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ORIGINAL SUBMISSION

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Procter & Gamble

The Procter & Gamble Company
Winton Hill Technical Center
6071 Center Hill Avenue, Cincinnati, Ohio 45224-1703

July 17, 2000

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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 a Phytosterol Ester-Enriched Vegetable Oil Product: GRAS Exemption Claim***

Dear Sir or Madame:

The Procter & Gamble Company (P&G) hereby notifies the Food and Drug Administration (FDA) that it has determined that the use of phytosterol esters to enrich common vegetable oil is "generally recognized as safe" (GRAS). This notification is pursuant to FDA's proposed GRAS notification policy described at 62 Federal Register 18938-18960 (April 17, 1997). This cover letter, which contains the required GRAS exemption claim, is attached to the document detailing the basis for the GRAS determination. Three copies of the materials are enclosed, as required.

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The GRAS determination was made via both scientific procedures and substantial equivalence to existing GRAS phytosterols. An expert scientific panel has reviewed the chemistry, anticipated exposure, safety assessment, and extensive literature assembled to support the safety of this phytosterol ester use. Phytosterol and phytosterol ester safety is well supported by clinical and laboratory studies, and has been demonstrated in previous GRAS notifications including McNeil (Benecol®), Lipton (Take Control®), Novartis (Phytrol®), and most recently, Cargill (Vegetable Oil Phytosterol Ester). P&G plans to enrich its vegetable oil product using a phytosterol ester product which has been shown to be substantially equivalent to the phytosterols found in Take Control™. [redacted]

This submission contains detailed information about the structure and composition of the notified substance, including its intended use, maximum and typical exposure levels, and analytical results from repeated heating under home use frying conditions. [redacted]

The notification also includes a comprehensive review of the scientific literature through March 2000, and a review of other key supporting documentation relevant to this class of food materials.

The following information is provided under proposed 21 CFR §170.36(c)(1):

Notifier:	The Procter & Gamble Company Winton Hill Technical Center 6071 Center Hill Avenue Cincinnati, OH 45224 Attn: Maury Bandurraga, Ph.D. Regulatory Affairs Manager Food and Beverage R&D
GRAS Substance:	Phytosterol Ester- Enriched Vegetable Oil
Intended Use:	The Procter & Gamble Company will incorporate phytosterol esters substantially equivalent to those already marketed in Take Control™ at a level up to 13.3%, by weight, as an ingredient in vegetable oil for home use applications such as baking, frying, and salad dressings. The phytosterol esters 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 the Procter & Gamble Company's GRAS determination are available for FDA's review and copying at reasonable times at the law firm of Covington & Burling, 1201 Pennsylvania Ave. NW, Washington, D.C. 20004-2401. The contact there is Mr. Peter Barton Hutt, Esq.

Please contact me at (513) 634-4137 if FDA has any questions or wishes to access these materials.

P&G plans, as for any new product, to conduct our usual post-market evaluation of consumer acceptance and use for this new product. This will include 800-line monitoring of comments from consumers and health professionals, and providing information to health professionals to answer questions about recommended dietary use. In addition, we typically do various types of consumer research to determine product acceptance and usage; the specific plans to gain additional consumer learning depend on many factors. As discussed, we will share any information on product

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usage and exposure that materially changes the GRAS determination presented in this review.

Respectfully submitted,

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Professional and Regulatory Services
Food and Beverage
The Procter & Gamble Company
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GRAS Notification

for

Phytosterol Ester-Enriched
Vegetable Oil

Submitted by:

The Procter & Gamble Company
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6071 Center Hill Avenue
Cincinnati, OH 45224

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1.0 INTRODUCTION

1.1 Background

Plant sterols or "phytosterols", which structurally resemble cholesterol but have a much lower absorption rate, are gaining popularity in consumer products. Recently marketed vegetable spreads such as Benecol™ and Take Control™ are two examples of phytosterol-containing products intended to promote healthy cholesterol levels. These two vegetable oil spreads contain up to 20% by weight added fatty acid esterified phytosterols. Phytosterols, which are found in a variety of plant sources, cannot be manufactured by the human body and are obtained exclusively through the diet. Because phytosterols can compete with cholesterol for absorption in the gastrointestinal tract, they are presumed to provide substantial health benefits by helping to maintain healthy blood cholesterol levels.

Benecol™ is a GRAS vegetable oil spread manufactured by McNeil Consumer Healthcare. It contains a blend of fatty acid esterified hydrogenated sterols derived from vegetable oil and tall oil, which is oil obtained from trees. Hydrogenation of this blend saturates the component sterols into phytostanols. The resultant phytostanol product is composed of campestanol (32%) and sitostanol (62%), as well as approximately 5% unsaturated sterols. Take Control™, which is manufactured by Unilever, contains fatty acid esterified phytosterols derived from vegetable oils such as canola, soybean, and corn oil; it is also considered to be GRAS. The composition of sterols in Take Control™ varies, depending on the oil used, but generally consists of approximately 14 to 23% stigmasterol, 20 to 28% campesterol, 40 to 55% β-sitosterol, and up to 8% of about 20 to 30 minor sterols. These sterols are commonly found in foods. Esterification of these sterols to vegetable oil fatty acids facilitates their incorporation into the matrix of common food items such as vegetable oil spread.

Both Benecol™ and Take Control™ have been self-affirmed by their respective manufacturers as Generally Recognized as Safe (GRAS). The U.S. Food and Drug Administration (FDA) is aware of and is not in disagreement with this opinion. Cargill Incorporated manufactures a phytosterol ester product with properties virtually identical

to the esterified phytosterols present in Take Control™ and similar to the phytostanols present in Benecol™. The Procter & Gamble Company has incorporated the Cargill phytosterol ester product into a vegetable oil intended for home use in cooking applications and salad dressing. This may be viewed as an add-on source of phytosterols to the diet, however, it is used in amounts comparable to current intake levels and is discussed in Section 6.0. Furthermore, no significant change in any potential oxidation products resulting from typical cooking applications of phytosterol enriched vegetable oil has been documented and is discussed in Section 3.0. The use of this product is intended to help maintain a healthy blood cholesterol level.

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 Procter & Gamble to determine the Generally Recognized as Safe (GRAS) status of a phytosterol ester enriched vegetable oil product. The panel of qualified experts has determined that the Procter & Gamble phytosterol ester enriched vegetable oil product may be considered GRAS based upon its substantial and demonstrable compositional equivalence to phytosterols in currently marketed products, particularly Take Control™, as well as on the basis that these phytosterols:

1) The substantial compositional equivalence of its phytosterol ester constituents to the phytosterol esters found in currently marketed products, particularly Take Control™;

2) Have an expected safety and physiologic activity profile equivalent to currently used phytosterols.

3) Are to be consumed at levels occurring from currently marketed products based upon an intake assessment demonstrating that the additional intake of phytosterol esters from home use of a salad and cooking oil would not present any additional risk to consumers of such products; and

4) The phytosterol component of the enriched vegetable oild does not pose any risk based upon the results of a chemical analysis of the phytosterol ester-enriched vegetable oil by GC and GC/MS methods which demonstrated the chemical stability of the phytosterols when the sterol-enriched oil is heated under conditions simulating typical frying conditions in the home.

[redacted]

The panel of qualified experts has reviewed the necessary technical, safety, and product information and has determined that the use of Procter & Gamble's phytosterol ester-enriched vegetable oil be considered GRAS based upon recognized scientific procedures. A written statement to that effect and signed by each member of the expert panel appears in Section 11 of this document.

1.2 History of Safe Use

The average American diet contains 700 to 1000 mg/d of sterols, including phytosterols and cholesterol. Approximately 30 to 40% of these sterols are phytosterols and 60 to 70% is cholesterol (Finocchiaro and Richardson, 1983). In the United States, the average intake of phytosterols is approximately 250 mg per day; vegetarians consume nearly twice as much. Research conducted over the past fifty years has indicated that increased phytosterol intake can contribute to the lowering of blood cholesterol levels in humans. Long-term clinical studies and studies with dose levels of up to 25,000 mg phytosterols per day (*i.e.*, 100 times the average dietary intake) have been conducted (Pollak and Kritchevsky, 1981; Pollak, 1985). Over 1,800 men, women, adolescents, and children have participated in these studies, some of which date back to the 1950s. Under the conditions of these studies, no significant adverse health effects have been reported (Lees *et al.*, 1977; Oster *et al.*, 1977).

Plant sources rich in phytosterols include rice bran, wheat germ, and oats, which can contain up to 4% phytosterols. The majority of phytosterols are found in seeds and seed oils. Most seed oils contain between 0.1 to 0.5% sterols. In contrast, the sterol content of fruits and vegetables typically ranges from 0.001% to 0.2% fresh weight; most of the higher values are for vegetable seedlings such as the pea and barley, as discussed by Weihrauch and Gardner (1978). Some crude vegetable oils contain 0.1% to 0.5% of phytosterols. Reduced fat and low fat spreads currently available in the marketplace may contain background phytosterol levels between 0.3% and 0.4%. The predominant phytosterol constituents present in the Procter & Gamble phytosterol-enriched vegetable oil are β-sitosterol, campesterol, and stigmasterol, all of which are commonly found in food products. Take Control™ and Benecol™ are two previously

mentioned phytosterol-containing spreads with up to 20% added esterified phytosterols by weight. Phytosterols are often esterified with C_{12} - C_{18} fatty acids to facilitate their incorporation into the fat matrix of common food items such as vegetable oil spreads.

Three of the most common plant sterols are β-sitosterol, campesterol, and stigmasterol. The predominant phytosterols in Take Control™, by weight, are β-sitosterol (40 to 55%), campesterol (20 to 28%), and stigmasterol (14 to 23%). Up to 4 to 8% of this product may be comprised of 20 to 30 different minor sterol components. The phytosterols in Take Control™ are derived from vegetable oils such as soybean, canola, and corn. Consequently, the principal sterol components can vary from batch to batch, depending on the type of vegetable oil used to obtain the phytosterols. Benecol™ spread, on the other hand, contains a blend of vegetable oil and tall oil phytosterols. Hydrogenation of this blend saturates the sterols into stanols, resulting in approximately 62% sitostanol and 32% campestanol. The remaining 6% is comprised primarily of unsaturated sterols. The phytosterol esters employed in the Procter & Gamble enriched vegetable oil product are derived from vegetable oils and are intended to be an alternative or additional source of esterified phytosterols for home use in maintaining a healthy cholesterol level in humans.

Take Control™ and Benecol™ are products recently marketed in the United States with the aim of achieving a healthy cholesterol level, although attempts to capitalize upon the purported benefits of increased phytosterol intake are not new. For example, Cytellin™, a preparation marketed by Eli Lilly between the 1950s and the 1980s, was available in the United States as a treatment for hypercholesterolemia. The same product was also available in Canada and sold under the brand name Positol™. Cytellin™ contained phytosterols derived from tall oil, with approximately 80% β-sitosterol, 10% sitostanol, 7% campesterol, and 2% campestanol. Recommended therapeutic doses ranged from 9,000 to 30,000 mg per day. No contraindications or adverse side effects were reported during repeated clinical investigations of Cytellin™ (Best *et al.*, 1955; Joyner and Kuo, 1955; Lesesne *et al.*, 1955; Farquhar *et al.*, 1956; Kuo, 1956; Duncan and Best, 1963).

In summary, the Cargill Inc. Phytosterols present in the Procter & Gamble phytosterol enriched vegetable oil are very similar to those naturally occurring in fruits, [redacted]

vegetables, and vegetable oils that are part of the human diet. No significant adverse effects have been associated with consumption of relatively high levels or long-term use of phytosterols during the course of clinical trials. The phytosterols present in the Procter & Gamble phytosterol-enriched vegetable oil are identical in composition to those present in Take Control™, a GRAS product. New analytical data of the Procter & Gamble product demonstrate chemical stability under the typical conditions associated with home use (*e.g.*, frying) and that the additional phytosterol intake would not pose any additional risk to consumers. All of this information has been reviewed and has been determined to be GRAS by an independent panel of qualified experts.

2.0 BASIS FOR REGULATORY APPROVAL OF GRAS AFFIRMATION

2.1 GRAS Regulatory Status

The intended use of the Procter & Gamble phytosterol ester-enriched vegetable oil product is to provide an alternative or additional source of phytosterol esters for home use by individuals in maintaining healthy levels of plasma cholesterol. The Procter & Gamble Company understands that this represents a new use of dietary phytosterols constituting a new source of consumption and may result in additional dietary intake.

Per Volume 62, Number 74 of the April 17, 1997 Federal Register, Pages 18937-18964 (Proposed Rules, 21 CFR Parts 170, *et al.*), The Procter & Gamble Company wishes to notify the U.S. Food and Drug Administration (FDA) that it has determined that the use of phytosterol esters manufactured by Cargill Incorporated at a level up to 13.3%, by weight, as an ingredient in vegetable oil for home use applications such as baking, frying, and salad dressings, is Generally Recognized as Safe (GRAS). The GRAS determination is based upon scientific procedure, including affirmation by a panel of qualified experts who reviewed the technical evidence of safety and concluded that the substance is not harmful under the intended conditions of use; i.e. it is safe. The expert panel further concluded that the Procter & Gamble product is safe for its intended

use. This would thereby warrant exemption from the pre-market approval requirements of the Federal Food, Drug, and Cosmetic Act for this product.

The phytosterol esters employed in this product, as manufactured by Cargill Inc., are equivalent in structure and function to the phytosterol esters contained in Unilever's Take Control™ vegetable oil phytosterol spread, and are derived primarily from soy. These phytosterols are also similar in nature to those in the Benecol™ hydrogenated vegetable oil / tall oil phytosterol spread. However, unlike Benecol™, the Procter & Gamble product contains no tall oil-derived phytosterols and contains only minor amounts of saturated phytostanols. Both Take Control™ and Benecol™ have been self affirmed as Generally Recognized as Safe (GRAS) and are currently marketed in the United States, with full U.S. FDA awareness.

In accordance with the specific requirements outlined in 21 CFR Section 170.36(c) of the Proposed Rule, this report provides the necessary information to support an evaluation by a panel of qualified experts, providing the basis for a conclusion that this product may be considered safe. The following table outlines the applicable sections, subsections, and specific requirements of the Proposed Rule.

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

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

[redacted]

The scientific and technical data presented herein is in support of a GRAS determination by a panel of qualified experts for the Procter & Gamble phytosterol-enriched vegetable oil product. These supportive materials were obtained from the following sources and are available for further FDA review:

1) A comprehensive review of the scientific literature through March of 2000 for data on the safety of sterols, particularly phytosterols was conducted;

2) Information from the recent GRAS self determination of the Cargill Incorporated phytosterol ester product Expert Panel;

3) Information from recent GRAS notifications for substantially equivalent products (e.g., Take Control™ and Benecol™);

4) An intake assessment for the consumption of phytosterol-enriched vegetable oil under the conditions of home use, such as in baking, frying, and salad dressing;

5) A comprehensive review of the scientific literature through March of 2000 for data on the expected reaction products that may arise from the heating of phytosterol esters under the conditions of home cooking applications; and

6) A chemical characterization (GC and GC/MS) of the Procter & Gamble phytosterol-enriched vegetable oil after heating under conditions simulating deep frying at home, compared to the unheated enriched oil and to a heated vegetable oil control.

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

2.2 Substantial Equivalence to a GRAS Substance

The phytosterol esters contained within the Procter & Gamble enriched vegetable

oil are manufactured by Cargill Incorporated. The Cargill phytosterol ester product is essentially identical in structure and function to the phytosterols contained in the Take Control™ vegetable oil spread and similar in nature to those in the Benecol™ vegetable oil / tall oil hydrogenated phytosterol spread, both of which are self-affirmed as Generally Recognized as Safe (GRAS). However, the Cargill phytosterol ester product is derived exclusively from vegetable oil and, unlike the Benecol™ product, contains no tall oil phytosterols. Chemical analyses of the Cargill phytosterol ester product have determined that its chemical composition and phytosterol profile are essentially identical to those in Take Control™. Chemical characterization of the component phytosterols in the Procter & Gamble vegetable oil product have demonstrated their stability under conditions typical of cooking (e.g., frying) at home. The potential safety considerations and physiological effects associated with increased intake of phytosterols above normal background levels are therefore the same for the Procter & Gamble phytosterol-enriched product as they are for Unilever's Take Control™. Consequently, because of the similarities between the Cargill phytosterol ester product found in the Proctor & Gamble phytosterol-enriched vegetable oil and the phytosterols found in the GRAS substance Take Control™, a qualified panel of experts has determined that these two products be considered substantially equivalent and should be treated in the same manner with respect to safety and efficacy.

2.2.1 Composition

The major phytosterol components of the Cargill phytosterol esters contained within the Procter & Gamble enriched vegetable oil product exhibit a profile that is essentially identical to those in Unilever's product Take Control™, but differ from the phytosterol components of the McNeil product Benecol™, which consist predominantly of stanols. Table 2 compares the approximate phytosterol composition of the three products. Some natural variation in the individual phytosterol components may occur, as the data in Table 2 indicate but, on the whole, the levels of the individual component phytosterols in Cargill's phytosterol ester product are comparable to the levels in the Unilever GRAS phytosterol products Take Control™. These variations stem from two

main factors: phytosterol source and use of hydrogenation processing. In each product, the phytosterols are esterified to vegetable oil fatty acids whose exact profile varies somewhat according to the choice of vegetable oil from which they are sourced.

2.2.1.1 Source and Hydrogenation

The Cargill phytosterol ester product contains significant levels of β-sitosterol, campesterol, and stigmasterol, similar to those occurring in Take Control™. Similarly, the Cargill product and Take Control™ contain only minor quantities of the naturally occurring saturated (stanol) compounds sitostanol and campestanol, found at high levels in Benecol™. 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 unsaturated congeners of the same saturated structures; hydrogenation may also reduce the diversity of minor components somewhat. However, Benecol™ still contains a range of minor phytosterols of up to 6% (ref. Benecol™ GRAS notification). The phytosterols in the Cargill phytosterol ester product and Take Control™ are not hydrogenated and contain up to 4% by weight of minor sterol and non-sterol components (see Table 2). The minor components represent primarily a variation in the position and/or number of double bonds within β-sitosterol (C_{29}) and campesterol (C_{28}) structures (see Section 3 for compositional details). The phytosterol esters present in the Procter & Gamble vegetable oil product has compositional elements which are equivalent to those in the Take Control™ GRAS product which, in aggregate, supports a determination substantial equivalence as evaluated by a panel of qualifies experts.

