2 ± 2.2	16.4 ± 2.1	15.7 ± 2.0†
PUFA	7.4 ± 1.3	7.7 ± 1.0	7.1 ± 1.2†
Linoleic acid	5.7 ± 1.1	6.0 ± 0.9	5.5 ± 1.1†
α-Linolenic acid	1.3 ± 0.2	1.3 ± 0.2	1.1 ± 0.2**†
Cholesterol (mg/MJ)	21 ± 4.5	21 ± 5.9	21 ± 4.5
Protein (energy%)	12.9 ± 1.5	12.7 ± 1.5	12.6 ± 1.4
Carbohydrates (energy%)	45.8 ± 1.2	46.1 ± 4.8	46.7 ± 5.3
Alcohol (energy%)	1.9 ± 1.8	1.6 ± 1.4	1.8 ± 1.9
Fiber (mg/MJ)	2.4 ± 0.5	2.5 ± 0.5	2.5 ± 0.5

<sup>a</sup>Values are means ± s.d. Thirty-nine subjects consumed no plant stanols (control period), 2.5 g plant stanols once a day (at lunch), or 2.5 g plant stanols divided over three meals (0.42 g at breakfast, 0.84 g at lunch and 1.25 g at dinner). Each period lasted 4 weeks. All plant stanols were transesterified with rapeseed oil fatty acids and were administered as its fatty acid esters.

<sup>b</sup>Estimated plant sterol and stanol intake as derived from the experimental margarines and shortenings. Dietary intakes were calculated from food frequency lists filled in during the last week of each period. SAFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.

\**P* < 0.001 compared with the control period. \*\**P* < 0.01 compared with the control period.

†*P* < 0.001 compared with the once per day period. ‡*P* < 0.01 compared with the once per day period.

During the control period, mean estimated daily margarine intake at breakfast was 10.1 ± 0.6 g, and at lunch was 18.8 ± 1.8 g, while the estimated shortening incorporated into the cakes and cookies consumed after dinner was 9.6 ± 0.8 g. For the once per day period, these values were respectively 10.1 ± 0.6, 19.3 ± 1.4 and 9.6 ± 0.8 g, and for the three times per day period respectively 10.2 ± 0.6, 18.8 ± 1.7 and 9.6 ± 0.6 g. Table 2 shows the estimated plant stanol intakes as derived from the margarines and shortening, as well as the cholesterol intakes as divided over breakfast, lunch and dinner, which were all as anticipated. The cakes or cookies prepared with the experimental shortenings were consumed approximately 22 ± 20 min after dinner with no difference between the three periods.

During the different periods of the study, changes in body weight were marginal. At the start of the study mean body weight was 64.5 ± 10 kg for women and 75.2 ± 9 kg for men. At the end of the control period body weight was 64.7 ± 10 kg for women and 75.7 ± 9 kg for men and at the end of the once per day diet and the three times per day diet, mean body weights were 64.2 ± 10 and 64.5 ± 10 kg for women and 75.3 ± 9 and 75.7 ± 9 kg for men, respectively. These values were not significantly different (*P* = 0.982 for the diet of diet for women and *P* = 0.993 for men).

Table 2 Plant stanol and cholesterol intakes at breakfast, lunch and dinner during the three different diets<sup>a</sup>

	Breakfast	Lunch	Dinner
<b>Control Period</b>			
Total plant stanols <sup>b</sup> (mg)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Cholesterol (mg)	40 ± 31	46 ± 27	122 ± 36
<b>Once per day period</b>			
Total plant stanols (mg)	0.0 ± 0.0	2468 ± 173*	0.0 ± 0.0
Cholesterol (mg)	34 ± 34	50 ± 35	119 ± 41
<b>Three times per day period</b>			
Total plant stanols (mg)	436 ± 26**†	806 ± 73**†	1215 ± 69**†
Cholesterol (mg)	38 ± 32	44 ± 32	113 ± 38

<sup>a</sup>See Table 1.

<sup>b</sup>Estimated plant stanol intake as derived from the experimental margarines and shortenings.

\**P* < 0.001 compared with the control period.

†*P* < 0.001 compared with the once per day period.

