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Original Submission

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January 28, 2000 VIA FACSIMILE AND MAIL

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

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

Dear Dr. Rulis:

Novartis Consumer Health, Inc. hereby submits this letter asking that the Food and Drug Administration (FDA) withdraw our original request for confidentiality submitted to you with our notification dated December 13, 1999. The entire submission may be made available to the public once filed, with the exception of Section 4.0-4.3: Production Methods. Novartis will submit a summary of the production methods as an addendum to the notification early next week.

Please confirm our understanding in writing that the Notification will be deemed filed as of today, January 28, 2000 and that review by the Agency will commence immediately. It is our understanding that if there are any significant issues we will hear from the agency as soon as possible, but in any case we will hear from the agency no later than 30 days from today.

If there are any questions or concerns, please feel free to contact me directly at 908-598-7048. Thank you for your attention to this matter.

Sincerely,

Judith A. Weinstein

cc: I. Scott Bass, Esq.
Sidley & Austin

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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 β-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_{24} position on the aliphatic side chain of the compound [Pollak and Kritchevsky, 1981]. When phytosterols are saturated at the 5-α 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

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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_{29}) and campesterol (C_{28}) structures (see Section 3 for compositional details). Also present are trace quantities of C_{15}-C_{25} 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

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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 [1]	2.7 g per day [1]	1.5 g/70kg/day[2]
Δ Total Cholesterol[3]	-8.3%	-7.3%	-9.1%
Δ LDL Cholesterol[3]	-13.0%	-13.0%	-15.5%
Δ HDL Cholesterol[3]	+0.6%	+0.1%	-4.4%

[1] 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.
[2] These data are from the Jones et al [1999] study conducted in males, only.
[3] 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; 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_{12} - C_{18} 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 Kritchevesky, 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: (3β)-Stigmast-5-en-3ol; $C_{29}H_{50}O$;

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: (3β, 5α)-Stigmastan-3-ol; $C_{29}H_{52}O$;

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: (3β,24R)-Ergost-5-en-3-ol; $C_{28}H_{48}O$;

Approximate percentage in Phytrol™: 9% to 18%

Mol. wt. 400.69

Melting point 157-158 degrees Celsius.

Soluble in acetone and ethyl acetate.

Campestanol: (3β, 5α, 24R)-Ergostan-3-ol; $C_{28}H_{50}O$;

Approximate percentage in Phytrol™: 2% to 14%

Mol. wt. 402.70

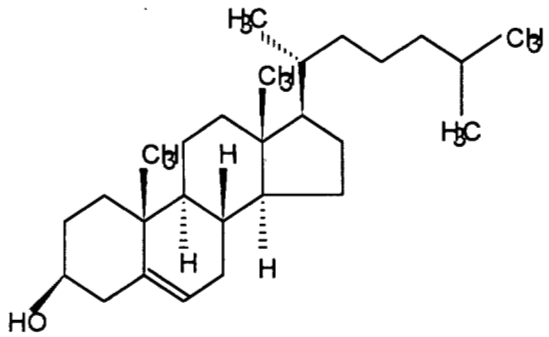
Melting point 146.5-147.8 degrees Celsius.

Soluble in acetone and ethyl acetate.

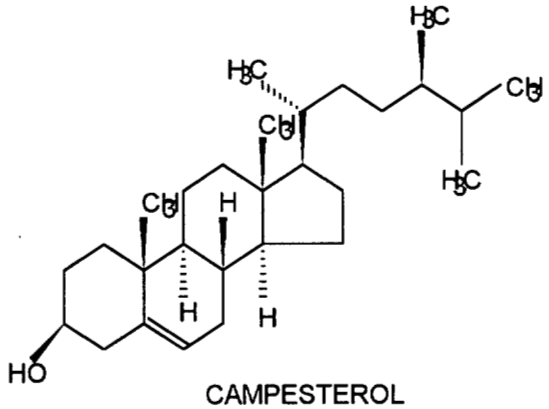
3.2 Chemical Structure of Component Phytosterols

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.