Table 2: Comparison of Approximate Phytosterol Compositions

Sterol	Cargill Phytosterol Ester Product[1]	Take Control™[2]	Benecol™[3]
β-Sitosterol	45	46	4
Campesterol	25	25	3
Stigmasterol	22	18	
Brassicasterol	1	4	
Sitostanol	2	2	64
Campestanol	1	1	23
Minor Sterols	4	4	6
Total Phytosterols	100	100	100

[1] As determined by Cargill (see Section 3.0);
[2] As determined in the Take Control™ GRAS notification;
[3] As determined in the Benecol™ GRAS notification.

2.2.1.2 Esterification

The Cargill phytosterols in the Procter & Gamble enriched vegetable oil product exist in an esterified form, similar to those in the two GRAS products. All 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 the Cargill phytosterol ester product to the other products. 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 consists of fatty acids. Accordingly, the phytosterol content of the Cargill phytosterol ester product will also be approximately 60% phytosterol by weight and approximately 40% by weight vegetable oil fatty acids.

2.2.2 Physiologic Properties

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The Cargill phytosterol esters employed in the Procter & Gamble phytosterol-enriched vegetable oil product are expected to be substantially equivalent in physiologic

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properties to those in Take Control™ with respect to their active form and their effects on blood LDL and HDL cholesterol, blood phytosterol levels, and absorption of vitamins and other nutrients.

2.2.2.1 Active Form

Esterified phytosterols 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. Swell (1953) determined that pancreatic cholesterol esterase hydrolyzes both cholesterol esters and phytosterol esters to their free forms. Cholesterol is not absorbed in the esterified form but must first be cleaved before it can be absorbed into the body. Hellman (1953) fed radio-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. It was reported that in rats, direct comparisons between free phytosterols and esterified phytosterols, the esterified forms were less effective as inhibitors of cholesterol absorption (Pollak & Kritchevsky, 1981). Mattson (1982) also reported lower efficacy of β-sitosteryl oleate than free β-sitosterol in inhibiting absorption of cholesterol in humans. However, in a previous rat study (Mattson *et al.*, 1977) indicated equal efficacy in lowering cholesterol absorption between free phytosterol and esterified phytosterols. The oleate esters of the phytosterols were prepared by reacting them with oleic acid isolated from olive oil. The addition of 3.3% of the oleate esters of either of the plant sterols, on a molar basis to 2% free sterols, brought about similar decreases in cholesterol absorption. Furthermore, the ability of the phytosterol esters to lower cholesterol were equally effective whether the carbon chain of the esterifying fatty acid was short (acetate), medium (decanoate), or long (oleate). 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.

2.2.2.2 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 several published clinical studies is provided in Section 10. The maximum lowering of LDL cholesterol observed in human studies with plant derived sterols are in the range of 13 to 15%. One study by Weststrate (1998) directly compared the Take Control™ product with that of the Benecol™ product in hyper-cholesterolemic patients over a 25 day treatment interval.

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

Product:	Take Control™	Benecol™
Dosage	3 g per day [1]	2.7 g per day [1]
Δ Total Cholesterol [2]	-8.3%	-7.3%
Δ LDL Cholesterol [2]	-13.0%	-13.0%
Δ HDL Cholesterol [2]	+0.6%	+0.1%

[1] Weststrate (1998).
[2] Values are corrected for the changes occurring in the control group.

In a review by Kritchevsky (1997), the suggested mechanisms of hypo-cholesterolemic properties of β-sitosterol were proposed. They consisted of: 1) Mixed crystal formation; 2) Adsorption aggregation; 3) Decreased solubility of cholesterol micelles; 4) Enhanced cholesterol excretion; 5) Interference with cholesterol synthesis; 6) Competitive esterification; 7) Competition for acceptor sites; and 8) Reduced membrane permeability.

2.2.2.3 Effects on Circulating HDL-Cholesterol

000025

There was no evidence of any significant effect on plasma HDL levels for either of the two currently available GRAS phytosterol ester products. Due to the compositional equivalence of the Cargill phytosterol ester product used to enrich the vegetable oil marketed by Procter & Gamble, no effect on circulating HDL cholesterol

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can be reasonably expected to occur.

2.2.2.4 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 β-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, as illustrated below in Table 4. Sitostanol, which is particularly poorly absorbed, appears to inhibit not only the absorption of cholesterol but also that of other sterols. The Cargill phytosterol ester product used to enrich the Procter & Gamble vegetable oil is expected to have an effect comparable to that displayed by the Unilever product Take Control™.

Table 4: Effect of Phytosterol Products on Blood Phytosterol Levels

	Take Control™	Benecol™	Benecol™
References	Weststrate et al 1998	Weststrate et al 1998	Gylling et al 1995
β-Sitosterol	+38.8%	-36.2%	-36.1%
Campesterol	+72.6%	-17.1%	-48.2%

2.2.2.5 Effects Upon Vitamin and Other Nutrient Absorption

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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™ (Benecol™ and Take Control™ GRAS Notification filings). Hendriks (1999) evaluated sterol ester consumption and observed statistically significant decreases in the absorption of α- and β-carotene and lycopene. No effect on lipid standardized plasma α-tocopherol and lycopene levels or upon plasma concentrations of the fat-soluble

vitamins K_1 and D was observed. Gylling (1996) evaluated phytostanol esters and reported a similar decrease in plasma β-carotene levels. In a comparative analysis of the cholesterol lowering effects of esterified phytosterols in Take Control™ and esterified phytostanols in Benecol™, Weststrate (1998) observed a lowering of plasma lycopene, α-, and β-carotene levels in all individuals consuming the fortified margarines. Lipid standardization of the carotenoid concentrations reduced the impact of both Take Control™ and Benecol™, and lipid standardization of the Take Control™ phytosterol ester data showed the decrease in lycopene not to be statistically significant. Hallikainen (2000) observed a dose dependant decrease in serum lycopene levels in women only and a dose dependant decrease in α- and γ-tocopherol levels in men and women when they consumed phytostanol esters in a margarine matrix. When compared to serum total cholesterol concentration, there were no significant difference in α- and γ-tocopherol among the different dose periods. No significant changes were reported in serum retinol, α-, and β- carotene levels.

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 the Cargill phytosterol esters contained within the enriched vegetable oil product have not been specifically tested in this regard, its substantial compositional equivalence and intended use level compared to Take Control™, assures that it presents no significant risk of adversely altering vitamin and nutrient absorption to any degree materially different from Benecol™ and Take Control™.

2.2.3 Safety

000027

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 Unilever product, Take Control™, has yielded substantial research into the safety of phytosterols, particularly β-sitosterol, campesterol, and stigmasterol as discussed by Baker (1999), Hepburn (1999), and Wallkens-Berendsen (1999). Similarly, the development of the McNeil product, Benecol™, has also yielded substantial research into the safety of

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phytosterols, particularly sitostanol, campestanol and stigmastanol as discussed by Slesinski (1999), Turnbull (1999a, b, c), and Whittaker (1999).

The information reviewed in determining the GRAS status of this product included extensive clinical and non-clinical data. Clinical data included a history of safe intake of these phytosterols as components of everyday foodstuffs. Safe exposure to β-sitosterol has further been demonstrated at levels of up to 30 grams per day for extended periods of use over the course of the marketing of the pharmaceutical agent Cytellin™. Results of direct clinical and nutritional investigations of products containing phytosterols in over 2000 subjects have been evaluated. Non-clinical studies included those designed to assess acute, short term and sub-chronic safety, genetic toxicity, estrogenic activity, developmental and reproductive safety, ADME (absorption, distribution, metabolism, and excretion), and nutrient effects.

Expert and FDA reviews have concluded in a reasonable certainty of no harmful effects and that phytosterols may be regarded as GRAS when used in the Benecol™ and Take Control™ vegetable oil spreads. The safety of the phytosterol ester-enriched vegetable oil marketed by Procter & Gamble for home use cooking applications is in part assured by the substantial compositional equivalence of the constituent Cargill phytosterol esters to those in the Unilever GRAS product Take Control™. This has been determined by scientific review of all available data by a panel of qualified experts.

2.3 Assessment of Additional Use and Intake

Since the phytosterol ester-enriched vegetable oil product represents a new source of added dietary phytosterols, an assessment of the expected additional dietary intake was conducted (CanTox, 2000) and reviewed by the panel of qualified experts in their determination of GRAS status for the Procter & Gamble phytosterol enriched vegetable oil. Calculations for the intake of vegetable oils consumed as cooking, baking, and salad oils from home use were based on the results of the U.S. Department of Agriculture 1989-1991 Continuing Survey of Food Intakes by Individuals (CSFII). Male teenagers were identified as the highest-end user population subgroup. The mean oil intake value for this group is estimated to be 6.45 g per day. Users in the 90th

percentile consumption bracket of this group would consume approximately 15.8 g per day of vegetable oil. This translates into an additional mean intake value of 0.86 g per day and 2.10 g per day of phytosterol esters for the mean and 90th percentile users in this group, respectively. The estimated intake of phytosterol esters, based upon the new application of phytosterol-enriched vegetable oil for home use, is comparable with that of the Take Control™ vegetable oil spread at 1.8 - 5.6 g/day of phytosterols esters (Take Control™ GRAS Notification). This results in a maximum estimated intake from both sources of 2.66 - 7.7 g per day of phytosterol esters, or 1.08 - 4.62 g per day of phytosterols, which is within the range covered by the current safety data base. Other constituent groups within the over-all population are likely to eat considerably lower levels of vegetable oil per person and therefore would consume lower levels of phytosterol esters when employed in home cooking applications. This is discussed at greater length in Section 6.0.

2.4 Assessment of Heated Phytosterol Esters

Hydrolysis, oxidation, polymerization, and dehydration reactions describe the chemistry of phytosterol esters and the reaction products that might form during the heating of cooking oil that is enriched with sterol esters at up to 13.3% (w/w). The chemistry and the expected oxidation products of phytosterol esters have been reviewed by Finocchiaro and Richardson (1983). Under the relatively mild (short time and low temperature) conditions of home cooking, little significant oxidation of phytosterols in oil is expected. The expected reaction products that may form from the heating of phytosterol esters in cooking oil are derived through well-documented oxidation reactions. These oxidation reactions are common to fatty acids, cholesterol, and phytosterols that are naturally occurring in vegetable oils and commonly available heated foods. Thus the degree of oxidation is expected to be on the order of 0.1% or less of the total phytosterol esters.

In order to determine the presence of significant phytosterol oxidation products under home cooking conditions, potatoes were deep-fried in the phytosterol enriched vegetable oil, the oil was allowed to cool to room temperature and frying was repeated.

The sterol fraction was then isolated and compared to control oils before frying and after the same frying conditions but without sterols. For this study, potatoes were selected as the model food because they are a common food fried in the home.

The chromatograms of the unheated sterol-containing oil, and the sterol-containing oil used for frying potatoes were virtually identical (Procter & Gamble, 2000). Even the minor peaks in the chromatograms showed excellent correspondence between the unheated sample and the one used for frying. There were no new peaks attributable to the frying conditions, and the peak heights on the two chromatograms were very similar. Smaller amounts of phytosterols and various other peaks could also be seen in the control vegetable oil used to fry potatoes. The chromatograms of the unheated phytosterol-enriched oil and the phytosterol-enriched oil used for frying potatoes indicate that no significant new oxidized species were formed by the heating. This information has been reviewed and taken into consideration by the panel of qualified experts in their determination of GRAS status for the Procter & Gamble phytosterol enriched vegetable oil product.

3.0 PHYTOSTEROL ESTER CHEMISTRY AND STABILITY

3.1 Compositional Equivalence of Constituent Phytosterols to a GRAS Product

3.1.1 Chemical Identity of Major Component Phytosterols

Plant sterols, or phytosterols, were originally thought to be cholesterol isomers. With the advent of modern analytical techniques such as chromatography and mass spectrometry, the empirical formulae and chemical structure of many phytosterols have been elucidated, including all the major components of the Cargill phytosterol ester product employed in the Procter & Gamble phytosterol enriched vegetable oil. The formula and structure of many phytosterols found in minute quantities throughout the plant kingdom are still poorly characterized. In large part, phytosterols, are similar to cholesterol at the basic level of skeletal structure. The difference exists on the aliphatic side chain of the compounds, where phytosterols contain methyl, ethyl, di-methyl, di-

ethyl or other groups next to the C_{24} position. (Pollak & Kritchevsky, 1981). A diagram of the chemical structure of the three major phytosterol constituents (β-sitosterol, campesterol, and stigmasterol) present in Take Control™ and in the Cargill phytosterol esters used to enhance the Procter & Gamble vegetable oil is provided in Figure 1. A diagram of the cholesterol molecule has also been provided to better illustrate the similarity of the sterol chemical structures. The precise chemical specifications of the three predominant phytosterol components found in the Cargill phytosterol ester product are provided in Table 5.

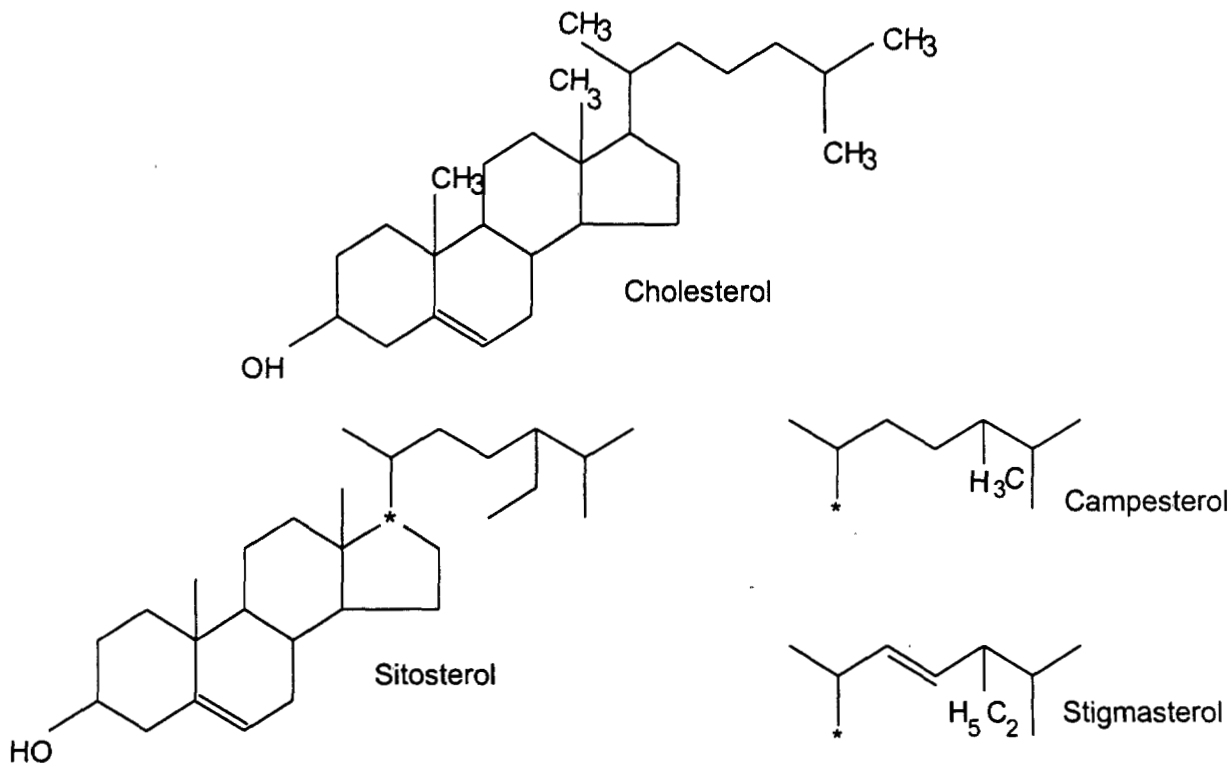

Figure 1: Major Phytosterols Present in the Cargill Product and Cholesterol

Table 5: Chemical Data of Constituent Cargill Phytosterols

	Chemical Name	Chemical Formula	Molecular Weight	Melting Point	Approximate Percentage
β-Sitosterol	(3β)-Stigmast-5-en-3ol	$C_{29}H_{50}O$	414.72	140° C	40-45%
Campesterol	(3β,24R)-Ergost-5-en-3-ol	$C_{28}H_{48}O$	400.69	157° C	20-25%
Stigmasterol	(3β,22E)-Stigmasta-5,22-dien-3-ol	$C_{29}H_{48}O$	412.7	170° C	20-25%

3.1.2 Minor Component Phytosterols

The saturation of phytosterols at the 5-α position results in the formation of phytostanols, or simply stanols. These are found in nature at very low concentrations and are present in the Cargill product at levels between 2 - 3% of the total phytosterols, which amounts to approximately 0.4% of the total Procter & Gamble phytosterol enriched vegetable oil. The other minor sterol components comprise approximately 3 - 5% of the total Cargill phytosterol esters and are approximately 0.66% of the total Procter & Gamble vegetable oil. While minor components were not identified, comparative gas chromatograph analysis between the Cargill product and the phytosterols present in Take Control™ demonstrated an identical compositional profile of the minor phytosterol components found in each product (Section 3.1.3). A copy of a representative gas chromatogram of the phytosterols present in the Take Control™ product is presented Figure 2.

