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Office of Nutritional Products, Labeling & Dietary Supplements
Food and Drug Administration
Center for Food Safety and Applied Nutrition
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Wheat germ-derived Ceramides

New Dietary Ingredient Notification
(under 21 C.F.R. sec. 190.6)

March 7, 2005

Submitted by:

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New Dietary Ingredient Notification:

For Wheat-Derived Ceramides

Introduction and Background

This New Dietary Ingredient (“NDI”) Notification is submitted pursuant to 21 C.F.R. § 190.6 and Section 8 of the Dietary Supplement Health and Education Act (“DSHEA”). This Notification concerns the new dietary ingredient wheat germ-derived Ceramides, a type of lipids which are constituents of sphingolipids, and for this Notification, derived from wheat, specifically from wheat germ oil (henceforth Ceramide or ceramides). The complete taxonomy for the botanical from which it is derived, wheat, is *Triticum aestivum* L. [*Poaceae*]. Currently, in its research and development for a dietary supplement containing this new dietary ingredient, Soft Gel Technologies, Inc. (“SGTI”) of Los Angeles, California is using Cennamides™ (a wheat germ-derived Ceramide, specifically, sourced from wheat germ oil). Cennamides™ is manufactured by Ennagram of Pantin, France, for SGTI, which intends to use this ingredient in a dietary supplement to promote and support healthy skin. Specifically, for its proposed new dietary supplement in soft gel form, SGTI intends to use these Ceramides derived from wheat germ oil, _____ SGTI has concluded that a new dietary supplement containing ceramides is reasonably expected to be safe, the required standard under DSHEA Section 8.

The safety evidence presented in this Notification will show that: 1a) Ceramides are naturally-occurring in many foods, including wheat flour, dairy, and vegetables; 2a) Ceramides are found in the human body, generated in the blood, the brain, spinal cord and nerve tissue; 2b) six forms of Ceramides are found in the skin, and these and the various forms of phyto-derived Ceramides are chemically identical or substantially equivalent; 3) an LD-50 test, detailed below, has been performed, using Cennamides™ (15% Ceramide in wheat germ oil) and concluding that the LD-50 is more than 5,000 mg per Kg.; 4) other animal studies also show no toxicity; 5) wheat germ oil and wheat germ, the sources of the NDI, have been sold and consumed in the U.S. before January 1, 1958 and thus are presumed safe; 6) human efficacy studies using orally-administered Ceramides have been done, including a recent study by SGTI, and they show no reported serious or continuous adverse events; 7) many studies increasingly show that Ceramides are necessary for human cellular processes and good health; and 8) nutritional supplements and functional foods containing Ceramide (derived from plant sources, both rice bran and wheat) are currently on the

market in Japan and have been for approximately 5 years. In this Notification, SGTI will present the scientific evidence and substantiation on which it bases its conclusion that a new dietary supplement containing wheat-derived Ceramides _____ is reasonably expected to be safe.

The Safety Standard to be Applied

SGTI believes that it is important to keep in mind, in both preparing and reviewing an NDI Notification, the precise standard of safety as set by Congress (in DSHEA) and by FDA (in the implementing regulation of 21 C.F.R. §190.6) for new dietary supplements, and how this differs from other safety standards for foods and for food ingredients and additives. Specifically, the standard to be met for the new dietary supplement containing the NDI is a “reasonable expectation of safety.” “Reasonable” is defined as “not conflicting with reason.”¹ Similarly, “to expect” is defined as “to consider reasonable” or to suppose or think that something will happen.² The legal definition of “reasonable” in Black’s Law Dictionary® is “Having the faculty of reason; rational; governed by reason; under the influence of reason; agreeable to reason.”³ Thus, a “reasonable expectation of safety” for a new proposed dietary supplement is a considerably lower standard than either the food additive standard or the GRAS standard.

To obtain FDA approval of a food additive, it must be shown to a reasonable certainty that no harm will result from the intended use of the additive. By contrast with “expect” above, “certainty” is defined as “the quality or state of being certain esp. on the basis of objective evidence,” with “certain” defined as “proved to be true,”⁴ and thus reasonable certainty is a far higher level of safety than a reasonable expectation. As you know, since GRAS substances are not considered food additives, GRAS substances are exempt from the premarket approval requirements for food additives. A substance may be determined to be GRAS if: (1) there is general recognition among experts that the particular substance is safe; (2) the experts are qualified by scientific training and experience; and (3) the experts based their safety judgment either on scientific procedures or the fact that

¹ Webster’s Ninth New Collegiate Dictionary, published in 1991, within a few years of when Congress was discussing and debating the bills that would become DSHEA (enacted in 1994), at p. 981. (“Webster’s”). Thus, in the early 1990’s the definitions and plain meaning of the relevant terms were as above.

² Webster’s at p. 436.

³ Black’s Law Dictionary®, 6th ed. West Publishing Co., 1990, at p. 1265.

⁴ Webster’s at pp. 222-223.

the substance was commonly used in food prior to January 1, 1958 (the date of the Food Additives Amendment). Thus, the GRAS standard requires both technical evidence of safety (e.g., from toxicology reports), and a general recognition of that safety by experts in the field. Thus, GRAS too is a higher standard for safety than what is required by law for new dietary supplements.

It is also significant to note that over the past few years there has been considerable confusion on the part of the industry and inquiry by the Agency itself as to the precise meaning of this standard and how it is to be consistently applied. That apparently was one of the factors prompting the Agency to issue a call for Comments on October 20, 2004 (in 69 Fed. Reg. 61680). One of the many questions posed by the FDA for comment was: What level of substantiation and scientific support is needed to meet this standard. As shown above, SGTI believes that the plain meaning of the words “reasonable expectation of safety” for the new supplement should be employed, and that this standard for NDIs be consciously and continuously distinguished from the GRAS standard during an evaluation of a company’s safety evidence.