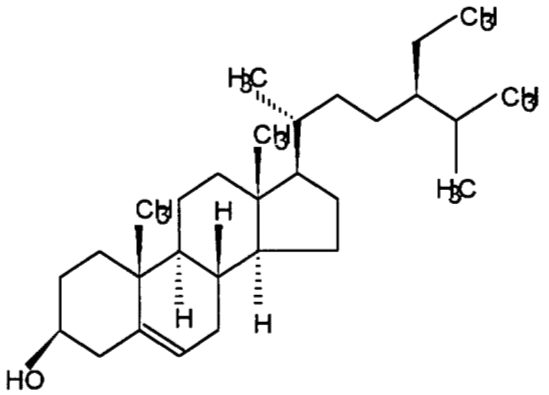
Figure 1: Cholesterol and Major Phytrol™ Constituent Phytosterols



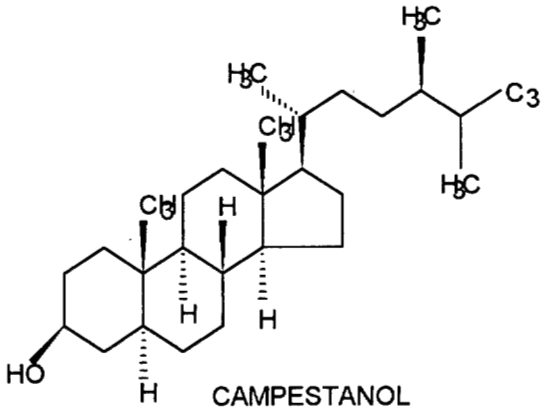
CHOLESTEROL



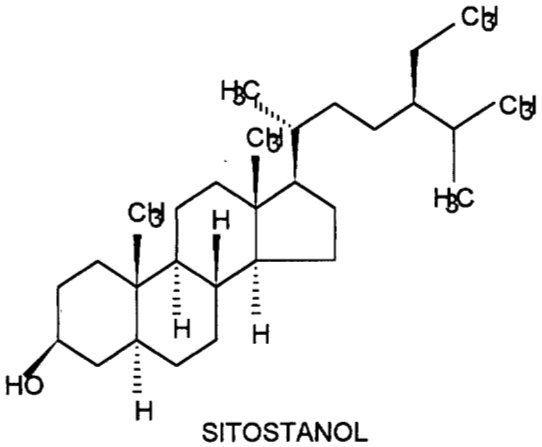
CAMPESTEROL



SITOSTEROL



CAMPESTANOL



SITOSTANOL

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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_{29}) and campesterol (C_{28}) structures. Also present in trace amounts are saturated long chain (C_{15} – C_{25}) 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_{28} compounds)
• Stigmasterol	• 24-Ethyl di- and tri- ene isomers (C_{29} compounds)
• Ergosterol	• Trace phytosterols and phytostanols
• C_{15} - C_{25} Aliphatic Alcohols (< 0.5%)	

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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.

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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.

7.0 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.

8.0 ABSORPTION, DISTRIBUTION, METABOLISM AND EXCRETION

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; Cerquira *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 *et al.*, 1969
Man	10	Borgstrom, 1968
Rat	5	Gould, 1955
Rat	5	Sylven & 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 5α-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 μmoles/L (1.62-12.72) and 9.31 μmoles/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 μmol/L)	0.42 mg/dL (105 μmol/L)
Hypercholesterolemic	361 (mg/dL)	0.55 mg/dL (13.3 μmol/L)	0.78 mg/dL (19.5 μmol/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 hypercholesterolemics, 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 (μmol/ L)		Campesterol (μmol/ L)	
			Control	Treated	Control	Treated
PHF9502	Hamster	680	19	37	15	18
PHF9401	Rat	1000	4.87 ± 0.65	20.30 ± 2.50	3.75 ± 0.50	13.91 ± 1.92
PHF9401	Rat	1000 (Soy Phytosterols)	5.78 ± 0.89	23.29 ± 2.93	7.07 ± 1.88	14.59 ± 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 μmol/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 *et al.*, 1970
5.65 g/d sitosterol	26.5	No data	
Control	8.0	17.5	Westestrate 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 *et al.*., 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 *et al.*, 1978
12.0 (Sterols)	1.48 ± 0.62	No data	
Control	13.3 ± 1.0	30.1 ± 2.4	Gylling *et al.*., 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 phytosterolemics, compared to about 0.4% in normal subjects.