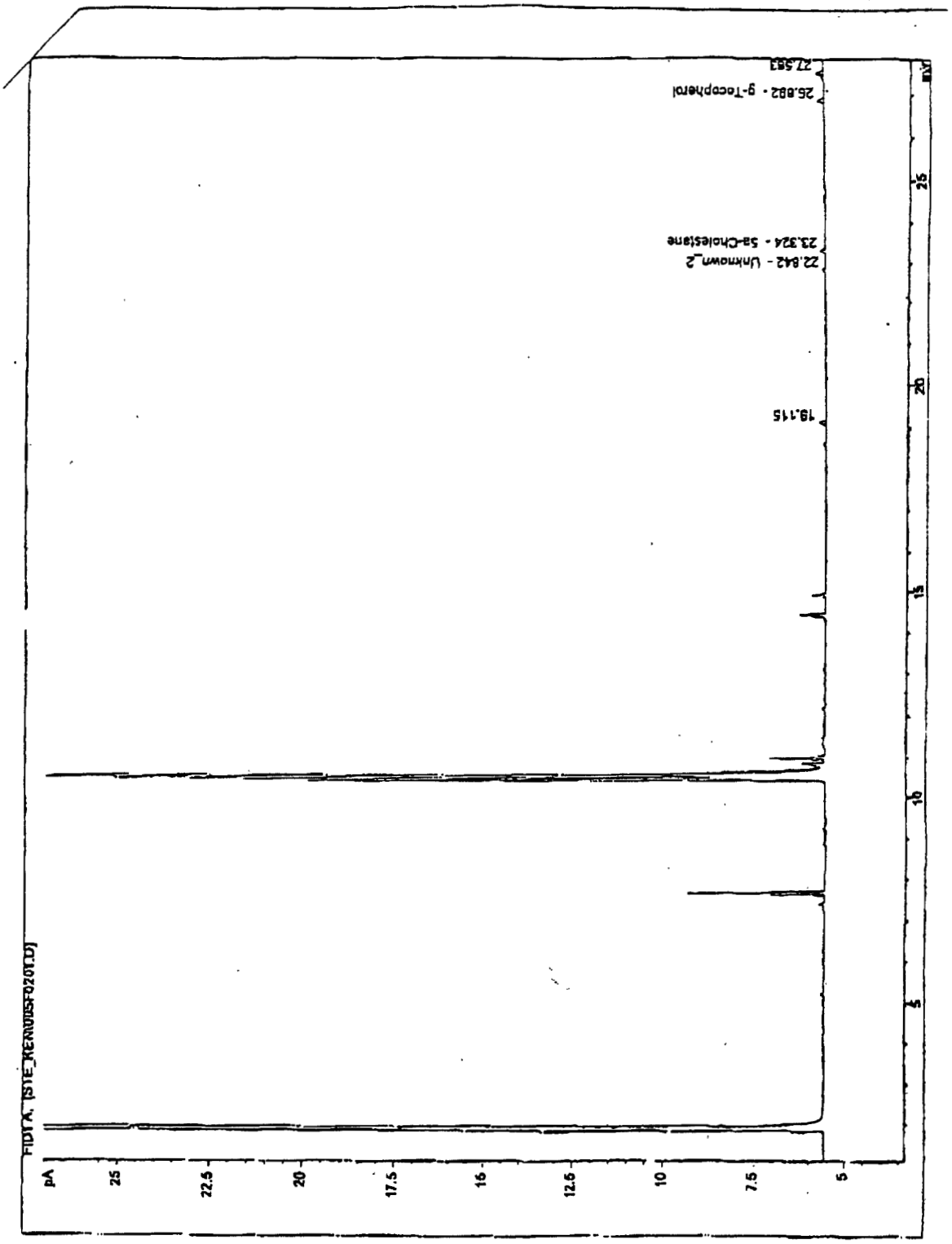

Figure 2: A Representative Gas Chromatogram of the Phytosterols in Take Control™

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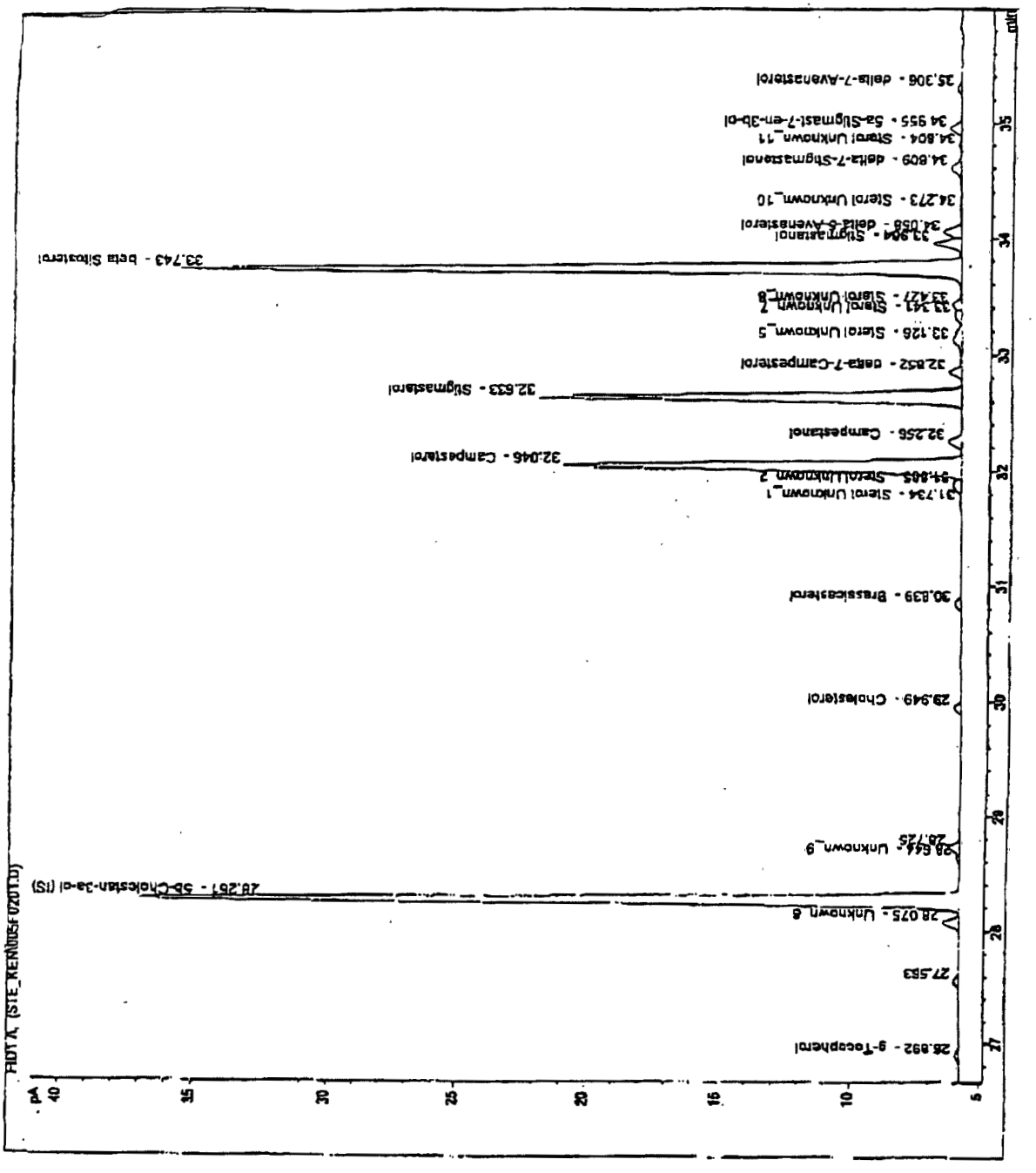

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Figure 2: A Representative Gas Chromatogram of the Phytosterols in Take Control™ (continued)

3.1.3 Comparative Analysis

As has been discussed in Section 2.2, one of the three principal reasons the Procter & Gamble phytosterol enriched vegetable oil product is considered GRAS by a consensus of qualified experts is the substantial chemical and compositional equivalence of its phytosterol ester component to the phytosterol esters found in the GRAS Unilever product Take Control™. To that end, Cargill, the manufacturer of the phytosterol ester component in the Procter & Gamble vegetable oil, has completed a comparative compositional analysis employing gas chromatography. As illustrated in Table 6a, a comparison of the major phytosterol concentrations using identified peaks in the Cargill product and in Take Control™ reveals them to be virtually identical, as does comparison of the unattributed peaks found Table 6b.

Table 6a: Comparison of Identified Peaks in Cargill Phytosterols and Take Control™

	Take Control™		Cargill Phytosterol Samples				
Peak ID	Sample 1 (wt %)	Sample 2 (wt %)	Lot# 3023 (wt %)	Lot# 3026 (wt %)	Lot# 3027 (wt %)	Lot# 3028 (wt %)	Lot# 3029 (wt %)
Total Tocopherols	0.38	0.34	0.5	0.44	0.27	0.41	0.48
Cholesterol	0.38	0.34	0.39	0.37	0.38	0.37	0.31
Brassicasterol	2.50	4.08	0.49	0.44	0.50	0.48	0.35
Campesterol	23.44	25.04	23.57	23.47	22.60	24.35	23.91
Campestanol	0.63	0.57	0.88	0.82	0.94	0.89	0.90
Stigmasterol	17.88	15.97	21.36	21.63	26.58	22.63	24.00
delta 7 Campesterol	0.52	0.40	0.66	0.72	0.75	0.71	0.69
β-Sitosterol	45.68	45.99	42.68	42.86	41.54	42.70	41.56
Stigmastanol	1.13	1.06	1.54	1.53	1.50	1.47	1.51
delta 5-Avenasterol	0.86	0.80	0.92	1.04	0.65	0.80	0.67
delta 7 Stigmasterol	0.88	0.75	0.90	0.82	0.62	0.62	0.62
Stigmast-7-en-3β-ol	0.88	0.75	0.90	0.82	0.62	0.62	0.62
delta 7 Avenasterol	0.20	0.14	0.29	0.26	0.19	0.30	0.28
Total %	95.36	96.22	95.09	95.23	97.13	96.36	95.90

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Table 6b: Comparison of Unattributed Peaks in Cargill Phytosterols and Take Control™

Peak ID	Take Control™ Sample 1 (wt %)	Take Control™ Sample 2 (wt %)	Cargill Phytosterol Samples Lot# 3023 (wt %)	Lot# 3026 (wt %)	Lot# 3027 (wt %)	Lot# 3028 (wt %)	Lot# 3029 (wt %)
Unknown 8	0.66	0.52	0.85	0.89	0.12	0.64	0.73
Unknown 9	0.71	0.51	0.31	0.16	0.52	0.43	0.67
Sterol Unknown 2	0.47	0.50	0.58	0.61	0.37	0.55	0.47
Sterol Unknown 5	0.75	0.57	0.66	0.56	0.42	0.75	0.52
Sterol Unknown 6	0.27	0.28	0.31	0.33	0.29	0.00	0.24
Sterol Unknown 7	0.42	0.33	0.54	0.49	0.21	0.46	0.43
Sterol Unknown 8	0.64	0.57	0.54	0.56	0.52	0.44	0.43
Sterol Unknown 10	0.24	0.12	0.27	0.21	0.13	0.00	0.09
Total %	4.17	3.38	4.06	3.82	2.58	3.27	3.58
Grand Total %	**99.53**	**99.60**	**99.15**	**99.05**	**99.71**	**99.63**	**99.48**

Please note that this data is normalized to weight % of all constituents found in the organic extract of the saponification of these products (essentially sterols, stanols, and tocopherols).

3.2 Phytosterol Stability Under Conditions of Use

3.2.1 Background

Hydrolysis, oxidation, polymerization, and dehydration reactions describe the chemistry of phytosterol esters and the reaction products that might form during heating of cooking oil that is enriched with sterol esters at up to 13.3% (w/w). The chemistry and the expected oxidation products of phytosterol esters have been reviewed by Finocchiaro and Richardson (1983). Under the relatively mild (short time and low temperature) conditions of home cooking, little significant oxidation of phytosterols in oil is expected. The expected reaction products that may form from the heating of phytosterol esters in cooking oil are derived through well-documented oxidation reactions. These oxidation reactions are common to fatty acids, cholesterol, and phytosterols that are naturally occurring in vegetable oils and commonly available heated foods. Thus the degree of oxidation is expected to be on the order of 0.1% of the total

phytosterol esters.

In order to confirm the expected lack of any significant phytosterol oxidation under home cooking conditions, potatoes were deep-fried on two consecutive occasions in the phytosterol enriched vegetable oil. The sterol fraction was then isolated and compared with isolated sterol fractions of phytosterol enriched vegetable oil before frying and control vegetable oil after undergoing the same frying conditions (Procter & Gamble, 2000). These samples represent oil that has been used to fry the most common fried food (potatoes), under home frying conditions (deep-frying with a single re-use). The 600 g batches of potatoes used for the experiment each represent approximately twice what a family of four would be expected to cook (84 g serving x 4 = 336 g), if the oil was re-used once and discarded.

3.2.2 Experimental Design

To document the effect of typical home use heating of phytosterol ester-enriched oil, the following experimental approach was used: Fresh cut French style potatoes were fried in vegetable oil containing 13.3% phytosterol esters or a control vegetable oil without the phytosterol esters (Crisco™). The phytosterol esters used for this study were vegetable oil fatty acid phytosterol esters purchased directly from Cargill Nutraceutical Department (Wayzata, Minnesota - lot number C3026). Approximately 1200 g of oil was placed in a home fryer and heated to frying temperature (~290° F). One 600 g batch of potatoes was then fried in each oil for a total of 12 minutes. The oils were cooled to approximately room temperature, then reheated to frying temperature and a second 600 g batch of potatoes was fried in each. The unsaponifiable fractions from fresh sterol-enriched oil, heated sterol-enriched oil, and the heated vegetable oil control were then extracted and the level of free sterols was quantitatively determined by capillary GC. The recovery of sterols in each sample was calculated, and the gas chromatographic profiles of all three samples were examined in detail for any new components or significant changes in component levels. The following samples were analyzed:

[redacted]

- 100% vegetable oil, heated (WHS 1554-103C-After 2nd Fry).
- 13.3% sterol ester in vegetable oil, heated (WHS 1554-103S-After 2nd Fry).
- 13.3% sterol ester in vegetable oil, unheated (WHS 1554-103S-Before Fry).

3.2.2.1 Sample Analysis

The baseline vegetable oil samples were initially analyzed for sterol and sterol ester content by a GC method in order to account for indigenous vegetable sterols and sterol esters. The expected amount of sterol was calculated and added to the measured free sterol to give an expected total sterol amount. Following saponification, the total sterol level was measured by the GC method described below, and recovery was determined by the ratio of measured to expected level.

Samples (0.5g), plus 2% tetracosane (internal standard), were saponified and extracted using procedures similar to Dutta (1997) except that ethyl ether was used as an extraction solvent instead of methylene chloride. This resulted in a clear sample, and produced excellent sterol recoveries. The final ethyl ether volume was 20 mL, and a 3 μL aliquot was injected into the GC injection port and analyzed under the following GC conditions:

- GC: HP-6890 with 7673 auto-injector
- Column: J&W Scientific, DB-1, 15M x 0.25mm, 0.25μM film thickness
- Column Temp Program: 190°C (2 min hold); 10°C/min to 345°C (1 min hold)
- Injection Mode: split with split ratio = 45:1
- Injection Port Temp: 325°C
- Detector/Temp: FID/345°C

GC/MS analyses were performed under similar conditions using a Finnigan MAT 95 mass spectrometer with HP-6890 GC. The mass spectrometer was operated in electron ionization mode (70 eV electron energy) and with a source temperature of 300°C.

3.2.3 Results

Figures 3, 4, and 5 (labeled as 1, 2, and 3) are GC/FID chromatograms of the unsaponifiable fraction extracts from the unheated phytosterol ester enriched vegetable oil, the heated phytosterol ester enriched vegetable oil, and the heated vegetable oil, respectively. Each chromatogram was normalized to the internal standard response, and then multiplied by the same factor to view minor peaks. The fact that the full scale responses (~150 pA) were very nearly the same indicates good precision in recovering the internal standard. In addition, the virtually identical chromatographic profiles of heated and unheated samples show that there was no significant change in the type or level of sterol components. The minor peak at retention time 11.309 minutes in Figures 3 and 4, is a peak whose level in the sample is approximately 50 ppm (by area comparison with the internal standard). The measured levels for the phytosterol ester enriched samples were are shown in Table 7:

Table 7: The Measured Concentration of the Phytosterol Ester Enriched Samples

	Measured	Expected	Recovery
13.3% sterol ester in oil (heated):	8.20%	8.97%	91.4%
13.3% sterol ester in vegetable oil (unheated):	8.28%	8.91%	92.9%

Figure 4 includes peak labels for many of the minor sterol components observed in this study. The major sterol peaks, labeled electronically, were identified by retention time comparison with standards and verified by GC/MS. The minor sterol peaks, labeled manually, were identified by GC/MS using NIST and Wiley mass spectral libraries and were not verified with standards.

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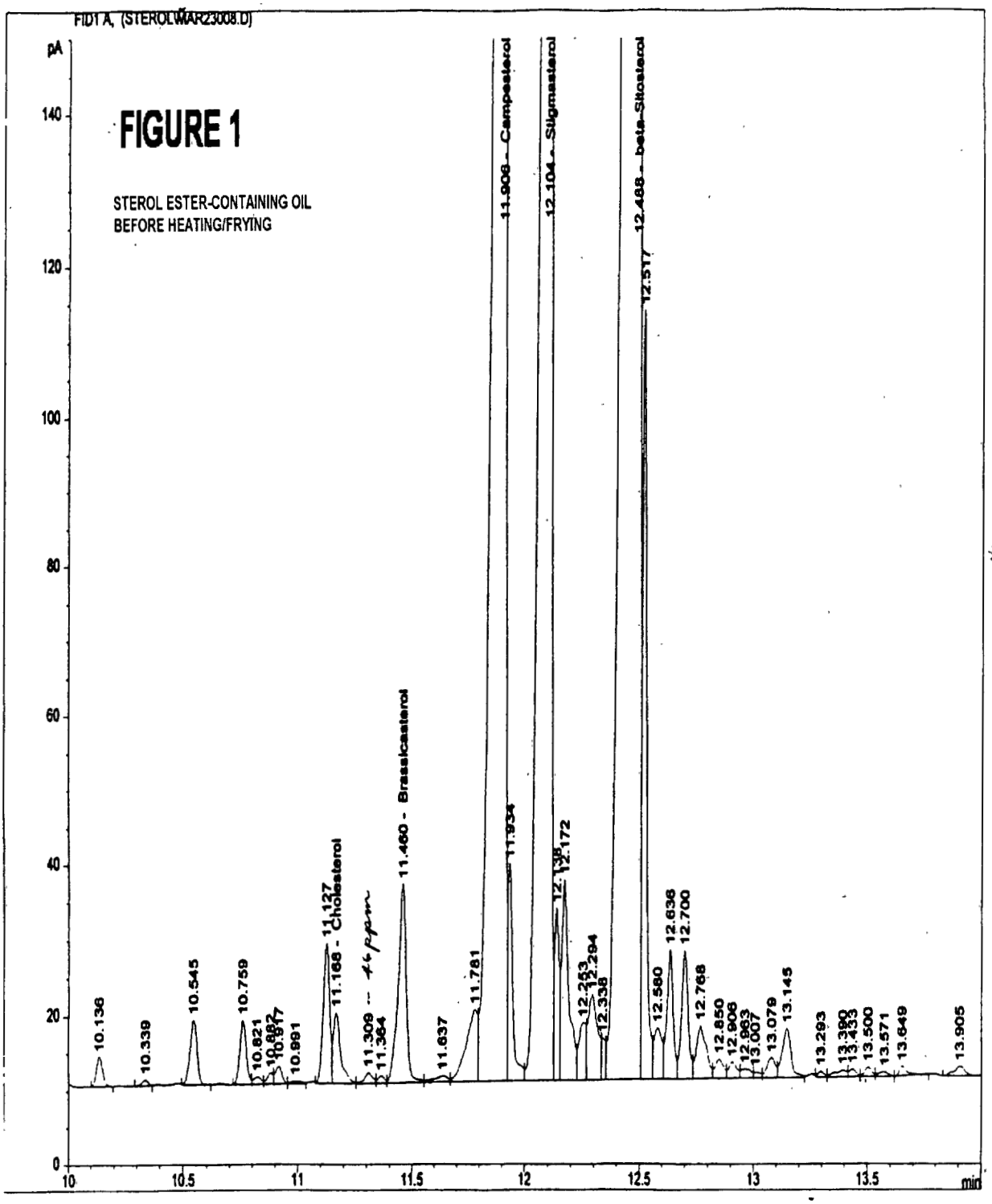