Ceramides Found in and Derived from Foods, Plants

The subject of this Notification, Ceramide, is derived from wheat germ oil, and both wheat germ and wheat germ oil have been on the market in the U.S. for over 40 years. Ceramides are found naturally in many foods, especially wheat flour.⁵ (Copies of all articles and reports cited or referenced in this Notification are attached.) The amounts of Ceramides in food vary considerably, but considering all the food sources, the per capita Ceramide consumption in the United States is estimated to be 0.3 to 0.4 grams per day.^{6,7,8} Sphingolipids, which contain Ceramides, as shown below, are found in large amounts in dairy products, eggs and soybeans,⁹ with up to 1 to 2 g/ kg.¹⁰

⁵ Schmelz, E.V., Uptake and Metabolism of Sphingolipids in Isolated Intestinal Loops of Mice, J. of Nutrition, Vol. 124(5), p. 702, 1994 (“Schmelz”).

⁶ Merrill, A.H., Jr. *et al.* Sphingolipid Uptake by Isolated Segments of the Rat Intestine, FASEB Journal, P. 3A, 469, 1989 (“Merrill 1989”).

⁷ Futerman, A. H., Ceramide Metabolism Compartmentalized in the Endoplasmic Reticulum and Golgi apparatus, Chapter 4, Current Topics in Membranes, Vol. 40, p. 93, 1994 (“Futerman”).

⁸ Lati, E., Special Topic: New Research and Development in Moisture Retention Mechanism and Moisturizing Agents: Phyto-ceramides and their Applications, Fragrance Journal, Vol. 23 (1), No. 81, pp. 1-14, at p.1, 1995 (“Lati”).

⁹ Vesper, H. *et al.* Sphingolipids in Food and the Emerging Importance of Sphingolipids to Nutrition, J. of Nutrition, Vol. 129: pp. 1239-1250, 1999 (“Vesper”); Berra, B. *et al.*, Dietary Sphingolipids in Colorectal Cancer Prevention, Eur. J. of Cancer Prev., Vol. 11, pp. 193-197, at p. 193, 2002.

¹⁰ Pfeuffer and Schrezenmeir (2003).

The structure of ceramides in soybean and wheat has been determined by mass spectrometry.¹¹ Ceramides are also found in rice, millet, and spinach.¹² By one estimate, sphingolipids account for .01 to .02% of the human diet.¹³ Wheat germ oil has been on the market in the U.S. and consumed by Americans for over 55 years; and wheat germ oil generally contains 6% glycolipids, which contains ceramides.¹⁴

Currently there are many types of Ceramides on the market (e.g., in Europe and Japan) and virtually all of them are commercially derived from plant sources. Originally Ceramides were derived from soybeans and bovine sources. However, unlike bovine-derived animal Ceramides, which can carry the problematic risk of viral infection or of conditions such as mad cow disease, plant-based Ceramides are preferable from a safety and low risk standpoint.¹⁵ Now, fermentation processes from yeast and glycerol are available. Two popular sources are brown rice and wheat germ extracts. These are the two common sources of commercially available Ceramide for dietary supplement and cosmetic applications.¹⁶

Specifically, the subject of this Notification is Ceramide derived from wheat germ oil. Ceramides as developed and consumed in other countries are derived from other wheat sources, from rice, and from Konjac (see Japanese brochures and websites, attached). But regardless of derivation, these phyto-based Ceramides are chemically very similar and are substantially equivalent for purposes of safety analysis.¹⁷ The complex plant sphingolipids that make up Ceramide have glucose, galactose, and inositol backbones. Wheat-derived ceramides are structurally similar to rice derived ceramides because they share common sugar components and only differ in the placement of the fatty acid component along the chain. This makes these two forms of plant ceramides

¹¹ Sullards et al. (2000).

¹² Lati at p. 2.

¹³ Hoang, T., Sphingolipids (a Powerpoint presentation), March 28, 2002, p. 12; also citing to Vesper. From the Internet.

¹⁴ YBS Corporation, Wheat Germ Oil, Containing Vegetable Ceramides, including "Characteristics of 'Wheat Germ Oil-S'" (at page 3 of 4). Available on the Internet at www.ybsweb.co.jp/exhf5.html.

¹⁵ Lati at p. 1-2.

¹⁶ Schmelz, Merrill 1989, Futerman, and Lati.

¹⁷ See, e.g., Lati.

interchangeable as functional components of a specific sphingoloid (ceramide) class.¹⁸ Thus, we have included certain evidence and indications of safety of rice-derived ceramides in this Notification, as further indicative of a reasonable expectation of safety of a new dietary supplement containing wheat germ-derived Ceramides.

Proposed Product and Recommended Conditions of Use

The proposed dietary supplement product containing Ceramide Oil will be in the form of a gel cap, and will also contain the following dietary ingredients: _____

The full list of the ingredients present in the proposed new dietary supplement is provided in the Report on the clinical trial performed for SGTI by RTL, Inc.¹⁹

There are no special requirements for use in dietary supplements containing Ceramides, such as the need to take it on an empty stomach. No special conditions for use of the proposed product are indicated by the chemical or safety profile of Ceramides. As shown herein, Ceramides are naturally-occurring in many foods, and have been safely consumed as a constituent of wheat from oil for decades. On the label of the proposed dietary supplement containing wheat-derived ceramides, in the listing of the dietary ingredient in the Supplement Facts box, the plant source of the ceramides will be declared, as Ceramides (from wheat germ oil). In the case of the use of wheat-derived ceramides, we note for your consideration that the source is wheat germ oil (rather than wheat flour or wheat gluten) which SGTI believes poses much less (if any) of a potential allergen problem. Nonetheless, even though the proposed new product will contain no wheat or wheat flour or wheat gluten or any derivatives of them, SGTI's proposed label for the new dietary supplement will contain the following cautionary language, printed in bold and prominent type: "WARNING: This product contains ceramides which are derived from wheat germ oil. Do not take this product if you are allergic to wheat in any form."