#### Serum lipids and lipoproteins

Table 3 shows that plant stanol ester consumption once a day lowered serum total cholesterol concentrations by 0.32 mmol/l or 12 mg/dl compared with the control period, a reduction of 6.3% ± 6.2% (*P* < 0.001; 95% confidence interval (CI), -0.20 to -0.44 mmol/l). Consumption of a similar amount of plant stanol esters, distributed

Table 3 Fasting lipid and lipoprotein concentrations at the end of the three different diets<sup>a</sup>

	Control period	Once per day period	Three times per day period
Total cholesterol	5.02 ± 0.88	4.70 ± 0.85*	4.69 ± 0.91*
LDL cholesterol	3.04 ± 0.86	2.74 ± 0.81*	2.73 ± 0.87*
HDL cholesterol	1.50 ± 0.39	1.48 ± 0.41	1.49 ± 0.37
Triacylglycerol	1.05 ± 0.44	1.04 ± 0.45	1.02 ± 0.43
Total to HDL cholesterol ratio	3.6 ± 1.5	3.4 ± 1.4†	3.4 ± 1.4*

<sup>a</sup>See Table 1. Concentrations are expressed in mmol/l, except for the total cholesterol to HDL cholesterol ratio. To convert values for total, HDL and LDL cholesterol to milligrams per deciliter, multiply by 38.67. To convert values for triacylglycerols to milligrams per deciliter, multiply by 88.54.

\**P* < 0.001 compared with the control period. †*P* < 0.01 compared with the control period.

over the day with the three meals, lowered total cholesterol concentrations by 0.33 mmol/l or 13 mg/dl, a reduction of  $6.6\% \pm 7.8\%$  compared with the control period ( $P < 0.001$ ; 95% CI,  $-0.21$  to  $-0.45$  mmol/l). The difference of 0.01 mmol/l for total cholesterol between the once per day period and the three times per day period was not significant ( $P = 0.808$ ; 95% CI,  $-0.11$  to  $+0.13$  mmol/l).

Effects of plant stanol esters on serum total cholesterol were mainly caused by effects on serum LDL cholesterol which were, compared with the control period, significantly decreased by 0.29 mmol/l or 12 mg/dl ( $-9.4\% \pm 9.1\%$ ;  $P < 0.001$ ; 95% CI,  $-0.19$  to  $-0.39$  mmol/l) after the once per day period and with 0.31 mmol/l or 12 mg/dl ( $-10.4\% \pm 11.9\%$ ;  $P < 0.001$ ; 95% CI,  $-0.20$  to  $-0.41$  mmol/l) after the three times per day period. As for total cholesterol, the difference of 0.02 mmol/l for LDL cholesterol concentrations between the once per day period and the three times per day period was not significantly different ( $P = 0.764$ ; 95% CI,  $-0.09$  to  $+0.11$  mmol/l). Serum HDL cholesterol and triacylglycerol concentrations were not changed by the diets. Therefore, the total to HDL cholesterol ratios were significantly lower at the end of the once per day period ( $3.4 \pm 1.4$ ;  $P = 0.002$ ) and at the end of the three times per day period ( $3.4 \pm 1.3$ ;  $P < 0.001$ ), compared to the control diet ( $3.6 \pm 1.5$ ).

**Fat soluble antioxidants**

Consumption of plant stanol esters, either once or three times a day, significantly lowered absolute  $\alpha$ -tocopherol and  $\beta$ -carotene concentrations (Table 4). The reduced lycopene and  $\beta$ -cryptoxanthin concentrations nearly reached significance after the once per day period ( $P = 0.044$  and  $0.032$ , respectively), while concentrations of both antioxidants were significantly lower after the three times per day period (both  $P = 0.001$ ). In addition, during

the three times per day period also phytofluene ( $P = 0.008$ ), and  $\beta + \gamma$  tocopherol ( $P = 0.007$ ) concentrations were significantly decreased, and changes in lutein/zeaxanthin concentrations nearly reached significance ( $P = 0.023$ ). Retinol concentrations were not affected by plant stanol ester consumption.

Although differences between the once and the three times per day period never reached statistical significance, changes for all antioxidants studied were more pronounced after the three times per day period. Also, changes were larger for the sum of the less polar hydrocarbon carotenoids (ie  $\alpha$ -carotene,  $\beta$ -carotene and lycopene) compared with reductions for the sum of the more polar oxygenated carotenoids (ie lutein/zeaxanthin and  $\beta$ -cryptoxanthin) and the sum of the tocopherols, which are more polar than the carotenoids.