Figure 3: Phytosterol Vegetable Oil Before Frying

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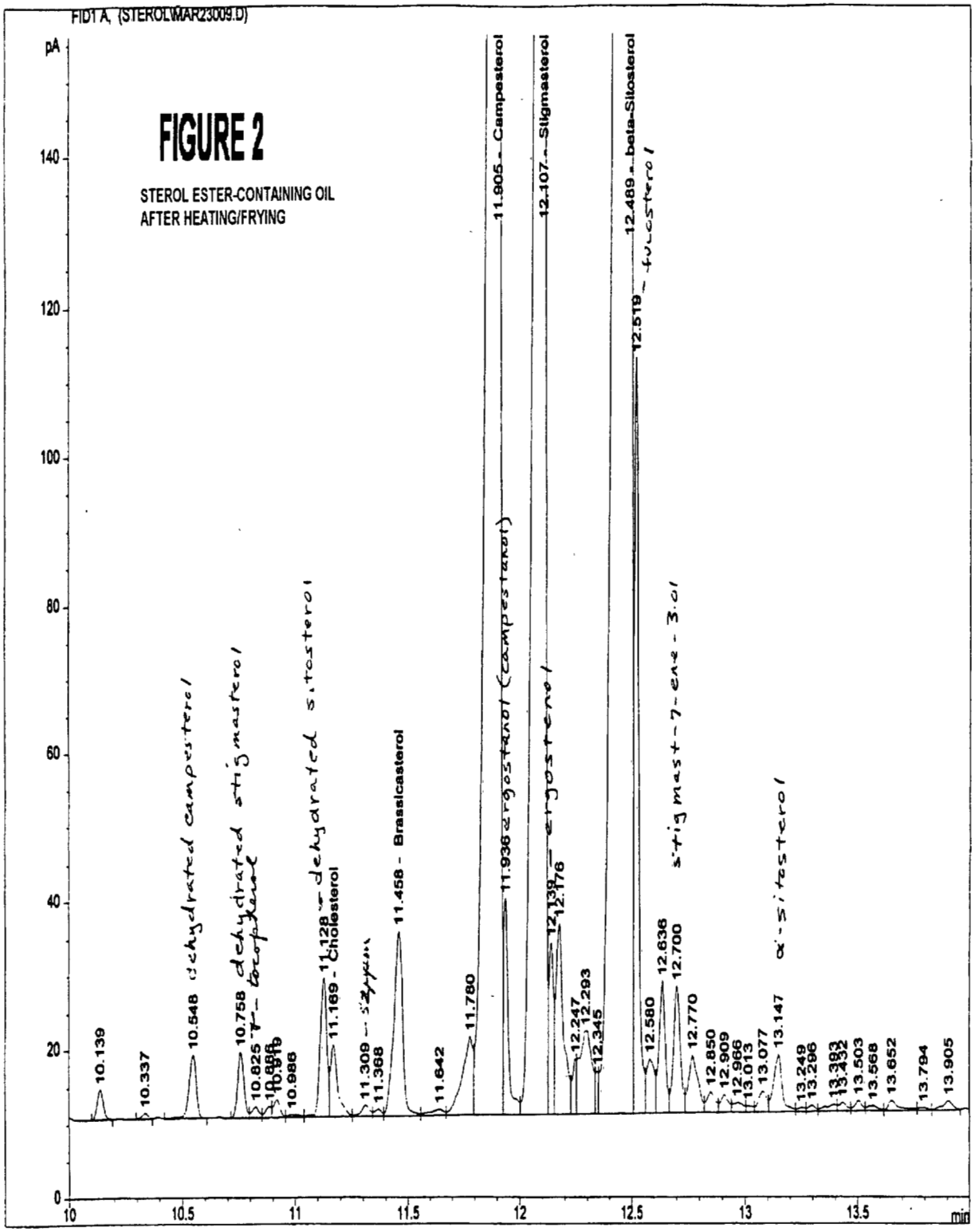

Figure 4: Phytosterol Vegetable Oil After Frying

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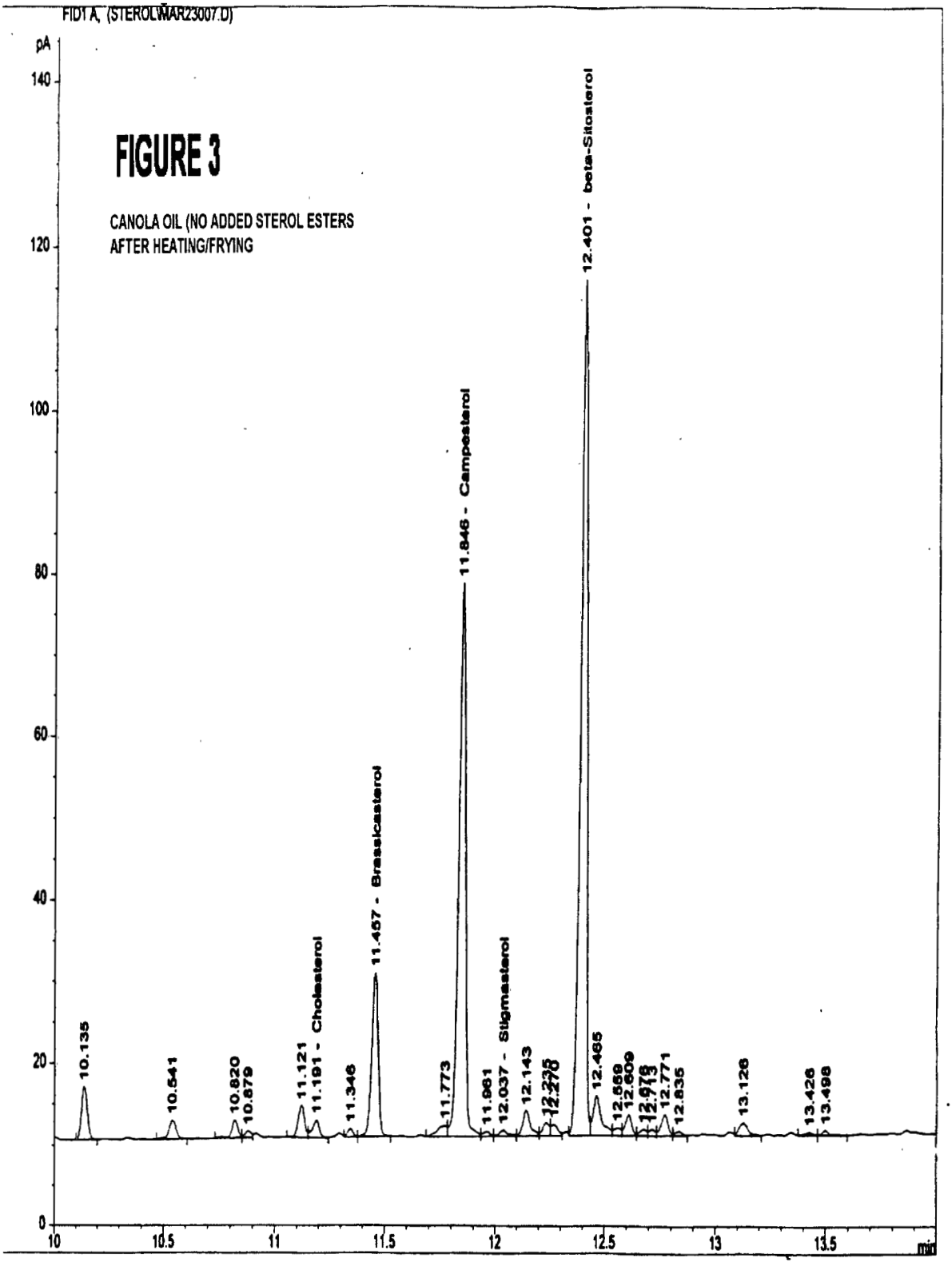

Figure 5: Control Vegetable Oil After Frying

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3.2.4 *Discussion and Conclusion*

These samples represent oil that has been used to fry the most common fried food (potatoes), under home frying conditions (deep-frying with one reuse). The 600 g batches of potatoes used for the experiment each represent approximately twice what a family of four would be expected to cook (84 g serving x 4 = 336 g), if the oil was re-used once and discarded. The fact that the chromatograms of the unheated sterol ester-containing oil, and the sterol ester-containing oil used for frying potatoes were the same, and the peak areas were the same, indicates that no significant new oxidized species were formed by the heating. This is illustrated below in Table 8 where retention times and area percentages of peak heights before and after frying are compared. Relevant peaks are labeled accordingly.

Table 8: Comparison of Peak Area Percent Before and After Frying

Approximate Retention Time (min)	Peak Area % Before Frying	Peak Area % After Frying	Identified Peaks
6.191	0.08		
6.247	0.05		
6.648	-	-	N-Tetracosane
7.345	0.02	0.02	
7.923	0.02		
8.568	0.02	0.03	
10.136	0.14	0.14	
10.339	0.03	0.03	
10.545	0.34	0.35	
10.759	0.32	0.31	
10.821	0.04	0.06	
10.882	0.05	0.05	
10.917	0.09	0.09	
10.991	0.02	0.02	
11.127	0.68	0.68	
11.168	0.40	0.39	Cholesterol
11.309	0.06	0.07	
11.364	0.03	0.03	
11.460	1.25	1.27	Brassicasterol
11.637	0.06	0.06	

000043

Table 8: Comparison of Peak Area Percent Before and After Frying (continued)

Approximate Retention Time (min)	Peak Area % Before Frying	Peak Area % After Frying	Identified Peaks
11.781	0.64	0.65	
11.906	23.69	23.70	Campesterol
11.934	0.80	0.80	
12.104	19.98	19.98	Stigmasterol
12.138	0.59	0.59	
12.172	1.11	1.12	
12.253	0.32		
12.294	0.62	0.94	
12.338	0.12	0.09	
12.488	42.75	42.78	β-Sitosterol
12.517	2.56	2.59	
12.580	0.31	0.32	
12.636	0.63	0.63	
12.700	0.65	0.65	
12.768	0.40	0.41	
12.850	0.12	0.13	
12.906	0.10	0.10	
12.963	0.07	0.10	
13.007	0.03		
13.079	0.12	0.13	
13.145	0.33	0.35	
13.293	0.03	0.03	
13.390	0.06	0.05	
13.433	0.04	0.05	
13.500	0.05	0.05	
13.571	0.03	0.03	
13.649	0.05	0.05	
13.905	0.08	0.07	

This information has been reviewed by a panel of qualified experts in their determination of GRAS status and determined by consensus to pose no additional risk under normal conditions of home use.

3.3 Proposed Food-Grade Specifications

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The Procter & Gamble Company intends their phytosterol ester enriched vegetable oil product to serve as an alternative source of phytosterols to consumers

[redacted]

seeking to incorporate phytosterols into their diet in order to promote and maintain a healthy cholesterol level. The proposed food grade specifications for this product are those normally associated with vegetable oil. Additionally, the food grade specifications for the constituent Cargill phytosterol esters contained within the enriched vegetable oil product are shown below:

Phytosterol content *	> 95%
β-Sitosterol	40% to 45%
Campesterol	20% to 25%
Stigmasterol	20% to 25%
Total major sterols	> 85%
Arsenic	< 0.1 mg/kg
Lead	< 0.1 mg/kg
Mercury	< 0.1 mg/kg
Cadmium	< 0.1 mg/kg
Total aerobic count	< 10,000 CFU/g
Combined molds & yeasts	< 100 CFU/g
Coliforms	negative
E. Coli	negative
Salmonella	negative

* The Cargill phytosterol ester product is approximately 60% by weight phytosterols and 40% by weight vegetable oil fatty acids to which they are esterified; percentages refer to non-fatty acid portion.

4.0 PRODUCTION OF PHYTOSTEROL ESTER-ENRICHED VEGETABLE OIL

The Procter & Gamble Company phytosterol-enriched vegetable oil is intended for home use in baking, frying, and salad dressings. This product is a blend of Cargill Incorporated's phytosterol ester product with standard vegetable oil to produce a phytosterol-enriched vegetable oil product containing 13.3% phytosterol esters. Home use of this phytosterol-enriched vegetable oil is expected to deliver approximately up to 1.8 g of phytosterol esters per day in one 14 g serving of oil. [redacted]

The Procter & Gamble phytosterol ester-enriched vegetable oil product is manufactured under conditions that are in accordance with Current Good Manufacturing Practice in Manufacturing, Packing, or Holding of Human Food, 21 CFR Part 110.

5.0 INTENDED USE IN FOOD

Phytosterols are naturally occurring plant sterols which resemble cholesterol in molecular structure but have a much lower absorption rate. Because they compete with cholesterol for absorption in the gastrointestinal tract, phytosterols are gaining popularity in consumer products intended to maintain blood cholesterol levels within acceptable healthy limits, as indicated by the recently marketed vegetable oil base spreads Benecol™ and Take Control™.

The Procter & Gamble Company has developed a phytosterol-enriched vegetable oil which has been determined by the consensus of an independent panel of qualified experts to be GRAS. The intended us of the Procter & Gamble product is for home use in cooking applications such as baking, frying, and salad dressings. This product is a standard vegetable oil blended with phytosterol esters supplied by Cargill at a level of 13.3%. This phytosterol-enriched vegetable oil product is intended to deliver up to approximately 1.8 g of phytosterol esters per day in one 14 g serving of oil. The Procter & Gamble phytosterol-enriched vegetable oil product is a new and alternative dietary source of phytosterols that, as with Take Control™ and Benecol™, is intended to help maintain healthy blood cholesterol levels.

6.0 ANTICIPATED USE AND INTAKE

6.1 Introduction

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The intake of vegetable oil from home-use as a cooking, baking, and salad oil in the United States was estimated in order to quantify the expected levels of phytosterol intake which may arise through use of the proposed product (CanTox, 2000). The calculations for intake of vegetable oils consumed as cooking, baking and salad oils

from home use were based upon the results of the U.S. Department of Agriculture (USDA) 1989-91 Continuing Survey of Food Intakes by Individuals (CSFII) (USDA, 1996). Calculations of the mean all-person intake, mean per-user intake, 90th percentile intake, and percent consuming were made for the intake of vegetable oil from each of the individually identified home uses. Similar calculations were performed for the total intake of vegetable oil from all home uses. In each case, the intake of vegetable oil per person and per kilogram body weight was calculated for the following population groups:

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

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6.2 Estimated Total Daily Intake of Vegetable Oil, Phytosterol Esters, and Phytosterols

The mean all-person and all-user daily intakes of vegetable oil, phytosterol esters, and phytosterols from all identified home uses as cooking, baking, and salad oil by population group are summarized in Tables 9 and 10. The number of users of vegetable oil from identified home uses ranged between 36% and 43% depending on population group. As a consequence, the mean and 90th percentile intakes for users are approximately 2 to 3 times the intake on an all person basis. The greatest mean per person user intake of vegetable oil from all identified home uses occurred in male teenagers aged 12 to 19 years with intakes of 6.45 g/person/day. The per person heavy consumer (90th percentile) intake of vegetable oil was also reported to be the highest in male teenagers aged 12 to 19 years (15.80 g/person/day), but on body weight basis, in infants aged 0 to 2 years (0.431 g/kg body weight/day). The mean per

[redacted]

person intake of vegetable oil among users from all identified home uses was 5.10 g/person/day or 0.096 g/kg body weight/day for the total U.S. population.

Table 9: Summary of Estimated Daily Per Person Vegetable Oil, Phytosterol Ester, and Phytosterol Consumption From All Identified Home Uses as Cooking, Baking and Salad Oil by Population Group

Group	Age Group	% Users	All-Users Oil Consumption		Expected All-User Phytosterol Ester Consumption [1]		Expected All-User Phytosterol Consumption [2]	
	(Years)		Mean (g)	90th Percentile (g)	Mean (g)	90th Percentile (g)	Mean (g)	90th Percentile (g)
Infant	0-2	36.8	2.39	5.55	0.32	0.74	0.19	0.44
Child	3-11	39.1	4.34	9.26	0.58	1.23	0.35	0.74
Female Teenager	12-19	43.0	4.56	9.48	0.61	1.26	0.37	0.76
Male Teenager	12-19	41.3	6.45	15.80	0.86	2.10	0.52	1.26
Female Adult	20 and Up	36.0	4.53	9.26	0.60	1.23	0.36	0.74
Male Adult	20 and Up	37.0	6.35	13.10	0.84	1.74	0.50	1.04
Total Population	All Ages	37.5	5.10	10.6	0.68	1.41	0.41	0.85

[1] Phytosterol ester consumption equals 13.3% w/w of oil consumption.
[2] Phytosterol consumption equals 60% w/w of phytosterol ester consumption.

Table 10: Summary of Estimated Daily Per Kilogram Body Weight Vegetable Oil, Phytosterol Ester, and Phytosterol Consumption From All Identified Home Uses as Cooking, Baking and Salad Oil by Population Group

Group	Age Group	% Users	All-Users Oil Consumption		Expected All-User Phytosterol Ester Consumption [1]		Expected All-User Phytosterol Consumption [2]	
	(Years)		Mean (g/kg)	90th Percentile (g/kg)	Mean (g/kg)	90th Percentile (g/kg)	Mean (g/kg)	90th Percentile (g/kg)
Infant	0-2	36.8	0.186	0.431	0.025	0.057	0.015	0.034
Child	3-11	39.1	0.168	0.371	0.022	0.049	0.013	0.029
Female Teenager	12-19	43.0	0.082	0.176	0.011	0.023	0.007	0.014
Male Teenager	12-19	41.3	0.120	0.309	0.016	0.041	0.010	0.025
Female Adult	20 and Up	36.0	0.070	0.140	0.009	0.019	0.005	0.011
Male Adult	20 and Up	37.0	0.080	0.158	0.011	0.021	0.007	0.013
Total Population	All Ages	37.5	0.096	0.211	0.013	0.028	0.008	0.017

[1] Phytosterol ester consumption equals 13.3% w/w of oil consumption.
[2] Phytosterol consumption equals 60% w/w of phytosterol ester consumption.