¹⁸ Vesper.

¹⁹ _____

Again, sphingolipids account for .01 to .02% of the human diet;²⁰ and Ceramides account for .3 to .4 mg per day in the human diet. The ingested amount of Ceramides in the proposed supplement is _____ We note that one of the many respected brands of wheat germ oil that has been on the market in the U.S. for many years, Solgar Wheat Germ Oil in soft gels contains 1140 mg of wheat germ oil. Given that several sphingolipids make up 6% of wheat germ oil, we calculate that this serving size of wheat germ oil, 1140 mg, would contain 68.4 mg of ceramides. Ceramides are neither hormones nor stimulants. Furthermore, there are no scientifically-determined limits on the duration of use of a ceramide-containing dietary supplement. Ceramides can be taken as a long-term supplementation regimen to keep the skin moist and healthy. And, indeed, ceramide supplements and functional foods have been on the market in Japan for over 5 years. Nonetheless, SGTI will place the following Caution on the label of the dietary supplement containing Ceramides: _____

Chemical Identification and Commercial Manufacturing Process

A general description of the new dietary ingredient can be found in the ingredient supplier's materials, specifically the Ennagram booklet entitled CENNAMIDES, in Sec. A – Structure and Formulas (original booklet attached).²¹ A complete and minutely detailed chemical description of Cennamide CERO15, the precise ceramide to be used in the new dietary supplement, as prepared by SGTI's supplier, Ennagram, is attached. This is entitled "Mass Spectrometry Detection and Structural Determination of the Ceramides and Digalactosyl Diglyceride Present in a Lipid Mixture of Plant Origin." The sub-title is "Proposal for an Assay Method for Total Ceramides and Assay of Digalactosyl Diglyceride."²²

The manufacturing process is as follows:²³ _____

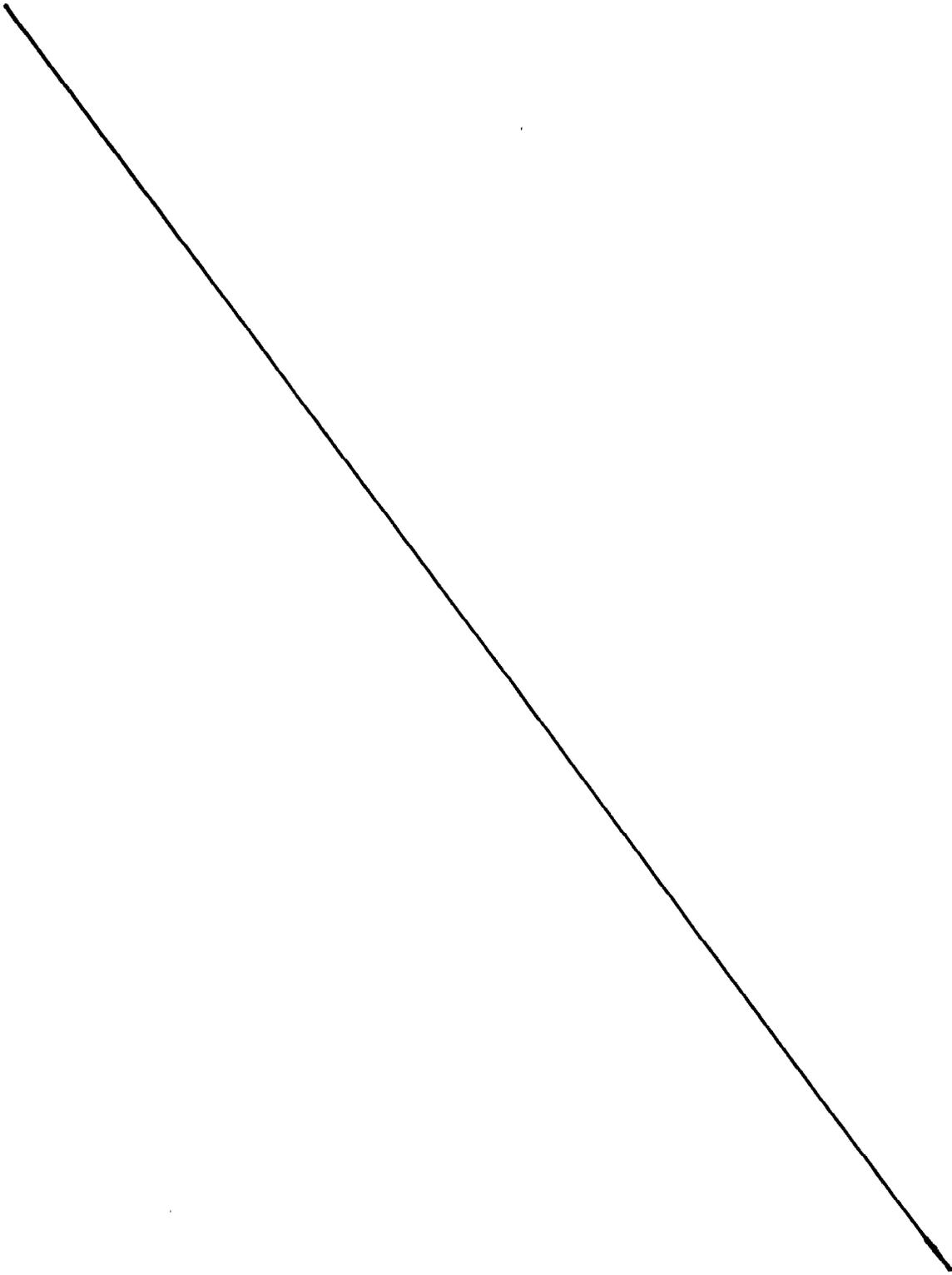
Here we will describe the production of _____

²⁰ Hoang.