After standardization of the antioxidant concentrations for LDL cholesterol (Table 5), none of the antioxidant concentrations was significantly different from the concentrations at the end of the control period. Changes in LDL cholesterol standardized hydrocarbon carotenoids were still slightly negative on the once per day diet ( $-0.017 \pm 0.018$   $\mu\text{mol}/\text{mmol}$  LDL cholesterol;  $P = 0.307$ ) and  $-0.032 \pm 0.016$   $\mu\text{mol}/\text{mmol}$  LDL cholesterol ( $P = 0.049$ ) on the three times per day diet. In contrast, after standardization for LDL cholesterol, changes were slightly positive for the oxygenated carotenoids and the tocopherols (Figure 3).

**Discussion**

Many studies have demonstrated that plant stanol esters, when consumed three times a day with each meal (Miettinen *et al*, 1995; Gylling *et al*, 1997; Plat & Mensink, 2000) or twice a day at lunch and dinner (Weststrate & Meijer,

**Table 4** Retinol and fat soluble antioxidant concentrations at the end of the three different diets<sup>a</sup>

	Control period	Once per day period	Three times per day period
Retinol	2.12 $\pm$ 0.41	2.10 $\pm$ 0.38	2.14 $\pm$ 0.41
$\delta$ -Tocopherol	0.21 $\pm$ 0.16	0.19 $\pm$ 0.09	0.17 $\pm$ 0.07
$\beta + \gamma$ -Tocopherol	2.57 $\pm$ 1.06	2.58 $\pm$ 0.95	2.32 $\pm$ 0.89 <sup>†</sup>
$\alpha$ -Tocopherol	24.40 $\pm$ 4.19	23.32 $\pm$ 3.78 <sup>†</sup>	22.58 $\pm$ 3.90 <sup>†</sup>
Phytofluene	0.37 $\pm$ 0.16	0.34 $\pm$ 0.21	0.32 $\pm$ 0.20 <sup>†</sup>
Lutein/zeaxanthin	0.43 $\pm$ 0.15	0.41 $\pm$ 0.12	0.40 $\pm$ 0.13
$\beta$ -Cryptoxanthin	0.33 $\pm$ 0.12	0.31 $\pm$ 0.14	0.30 $\pm$ 0.14 <sup>†</sup>
Lycopene	0.72 $\pm$ 0.28	0.64 $\pm$ 0.27	0.60 $\pm$ 0.28 <sup>†</sup>
$\alpha$ -Carotene	0.05 $\pm$ 0.04	0.04 $\pm$ 0.03	0.04 $\pm$ 0.03
$\beta$ -Carotene	0.32 $\pm$ 0.18	0.26 $\pm$ 0.13 <sup>*</sup>	0.25 $\pm$ 0.13 <sup>*</sup>

<sup>a</sup>See Table 1. Concentrations are expressed in  $\mu\text{mol}/\text{l}$ , except for phytofluene, which is expressed in  $\text{mV}^*\text{min}/\mu\text{l}$  (amplification 100).

<sup>\*</sup> $P < 0.001$  as compared with the control period. <sup>†</sup> $P < 0.01$  as compared with the control period.

**Table 5** LDL cholesterol standardized antioxidant concentrations at the end of the three different diets<sup>a</sup>

	Control period	Once per day period	Three times per day period
$\delta$ -Tocopherol	0.07 $\pm$ 0.04	0.07 $\pm$ 0.03	0.07 $\pm$ 0.03
$\beta + \gamma$ -Tocopherol	0.92 $\pm$ 0.32	0.96 $\pm$ 0.32	0.91 $\pm$ 0.36
$\alpha$ -Tocopherol	8.68 $\pm$ 2.30	8.91 $\pm$ 2.32	9.05 $\pm$ 2.51
Phytofluene	0.13 $\pm$ 0.06	0.14 $\pm$ 0.08	0.12 $\pm$ 0.08
Lutein/zeaxanthin	0.15 $\pm$ 0.06	0.16 $\pm$ 0.07	0.16 $\pm$ 0.07
$\beta$ -Cryptoxanthin	0.12 $\pm$ 0.06	0.13 $\pm$ 0.08	0.13 $\pm$ 0.08
Lycopene	0.25 $\pm$ 0.11	0.25 $\pm$ 0.12	0.23 $\pm$ 0.11
$\alpha$ -Carotene	0.02 $\pm$ 0.02	0.02 $\pm$ 0.02	0.02 $\pm$ 0.02
$\beta$ -Carotene	0.12 $\pm$ 0.07	0.10 $\pm$ 0.07	0.10 $\pm$ 0.07