Table 8-9 Plasma Levels of Phytosterols in Phytosterolemia

Sterol	Average Plasma Level (μmol/L)	Range (μmol/L)	References
Sitosterol	850	340 - 1570	Salen *et al.*, 1985
Campesterol	425	186 - 596	
Sitostanol	100	46 - 144	Bhattacharyya *et al.*, 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 5α-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 7α-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}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 C_{24} bile acids are formed, C_{21} bile acids are formed from sitosterol in the

rat. Two bile acids have been tentatively identified as having hydroxyl groups at C_3 and C_{15}. 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 [2] mg/kg/day	Treatment Time (days)	% Change in Food Intake [1]	% Change in Body Weight [1]
Subchronic Studies							
PHF9501	Hamster [3] 20 M & F	0.25%	0.5% 1.0%	340 680	90	+10.2% NS	-3.25%
PHF9601	Mouse [4] 10 M	0.15%	2.0%	3340	126	NS	+6.13% $p<0.05$
PHF9602	Mouse [4] 8 M	0%	2.0%	3340	140	NS	NS
PHF9703	Mouse [4] 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 [2] mg/kg/day	Treatment Time (days)	% Change in Food Intake [1]	% Change in Body Weight [1]
Subacute Studies							
PHF9401	Rat [4] 6 M	1.0%	1.0%	1000	10	-2.56% NS	-1.72% NS
PHF9402	Hamster [3] 8 M	0.25%	0.25% 0.50% 1.00%	170 340 680	34	-1.5% $p<0.05$	-13.07% NS
PHF9502	Hamster [3] 10 M	0.25%	1.0%	680	45	+0.38% NS	+0.61% NS
PHF9503	Rabbit [5] 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 osestradiol, 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
Subchronic Studies							
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.
Subacute Studies							
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.
Reproductive Studies							
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

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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
Acute Studies			
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.
Subchronic Studies			
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.

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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 phytostanols. 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 xanthamatous 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 μM. 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_1 or F_2 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_1 and F_2 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_0- and F_1-generation Wistar rats or the development of F_1 and F_2 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 μg 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 *et al.*, 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 *et al.*, 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	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. 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. 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). 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. 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.

000060

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 *et al.*, 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 *in vivo* 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 utherotrophic 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 *et al.*, 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.

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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 *et al.*, 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 *et al.*, 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 bwt/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 *et al.*, 1999.	Ames assay (s typhimurium) bacterial cell genotoxicity test L5178Y 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 microsome 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.

0000062

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 *et al.*, 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 *et al.*, 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 *et al.*, 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 et al., 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.

000063

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 *et al.*, 1993.	Wistar albino rats 10 F	Sitosterol	Anacardium occidentale	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 *et al.*, 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 *et al.*, 1991.	Wistar albino rats 10 M	Sitosterol	Anacardium occidentale	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.

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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 ± 0.2 mmol/l) was lower ($p<0.05$) than that of post placebo treatment (4.3 ± 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 about1800 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[2]	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™) Soy PS ester	Tall oil Soy	Margarine suspension	2.74 3.24	24-25	6377 7541	None except effects on vitamin and nutrient levels in plasma (See Table 10-1).
Plat J and Mensink R, 1998.	Healthy volunteers	112		SITO 70 SITO 90	Vegetable Tall oil	Margarine suspension	3.8 4.9	56	23833.6 25088	Hematology and blood chemistry parameters remain within normal range.
Kris-Etherton PM *et al.*, 1998.	HC	35M & 23F		Sitostanol mixture	Vegetable	Margarine suspension	3	28	4872	none
Cobb MM *et al*, 1997.	Sitosterolemic homozygote	1F	9	Sitosterol	Soybean oil Sesame oil	Oil suspension	0.06 0.09 0 122	56	34-67	none
Gylling *et al.*, 1997.	Woman with angiographically documented CAD Women treated with simvastatin for more than 1 year	22F 10F		Sitostanol ester (Benecol™)	Tall oil	Margarine suspension	3 3	49 90	3234 2700	None
Gylling *et al*, 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