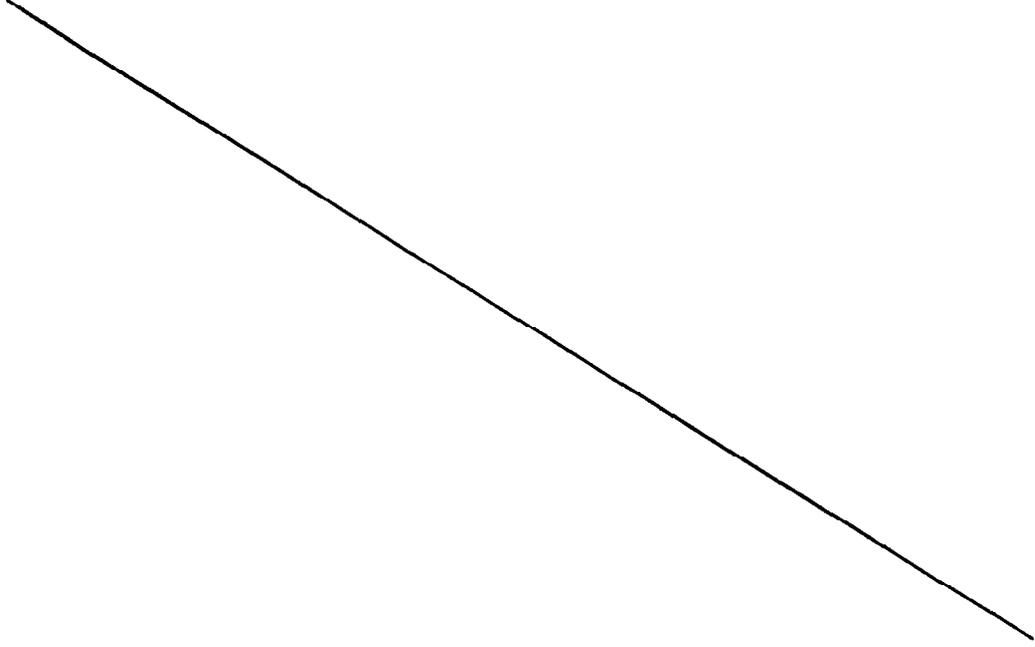
²¹ Ennagram, CENNAMIDES: ENNAGRAM'S VEGETAL CERAMIDES [no date]; original attached.

²² Ennagram, "Mass Spectrometry Detection and Structural Determination of the Ceramides and Digalactosyl Diglyceride Present in a Lipid Mixture of Plant Origin." For Cennamide CERO15 (Sept. 24, 2004).

²³ A short step-by-step summary of the process can be found in Ennagram, CENNAMIDES: ENNAGRAM'S VEGETAL CERAMIDES [no date] at Section B; original attached.



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History on the Discovery and Safe Use of Ceramides

As far back as 1853, scientific papers were published explaining the role of internal lipids (fats) in the skin.²⁸ Nearly twenty years later, classes of lipids named Sphingolipids were discovered. Specifically, the presence of ceramides as a category within Sphingolipids was “first detected in the animal (human) brain by the German physician L.T. W. Thudichen in 1884, and subsequently these substances were extracted from sources such as the bovine brain for use in cosmetics, etc.”²⁹ Over a century later, much of the biochemistry of lipids in the skin has received significant attention and is much better understood. As discussed below, Ceramides are a special class of sphingolipids and are found in the stratum corneum, the outermost skin layer. They account

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²⁸ Vesper, Oryza Brochure.

²⁹ Kajimoto, O. *et al.*, Clinical Investigation of the Skin Beautifying Effects of a Beauty Supplement Containing Rice Derived Ceramides-Objective Assessment of Dry Skin through Analysis of Three Dimensional Microscopy Images. Authors are affiliated with the Health Management Center, Osaka University of Foreign Studies; Dermatology Department, Faculty of Medicine, Osaka City University; and the Institute of Comprehensive Medical Science (undated publication) at p. 2 (“Kajimoto”).

for about 40-65% of lipids in the stratum corneum. There are six kinds of Ceramides found in the skin, all serving different purposes. Ceramides are not very soluble, thus making them difficult to manufacture. They work when absorbed into the stratum corneum, and that mechanism necessitates special processing procedures to derive a viable product for human ingestion and topical use.³⁰

Much has been written about "anti-aging" creams and lotions, and more topicals have been sold in recent years based on the current marketing of beauty creams, which promise youthful skin. Aggressive marketing and promising cosmetic formulas have characterized much of this sales boom. The term "anti-aging" is important in establishing the market for Ceramide products. In the early 1990's, major cosmetic companies such as Elizabeth Arden formulated and branded products based on the safe and effective use of Ceramides for treating aging problems such as fine lines, wrinkles, and dryness. These were often in the form of expensive topical creams and potions. Because of its moisture retaining properties, Ceramides were eventually included in cosmetic products such as foundations and lipsticks to increase outer moisture and to provide a benefit to the skin while protecting it from damaging elements.³¹ For the same purpose of healthier skin as one ages, but via a different route—ingested--SGTI is developing Ceramide-containing dietary supplements.