<sup>a</sup>See Table 1. Concentrations are expressed in  $\mu\text{mol}/\text{mmol}$  LDL cholesterol, except for phytofluene which is expressed in  $\text{mV}^*\text{min}/\text{mmol}$  LDL cholesterol (amplification 100).

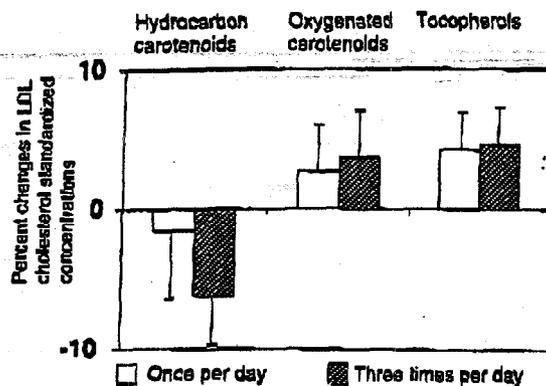


Figure 3 Percentage changes of LDL cholesterol standardized plasma hydrocarbon carotenoid, oxygenated carotenoid and tocopherol concentrations ( $\mu\text{mol}/\text{mmol}$  LDL cholesterol) at the end of the once per day period and the three times per day period, both compared with the concentrations at the end of the control period (means  $\pm$  s.e.). Hydrocarbon carotenoids were calculated as the sum of  $\beta$ -carotene,  $\alpha$ -carotene and lycopene, oxygenated carotenoids as the sum of lutein/zeaxanthin and  $\beta$ -cryptoxanthin, and tocopherols as the sum of  $\alpha$ -tocopherol,  $\beta + \gamma$ -tocopherol and  $\delta$ -tocopherol.

1998), lower serum total and LDL cholesterol concentrations. We have now demonstrated that a daily intake of 2.5 g plant stanols as its fatty acid esters, either consumed once per day (at lunch) or divided over three meals (0.4 g at breakfast, 0.8 g at lunch and 1.2 g at dinner), resulted in a similar decrease in serum total and LDL cholesterol. The amount of plant stanols in the latter period was divided over the three meals in such a way that the largest intake was at dinner and the lowest intake at breakfast. This differentiation is largely in correspondence with the distribution of cholesterol intake over the day (Ministeries van Welzijn, Volksgezondheid en Cultuur en van Landbouw, Natuurbeheer en Visserij, 1993; Table 2). Our findings therefore demonstrate that it is not necessary to consume plant stanol ester products simultaneously with dietary cholesterol or with each meal. This provides variety and may increase compliance for potential consumers. Like in other studies, serum HDL cholesterol and triacylglycerol concentrations were not affected. As a result, the total to HDL cholesterol ratio was significantly lower at the end of both the once per day and the three times per day period, as compared with the control period.

The mechanism by which plant stanol esters affect lipoprotein metabolism and lower serum cholesterol concentrations has only partly been elucidated. It is, however, generally assumed that the intestinal absorption of both dietary and biliary cholesterol is reduced in the presence of plant stanols, since the micellar solubility of cholesterol is lowered (Ikeda *et al.*, 1989). Therefore, it has been suggested that plant stanols, which also lower the micellar solubility of cholesterol, should be consumed at each cholesterol-containing meal to achieve an optimal effect (Mattson *et al.*, 1982). However, this suggestion is not supported by our findings. We therefore hypothesize that plant stanols, or plant stanol esters, remain in the intestinal lumen or in the enterocytes for a while. Indeed, only 70% of an orally administered single bolus of  $^{14}\text{C}$  labeled sitostanol to male Wistar rats is found in the feces after 24 h (Ikeda & Sugano, 1978). After 2 and 3 days the cumulative fecal excretions were 90% and 97%, respectively. Thus, when the low absorption of sitostanol into the circulation (Hassan & Ramponc, 1979) is neglected, at least