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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[2]	Duration (days)	Extent g/d x days x subjects	Adverse Events Reported
Gylling *et al*, 1995	FH	7 M 7 F	9.1±1.1	Sitostanol ester	Tall Oil	Margarine suspension	3.0	42	1764	none
Gylling *et al*, 1995	NIDDM with HC	6 M	63.2±1.2	Sitostanol ester	Tall Oil	Margarine suspension	3.0	28	504	none
Pelletier *et al*, 1995	Healthy volunteers	12M	22.7±2.6	Sitostanol ester	Soybean Phytosterol	Margarine suspension	0.740	28	249	none
Miettinen *et al*, 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 *et al*, 1995	HC	33 M	31-70	Sitostanol	Tall Oil	Margarine suspension	3.0	30	2970	none
Gylling *et al*, 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 *et al*, 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 *et al*, 1993	HC	47M 20F	25-60	Sitostanol Ester	No data	Margarine suspension	3.4 (n=34)	42	9568	none
Becker *et al*, 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

000069

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[2]	Duration (days)	Extent g/d x days x subjects	Adverse Events Reported
Becker *et al*, 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 Sitostanol	No data	Margarine suspension	0.625 (n=8) 0.630 (n=8)	54 54	270 272	none
Heinemann *et al*, 1986	HC and FH	3 M 3 F	27-59	Sitostanol	No data	Capsule	1.5	28	252	none
Weisweiler *et al*, 1984	FH (type IIa)	6M 4F	29-67	Sitosterol	No data	Capsule ?	6.0	56	3360	none
Mattson *et al*, 1982	Unknown cholesterol status	9 M&F	adults	Sitosterol	No data	Aqueous Suspension (Cytellin™)	1.0	30	270	none
Schlierf *et al*, 1978	FH (type II)	12 M&F	8-20	Sitosterol	No data	Granule	12.0	56	8064	none
Lees *et al*, 1977	FH (type II)	9M 3F	Adults	Sitosterol Campesterol	Soybean	Capsule	18.0	280 (average) (364-728)	60480	none
Lees *et al*, 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

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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[2]	Duration (days)	Extent g/d x days x subjects	Adverse Events Reported
Lees *et al*, 1977	FH (type II)	9 M	adults	Campesterol	Tall Oil	Capsule	3.0	Ave 196	5292	none
Lees *et al*, 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 *et al*, 1977	FH (type II)	5 M 13 F	adults and children	Phytosterol mixture	Tall Oil	Capsule	6.0	Ave 140	15120	
Duncan *et al.*, 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 *et al*, 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

000071

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[2]	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 *et al.*, 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 *et al.*, 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 *et al.*, 1955	Coronary artery disease	18M 8F	unknown	Sitosterol	Unknown	palatable biscuit	9	147	34398	none
Joyner *et al.*, 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 *et al.*, 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.

000072

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 α+β 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 et al., 1998.	Stanol esters	α tocopherol / LDL ratio unchanged	β-carotene/LDL ratio – 17.6%	No change in Vit. A or D	-
Gylling et al., 1996	Stanol esters	-9.8% ($p<0.001$)	-30% ($p<0.001$)	-	-

Data summarized above is from phytosterols administered in margarine.

000073

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)

Sterol	Take Control™ (phytosterols from Soybean Oil)	PHYTROL™ (Forbes Medi-Tech natural Tall Oil Phytosterols)	Benecol™ (Hydrogenated Tall and Soybean Oil Phytosterols)
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_{15}-C_{25} 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 ($C_{29}H_{46}$), 24-methylene lophenol ($C_{29}H_{48}O$), 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

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

[1] 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.
[2] These data are from the Jones et al [1999] study conducted in males, only.
[3] 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.

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

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Adjunct Professor, Georgetown University
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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.

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End Submission

Linda -
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NOVARTIS

DUPLICATE

AM

January 28, 2000 **VIA FACSIMILE AND MAIL**

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

2000 FEB -1 P 12: 11

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

Dear Dr. Rulis:

Novartis Consumer Health, Inc. hereby submits this letter asking that the Food and Drug Administration (FDA) withdraw our original request for confidentiality submitted to you with our notification dated December 13, 1999. The entire submission may be made available to the public once filed, with the exception of Section 4.0-4.3: Production Methods. Novartis will submit a summary of the production methods as an addendum to the notification early next week.