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[redacted]

6.3 Conclusions

Consumption data and information pertaining to the identified home uses of vegetable oil as cooking, baking and salad oils were used to provide an estimate of the intake of vegetable oil, per-user, by demographic group and by the total U.S. population. The approach used in this study to provide estimates for the intake of vegetable oil from home use as cooking, baking and salad oil is considered to be 'worst case' since a number of conservative assumptions were used in the calculations. The calculated intakes of vegetable oil would be considered overestimates for a number of the following reasons:

- Several mixed food dishes made with vegetable oil were assumed to be home uses based on the assumption that commercial products would be made with hydrogenated vegetable oil, however some commercial food products may be included that were formulated with vegetable oil.

- Vegetable oil intake calculations were based on 3-day food consumption data which are known to overestimate the chronic consumption of food ingredients or nutrients by individuals, that is, consumption over a longer time period which would be more representative of a chronic intake.

On an all-user basis, the estimated mean total intake of vegetable oil by the total population from all identified home uses as cooking, baking and salad oils was determined to be 5.10 g/person/day (0.096 g/kg body weight/day). These levels of intake would correspond to a mean expected all-user phytosterol ester intake of 0.68 g/person/day (0.013 g/kg body weight/day). Taking into account the 60% phytosterol content, this results in a mean expected all-user phytosterol intake of 0.41 g/person/day (0.008 g/kg body weight/day). The heavy consumer (90th percentile) per-user intake of vegetable oil for the total population was 10.60 g/person/day (0.211 g/kg body weight/day). These high levels of intake would correspond to phytosterol ester consumption of 1.41 g/person/day (0.028 g/kg body weight/day) or a phytosterol consumption of 0.85 g/person/day (0.017 g/kg body weight/day).

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Male teenagers were subject to the largest per-user intake of vegetable oil from all identified home uses, exhibiting a mean oil intake of 6.45 g/person/day (0.12 g/kg

[redacted]

body weight/day). This corresponds to a mean phytosterol ester intake of 0.86 g/person/day (0.016 g/kg body weight/day) or a phytosterol intake of 0.52 g/person/day (0.010 g/kg body weight/day). Heavy vegetable oil consuming (90th percentile) male teenagers per user intake was determined to be 15.80 g/person/day (0.309 g/kg body weight/day). This high consumption corresponds to a phytosterol ester intake of 2.10 g/person/day (0.041 g/kg body weight/day) or a phytosterol intake of 1.26 g/person/day (0.025 g/kg body weight/day).

High intake (90th percentile) infants and children would consume the largest amounts of vegetable oil on a body weight basis, up to 0.431 g/kg body weight/day. This is a result of consuming larger amounts of food or energy on a body weight basis. The corresponding phytosterol ester intake level in this group would be 0.057 g/kg body weight/day or a phytosterol intake of 0.034 g/kg body weight/day. This information was evaluated by the independent panel of qualified experts in their determination of GRAS status for the Procter & Gamble phytosterol-enriched vegetable oil. Their consensus indicated that the additional intake of phytosterol esters from home use of a salad and cooking oil would not present any additional risk to consumers of such products.

7.0 STRUCTURE-ACTIVITY RELATIONSHIPS

Analysis of structure-activity relationships is suggested as a useful approach to correlating the molecular structure of a chemical with its biological activity (Food and Drug Administration, 1982). Due to their low toxicity potential, the Cargill vegetable oil phytosterols present in the Procter & Gamble product belong in Structure Category A. However, since the FDA has classified mixtures as belonging to Structure Category B, the phytosterols mixture must be placed into Category B. Structure category notwithstanding, phytosterols are structurally similar to cholesterol and the intermediate products of lipid metabolism in humans. It is therefore unlikely that the Procter & Gamble vegetable cooking oil containing Cargill Incorporated phytosterol esters would cause any adverse health effect in humans, based on the this type of structure-activity relationship.

[redacted]

8.0 PHYTOSTEROL ABSORPTION, DISTRIBUTION, METABOLISM AND EXCRETION (ADME) PROFILE

Phytosterols are plant sterols that are structurally related to cholesterol; however, differences exist in the configuration of their side chains. A wide variety of phytosterol structures exist in nature but the most abundant phytosterols are β-sitosterol, campesterol, and stigmasterol. Less abundant are the saturated phytosterols or stanols. Sitostanol and campestanol are formed by hydrogenation at the 5-α position of β-sitosterol and campesterol, respectively. Sitostanol is also produced by the complete hydrogenation of stigmasterol. Stanols are found in the Procter & Gamble phytosterol-enriched vegetable oil only at very low levels (< 0.4%) and are not significant to the ADME profile of the sterols found therein.

Foods prepared with phytosterol-enriched oils such as the Procter & Gamble product are expected to contain a higher level of phytosterols than the same foods prepared with conventional oils containing natural levels of phytosterols. Because the commercially prepared phytosterols present in the Procter & Gamble product are structurally identical to naturally occurring phytosterols, they will undergo the same chemical reactions and will be equally affected by higher temperature processing, such as frying, baking, etc. Some of the reaction products that might form during heating of phytosterol-containing cooking oils include products of hydrolysis, oxidation, polymerization, and dehydration. Oxidation products of phytosterols are of particular interest because oxidation products of cholesterol, a structurally similar molecule, have been implicated in the etiology of a variety of human diseases (Finocchiaro and Richardson, 1983).

Chemical analysis of the Procter & Gamble phytosterol-enriched oil, discussed in more detail in Section 3.0, demonstrated that no significant new oxidized phytosterol species were formed after heating under conditions simulating deep frying at home that were not also present in the unheated enriched oil or in 100% canola oil (control). Therefore, no significant safety issues are expected to arise from use of this product under normal conditions. Data on the absorption, distribution, metabolism, and excretion characteristics of phytosterol esters is discussed herein for completeness.

8.1 Absorption

The level of human phytosterol intake is affected by natural variations in the phytosterol content of plants in the diet and that of plant-derived foods. Sources of phytosterols usually come from corn, beans, nuts, and plant oils, which are common components of diet. Research by Cerqueira *et al.* (1979) into vegetarian diets confirms that vegetarians consume higher amounts of phytosterols compared to the conventional Western diet, and that saturated phytosterols are present only in trace amounts in the Western diet.

Phytosterol oxides and their occurrence in foods have been studied (Daly *et al.*, 1983; Finocchiaro and Richardson, 1983; Blekas and Boskou, 1989; Dutta, 1997), but there is only limited information about their absorption and possible physiological effects (Grandgirard *et al.*, 1999). Few effects possibly related to phytosterol oxides have been described in the literature, such as abortifacient effects in mice (Pakrashi and Basak, 1976) and cytotoxicity in mealworms (Salen *et al.*, 1970); an increase in phytosterol oxides was observed in aging *Chenopodium rubrum* cell cultures (Meyer and Spiteller, 1997).

8.1.1 Absorption in Experimental Animals

In a series of experiments to determine the effectiveness of plant sterols, Mattson (1977) administered a series of test diets to thoracic duct-cannulated male rats. The phytosterols β-sitosterol, campesterol, and stigmasterol, as well as oleate esters of each, were administered by stomach intubation either individually or as mixtures in conjunction with [4-^{14}C]cholesterol dietary fat. The oleate esters of the phytosterols were prepared by reacting them with oleic acid isolated from olive oil. Each rat was administered an emulsion type diet that contained 15 mg of [4-^{14}C]cholesterol as a 60-40 mixture of cholesterol and cholesteryl oleate. The total cholesterol equivalent was 1% of the dietary fat while the total phytosterol equivalent was 2%, whether added as free phytosterol or at an appropriately higher level of sterol ester. The addition of 3.3% of the oleate esters of either of the plant sterols, on a molar basis to 2% free sterols, [redacted]

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brought about similar decreases in cholesterol absorption. Furthermore, the ability of the phytosterol esters to lower cholesterol were equally effective whether the carbon chain of the esterifying fatty acid was short (acetate), medium (decanoate), or long (oleate). 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.

Phytosterols are poorly absorbed from the gastrointestinal tract. Sylven and Borgstrom (1969) determined the absorption of tritium-labeled β-sitosterol fed to rats. Feeding of 1.5, 50, or 100 μmoles of β-sitosterol resulted in a transfer of 3 to 6% of the dose to the lymph within the first 24 hours. This was largely independent of the dose fed to the animals. By contrast, 30% of the dose of labeled cholesterol was transferred to the lymph in the first 24-hour period. Most of the studies indicate an absorption rate of approximately 5%. Further investigation revealed that β-sitosterol had no effect upon lymphatic transport of cholesterol and that the same percentage of each sterol was always absorbed. Absorption of one compound did not appear to be affected by the other and it was concluded that the mechanisms for specificity within the intestinal mucosa cells in sterol absorption must lie in the early transport of sterols.

In a study by Ikeda and Sugano (1978), the rate of dietary absorption of [^{14}C]β-sitosterol was compared to that of [^{14}C]β-sitostanol by examining excretion levels in rats. Whereas 97% of the β-sitostanol was recovered from the feces, only 88% of the β-sitosterol was recovered. In subsequent studies, Ikeda *et al.* (1988a, 1988b) determined that two major sites of distinction existed between absorbable and non-absorbable sterols. Differential uptake occurs in the brush border cell membranes, with uptake of β-sitosterol one-eleventh that of cholesterol. There is also a differential sterol affinity, depending on the composition of bile salt micelles. The data suggest that plant sterols displace cholesterol from taurocholate bile salt micelles. Other minor sites of discrimination may exist intracellularly since cholesterol and β-sitosterol are processed differently with respect to esterification and chylomicron incorporation.

In a recent study, Grandgirard *et al.* (1999) examined the lymphatic absorption of chemically-synthesized phytosterol oxides in rats by measuring the levels of sitosterol

and campesterol oxides (*e.g.*, 7-keto and epoxides) in lymph following intragastric administration. Lymphatic recovery (*i.e.*, absorption) of phytosterol oxides was low, with mean absorption values of 1.5% of the given dose for 7-keto compounds and 4.7% for epoxy derivatives. The campesterol oxides were better absorbed than the sitosterol oxides.

8.1.2 Basal Plasma Phytosterol Levels Absorption in Humans

Phytosterols detected in the human body at any particular time can be presumed to have come from dietary sources, since phytosterols are not synthesized in the body endogenously. Salen *et al.* (1970) demonstrated that in diets devoid of plant sterol content, human plasma and feces became free of β-sitosterol and other phytosterols. Salen *et al.* also determined the basal level of β-sitosterol in human plasma to range between 0.3 and 1.02 mg/dl in individuals consuming a typical Western diet.

Gould *et al.* (1969) measured β-sitosterol concentrations of 0.47 and 0.76 mg/dl in the blood of two patients. This is consistent with a determination made by von Bergmann and Lutjohann (1998), who compared basal phytosterol levels in normocholesterolemic and hypercholesterolemic subjects. Normocholesterolemic subjects were found to have an average basal concentration of 0.3 mg/dl for β-sitosterol and 0.42 mg/dl for campesterol. Hypercholesterolemic patients maintained elevated β-sitosterol and campesterol levels in the average range of 0.55 mg/dl and 0.78 mg/dl, respectively. A comparison between the two groups revealed a similar phytosterol to cholesterol ratio. In the normal population, the campesterol / cholesterol ratio was 2.33 and in hypercholesterolemic patients it was 2.18. The β-sitosterol / cholesterol ratio was 1.7 in normocholesterolemic subjects and 1.54 in the hypercholesterolemic group.

In humans, the absorption of dietary cholesterol is approximately 40%, whereas the absorption of dietary phytosterols is in the range of 5% or less, as indicated by Salen *et al.* (1989) and Miettinen *et al.* (1990). A literature review by Pegel (1997) confirms this, stating that the absorption of cholesterol is approximately 50% while that of β-sitosterol is approximately 5%.

The absorption of phytosterols is somewhat selective along the gastrointestinal tract. Heinemann *et al.* (1993) conducted a study in 10 healthy subjects using an intestinal perfusion technique to compare the absorption of various phytosterols over a 50-cm segment of the upper jejunum. The respective absorption percentages of β-sitosterol, campesterol, stigmasterol, and campestanol, were 4.2%, 9.6%, 4.8%, and 12.5%. Sitostanol was also examined and was not absorbed to any significant extent.

Mattson *et al* (1982) conducted an assay designed to optimize the effect of plant sterols on cholesterol absorption in humans. Over three experimental sessions, with one-month intervals, 9 adults were fed a meal containing 500 mg cholesterol. In addition, the meal contained 1) no additive; 2) 1 g β-sitosterol; or 3) 2g β-sitosteryl oleate. Cholesterol absorption through stool samples over the following 5 days was analyzed. The addition of β-sitosterol resulted in a 42% decrease in cholesterol absorption whereas β-sitosteryl oleate caused only a 33% reduction. It was hypothesized that this is a reflection of the facility with which the micellar phase is formed and absorbed, possible as a consequence of differential rate of introduction into the intestinal lumen of bile acids, a necessary component of these micelles. However, the esters, although not as effective as the free sterols, had the advantage of ready incorporation into the diet.

It has been suggested that absorption of dietary phytosterols may be higher during infancy and childhood, based on observations of accumulated plant sterols in the aortic tissues of vegetable oil formula-fed infants (Mellies et al., 1976a). Elevated total plasma phytosterol levels have been reported in infants on commercial formulas, which are poor in animal fats and enriched with vegetable oils, and in hypercholesterolemic children on a low-cholesterol, phytosterol-rich diet (Mellies et al., 1976b). Mean plasma levels of phytosterols (campesterol, stigmasterol, β-sitosterol), normally in the 2 mg/100 ml range, were increased three- to five-fold to 9 mg/100 ml.

8.2 Distribution

8.2.1 Distribution in Experimental Animals

[redacted]

Bhattacharyya and Lopez (1979) fed rabbits a low-cholesterol diet containing 2% plant sterols from an unidentified source over a 10-week period. The mean plasma levels of β-sitosterol and campesterol were 0.76 and 8.9 mg/100 ml, respectively. The blood levels of β-sitosterol and campesterol reached a plateau after about five weeks. The initial blood levels of phytosterols were below 0.01 mg/100 ml. In an analysis of distribution, campesterol and β-sitosterol were found to have accumulated in the abdominal organs, liver, intestine, kidney, adrenals, and ovary. Campesterol was also found in numerous peripheral tissues. No esterified campesterol or β-sitosterol were found in any of the tissues examined.

Subbiah and Kuksis (1973) reported that, following the intravenous injection of [4-^{14}C]cholesterol and β-[22,23-^{3}H]sitosterol, β-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. Leikin and Brenner (1989) determined that the amount of phytosterols in rat liver microsomes was only 5% that of cholesterol.

Phytosterols are very similar to cholesterol in structure (see Figure 1). Cholesterol interacts with cell membrane phospholipids and affects membrane lipid composition. Similarly, high intakes of dietary phytosterols have been reported to decrease liver cell membrane fluidity when incorporated into rat liver microsomes (Leikin and Brenner, 1989). No changes in phospholipid or total sterol content of the liver cell membranes were noted following elevated phytosterol consumption.

8.2.2 Distribution in Humans

Phytosterols (1 to 42 μmol/L) are found in human serum under normal conditions with dietary intakes of 160 to 360 mg/day. However, work by investigators such as Connor (1968), Salen *et al.* (1970), and Cerqueira *et al.* (1979) has shown that plasma levels may increase up to two-fold through dietary supplementation.

The effect of orally administered phytosterols on plasma phytosterol content is dependent on the nature and composition of the sterols administered. Elevated dietary β-sitosterol intake elevates plasma β-sitosterol and depresses plasma levels of campesterol. Weststrate (1998) observed that Unilever soy-derived phytosterol esters,

which have elevated β-sitosterol and campesterol content, raised the concentration of both phytosterols in the plasma.

Becker *et al.* (1992, 1993) further confirmed the elevation of plasma levels from dietary phytosterol intake by comparing the effects of a phytosterol preparation high in β-sitosterol to a low-cholesterol diet in hypercholesterolemic children. In each assay, β-sitosterol was observed at elevated levels compared to the control diets.

Tilvis and Miettinen (1986) determined that, compared to cholesterol, free and esterified phytosterols in serum tended to be accumulated in HDL where the phytosterol / cholesterol ratios were almost 40% higher than in LDL and VLDL. The serum phytosterol concentrations, the phytosterol / cholesterol ratios, and the fractional absorption of cholesterol were lower in men than in women. However, serum levels of noncholesterol sterols are effectively determined by their absorption which is in turn proportionate to the fractional absorption of cholesterol.

Ling and Jones (1995) established that there is little information available regarding the distribution of phytosterols in various tissues of the body. The liver is the major organ involved in cholesterol homeostasis; however, whole liver and liver microsomes of normal subjects contain only trace levels of phytosterols (Nguyen *et al.*, 1990). High phytosterol levels were found in the liver microsomes of subjects with sitosterolemia, a condition which is discussed further in the Human Clinical Safety section, 10.5.

Bhattacharyya *et al.* (1983) found that in providing patients with a sterol free diet, β-sitosterol gradually decreased and eventually disappeared altogether from the skin as well as the feces. Upon reintroduction of phytosterols into the diet, β-sitosterol reappeared in the skin surface lipids within one week. The results demonstrated clearly that plant sterols absorbed into the plasma from the diet were distributed into the skin surface lipids.

8.3 Phytosterol Metabolism

Phytosterols are not endogenously synthesized in the body and they are not converted to cholesterol in humans or other mammals, nor is cholesterol converted to

any of the phytosterols (Pollak, 1987; Salen *et al.* 1970).

Subbiah and Kuksis (1973) determined that, when given intravenously to rats, β-[22,23-^{3}H]sitosterol was esterified with fatty acids in a reaction mediated by cholesterol-lecithin acyltransferase. Cholesterol is esterified in the same manner. However, unlike cholesterol, which is metabolized into C_{24} bile acid, β-sitosterol is converted into di- and trihydroxylated C_{21} bile acids in the rat, (Subbiah and Kuksis, 1973; Skrede *et al.*, 1985; Boberg *et al.*, 1990a; and Lund *et al.*,1991). Boberg *et al.* (1990b) tentatively identified two of the bile acids as having hydroxyl groups at the C_3 and C_{15} positions. Additionally, one bile acid has a keto group and the other a hydroxyl group, although their exact position on the molecule remains undefined.