Ceramides, Sphingolipids and Glycosphingolipids

Ceramides are a type of sphingolipids, which are lipid substances present in all cell structures. One researcher³² describes the relation between sphingolipids and ceramides in this way:

Sphingolipids are present in every human cell as a constituent of the biological cell membrane. They are also present in most foods, especially dairy products. The backbone of this group of phospholipids is a long chain sphingoid base, called

³⁰ Imokawa, G., Structures and Functions of Stratum Corneum Lipids in the Skin, Journal Japan Oil Chem. Society, Vol. 44, No. 10, pp. 1-23, esp. pp. 1, 6 (1995) ("Imokawa"); Lee, M., Analysis of Ceramides in Cosmetics by Revised-Phase Liquid Chromatography/ Electrospray Ionization Mass Spectrometry with Collision-Induced Dissociation, Rapid Commun. Mass Spectrom, 17:64-75 (2003) ("Lee"); Goldstein, A., Ceramides in the Stratum Corneum: Structure, Function, and New Methods to Promote Repair, Int. J. of Derma., Vol. 42, p. 256-259, 2003 ("Goldstein"); and Chamlin, S.L., *et al.* Ceramide-Dominant Barrier Repair Lipids Alleviate Childhood Atopic Dermatitis: Changes in Barrier Function Provide a Sensitive Indicator of Disease Activity, Journal of Am. Acad. Dermatol, Volume 47, No. 2, PR 198-208, 2002 ("Chamlin").

³¹ See, e.g., Zettersten, E., Optimal Ratios of Topical Stratum Corneum Lipids Improve Barrier Recovery in Chronologically Aged Skin, Journal of American Academy of Dermatology, Volume 37, Number 3, Part 1, 1997.

³² Possemiers, Sam. Research: 1. Microbial metabolism of dietary sphingolipids and its implications for human health, p. 1 of 4. [no date] Report of Lab MET, the Laboratory of Microbial Ecology and Technology. Ghent University, Belgium. Available via Internet.

sphingosine. The free amino group of this compound is usually substituted with a long-chain fatty acid to produce ceramide.

Ceramides are constituents of cells in the cytoplasmic membranes. Thus, they are naturally found in the skin, central nervous system, and spinal marrow.³³ As shown above, Ceramides are also present in the plant world, with the main sources being wheat, rice, soy, and spinach. These compounds are the result of the formation of an amide bond between a fatty acid and sphingosine, or phytosphingosine.

Glycosphingolipids (GSLs) are a varied and diverse class of molecules which consist of a sugar attached to Ceramide moieties. In other words: GSLs are “compounds consisting of oligosaccharides and ceramides, [which] are characteristic components of the plasma membranes of vertebrates. The ceramide portion of the GSL molecule is embedded in the fluid phase of the plasma membrane, and a sugar chain faces the external environment.”³⁴ More than 400 species of ceramides are known, though only seven monosaccharides are found in vertebrates. Distributed mainly at the surface of the cell, they participate in the regulation of the interactions of cells with their environment.³⁵ These lipids serve as distinguishing markers for cells and mediate cell-to-cell recognition and communication. They are essential for the development and growth of organisms, and their decrease has been implicated in skin disease,³⁶ and in a number of serious diseases such as cancer and viral infections.^{37,38,39}

The separation of GSLs into groups and subgroups is made according to the different kinds of carbohydrates, acyl, or sphingosine structures which can attach to form a variety of compounds. GSLs are commonly divided into two main groups, Neutral GSLs and Acidic GSLs. Acidic GSL compounds reflect the type of structures which form the basis of the Ceramide compounds which are the subject of this NDI Notification.

³³ Lati at p. 1.

³⁴ Horibata, Yasuhiro *et al.*, Unique Catabolic Pathway of Glycosphingolipids in a Hydrozoan, Hydra magnipapillata, Involving Endoglycoceramidase, J. Biol. Chem., Volume 279, Issue 32, pp. 33379-33389 (August 6, 2004). Introduction.

³⁵ Goldstein.

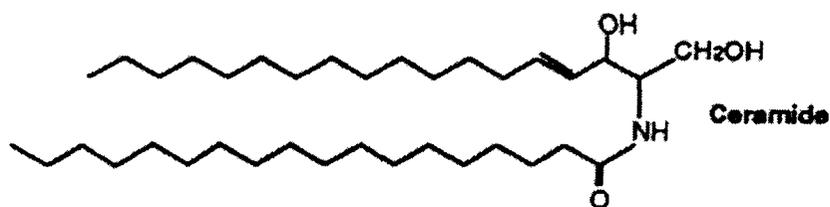
³⁶ Kajimoto at p. 1.

³⁷ Goldstein, p. 256.

³⁸ Yamamura, T. and Tekezuka, T., Change in sphingomyelinase activity in human epidermis during aging, Journal of Dermatological Science, 1(2): p. 79-84, 1990 (“Yamamura”).

³⁹ Merrill, A.H., Jr. *et al.*, Importance of Sphingolipids and Inhibitors of Sphingolipid Metabolism as Components of Animal Diets, Journal of Nutrition, Vol. 127, pp. 830S- 833S, 1997 (“Merrill 1997”).

Ceramides are the simplest sphingolipids and situated at the center of sphingolipid metabolism. Thus, the transfer of a phosphorylcholine head group from phosphatidylcholine to Ceramide yields another phospholipid, sphingomyelin (also sphingolipid); and the addition of carbohydrate groups from the sugar donor, UDP-hexose, yields complex Glycosphingolipids (cerebrosides, sulfatides, gangliosides). These compounds can be converted back to Ceramide by the removal of sugars (glycosidases) or phosphorylcholine by sphingomyelinases. An enzyme (ceramidase) is able to cleave the amide-linked fatty acid of ceramide and free sphingosine.