Please confirm our understanding in writing that the Notification will be deemed filed as of today, January 28, 2000 and that review by the Agency will commence immediately. It is our understanding that if there are any significant issues we will hear from the agency as soon as possible, but in any case we will hear from the agency no later than 30 days from today.

If there are any questions or concerns, please feel free to contact me directly at 908-598-7048. Thank you for your attention to this matter.

Sincerely,

Judith A. Weinstein

cc: I. Scott Bass, Esq.
Sidley & Austin

AM

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Judith A. Weinstein
Associate General Counsel

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Linda Kahl

FAX

Date:	2/1/00
To: Company:	Alan Rulis FDA 202-418-3131
From:	Judith A. Weinstein
# of Pages (Including Cover)	3

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CONFIDENTIALITY NOTE

This facsimile cover sheet and any documents accompanying this facsimile transmission contain information from Novartis Consumer Health, Inc. (NCHI) which is confidential and or legally privileged. The information is intended only for the use of the individual or entity named on this transmission sheet. If you are not the intended recipient, you are hereby notified that any disclosure, copying, distribution or the taking of any action in reliance on the contents of this information is strictly prohibited, and that the documents should be returned to NCHI immediately. In this regard, if you have received this facsimile in error, please notify us by telephone immediately so that we can arrange for the return of the original documents to us at no cost to you.

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Judith A. Weinstein
Associate General Counsel

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February 1, 2000

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

VIA FACSIMILE AND MAIL

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

Dear Dr. Rulis:

On January 28, 2000 Novartis Consumer Health, Inc. requested via letter and facsimile that the Food and Drug Administration (FDA) withdraw our original request for confidentiality submitted to you with our notification dated December 13, 1999. We noted that the entire submission could be made available to the public once filed, with the exception of Section 4.0-4.3: Production Methods. We committed to file an addendum regarding production methods early this week.

Novartis hereby submits to the Agency a summary of the production methods as an addendum to the notification. This document should take the place of the original pages 23-24 in the notification. This information may be made available to the public.

If there are any questions or concerns, please feel free to contact me directly at 908-598-7048. Thank you for your attention to this matter.

Sincerely,

Judith A. Weinstein

Cc: I. Scott Bass, Esq.

David Stewart
Forbes Medi-Tech, Inc.

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 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.

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.

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 recrystallized a second time.

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November 21, 2000

Alan M. Rulis, Ph.D.
Office of Premarket Approval
Center for Food Safety & Applied Nutrition
Food and Drug Administration
200 C Street, SW
Washington, DC 20204

Re: GRAS Notification: GRN 000039, Tall Oil Phytosterols

Dear Dr. Rulis

Novartis' supplier for its tall oil phytosterols has scaled up its manufacturing process to prepare for commercial marketing of the product described in our December 13, 1999 GRAS notification. As part of the scale-up, the manufacturing process was slightly modified to enhance production efficiency. The product retains its fundamental characteristics and no impurities or potentially deleterious substances have been added. Novartis' GRAS panel has reviewed the minor changes and issued an updated GRAS expert report, which we attach.

Respectfully,

Judith Weinstein

Enc: GRAS Panel Report

cc: I. Scott Bass

ADDENDUM TO THE 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 in 1999 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.

Based on its critical evaluation, the Expert Panel in 1999 determined that Phytrol™, meeting the specifications cited at that time, is Generally Recognized as Safe 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. Novartis Consumer Health Inc. then submitted to FDA a notification (GRN39) that it had determined that tall oil phytosterols are Generally Recognized as Safe 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.

The Expert Panel has been recently provided with new information regarding changes in the production and phytosterol specification ranges for the product Phytrol™. The Expert Panel was asked to evaluate these changes with regard to any inconsistency with their original GRAS determination.