According to Grandgirard *et al.* (1999), few studies have examined the metabolism of phytosterol oxides. Some have described the possible conversion of plant stanol (*i.e.*, hydrogenated phytosterol) epoxides in triols (compound with three hydroxyl groups) (Aringer and Eneroth, 1974); a similar process has been described for cholesterol epoxides *in vitro* (Maerker *et al.*, 1988) and *in vivo* (Fioriti *et al.*, 1970). Other possible mechanisms for epoxide metabolism include possible modification by enzymes present in human intestinal microflora (*e.g.*, epoxide hydrase) or inside intestinal cells (*e.g.*, microsomal hydrolase) (Grandgirard *et al.*, 1999).

8.4 Phytosterol Excretion

The elimination of absorbed phytosterols from the body takes place primarily through the biliary route. This was confirmed by Boberg and Skrede (1988), who reported that in humans, β-sitosterol is not metabolized into a C_{24} bile acid but rather into a C_{21} bile acid. This is consistent with observations made by Boberg *et al.* (1990) in other mammalian species. Lin et al. (1984) further indicated the possibility of selectively greater excretion of other sterols relative to cholesterol. In an analysis of sterol excretion after prolonged consumption of stigmasterol in rats, Andriamiarina *et al.* (1989) concluded that stigmasterol induces increased fecal neutral sterol levels in a fashion similar to β-sitosterol. Ling and Jones (1995) proposed that there is a correspondingly small pool of endogenous phytosterols relative to cholesterol due to

poor absorption in the intestine and faster excretion via the bile. Once in the anaerobic lumen of the lower intestine, unabsorbed phytosterols may be converted by normal bacterial flora to other naturally occurring metabolites.

Weststrate *et al.* (1999) conducted an evaluation in 12 male and 12 female normolipidemic volunteers who, following a run-in period, consumed 40 g of a vegetable oil spread for 21 (males) to 28 days (females). The vegetable oil spread, Take Control™, which contained 8.6 g of vegetable oil phytosterols (46% β-sitosterol, 26% campesterol, 20% stigmastanol by weight), increased fecal neutral sterol concentrations from 40 to 190 mg/g, dry weight. Fecal neutral sterol metabolites increased from 30 to 50 mg/g. Of the total fecal neutral sterols, β-sitosterol, campesterol and stigmasterol comprised 28%, 15%, and 12%, respectively. This reflects the composition of the phytosterol ester-enriched vegetable oil spread. Major sterol metabolites excreted were predominantly formed by oxidation at the 3-position or saturation at the 5,6-position in a β-configuration. Secondary bile acid was reduced from 7.6 to 6.0 mg/g in dry feces. Fecal concentrations of the mutagen 4-cholesten-3-one remained low and no sterol oxides could be detected. The authors concluded that increased vegetable oil phytosterol ester consumption increased the levels of neutral sterols in the feces but did not result in increased bile acid or sterol metabolite formation.

Ayesh et al. (1999) conducted an identical study employing Take Control™. The test group showed an 18% reduction in total cholesterol and a 23% reduction in LDL-cholesterol levels. A statistically significant decrease in fecal lactic acid was observed. When compared to baseline measurements, the test group had a reduced fecal lactic acid concentration and lactobacilli content, as well as a lower ratio of acetic acid to total short-chain fatty acids. In female subjects, none of whom took oral contraceptives, decreased serum progesterone was observed. No other significant treatment-related effects were noted in the test group. No other changes in clinical toxicological endpoints were noted. It was concluded that the daily consumption of the phytosterol esters found in Take Control™ had no effect on the gut microflora in terms of bacterial profile or metabolic activity. The decrease in serum sex hormone levels in females was not considered to be biologically relevant due to numerous factors which may cause variation in the pattern of female sex hormones. Daily consumption of this product was

well-tolerated by both male and female volunteers, and was not associated with any effects other than a significant reduction of total and LDL cholesterol levels.

9.0 PRECLINICAL TOXICOLOGY

The safety of this product, as determined by a panel of qualified experts, is based upon the chemical similarity and substantial equivalence of its constituent phytosterol esters to other phytosterol ester-containing products for which preclinical safety has been investigated and documented. Therefore, no separate preclinical safety studies of the Procter & Gamble phytosterol-enriched vegetable oil have been conducted.

Foods prepared with phytosterol-enriched oils such as the Procter & Gamble product are expected to contain a higher level of sterols than the same foods prepared with conventional oils containing natural levels of phytosterols. Because the commercially prepared phytosterols present in the Procter & Gamble product are structurally identical to naturally occurring phytosterols, they will undergo the same chemical reactions and will be equally affected by higher temperature processing, such as frying, baking, etc. Some of the reaction products that might form during heating of phytosterol-containing cooking oils include products of hydrolysis, oxidation, polymerization, and dehydration. Oxidation products of phytosterols are of particular interest because oxidation products of cholesterol, a structurally similar molecule, have been implicated in the etiology of a variety of human diseases (Finocchiaro and Richardson, 1983).

Chemical analysis of the Procter & Gamble phytosterol-enriched oil, discussed in more detail in Section 3.0, demonstrated that no significant new oxidized phytosterol species were formed after heating under conditions simulating deep frying at home that were not also present in the unheated enriched oil or in 100% canola oil (control). Thus, no significant safety issues are expected to arise from use of this product under normal conditions.

9.1 Genotoxicity

The phytosterols contained within the Procter & Gamble phytosterol-enriched oil are not genotoxic. The safety of phytosterols with regard to genotoxicity has been well documented in the two currently available GRAS dossiers for Benecol™ and Take Control™. Specifically, the phytosterols in the Unilever Take Control™ product, with their similar nature and composition to the phytosterols in the Procter & Gamble phytosterol-enriched oil, demonstrate a lack of genotoxicty. The Take Control™ dossier contains unpublished reports of an Ames bacterial mutagenicity assay and a metaphase chromosome analysis of human lymphocytes cultured *in vitro*, both in the presence or absence of an S9 Aroclor-induced rat liver microsome fraction for metabolic activation. No evidence of genotoxicity was observed in either study.

One incidence of mutagenicity has been observed in the case of β-sitosterol. An investigation by Zaied (1996) reported that subcutaneous injection of β-sitosterol in rats at a low and high dose of 5 and 7 mg/kg, respectively, induced the development of micronucleated erythrocytes in their progeny, which the author attributes as possibly due to a phytoestrogen effect. Newborn rats were affected when either parent was treated individually but particularly, when both parents were injected with β-sitosterol. This study does not take into account the normal metabolic pathway following typical ingestion of phytosterols and has been included for completeness. The topic of potential estrogenic responses following phytosterol exposure is addressed in the reproductive toxicology subsection.

An assay conducted by Ansari *et al.* (1982) further supports the lack of genotoxicity from the Procter & Gamble phytosterol-enriched product. In this assay, the mutagenicity of oxidized commercially available sterols was examined using the Ames test. Autoxidized β-sitosterol, stigmasterol, brassicasterol and lanosterol, as well as 38 individual cholesterol derivatives were all found to be nonmutagenic in the presence or absence of metabolic activation.

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Furthermore, an evaluation of the genotoxic potential of vegetable oil-derived stanol esters, some of which are found at very low concentrations in the Procter & Gamble product, has been conducted using the Ames test. *S. typhimurium* strains

TA98, TA100, TA1535 and TA1537 were assayed with and without metabolic activation. Turnbull *et al.* (1999b) concluded that the plant stanol esters were not toxic to the cells, nor were they mutagenic. A chromosome aberration segment of this study conducted on Chinese hamster ovary (CHO) cells and on mouse lymphoma L5178Y cells revealed no mutagenic potential in the presence or absence of metabolic activation.

9.2 Subchronic and Chronic Toxicity

Procter & Gamble has not instituted any new toxicological evaluations of their phytosterol-enriched vegetable oil product. The safety of the phytosterol-enriched product, as determined by a panel of qualified experts, is assured by the substantial equivalence of its component Cargill phytosterol ester fraction to phytosterols present in other GRAS products for which ample evidence of safety is available in the published literature. This evidence includes toxicological evaluations conducted to assess the potential effect of elevated levels of dietary β-sitosterol in animals; β-sitosterol is one of the primary phytosterol components in the Procter & Gamble product. The results of these studies, discussed below, demonstrate that elevated phytosterol intake can be considered safe.

9.2.1 Toxicity Studies of Vegetable Oil Phytosterol Esters

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A 90-day oral toxicity assay conducted by Hepburn *et al.* (1999) is relevant to establishing the safety of the Procter & Gamble phytosterol-enriched product due to similarities in phytosterol composition. Their analysis of the material employed in this toxicity assay revealed a mixture (w/w) with a compositional breakdown of 48.7% β-sitosterol, 25.8% campesterol, and 21.6% stigmasterol. Only 8.4% (w/w) of the phytosterol content existed as free sterols; the remaining sterols were esterified. The phytosterol ester fraction of the Procter & Gamble phytosterol-enriched vegetable oil is primarily composed of 45% β-sitosterol, 25% campesterol, and 25% stigmasterol. During this study, 20 male and 20 female rats per dose group received soy-derived phytosterol esters in the diet at levels of 0, 0.16, 1.6, 3.2, and 8.1% (w/w). Clinical

observations made throughout the study did not reveal any changes in behavior, body weight, or water consumption. Food consumption was slightly elevated, probably because of lower energy content in the diets due to the phytosterol component. This effect was considered to be of no toxicological significance. A thorough post-mortem examination did not reveal any changes in organ weights or gross macroscopic observation at necropsy. No histopathological changes of toxicological significance were observed. The 8.1% phytosterol ester concentration was therefore considered to be the no observed adverse effect level (NOAEL) following daily oral administration over 90 days. The equivalent dose of was calculated to be 6.3 g phytosterol esters per kg body weight per day and 6.8 g phytosterol esters per kg body weight per day for males and females respectively. While not stated within the study, extrapolation of this data was used to determine the equivalent dose of phytosterol esters in the other dose groups. In rats receiving 0.16% phytosterol esters, the equivalent dose of was determined to be 0.124 g phytosterol esters per kg body weight per day and 0.134 g phytosterol esters per kg body weight per day for males and females respectively. In rats receiving 1.6% phytosterol esters, the equivalent dose of was determined to be 1.24 g phytosterol esters per kg body weight per day and 1.34 g phytosterol esters per kg body weight per day for males and females respectively. In rats receiving 3.2% phytosterol esters, the equivalent dose of was determined to be 2.49 g phytosterol esters per kg body weight per day and 2.69 g phytosterol esters per kg body weight per day for males and females respectively.

9.2.2 Toxicity Studies of Cytellin™

Eli Lilly Research Laboratories marketed a mixture of phytosterols derived from pine tall oil in the United States between 1954 and 1982. It was sold as Cytellin™ in the United States and as Positol™ in Canada. This product was marketed as an anti-hypercholesterolemic agent and was available either as a powder or liquid suspension. Its reported composition was 80% β-sitosterol, 10% sitostanol, 7% campesterol, and 2% campestanol. As previously stated, the phytosterol ester fraction in the Procter & Gamble phytosterol-enriched vegetable oil is primarily composed of 45% β-sitosterol,

25% campesterol, and 25% stigmasterol. Although Cytellin™ was eventually withdrawn from the market due to business considerations, several toxicity studies had been conducted with the product.

Little or no toxicity was observed following administration of large single oral doses to mice. Administration of a single dose of 5 g/kg β-sitosterol in sesame oil to 565 albino mice by stomach tube produced few or no signs of toxicity. This is consistent with the findings of Gupta *et al.* (1980), who reported an intraperitoneal LD_{50} for β-sitosterol greater than 3 g/kg in mice.

Thirty female rats were also administered 1% and 5% β-sitosterol in their diet for a period of 18 months. Both dose groups of rats 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. Another study employed female mongrel dogs given capsules of Cytellin™. Four dogs received 500 mg/kg/d and 4 dogs 1000 mg/kg/d each for 18 months. Dogs that received daily doses of 500 mg/kg/d or 1000 mg/kg for 18 months survived, gained weight and had no hematological or visceral damage. No changes in serum cholesterol, calcium, phosphorus, total lipids, lipid phosphorus, vitamin A and blood protein fractions were observed. Total lipid and total cholesterol values in the livers were unchanged.

9.2.3 Other Non-Clinical Studies

Phytosterols have been studied extensively. Many studies in the scientific literature discuss the non-clinical safety of phytosterols indirectly while focusing upon other known or suspected properties. This section seeks to document the safety of phytosterols by reviewing scientific publications which discuss the safety of phytosterols in general terms.

In a series of evaluations conducted by Shipley *et al.* (1958), the safety of β-sitosterol was assessed. Three groups of 10 rats were fed a mixture of rat chow and 5% β-sitosterol derived from tall oil for periods of 8, 18, or 22 months. Parallel groups received 5% dose levels of β-sitosterol derived from cottonseed oil for 18 and 22 months. No alterations in growth, blood cell counts, blood urea nitrogen, or serum

proteins were observed. No unusual gross or microscopic organ or tissue findings were reported. Dogs were also fed β-sitosterol at levels of 0.5 and 1 g/kg for periods of 8 to 22 months. All dogs maintained their weight and had normal values for serum composition and formed blood elements. No gross or microscopic pathological changes attributable to β-sitosterol were observed in any dog. An evaluation was also conducted in New Zealand white rabbits using 4% soy-derived β-sitosterol along with 3% cottonseed oil, which is also high in phytosterol content. Rabbits were maintained on this diet for as long as 842 days and showed no gross or microscopic pathological abnormalities. Furthermore, the total liver and aortic lipid concentration, and the aorta sterol concentrations were comparable to controls.

In an attempt to determine the effect of β-sitosterol on aorta lipid, cholesterol, and plaque regression, Beher *et al.* (1956) fed 4 groups of 10 female albino rabbits a high cholesterol test diet for three months. As part of a regression analysis, the rabbits were then taken off the high cholesterol diet and one experimental group received a 2.5% β-sitosterol supplement to their diet. Incorporating 2.5% β-sitosterol into the feed of atherosclerotic rabbits slightly increased the regression of plasma and liver cholesterol but had no effect on aorta lipid, cholesterol, or plaque regression. No significant toxicological effects were reported.

In second study, Beher *et al.* (1957) administered a high cholesterol diet supplemented with an additional 1% dietary cholesterol to female rabbits over a period of 3-months. This resulted in the formation of aortic plaques and a general increase in all lipid fractions. Histologic examination gave evidence of advanced cirrhosis of the liver. In a satellite study, 2 experimental groups received the high cholesterol diet which which was supplemented with either 2% β-sitosterol or 2% dihydrocholesterol. Administration of β-sitosterol did not result in aortic plaque formation or any changes in liver or aortic lipid fractions and was proven to be as effective as dihydrocholesterol in lowering total plasma cholesterol.

Sugano *et al.* (1977) conducted a 28-day assay in male Wistar rats wherein the effect β-sitosterol, sitostanol, and cholesterol on the concentration of plasma and liver lipids were compared when administered as 0.5% of the basal diet. In different test groups, dietary fat was provided in the form of safflower oil, corn oil, or lard. In all, 24

test groups, comprising of 6 - 8 rats, were evaluated. No differences in the effects of the sterol or stanol were noted in the concentration of liver cholesterol and triglyceride. Increases in plasma triglyceride due to feeding β-sitosterol were not observed in sitostanol and incorporation of dietary sitostanol into plasma, liver, other tissues was negligible. No demonstrable negative effects on growth or on the weight of visceral tissues were observed in either test group.

Laraki *et al.* (1991) studied the effects of dietary phytosterols on plasma cholesterol by feeding 12 Wistar rats per dose group a diet containing a cholesterol overload of 24 mg/day, excluding control, over a period of 3-weeks. In 2 groups, an additional amount of phytosterols (72.5% β-sitosterol, 20.5% campesterol, 7% stigmasterol) was administered at either 24 or 96 mg/day. The phytosterol rich diet reduced the effect of the cholesterol overload and no adverse toxicological effects were associated with elevated phytosterol intake.

Ambrosova (1999) fed a diet containing 21 mg/kg phytosterols (65% β-sitosterol, 18% stigmastanol, 14% campesterol and 3% campestanol) to two groups of rats, one normal and the other PHHC (an experimental model of hypercholesterolemia) for 60 days. No clinical signs of toxicity were observed in the normal group and a positive effect on the biochemical lipid profile and on endothelial physiology was noted in the PHHC group. However, an increase in free unsaturated fatty acids was noted in both groups indicating a possible advancement in the process of atherogenesis. No causal relationship between phytosterol treatment and development of atherosclerosis could be established.

Malini and Vanithakumari (1990) administered β-sitosterol to rats by daily subcutaneous injection at dose levels of 2.5, 5, and 10 mg/kg/day over 60 days. No evidence of gross microscopic lesions in the kidney or liver was observed. β-sitosterol was well tolerated and all biochemical parameters were within normal range with the exception of reduced serum cholesterol.

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Iyer *et al.* (1998) conducted a study in which neonatal piglets were given intravenous injections of soy phytosterols at 18 nM/kg per day for 14 days and the presence of phytosterols in plasma, bile and the liver examined. Serum bile acid levels

increased and bile acid-stimulated bile flow was reduced. Liver function tests remained normal, as did liver histology.

Raicht (1980) conducted an assay to determine whether β-sitosterol is involved in the finding that a diet rich in vegetables is associated with a reduced risk of colon cancer. The known carcinogen N-methyl-N-nitrosurea (MNU), administered to rats intracolonically. In conjunction 0.2% β-sitosterol was included in the diet and the rats were observed for 28 weeks. A significant decrease in the proportion of tumor-bearing animals was observed following 28 weeks of β-sitosterol feeding. No adverse effects related to long term β-sitosterol feeding were noted.

In an assay by Janezic and Rao (1992), the effect of dietary phytosterols on the proliferative status of the intestinal epithelium was investigated in inbred C57B1/6J mice. Their diet consisted of 0, 1, or 2% of a phytosterol blend consisting of 60% β-sitosterol, 30% campesterol, and 5% stigmasterol, administered over 2 weeks. Phytosterol intake significantly reduced the enhanced labeling index and the position of the highest labeled cell in a dose-dependent manner. The mitotic index was also reduced but no dose-dependent relationship was observed. The overall results indicate that phytosterols influence colonic epithelial cell morphometrics, which are important preneoplastic events in colonic carcinogenesis, and may therefore contribute to a reduction in the risk of cancer.