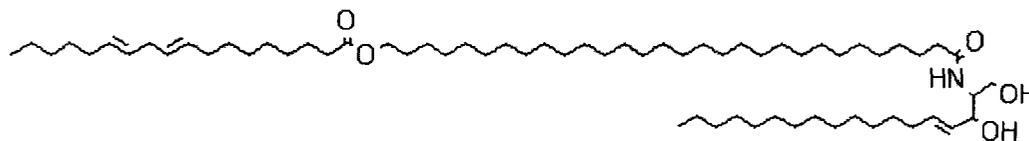
Free ceramides are separated with the neutral lipid fraction on a silica column; and the minute amounts present in cell extracts are best analyzed after derivatization before HPLC. Their chromatographic behavior is not very different from that of diacylglycerols which are also structurally similar. The amide linkage is resistant to hydrolysis but is disrupted by prolonged heating in an alkaline medium (although better with an acidic reagent). Ceramide can be prepared conveniently from complex Glycosphingolipids by chemical degradation.⁴⁰

The stratum corneum of the skin (the upper-most layer made of dead cells) has a unique lipid composition which comprises mostly free fatty acids, cholesterol and ceramides (O-acylceramides). These are different molecular species, differing by the head group architecture and by the mean fatty acid chain length. The fatty acid esterified to the amide of the (phyto)sphingosine head group can be either α -hydroxy or nonhydroxy fatty acids. The fatty acid chain length varies from 16 to 34 carbon atoms.⁴¹ One Ceramide (figure below) contains linoleic acid linked to the long chain (n=30) ω -hydroxy fatty acid. This molecule is thought to be of importance for proper skin barrier function.⁴²

⁴⁰ Orgretmen, B. *et al.*, Biochemical mechanisms of the generation of endogenous long chain ceramide to exogenous short chain ceramide in A549 human lung adenocarcinoma cell line, J. of Bio. Chem., Vol. 227, No. 15, 2002 (“Orgretmen”).

⁴¹ Bouwstra, J.A. *et al.*, Role of ceramide 1 in the molecular organization of the stratum corneum lipids. J. Lipid Res., 39(1), pp. 186-96, Jan. 1998 (Abstract from PubMed).

⁴² Brochure entitled Oryza Ceramide, Nutritional Supplement, from Oryza Oil & Fat Chemical Co. Ltd, called in the footer: ORYZA CERAMIDE CATALOG ver. 1.0EZ (“Oryza brochure”).



Orgretmen explains how endogenous ceramide found in skin mediates the action of exogenous ceramide. This action is thought to work via a recycling process where the sphingosine backbone of Ceramide is thought to be recycled. This provides the link which allows phyto-derived ceramide compounds to be used to replace degraded ceramide which is depleted in the stratum corneum.⁴³ As stated above, Ceramides are commercially extracted from rice bran or wheat germ. These plant sources naturally contain a large amount of glycosphingolipids. The glycosphingolipids of rice bran are similar to the animal glycosphingolipids, in which the backbone of the Ceramide including sphingoid bases with fatty acids is an amide linkage, and the terminal hydroxyl group is substituted by glucose. Glycosphingolipids have a great variety of molecular species because of the partial structure of sphingoid bases.⁴⁴

Ceramide Functionality and Efficacy in the Skin

Ceramides are also natural components of human skin; in fact, Ceramide is the predominant lipid in the skin.⁴⁵ There are six Ceramide species or structures commonly found in skin.⁴⁶ These Ceramides are formed via a biosynthetic mechanism in the epidermis. The four layers of the epidermis contain Ceramides, and they play an important role by creating a barrier which reduces infection and helps to retain the skin's moisture.⁴⁷ Reduction in the amount of Ceramides in the epidermal layers results in dry skin conditions, dermatitis, and is believed to be a major cause of wrinkles.⁴⁸ Studies have shown that a proper amount of Ceramides in the internal epidermal layer is necessary to maintain healthy skin.^{49,50,51}

⁴³ Orgretmen.

⁴⁴ Schmelz, Merrill 1989, Futerman, and Lati.

⁴⁵ Imokawa.

⁴⁶ Imokawa at p. 3.

⁴⁷ Imokawa at pp. 6-9.

⁴⁸ See, e.g., Lati at pp. 5-6.

⁴⁹ Futerman and Lati; Zetterstern, E., Optimal Ratios of Topical Stratum Corneum Lipids Improve Barrier Recovery in Chronologically Aged Skin, *Journal of American Academy of Dermatology*, Volume 37, Number 3, Part 1, 1997 ("Zetterstern").

⁵⁰ Chamlin.

⁵¹ Yamamura.

The stratum corneum is the outermost layer of mammalian skin. This layer primarily acts as a barrier to protect us from external environmental stresses and to prevent excessive transcutaneous water loss. The cells of the stratum corneum, corneocytes, and the lipids between them, Ceramides, accomplish this important function. Disruptions of this barrier, through either physical trauma caused by dermatitic conditions like eczema or by simple aging, results in this important function being compromised. The result is decreased elasticity, increased susceptibility to infection, and increased water loss which can lead to aging conditions such as wrinkles and fine lines, as well as an overall dullness to the skin's texture.^{52,53}

Plants contain structures which are chemically similar to human Ceramides. These plant-derived, or phyto-derived, Ceramide products can aid in creating the protective barrier in the epidermis. Supplementation with an oral agent of Ceramide replaces the components lost through aging, and has hydration effects.⁵⁴ The moisturizing effect comes from the Ceramides being carried directly to the stratum corneum via the blood. This direct method improves the functionality of the Ceramides and produces results not seen in cosmetic topical applications.⁵⁵

Ceramides have become an important compound for skin protection. Lipid depletion and replenishment studies have shown that Ceramides play an essential role in establishment and maintenance of the water-retaining properties of skin. Since it is known that Ceramides decrease with age, it has been suggested that increased transepidermal water loss is the result of their reduced presence in the skin. In short, Ceramides play an important role in preventing moisture loss which can be caused by physical trauma or aging.⁵⁶

It is also known that Ceramides inhibit Melanogenesis and are thought to promote a pigment lightening effect. Melanogenesis is a process by which the skin produces pigments that give our skin its unique tone and color. This is particularly helpful for hyper-pigmentation which causes age spots and other discolorations of the skin. In an *in vitro* study using B-16 melanoma cells, the

⁵² Imokawa, Goldstein, and Yamamura.