25–30% of the sitostanol is still in the intestinal tract after one day. However, when rats were fed 0.5% cholesterol and 0.5% sitostanol (W/W) for 18 days, the daily fecal excretion of sitostanol showed a recovery of approximately 100% (Sugano *et al.*, 1977). This implies that in rats, at least within 18 days, a steady state was reached and sitostanol intake equaled sitostanol excretion. This still does not elucidate whether sitostanol remains in the intestinal lumen, and if so, in which part, or in the enterocytes. It also does not answer the question of how long plant stanols are active in the intestine. Studies with caco-2 cells have addressed the question whether micellar  $^{14}\text{C}$ -labeled sitostanol could be taken up in the enterocyte and subsequently be excreted across the basolateral membrane (Field *et al.*, 1997). To our knowledge no such studies with sitostanol have been published. It appeared that sitosterol was indeed associated with the caco-2 cells. It was, however, not esterified intracellularly and not excreted to the basolateral medium. This implies that sitosterol can indeed remain in or can be associated with enterocytes. The functional significance of these findings, however, is unknown. Theoretically sitosterol could remain associated with the enterocytes only temporarily, be released into the lumen after several hours, and consequently affect micellar solubility of intestinal cholesterol at that moment. It can, however, also be speculated that plant sterols or stanols not only affect micellar solubility of cholesterol, but have additional effects on intestinal lipoprotein metabolism as well.

In this study, serum LDL cholesterol concentrations were significantly reduced by 9–10%, when plant stanol esters were consumed. In a previous study, also in a normocholesterolemic and mildly hypercholesterolemic population, serum LDL cholesterol concentrations decreased by 11–13%, when 3.8 or 4.0 g plant stanols as its fatty acid esters were consumed (Plat & Mensink, 2000). As already discussed (Mensink & Plat, 1998; Wester, 1999), hardly any additional benefit is obtained when daily intake of plant stanols exceeds 2.2 g.

Although total fat consumption during the three diet periods was similar, the fatty acid compositions of the diets were not entirely comparable. This was due to the slightly lower absorbable fat content of the stanol ester shortening compared with the control shortening. However, the marginal differences in the dietary fatty acid compositions were too small to have a major impact on serum lipoproteins. The LDL-cholesterol-lowering effect of the once per day period might have been overestimated by 0.02 mmol/l compared with the control period, while the LDL-cholesterol-lowering effect of the three times per day diet might have been underestimated by 0.01 and 0.04 mmol/l, when compared with the control period and the once per day period, respectively (Mensink & Katan, 1992).

Consumption of 2.5 g plant stanols three times a day significantly lowered most of the carotenoid and tocopherol isomers studied. In contrast, consumption of a similar amount of plant stanols once a day at lunch only resulted in reduced absolute  $\alpha$ -tocopherol and  $\beta$ -carotene concentrations. In addition, all antioxidants studied showed slightly lower concentrations at the end of the three times per day period compared with the concentrations at the end of the once per day period (Tables 4 and 5). These absolute reductions can be explained largely by a reduced number of LDL particles in the circulation, which are major carriers of the fat-soluble antioxidants. Therefore, the differences were no longer significant after standardization for LDL chole-

terol. Furthermore, we have shown that, in particular, the most lipophilic hydrocarbon carotenoid concentrations (ie  $\alpha$ -carotene,  $\beta$ -carotene and lycopene) were lowered by plant stanol ester consumption. The mechanism and the biological significance of these effects, however, remain to be elucidated.

From our results we conclude that a daily consumption of 2.5 g plant stanols as fatty acid esters either at lunch or divided over the three meals does not affect its serum LDL-cholesterol-lowering efficacy. This implies that it is not necessary to consume plant stanol esters simultaneously with dietary cholesterol or with each meal. We therefore hypothesize that plant stanols, or plant stanol esters, remain in the intestinal lumen, or possibly in or associated with the enterocytes. It can also be speculated that plant stanols not only affect micellar solubility of cholesterol, but have other intestinal effects on lipoprotein metabolism as well. Therefore, further research will be necessary to elucidate the mechanism by which plant stanols lower LDL cholesterol.