9.3 Reproductive Toxicity and Estrogenic Activity

9.3.1 Reproductive Toxicity

Studies in experimental animals confirm the reproductive safety and the lack of estrogenic activity associated with elevated levels of dietary phytosterols. In a study investigating the effects of soy phytosterol esters on rat reproductive parameters, Waalkens-Berendsen (1999) incorporated levels of 0, 1.6, 3.2, or 8.1% (w/w) 000067 phytosterol esters into the diet over the course of two successive generations. A wide range of reproductive and developmental parameters was evaluated, including sexual maturation and estrous cycle length. No effect on the reproductive performance of parental F_0- and F_1-generation Wistar rats or on the development of F_1 and F_2 pups

were reported, nor were any histopathological effects on selected organs observed. The high dose of 8.1%, calculated to approximately 2.3 to 9.1 g of phytosterol ester per kilogram per day, was considered to be the no-observed-adverse-effect-level (NOAEL) in rats following daily oral administration of phytosterol ester over two successive generations.

Few effects possibly related to phytosterol oxides have been described. Among them are abortifacient effects in mice reported by Pakrashi and Basak (1976) following oral gavage administration of a number of oxidized sterols as a single dose of 30 mg/kg body weight. When administered to pregnant mice on day 1 or day 6 to 7 of pregnancy, the most effective abortifacient compounds were 7-hydroxysitosterol, sitostane-triol, and ergosterol 5α, 8α-peroxide. Sitosterol and 7-ketositosterol were only slightly effective.

9.3.2 Estrogenic Activity

A number of reports have suggested that β-sitosterol may have estrogenic properties. Ghannudi (1978; 1979) conducted research in immature male and female rabbits, subcutaneously injecting them with β-sitosterol daily for 30-days. Male rabbits received doses of 10.0, 59.0, 144, and 350 μg per day with testicular and epididymal samples taken at intervals of 3, 10, and 30 days. Females received the same dose regimen, 10.0-350 μg per day, but were only evaluated on day 30 of the experiment. Estrogenic responses were noted in both assays. El Samannoudy (1980) also noted an estrogenic response when 0, 0.5, 1.0, 5.0, 10.0, 15.0, or 20.0 mg of β-sitosterol was injected subcutaneously to groups of six immature female sheep over a period of 20 days. A pair of articles by MacLatchy (1995; 1997) indicated that exposure to elevated levels of β-sitosterol may be a contributing factor to reproductive dysfunctions observed in goldfish. Burck (1982) even determined that β-sitosteryl sulfate, when released in the uterine horn of female rabbits induced a significant reduction in the number of embryos. β-Sitosterol sulfate, but not β-sitosterol, has an acrosin inhibitory activity, which would reduce the sperm efficiency in ova fertilization. Implantation of silicone rods containing β-sitosterol sulfate into uterine horns of rabbits for 16 days, significantly reduced the

number of embryos present in those horns. This treatment did not effect the number of corpora lutea.

Malini (1991) investigated and noted effects on male rat reproductive tissue by injecting 0.5 mg/kg or 5 mg/kg β-sitosterol subcutaneously for 16, 32, and 48 days. An antifertility effect of β-sitosterol was pronounced only at the high dose but there was significant decrease in testicular weight and sperm concentrations after long-term treatment with the low dose of β-sitosterol. The weights of all accessory sex tissues except caput epididymis increased following low dose β-sitosterol treatment. High dose treatment reduced sperm concentrations as well as the weights of testis and accessory sex tissue in a time-dependant manner. Withdrawal of treatment for 30 days restored the weights of the accessory sex tissue but not the testis. Further study was indicated to elucidate the exact site and mechanism of action.

Malini (1993) also investigated and noted effects on female rat reproductive tissue by subcutaneously injecting 50, 250, or 500 μg/100g body weight of β-sitosterol to ovariectomized rats for 10 days. Uterine growth response to β-sitosterol was found to be dose-dependant and only the high dose produced an increase in uterine weight with massive edema comparable to that of an estradiol positive control. Test results therefore indicate a uterotrophic effect of β-sitosterol in ovariectomized rats and a potential synergism with estradiol.

The relevance of the fish model, or models which employ subcutaneous or intravenous injection, to the normal route of human phytosterol intake is questionable. The kinetics of phytosterols administered in this manner differ from those of oral consumption. Administered orally, approximately 5% of phytosterols are absorbed. Those which become absorbed may be incorporated into very low density lipoproteins (VLDL) or chylomicrons before entering the blood stream whereas subcutaneously or intravenously administered phytosterols would more likely be available as free sterols.

In order to more thoroughly evaluate the possible estrogenic effects of phytosterols, Baker (1999) carried out a combination of *in vitro* (2) and *in vivo* (1) assays employing a combination of 47.9% β-sitosterol, 28.8% campesterol and 23.3% stigmasterol. The phytosterols were sourced from a variety of vegetable oil distillates

such as soy or sunflower. The phytosterols employed were then esterified with fatty acids from sunflower oil.

In the first of two *in vitro* assays, competitive binding of the phytosterol esters was compared with [2,4,5,6-^{3}H]estradiol for the ability to bind to immature rat uterine estrogen receptors. As a positive control, β-estradiol was employed. This assesses the ability of the phytosterols to bind to estrogen receptors. The phytosterols were negative in the receptor binding assay and did not compete with [2,4,5,6-^{3}H]estradiol for binding to the estrogen receptors at concentrations of to 10^{-4} M. The β-estradiol positive control demonstrated an EC_{50} value of $1.3X10^{-10}$ M. This is the concentration required to reduce β-estradiol binding by 50% and is indicative of the low estrogenic response of phytosterols in this assay.

In the second in vitro assay, a recombinant yeast strain was used to directly assess the ability of the phytosterol mixture to stimulate the transcriptional activity of human estrogen receptor. In this case, the yeast responded positively to a known phyto-estrogen , coumestrol providing an EC_{50} value of $3.3x10^{-8}$ M whereas the β-estradiol positive control yielded 3.2 M as an EC_{50} value. By contrast, at $2x10^{-4}$ M, the highest dose tested, neither the phytosterol mixture nor β-sitosterol stimulated the transcriptional activation of human estrogen receptor in yeast.

The third *in vivo* immature rat uterotrophic assay was conducted in order to evaluate the effects of phytosterols on estrogen-responsive tissue. Over a three day period, 0, 5, 50, and 500 mg/kg/day were administered by oral gavage to groups of 10 animals. A weak positive control, the phyto-estrogen coumestrol, was administered to parallel groups at doses of 0, 5, 20, 40, and 80 mg/kg/day. Furthermore, a 0.4 mg/kg/day β-estradiol positive control was administered to one group of animals. The group mean uterus weight in all dose groups treated with the phytosterol ester mixture was unaffected whereas the coumestrol induced a statistically significant dose-response increase in uterine weight in the 20, 40, and 80 mg/kg/day groups. As expected the β-estradiol positive control produced a significant increase in uterine weight when compared to each test substance.

Awad (1998) investigated the effect of dietary phytosterols on the activity of 5-α-reductase and aromatase enzymes in rat liver, testis and prostate. Testosterone can be

activated by reduction to dihydrotestosterone and other reduced metabolites or aromatization to estrogen. The end products of these two pathways are implicated in prostate cancer (Stone *et al.*, 1987; Montie & Pienta, 1994). Elevated levels of estrogens and androgens are risk factors in the development of prostate cancer. After feeding male rats for 22 days a 2% mixture of phytosterols (56% β-sitosterol, 28% campesterol, 10% stigmasterol and 6% dihydrobrassicasterol) with 0.2% cholic acid, the liver, testes, and prostrate were evaluated. No phytosterol effect was noted in the testes but in the liver and prostate, 5-α-reductase activity was reduced by 41% and 33%, respectively. This resulted in a net reduction of 33% in serum testosterone and a 55% decrease in aromatase activity on the prostate. It was concluded that dietary phytosterols feeding may reduce the risk of prostate cancer by lowering the activities of the enzymes associated with testosterone metabolism.

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.

9.4 Safety of Fully Saturated Phytosterols (Stanols)

The safety of fully saturated phytostanol esters has also been extensively studied. Phytostanols are contained within the phytosterol fraction of the Procter & Gamble product at very low levels, approximately 3% in aggregate. Due to the presence of these low levels of phytostanols and the similarity between stanols and sterols in molecular structure, a brief review of recent studies of stanol safety is warranted to further establish the safety of the Procter & Gamble phytosterol-enriched product.

Turnbull (1999c) carried out a 13-week oral toxicity study in groups of 20 male and 20 female rats employing vegetable oil or wood (tall oil) derived stanol esters in dietary concentrations of 0, 0.2, 1, and 5%. This resulted in a daily wood derived phytostanol ester intake of 0, 174, 872, and 4579 mg/kg/day in males and 0, 193, 979, and 5093 mg/kg/day in females. The vegetable oil derived phytostanol intake was 187,938, and 4934 mg/kg/day in males and 206, 1049, and 5509 mg/kg/day in females. Both the tall oil

and vegetable oil preparations were well tolerated as evidenced by the absence of clinical changes or major abnormalities in growth, food and water consumption, ophthalmoscopic observation, hematological and clinical chemistry values, renal concentrating ability, organ weight, gross necropsy finding and histopathological evaluation. In each case, a no observed adverse effect level was set at the mid dose level. Plasma levels of vitamins E, K and to a lesser extent, vitamin D, were decreased in males and females fed the high dose diet. Hepatic levels of vitamin D and E also showed similar changes. Compared to control, male rats fed stanol esters showed slightly lower liver weights and more pronounced glycogen depletion. These hepatic changes were thought to reflect an altered nutritional condition and not a pathological condition.

Turnbull *et al.* (1999a) also investigated the potential for estrogenic activity in plant stanols (soy or tall oil) and plant stanol esters using the *in vitro* E-screen test, which determines the ability of a compound to cause induction and proliferation of estrogen-responsive human breast MCF-7 adenocarcinoma cells. An *in vivo* assay to determine uterotrophic activity in immature female rats fed the phytostanol and phytostanol ester test substances was also conducted. Under the conditions of this test, neither the stanol esters nor the free stanols displayed any evidence of uterotrophic or estrogenic activity.

Slesinski *et al.* (1999) carried out a developmental toxicity assay in female rats employing a vegetable oil-derived stanol fatty acid ester. The test article was administered at concentrations of 0, 1, 2.5 and 5% total stanols in the diet from days 0 to 21 of gestation. Phytosterol ester test substance intake was calculated to be 6.2 g/kg/day between days 0-14 of gestation and 4.2 g/kg/day from days 14-21 of gestation. A statistically significant increase in food consumption was noted in the high dose group during days 7 to 21 of gestation. However, there were no statistically significant differences in uterine, placental, or fetal weights. The number of fetuses, implantation sites, corporea lutea and early/late resorptions were similar between test and control groups. Visceral and skeletal examination of the fetuses did not reveal any incidence of malformation, anomaly or variation which could be considered treatment-related. Dietary stanol esters had no adverse effect on reproduction or development at the highest concentrations administered.

In the fourth and final assay reviewed herein to establish the safety of phytostanols, Whittaker (1999) carried out a two-generation reproductive toxicity study employing vegetable oil stanol esters at concentrations of 0, 1, 2.5, and 5% total stanols in the diet. No treatment-related adverse effects on the reproductive performance of either male or female rats were observed in any of the dose groups. Increased food consumption was noted in both the F_0 and F_1 high-dose males and females and was considered to be the result of a reduced caloric value of the test diet. In the F_1 or F_2 pups, no adverse developmental effects were noted in either the low- or mid-dose groups. Lower body weights and body weight changes were observed in the F_1 and F_2 pups of the high-dose group during the later stages of lactation. This was also attributed to a reduced caloric intake from the test diet as compared the control diet.

10.0 CLINICAL SAFETY

The safety of this product, as determined by a panel of qualified experts, is based in part upon the chemical composition of the constituent Cargill phytosterol esters, which are essentially identical to the phytosterol esters found in Take Control™ and substantially similar in nature to those in Benecol™. These products have been investigated and documented for clinical safety and GRAS status has been determined. Furthermore, the safe clinical use history of Cytellin™ further indicates the general safety of phytosterol consumption. Therefore, no clinical studies of the Procter & Gamble phytosterol enriched vegetable oil have been conducted.

Foods prepared with phytosterol-enriched oils such as the Procter & Gamble product are expected to contain a higher level of sterols than the same foods prepared with conventional oils containing natural levels of phytosterols. Because the commercially prepared phytosterols present in the Procter & Gamble product are structurally identical to naturally occurring phytosterols, they will undergo the same chemical reactions and will be equally affected by higher temperature processing, such as frying, baking, etc. Some of the reaction products that might form during heating of phytosterol-containing cooking oils include products of hydrolysis, oxidation, polymerization, and dehydration. Oxidation products of phytosterols are of particular

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interest because oxidation products of cholesterol, a structurally similar molecule, have been implicated in the etiology of a variety of human diseases (Finocchiaro and Richardson, 1983).

Chemical analysis of the Procter & Gamble phytosterol-enriched oil, discussed in more detail in Section 3.0, demonstrated that no significant new oxidized phytosterol species were formed after heating under conditions simulating deep frying at home that were not also present in the unheated enriched oil or in 100% canola oil (control). Although no significant safety issues are expected to arise from use of this product under normal conditions, any existing data on the clinical safety of thermal products will be discussed herein for completeness.

10.1 Literature Review of Clinical Studies Employing Unilever's Phytosterol Ester Product Take Control™

The safety of the constituent Cargill phytosterol esters in the Procter & Gamble phytosterol-enriched product, as determined by a panel of qualified experts, is supported through its identical chemical and compositional makeup to the GRAS phytosterol esters in Unilever's Take Control™ product. The compositional comparability of the phytosterol esters in the two products has been established in Section 3 of this document. Additionally, some recently published studies documenting the safety of the Unilever phytosterol ester product Take Control™ are also available.

In an assay by Weststrate (1998), the effect on plasma total, LDL-, and HDL-cholesterol concentrations was observed in control and test vegetable oil spreads containing phytosterols from sheanut, ricebran, plus the products Take Control™ and Benecol™. Take Control™ and Benecol™ represented the vegetable oil spreads containing the most significant quantity of phytosterol esters, containing 5.03 g/100 g β-sitosterol and 7.45 g/100 g sitostanol, respectively. The test was carried out in 100 male and female subjects, with plasma total cholesterol levels below 8 mmol/l at entry, over the course of 5 treatments and 4 periods employing an incomplete Latin square design. Subjects received in four consecutive periods of 3.5 weeks 30 g/day of a test margarine for consumption. None of the vegetable oil spreads induced any adverse changes in blood chemistry, serum total bile acids or hematology. Compared to control,

plasma total and LDL cholesterol concentrations were reduced in subjects consuming spreads containing phytosterol esters and phytostanol esters. No effect on HDL cholesterol concentration occurred. The LDL to HDL cholesterol ratio was reduced by 0.37 and 0.33 units, respectively. It was concluded that a vegetable oil spread containing phytosterol esters was as effective in lowering blood total and LDL-cholesterol as the Benecol™ spread containing primarily phytostanol esters.

Hendriks (1999) conducted an investigation to determine any dose response relationship between cholesterol lowering and three different intake levels of esterified phytosterols in a margarine matrix. The impact of esterified phytosterol intake upon plasma vitamin and nutrient levels was also investigated. Relative phytosterol ester levels were set at (w/w) 3.37%, 6.47%, and 13.06% which provided approximately 0.83, 1.61, and 3.24 g per day of plant sterols. No statistically significant difference between dose levels could be determined in the cholesterol lowering effect. No side effects were reported as determined by measuring the liver enzymes ALP, ALT, AST and gamma-GT. Minor effects on vitamins and nutrient levels were observed and are discussed in greater detail in Section 10.4. No adverse events were reported. It was concluded that a dose level of 1.6 g/day of phytosterols would have a beneficial effect upon plasma cholesterol concentration without any serious impact upon carotenoid concentration in the plasma.

In a study previously discussed in the ADME section of this document (Section 8.4), Weststrate (1999) conducted an evaluation in 12 male and 12 female normolipidemic volunteers who consumed 40 g of the vegetable oil spread Take Control™. Phytosterols levels where comparable to the Cargill phytosterol ester product at 46% β-sitosterol, 26% campesterol, and 20% stigmasterol (w/w). Fecal concentrations of the mutagen 4-cholesten-3-one remained within the normal range indicating that vegetable oil phytosterols would not be expected to increase the risk of colon cancer; no sterol oxides were detected. The study concluded that increased vegetable oil phytosterol ester consumption increased the levels of neutral sterols in the feces but did not result in increased bile acid or sterol metabolite formation. No effects in clinical toxicological endpoints were noted.

Also discussed in the ADME discussion section (Section 8.4) was an identical investigation by Ayesh (1999). The test group showed an 18% reduction in total and a 23% reduction in LDL-cholesterol levels. Fecal lactic acid was also decreased, as was serum progesterone in female subjects, none of whom took oral contraceptives. When compared to baseline measurements, the test group showed a reduced fecal lactic acid concentration and acetic acid to total short-chain fatty acids ratio, as well as lactobacilli content. No other significant treatment related effects were noted in the test group. No effects on clinical toxicological endpoints were noted. It was concluded that the daily consumption of the phytosterol esters found in Take Control™ did not affect the bacterial profile or the metabolic activities of the gut microflora. This product was well tolerated by both male and female subjects, and daily consumption did not result in any biologically relevant effects on serum female sex hormone levels.

10.2 Literature Review of Other Clinical Studies Employing Phytosterols

Humans are continually exposed to phytosterols in the diet. The average dietary phytosterol intake has been estimated to be about 250 mg per day, while vegetarians may consume twice that amount. There is ample scientific literature on the effects of human exposure to elevated phytosterol intake dating back as far as the 1950's. Pollak and Kritchevsky (1981) published an extensive review of the scientific data available at the time and estimated that clinical data on the cholesterol-lowering action of phytosterols was available in approximately 1800 subjects at the time of their review. They concluded that alimentary use of β-sitosterol could be employed as part of a sensible regimen to reduce elevated plasma cholesterol levels to a mean normal range. Among other more recently conducted reviews of phytosterol safety are those written by Ling (1995), Jones (1997a, b), Kritchevsky (1997), Plat (1998), Jenkins (1999), and Moghadasian (1999). Other peer reviewed scientific analyses also confirm the safety of phytosterols for human consumption.