⁵³ Lee.

⁵⁴ Lati at p. 6.

⁵⁵ Imokawa, Lee, Goldstein, and Yamamura.

⁵⁶ Imokawa, Lee, Goldstein, and Yamamura.

melanogenic effect of Ceramides was observed. Ceramides exhibited a stronger whitening effect when compared to ascorbic acid, arbutin, and ellagic acids.^{57, 58}

The moisturizing effect of Ceramide was established in several clinical studies. These studies show that Ceramide is absorbed via the digestive system and carried to the stratum corneum by blood, where it circulates, and produces a significant effect in promoting a water barrier in the layers of the skin. Therefore, Ceramides in the stratum corneum of the skin play important roles for maintaining a barrier function and protecting the skin from harm.⁵⁹

Wheat Germ and Wheat Germ Oil, both Containing Ceramide Sold in U.S. Pre-1958

The subject of this NDI Notification, Ceramide, is derived from wheat germ oil, which in turn is derived from wheat germ. Both of those ingredients were on the market in the U.S. as foods or as “nutritional supplements” before January 1, 1958. Thus, under the Food Additive Amendment, the source materials for this NDI are not only safe, but indeed GRAS. Currently, the major brand of wheat germ is sold by Kretschmer, and the serving size is 2 Tbs., or 13 g. (Product label attached under Kretschmer.) Kretschmer Wheat Germ, manufactured by The Quaker Oats Company, is the oldest and most widely available wheat germ (Quaker Oats acquired Kretschmer in 1986.). There is a useful report entitled: "Search for the Competitive Edge: A History of Dietary Fads and Supplements" The Journal of Nutrition Vol. 127 No. 5, May 1997, pp. 869S-873S. The above mentioned report references studies on Wheat Germ and its uses before 1958. Use of wheat germ oil, starting decades ago, is documented in this article: "Other items used by athletes may be called ergogenic aids, among them gelatin, ginseng, sodium bicarbonate (baking soda) and wheat germ oil. Research on these products dates to the 1930s, 1940s and 1950s (Barron and Vanscoy 1993, Burke and Read 1993, Cureton 1954, Cureton and Pohndorf 1955, Grunwald and Bailey 1993, Williams 1989b and 1994)." (Emphasis added.)⁶⁰

Two pre-1958 scientific studies specifically discuss wheat germ oil being used as a dietary supplement in this same time period of the 1940s and the 1950s. These are: Cureton, T. K. (1954)

⁵⁷ Schmelz, Merrill 1989, Futerman, and Lati.

⁵⁸ Motta, S. *et al.*, Ceramide Compositions of the Psoratic Scale, *Biochimica et Biophysica Acta*, 1182: pp. 147-151 (1993).

⁵⁹ Imokawa, Lee, Goldstein, Lati and Chamlin.

⁶⁰ Applegate, Elizabeth and Grivetti, Louis, Search for the Competitive Edge: A History of Dietary Fades and Supplements, *The Journal of Nutrition*, 77:869S-873S (1997) (emphasis added). Available at <http://www.nutrition.org/cgi/content/full/127/5/869S>.

Effect of wheat germ oil and vitamin E on normal human subjects in physical training programs. Am. J. Physiol. 179: 628 (abs.); and Cureton T. K., Pohndorf R. H. Influence of wheat germ oil as a dietary supplement in a program of conditioning exercises with middle-aged subjects. Res. Quarterly 1955; 26:391-407. These articles from 1954 and 1955 are not readily available, and thus are not attached, but the fact that wheat germ oil and wheat germ were being researched and were the subjects of articles in nutrition journals published in the mid-1950s demonstrates that they were on the market in the U.S. and consumed before January 1, 1958. Finally, the fact that SGTI's wheat-derived Ceramide is sourced from ingredients or foods that were sold and consumed in the U.S. before 1958, and thus are "grandfathered" as GRAS, and at amounts much larger _____ is a strong indication that the proposed new supplement is reasonably expected to be safe.

Ceramide Toxicity and Safety Data and Animal Studies

There have been numerous toxicology and animal studies of ingested Ceramide. The available toxicology and safety profile of this product offer ample assurances of the safety of Ceramide in clinical, ingested use. This conclusion can be drawn from the work of several researchers who examined both acute and chronic toxicity in animals.

Acute Toxicity

An LD 50 test was performed by SGTI's supplier, Ennagram, dated July, 1999 (copy attached). After administering 5000 mg of Cennamides™ to rats for 18-24 months, no toxic effects were observed. Cennamides™ is the identical ingredient that will be used in SGTI's new dietary supplement. Thus, the LD50 (for mice) is more than 5000 mg/kg.⁶¹ Further, Ennagram's Cennamides™ publication also reports that Ennagram's 3% vegetal ceramide product (batch 120991), fed by oral route to rats, did not cause any mortality. Similarly, when Oryza administered 5,000 mg of Ceramides (in this case rice-based) to mice for 18-24 months, "no toxic effects were observed, thus the LD50 (mouse) is more than 5000 mg/kg."⁶² Again, by contrast, the amount of Ceramides in SGTI's proposed dietary supplement _____

In addition to the LD 50, other animal tests were conducted on the ingredient, for example, an evaluation of acute toxicity in rats by oral route. In the retained experimental conditions, a 3%

⁶¹ Ennagram's Vegetal Ceramides: LD-50 Study, dated July 1999; and Ennagram Report dated November 1997.