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## References

- Field FJ, Born E & Mathur SN (1997): Effect of micellar  $\beta$ -sitosterol on cholesterol metabolism in Caco-2 cells. *J. Lipid Res.* 36, 348–360.
- Friedewald WT, Levy RI & Fredrickson DS (1972): Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin. Chem.* 18, 499–502.
- Gylling H & Miettinen TA (1999): Cholesterol reduction by different plant stanol mixtures and with variable fat intake. *Metabolism.* 48, 575–580.
- Gylling H, Radhakrishnan R & Miettinen TA (1997): Reduction of cholesterol in postmenopausal women with previous myocardial infarction and cholesterol malabsorption induced by dietary sitostanol ester margarine. *Circulation* 96, 4226–4231.
- Hassan AS & Rampone AJ (1979): Intestinal absorption and lymphatic transport of cholesterol and beta-sitostanol in the rat. *J. Lipid Res.* 20, 646–653.
- Heinemann T, Kullak-Ublick GA, Pietruck B & Von Bergmann K (1991): Mechanisms of action of plant sterols on inhibition of cholesterol absorption; comparison of sitosterol and sitostanol. *Eur. J. Clin. Pharmacol.* 40(Suppl 1), s59–s63.
- Hess D, Keller HE, Oberlin B, Boufanti R & Schöpp W (1991): Simultaneous determination of retinol, tocopherols, carotenes and lycopene in plasma by means of high-performance liquid chromatography on reversed phase. *Int. J. Vit. Res.* 61, 232–238.
- Ikeda I & Sugano M (1978): Comparison of absorption and metabolism of  $\beta$ -sitosterol and  $\beta$ -sitostanol in rats. *Atherosclerosis* 30, 227–237.
- Ikeda I, Tanabe Y & Sugano M (1989): Effects of sitosterol and sitostanol on micellar solubility of cholesterol. *J. Nutr. Sci. Vitaminol.* 35, 361–369.
- Law M (2000): Plant sterol and stanol margarines and health. *Br. Med. J.* 320, 861–864.
- Manson FH, Grundy SM & Crouse JR (1982): Optimizing the effect of plant sterols on cholesterol absorption in man. *Am. J. Clin. Nutr.* 35, 697–700.
- Mensink RP & Katan MB (1992): Effect of dietary fatty acids on serum lipids and lipoproteins. *Arterioscler. Thromb.* 12, 911–919.
- Mensink RP & Plat J (1998): Efficacy of dietary plant stanols. *Postgrad. Med.* (a Special Report), 27–31 November.
- Miettinen TA, Puska P, Gylling H, Vanhanen H & Vartiainen E (1995): Reduction of serum cholesterol with sitostanol-ester margarine in a mildly hypercholesterolemic population. *New Engl. J. Med.* 333, 1308–1312.
- Ministeries van Welzijn, Volksgezondheid en Cultuur en van Landbouw, Natuurbeheer en Visserij (1993): *Zo eet Nederland, 1992. Voedingsrichtingen voor de voeding.*
- Oostenbrug GS, Mensink RP, Hardeman MR, de Vries T, Brouns F & Hornstra G (1997): Exercise performance, red blood cell deformability, and lipid peroxidation: effects of fish oil and vitamin E. *J. Appl. Physiol.* 83, 746–752.
- Plat J & Mensink RP (1999): Dietary plant stanol ester mixtures; effects on safety parameters and erythrocyte membrane fatty acid composition in non-hypercholesterolemic subjects. *Eur. Heart J.* 1(Suppl), s58–s63.
- Plat J & Mensink RP (2000): Vegetable oil based versus wood based stanol ester mixtures; effects on serum lipids and hemostatic factors in non-hypercholesterolemic subjects. *Atherosclerosis* 148, 101–112.
- SAS Institute Inc. (1985): *SAS: User's Guide Statistics, Version 5 Edition.* Cary, NC: SAS Institute Inc.
- Sugano M, Morioka H & Ikeda I (1977): A comparison of hypocholesterolemic activity of  $\zeta$ -sitosterol and  $\zeta$ -sitostanol in rats. *J. Nutr.* 107, 2011–2019.
- Wester L (1999): Dose responsiveness to plant stanol esters. *Eur. Heart J.* 1(Suppl), s104–s108.
- Weststrate JA & Meijer GW (1998): Plant sterol-enriched margarines and reduction of plasma total- and LDL-cholesterol concentrations in normocholesterolaemic and mildly hypercholesterolemic subjects. *Eur. J. Clin. Nutr.* 52, 334–343.