The Expert Panel was informed that 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 hydrogenation process component to restore the stanol

concentrations. Consequently, this modification to the method of Phytrol™ manufacture at the Quest facility has resulted in a slight change in the chemical composition of the Phytrol™ product. Forbes Medi-Tech Inc. conducted a comparative GC-MS analysis between Phytrol™ manufactured at the Quest facility and the original Phytrol™ material detailed in the original report to the Expert Panel. Based upon the revisions in the Phytrol™ method of manufacture, new product specifications were developed and provided for review by the Expert Panel. A report by CANTOX U.S. Inc., amending its original report to the Expert Panel and providing detailed information regarding production changes, chemical composition and proposed new specification ranges for Phytrol™ content of sitosterol, sitostanol, campesterol and campestanol aided and facilitated the work of the Expert Panel. The Expert Panel noted FDA's recent publication of an Interim Final Rule which authorizes, with certain conditions, the use of a coronary heart disease health claim for plant sterol esters and plant stanol esters. The Interim Final Rule, which is currently undergoing a comment period, authorizes the health claim for several product forms, including spreads, wherein a single product serving contains at least 0.65 grams of plant sterol esters or 1.7 grams of plant stanol esters. The Expert Panel considers this to represent important evidence supporting the safety and effectiveness of consuming phytosterols and stanols for the purpose of maintaining healthy blood cholesterol levels. The Expert Panel noted further that the proposed use of Phytrol™ in a Novartis spread would contain phytosterols within the range described for the health claim criteria.

The Expert Panel evaluated the results of detailed chemical analyses of three production lots of Phytrol™ manufactured by Quest and determined that the composition remains intermediate between that of the phytosterol/stanol ingredients of Take Control™ and Benecol™. Compared to the original composition, new Phytrol™ contains a higher percentage of sitosterol and lower percentages of sitostanol, campesterol and campestanol such that the ratio of total phytosterols to total stanols is increased yet remains intermediate between that occurring in Take Control™ and in Benecol™. The Phytrol™ product specification ranges have been appropriately modified with respect to sitosterol (36% to 79%), sitostanol (6% to 34%), campesterol (4% to 25%) and campestanol (0% to 14%) to accommodate the change in composition; all other specifications remain as initially established.

With respect to the occurrence of minor product components, the Expert Panel determined there to be no significant differences between Phytrol™ manufactured by Quest and the original

Phytrol™ product. Similarly, the hydrogenation component in the Quest manufacturing process was determined to be representative of standard food oil industry practice and to introduce no issues regarding Phytrol™ safety.

Based on the critical evaluations discussed above, the Expert Panel concluded that the composition of Phytrol™ as manufactured by Quest is equivalent to the original composition of Phytrol with respect to safety.

The Expert Panel also evaluated the change in Phytrol™ composition with regard to effects on blood cholesterol parameters, blood phytosterol levels and absorption of vitamins and nutrients. Based on this evaluation, the Expert Panel concluded that the moderate increase in phytosterol/stanol ratio would not alter Phytrol's effectiveness in decreasing blood cholesterol. The Expert Panel concluded similarly that ingestion of new Phytrol™ would be expected to continue to produce blood phytosterol concentrations which remain similar to those produced by Take Control™ and Benecol™. Lastly, the Expert Panel, after considering the substantial compositional equivalence between the Phytrol™ manufactured at the Quest facility and the original GRAS Phytrol as well as its intermediate composition between Take Control™ and Benecol™, concluded that the revised Phytrol™ presents no significant risk of adversely altering vitamin and nutrient absorption to any degree materially different from Benecol™, Take Control™, or original Phytrol™.

Based on the critical evaluation discussed above, the Expert Panel has determined that Phytrol™, manufactured at Quest using the process modifications and meeting the new specifications cited above, is Generally Recognized as Safe 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 (21CFR § 182.1(b)) in an amount not to exceed 12% phytosterol plus stanol in the finished spread.

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

Walter H. Glinsmann, M.D.
Adjunct Professor, Georgetown University
President, Glinsmann Inc.

K.C. Hayes, D.V.M., Ph.D.
Professor Biology (Nutrition)
Director, Foster Biomedical Research Laboratory
Brandeis University

Based on the critical evaluation discussed above, the Expert Panel has determined that Phytrol™, manufactured at Quest using the process modifications and meeting the new specifications cited above, is Generally Recognized as Safe 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 (21CFR § 182.1(b)) in an amount not to exceed 12% phytosterol plus stanol in the finished spread.

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Based on the critical evaluation discussed above, the Expert Panel has determined that Phytrol™, manufactured at Quest using the process modifications and meeting the new specifications cited above, is Generally Recognized as Safe 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 (21CFR § 182.1(b)) in an amount not to exceed 12% phytosterol plus stanol in the finished spread.

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