As discussed previously, Mattson *et al* (1982) conducted an assay designed to optimize the effect of plant sterols on cholesterol absorption in humans. Over three experimental sessions, with one-month intervals, 9 adults were fed a meal containing

500 mg cholesterol. In addition, the meal contained 1) no additive; 2) 1 g β-sitosterol; or 3) 2g β-sitosteryl oleate. Cholesterol absorption through stool samples over the following 5 days was analyzed. The addition of β-sitosterol resulted in a 42% decrease in cholesterol absorption whereas β-sitosteryl oleate caused only a 33% reduction. It was hypothesized that this is a reflection of the facility with which the micellar phase is formed and absorbed, possible as a consequence of differential rate of introduction into the intestinal lumen of bile acids, a necessary component of these micelles. However, the esters, although not as effective as the free sterols, had the advantage of ready incorporation into the diet.

In two previously mentioned studies conducted by Becker (1992, 1993), children with heterozygous familial hypercholesterolemia were treated over the course of 3 months with β-sitosterol pastils at 6 g / day. In the 1992 study, the cholesterol lowering drug benzafibrate was then administered separately and concomitantly with β-sitosterol. Combined treatment reduced the necessary dosage of each agent and was as effective as elevated doses of benzafibrate, resulting in an average 40 and 50% lowering of total and LDL cholesterol values, respectively. The combined regimen was then maintained for 24-months as was the lipid lowering effect. Clinical safety parameters and physical examination revealed no obvious side effects. In the 1993 evaluation, sitostanol was administered for 7 months following the 3-month β-sitosterol feeding regimen. No physical or clinical toxicological effects were observed in either case. However, sitostanol was determined to be more effective than β-sitosterol at reducing LDL cholesterol levels.

Vanhanen and Miettinen (1991) conducted an assay wherein 50 g per day of rapeseed oil spread was administered to 24 hypercholesterolemic patients for 6 weeks. Following this β-sitosterol or sitostanol was added to the rapeseed oil spread divided into 3 subgroups, a control, β-sitosterol and sitostanol. This continued for 9 weeks. While differences in the absorption between β-sitosterol and sitostanol where noted, as well as changes in the respective proportions of cholesterol and campesterol, no clinical or toxicological effects where noted.

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Miettinen and Vanhanen (1994) also conducted a 9-week study in which 31 hypercholesterolemic men and women were fed rapeseed oil spread in which an equivalent 1 g per day free sterol of β-sitosterol, sitostanol, or sitostanol esters had been dissolved. Results in lowering LDL cholesterol were observed and compared across different apo lipoprotein E phenotypes. In each phytosterol regimen, dietary plant sterols were observed to decrease serum total and LDL cholesterol by a proportional decrease in cholesterol absorption. This was in turn associated with a compensatory increase in cholesterol synthesis. The effects where deemed most consistent in those individuals with the E4 allele and insignificant with the apo E3/3 phenotype. No change in body weight or serum lipid profile were observed.

Pelletier (1995) also evaluated the effect of soy derived phytosterol over the course of a 9-week feeding study in which a phytosterol blend was mixed with butter and ingested by 12 normolipidemic subjects at 740 mg/day. Similar to the other studies evaluated, a significant lowering of the plasma total and LDL cholesterol was observed. No clinical or toxicological side effects were observed.

In studies conducted by Berges (1995) and Klippel (1997), the effect of β-sitosterol on benign prostatic hyperplasia (BHP) was evaluated. In the Berges study, 200 patients were administered 20 mg capsules of β-sitosterol three times per day for six months. In the Klippel study, 177 patients received 130 mg capsules of β-sitosterol twice per day for six months. In each case, while no clear mechanism of action could be determined, significant improvement in symptoms and urinary flow parameters showed the effectiveness of β-sitosterol in treating BPH. No adverse events were reported in either study.

It has been suggested that absorption of dietary phytosterols may be higher during infancy and childhood. In a study of the relationships between aortic tissue phytosterols and cholesterol on five adults, five infants, two neonates, and one 30-week abortus, Mellies et al. (1976a), observed mean tissue stigmasterol to be 9.2 ± 2.7 and β-sitosterol to be 8.9 ± 1.3 in 11 aortic sections from the five infants when compared to the mean levels (0.51 ± 0.18 and 1.8 ± 0.6) in 4 aortic sections from the abortus and two neonates. It was concluded that prior to birth, some phytosterols may cross the

placenta. In the first several months of life, vegetable-oil formula-fed infants may accrue phytosterols in their aortic tissues.

A study of 26 infants on various commercial formulas, 36 infants on breast or cow's milk formulas, 32 hypercholesterolemic children on a low-cholesterol diet, and 101 normal and 22 hypercholesterolemic children on a free diet revealed elevated total plasma phytosterol levels in infants on commercial formulas which are poor in animal fats and enriched with vegetable oils, and in hypercholesterolemic children on a low-cholesterol, phytosterol-rich diet (Mellies et al., 1976b). Mean plasma phytosterol levels of phytosterols (campesterol, stigmasterol, β-sitosterol), normally in the 2 mg/100 ml range, were increased three- to five-fold to 9 mg/100 ml.

10.3 Literature Review of Clinical Studies Employing Phytostanols

Although they are only a minor component of the phytosterol fraction in the Procter & Gamble phytosterol-enriched oil, it is worth noting that the safety of phytostanol esters has also been extensively evaluated, as illustrated by the GRAS status of Benecol™. Several clinical studies have been conducted using phytostanols, as reflected below in Table 11.

Table 11: Clinical Evaluations Reporting the Safety of Phytostanol Consumption

Reference	Disease State [1]	Number & Sex	Age Range or Mean (yr)	Phytosterol	Plant Source	Dosage Form [2]	Dosage (g/day)	Duration	Safety Endpoints	Adverse Events Reported
Heinemann *et al*, 1986	HC and FH	3 M 3 F	27-59	Sitostanol	No Data	Capsule	1.5	4 weeks	- Total cholesterol - Total phytosterol(s) - Triglycerides - HDL cholesterol - LDL cholesterol	None
Vanhanen *et al*, 1993	HC	47 M 20 F	25-60	Sitostanol Ester	No Data	Mayonnaise Suspension	3.4 (n=34)	6 weeks	- Total cholesterol - Total phytosterol(s) - Triglycerides - HDL cholesterol - LDL cholesterol - ApoE phenotype	None
Gylling *et al*, 1994	NIDDM with HC	11 M	57.8±1.9	Sitostanol Ester	Tall Oil	Margarine Suspension	3.0	6 weeks	- Total cholesterol - Total phytosterol(s) - Triglycerides - apolipoproteins A I, A II, & B	None
Vanhanen *et al*, 1994	HC	11 M 4 F	33-60 M 37-55 F	Sitostanol Ester	No Data	Mayonnaise Suspension	0 (n=8) 0.8 (n=7) followed by 2.0 (n=7)	15 weeks 9 weeks followed by 6 weeks	- Total cholesterol - Total phytosterol(s) - - HDL cholesterol - LDL cholesterol	None
Denke, 1995	HC	33 M	31-70	Sitostanol	Tall Oil	Capsules	3.0	90 days	- Total cholesterol - Triglycerides - HDL cholesterol - LDL cholesterol - VLDL cholesterol	None
Miettinen *et al*, 1995	HC	64 M 89 F	25-64	Sitostanol Ester	Tall Oil	Margarine Suspension	0 (n=51) 1.8 (n=51) 2.6 (n=51)	1 year	- Body weight - Total cholesterol - Triglycerides - HDL cholesterol - LDL cholesterol - Total phytosterol(s)	None

[1] M - male; F - female; HC – hypercholesterolemic; NIDDM - non-insulin-dependent diabetes mellitus; FH - familial hypercholesterolemia.

[2] Unless otherwise stated, pretreatment and (or) control diets were also consumed during treatment phases.

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10.4 Clinical Vitamin and Other Nutrient Uptake Levels

Weststrate (1998) and Hendriks (1999) evaluated sterol ester consumption and reported a decrease in the absorption of certain carotenoids, such as β-carotene and lycopene. Gylling (1996) studied phytostanol esters and reported a similar decrease in β-carotene levels. According to Shipley (1958), β-sitosterol had no effect on vitamin D absorption in rats and dogs. No other significant effects on the availability of fat-soluble vitamins have been noted for plant sterol or sterol esters.

Hendriks (1999) observed a decrease in plasma concentrations of α- and β-carotene of 12%, 11%, and 19% at three dose levels of phytosterol esters in a vegetable oil spread matrix. Lycopene plasma concentration was decreased by 11-15%, although no difference was observed among dose levels. Lycopene concentration per total lipid in plasma was not affected by the consumption of phytosterol esters. After consumption of esterified phytosterols, plasma concentrations of α-tocopherol were decreased in the mid- and high dose groups by 6% and 8%, respectively. The concentration of α-tocopherol per total lipid in plasma was unaffected. No effect on the plasma concentrations of fat-soluble vitamins K_1 and D was observed.

Hallikainen (2000) observed no significant changes in serum retinol, α- and β-carotene nor their ratios to serum total cholesterol concentrations over different dose levels of a phytostanol ester administered to a hypercholesterolemic test population. Serum lycopene concentrations differed significantly between men and women. There were no significant differences in the serum lycopene concentrations in the men throughout the study. However, in women, serum lycopene concentrations were significantly lower at the end of all experimental dose periods than at the end of the control period. In addition, serum α-tocopherol concentration was significantly lower at the end of the high dose 3.2 g period than at the end of the 0.8 g low dose period. Serum γ-tocopherol concentration was significantly lower only at the end of the 3.2 g and the 2.4 g dose periods than at the end of the control period. The changes in the α + γ-tocopherol concentration were parallel to the changes in the serum α-tocopherol concentration during the trial. However, after relating the serum α-, γ-, and α + γ-

tocopherol to the serum total cholesterol concentration, there were no significant differences among the different dose periods.

In a comparative analysis of the cholesterol lowering effects of esterified phytosterols in Take Control™ and esterified phytostanols in Benecol™, Weststrate (1998) observed a lowering of plasma lycopene, α-, and β-carotene levels in all individuals consuming the fortified margarines. Lipid standardization of the carotenoid concentrations reduced the impact of both Take Control™ and Benecol™, and lipid standardization of the Take Control™ phytosterol ester data showed the decrease in lycopene not to be statistically significant.

It is possible that esterified phytosterols may interfere with the uptake of fat-soluble vitamin and other nutrients in the intestine. This is primarily true for carotenoids. 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 for fat-soluble vitamins. The impact on human safety is not thought to be significant.

10.5 Phytosterolemia in Humans

Phytosterolemia, also known as sitosterolemia, is an extremely rare disease involving lipid storage. In a review of the literature, Berger (1998) could only identify 34 cases of individuals with this condition. It is characterized by elevated levels of plant sterols and 5α-saturated stanols in plasma and tissue. It is associated with xanthomatosis and premature atherosclerosis. In individuals with this condition, phytosterols account for an average of 13% of the total plasma sterols, compared to about 0.4% in non-phytosterolemic subjects. Salen (1985) measured the plasma sterol composition in 14 subjects with this condition and found plasma β-sitosterol and campesterol levels to be elevated to 35 ± 16 mg/dl and 16 ± 7 mg/dl, respectively. Normal to moderately high cholesterol levels of 258 ± 96 mg/dl were also noted.

Phytosterolemia is inherited as a recessive trait. Salen (1992) and Cobb (1997) determined that heterozygous individuals are biochemically and clinically normal.

However, the plasma phytosterol levels in some heterozygous individuals may be slightly elevated.

The pathophysiological basis for this disease involves abnormalities in both the absorption and excretion of phytosterols. In phytosterolemic persons, the β-sitosterol absorption rate is very high. In the intestine, the sterol uptake mechanism does not distinguish between phytosterols and cholesterol. Therefore relatively equal proportions of are absorbed. Shellfish sterols are also absorbed in excess. In addition, Bhattacharyya (1974) observed that less cholesterol is secreted into the bile. Bhattacharyya (1991) confirmed this by determining the ratio of β-sitosterol in the bile was very low compared to plasma, indicating a slow turn-over of β-sitosterol, low excretion of β-sitosterol into the duodenal bile and feces, and low cholesterol synthesis. Shefer (1994) determined that the large quantities of β-sitosterol and cholestanol in the liver of phytosterolemic subjects competitively inhibited cholesterol 7α-hydroxylase mediated bile acid synthesis.

In most cases, a rigorous low-sterol diet to curtail input, combined with bile acid resins to enhance sterol excretion, is the current treatment recommendation for this disorder. Cobb (1997) has identified at least one case wherein a very low β-sitosterol diet to curtail sterol input was of minimal therapeutic benefit. Regardless, in those individuals afflicted with sitosterolemia, consumption of any product containing phytosterol esters would be contraindicated.

11.0 DETERMINATION OF THE GRAS STATUS OF a PROCTER & GAMBLE PHYTOSTEROL-ENRICHED VEGETABLE OIL

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 Procter & Gamble, to determine the Generally Recognized as Safe (GRAS) status of a phytosterol-enriched vegetable oil product. The Expert Panel statement follows:

[redacted]

OPINION OF AN EXPERT PANEL ON THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF A PHYTOSTEROL ESTER ENRICHED VEGETABLE OIL MARKETED BY PROCTER & GAMBLE FOR HOME COOKING APPLICATIONS

An independent panel of internationally recognized experts (the Expert Panel), qualified by their scientific and/or medical training and relevant national and international experience to evaluate the safety of food and food ingredients, was requested by the Procter & Gamble Company to determine the Generally Recognized As Safe (GRAS) status of a phytosterol ester enriched vegetable oil for home cooking applications. The phytosterol ester ingredient has been affirmed as GRAS for uses that are similar to the phytosterol product Take Control™ by its manufacturer, Cargill Inc., and is the subject of a pending GRAS Notification to the U.S.F.D.A.

A comprehensive search of the scientific literature for safety and toxicity information through March 2000 was conducted and was made available to the Expert Panel. In addition, the members of the Expert Panel considered other information deemed appropriate or necessary. The information considered essential by the Expert Panel is summarized in the accompanying Report to the Panel. The members of the Expert Panel independently critically evaluated the available information on phytosterol esters, conferred by telephone several times, and then conferred via conference call with members of Cargill and Cantox U.S., Inc. The members of the Expert Panel then conferred privately to determine the safety and the GRAS status of the phytosterol ester product (Cargill phytosterol ester product) used to enrich vegetable oil to be marketed by Procter & Gamble for home cooking applications.

The Expert Panel critically evaluated the analytical chemical data provided and concluded the soy-based Cargill phytosterol ester product to be compositionally equivalent to the phytosterol esters present in a currently marketed and affirmed GRAS product, Take Control™. The Expert Panel further noted that the phytosterols found in this phytosterol ester product and in Take Control™ are similar to those naturally occurring in fruits and vegetables. Food-grade specifications for this phytosterol ester product were provided together with the analysis of five non-sequential batches. The data indicate that food-grade specifications can be consistently provided. All raw materials and processing aids used in the production of this phytosterol ester product are approved for use in food processing.

The Cargill phytosterol ester product is to be used by the Procter & Gamble Company to enrich vegetable oil for home cooking applications as an add-on source of dietary phytosterols. The Expert Panel therefore specifically considered aspects related to estimated consumer incremental phytosterol intake and phytosterol stability during typical home cooking conditions.

The estimated maximum incremental consumer intake of phytosterols from home cooking use of this phytosterol-enriched vegetable oil was comparable in amount to that from currently marketed and affirmed GRAS products such as Take Control™; combined maximum intake from both product sources was within the range covered by the current safety data base established at the time of the GRAS determination for Take Control™.

The thermal stability of the phytosterol-enriched vegetable oil was evaluated in studies that simulated home use (~290°F, 2 heatings, 600 g of potatoes per heating). The analytical data clearly indicate that no significant oxidized species were formed during the heating.

Most animal and human studies indicate that the absorption of phytosterols is about 5 % or less of dietary intake levels (cholesterol absorption is about 40 %). The concentration of phytosterols in serum is a function of dietary intake and may be increased through dietary supplementation. Phytosterols are not endogenously synthesized and are not converted to cholesterol and cholesterol is not converted to phytosterols. Phytosterols are esterified but at a slower rate than cholesterol in the rat. Phytosterols that are absorbed are eliminated through the biliary route. Anaerobic bacteria in the lower intestine may metabolize unabsorbed phytosterols.

There were no reported preclinical or clinical toxicity studies on the Cargill phytosterol ester product or on the Procter & Gamble vegetable oil product. However, since the Expert Panel concluded that Take Control™ and the Procter & Gamble vegetable oil product are compositionally equivalent with respect to phytosterols, the Expert Panel critically evaluated the clinical studies with Take Control™. The Expert Panel also critically evaluated the preclinical data on Cytellin™, a product marketed by Eli Lilly in the U.S. between 1954 and 1982. This product was a mixture of phytosterols derived from tall oil and was marketed as an anti-hypercholesterolemic agent. Other clinical studies were also evaluated. There were no consistent dose-dependent phytosterol-related significant adverse effects reported in either the preclinical or clinical studies.

Esterified phytosterols can variably interfere with the absorption of fat-soluble nutrients, particularly carotenoids. The literature bearing on the effects of phytosterol products on plasma levels of alpha- and beta- carotene, lycopene, alpha-tocopherol, and vitamins K1 and D was reviewed. As with other phytosterol products, it is anticipated that plasma carotenoids may be somewhat reduced. The significance of such an effect is not known. Recent studies show that a high intake of phytosterol esters increases the amounts of neutral steroids in the feces but is without increased formation of bile acids or sterol metabolites.

Based upon its independent and collective critical evaluation of the information summarized in the attached report, the Expert Panel concluded that the Procter & Gamble enriched vegetable oil product containing the Cargill phytosterol ester product, meeting appropriate food grade specifications and produced in accordance with current Good Manufacturing Practice is GRAS by scientific procedures for home use.

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END OF SUBMISSION

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Procter&Gamble

The Procter & Gamble Company
Winton Hill Technical Center
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July 26, 2000

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 a Phytosterol Ester-Enriched Vegetable Oil Product: GRAS Exemption Claim***

Dear Sir or Madame:

The Procter & Gamble Company (P&G) hereby provides a clarification to our original letter dated July 17, 2000 to the Food and Drug Administration (FDA) regarding Notification of GRAS Determination for a Phytosterol Ester-Enriched Vegetable Oil Product: GRAS Exemption Claim.

In the original letter, we noted that we would make available to the FDA the data and information that are the basis for The Procter & Gamble Company's GRAS determination at the law firm of Covington & Burling (Mr. Peter Barton Hutt, Esq). In addition, we intended our July 17 letter to agree to send this data and information to FDA upon request. We apologize for the omission.

Respectfully submitted,

2000 JUL 31 P 2:46

Dr. Maury Bandurraga
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