⁶² Oryza Brochure at p. 8.

vegetal Ceramide product (derived from wheat) did not cause mortality. “The autopsies performed on the animals at the end of testing did not show any macroscopic lesion which could be related to any toxic effect of the product.”⁶³ This wheat Ceramide safety brochure also contained toxicity data, and the results were very similar to the rice-derived Ceramide studies because of the similarity between the components.

In a 1996 report, Richeux discusses administering CENNAMIDE CERO 15, a product name for a vegetal ceramide produced by Ennagram, to a group of 6 Sprague Dawley rats (3 males and 3 females) at a single dose of 5,000 mg/kg body weight per day for a week. This study was performed according to the experimental protocol established on the basis of the official method as defined in the O.E.C.D. guideline No. 423 dated March 22nd, 1996 and the test method B.1ter of the Directive No. 96/54/EC dated July 30th, 1996. The animals were administered the product by force-feeding under a volume of 5 ml/kg body weight using a suitable syringe (graduated), fitted with an oesophageal metal canula. No mortality occurred during the study. The conclusion was: LD₅₀ of the product (REF.03.749 CENNAMIDE CERO 15) is higher than 5 g/mg by oral route in the rat. ⁶⁴

In 1989 Merrill reported the digestion and absorption of sphingomyelin, ceramide, and sphingosine using an *in vivo* intestinal loop technique with rats. This abstract reports that essentially no sphingomyelin or ceramide was hydrolyzed or lost from the small intestinal segments; however, digestion and uptake from the colon was evident. In addition, later work by Merrill, in 1997, confirmed and refined these results.⁶⁵

Schmelz, in 1994, reported that radio-labeled sphingolipids were placed in isolated intestinal segments of female mice, and the metabolism and distribution of the radiolabel were followed.⁶⁶ The aim of the study was to determine if sphingomyelin can be digested and taken up by different regions of the intestine, with particular interest in determining if ceramide and sphingosine are formed. The study documents that a substantial portion of the sphingomyelin was cleaved to

⁶³ Kenko Corporation In-house Report.

⁶⁴ Richeux, F., BioHC Appendix 2, Report to the Investigator TA0423-PH-03/0307 (1996); Appendix 2 (1997). [See cover letter of Report.]

⁶⁵ Merrill, A.H., Jr. *et al.*, Sphingolipid Uptake by Isolated Segments of the Rat Intestine, FASEB Journal, P. 3A, 469, 1989. Merrill, A., Importance of Sphingolipids and Inhibitors of Sphingolipid Metabolism as Components of Animal Diets, Journal of Nutrition, Vol. 127, pp. 830S- 833S, 1997 (“Merrill 1997”).

⁶⁶ Schmelz.

ceramide and sphingosine. Similar to information presented at the beginning of this Notification, this study reports that sphingomyelin is present in substantial amounts in milk (123 $\mu\text{mol/L}$), salmon (160 nmol/g), pork and beef tissue (350-390 nmol/g), and chicken (530 nmol/g). The study reports that there was substantial metabolism of sphingomyelin throughout the intestine. Ceramide was the primary breakdown product of sphingomyelin found in almost all regions of the intestine, accounting for up to 9.5% of the administered dose. No safety data is reported in the study.

Most recently, in 2002, Berra reports that sphingomyelin is hydrolyzed by a sphingomyelinase in response to extracellular stimuli, generating the lipid moiety ceramide and the water-soluble moiety choline-phosphate. The study also reports that milk (lyophilized) contains several species of glycosphingolipids, such as Mono-hexosylceramide, Lactosylceramide, and Tetra-hexosylceramide. No safety data is reported in the study, but its import lies in the careful documentation of the ubiquitous nature of Ceramides, in various forms, in conventional foods.⁶⁷

Clinical Trials and Safety

Human clinical trials have been conducted on Ceramides as well. A double-blind placebo-controlled study was conducted at Osaka City University to evaluate the effects of oral administration of supplements containing rice ceramides in 33 patients with a habitual tendency toward dry, rough skin. The 33 patients were comprised of 6 men and 27 women, and the study was performed in keeping with the tenets of the Helsinki Accord, with written Informed Consent forms. This study was performed as a double-blind trial involving 6-week oral administration of “functional supplements” containing rice-derived Ceramides or a placebo.⁶⁸

The test supplement was given in the form of soft capsules provided by Oryza Oil & Fat Chemical Co., Ltd., with a daily consumption of rice-derived Oryza Ceramide, 40 mg/day , containing rice-derived sphingolipids of 1.2 mg/day . Placebo capsules were administered that were identical in appearance and smell, but containing 0 mg/day of rice-derived Oryza Ceramide and 0 mg/day of rice-derived sphingolipids. After the 6-week trial period, no subjects had dropped out and no adverse events were reported. Other evidence of the safety of ingested Ceramides is also presented in this study:

⁶⁷ Berra, B. *et al.*, Dietary Sphingolipids in Colorectal Cancer Prevention, Eur. J. of Cancer Prev., Vol. 11, pp. 193-197, 2002.

⁶⁸ Kajimoto, esp. at p. 2.

Experiments have shown that after oral administration plant-based ceramides are absorbed unchanged in the small intestine or are broken down into sphingosine and fatty acids for absorption and are then reconfigured as ceramides. The substance is then transported by the capillaries into the horny layer and the keratin intercellular spaces. The supplements containing rice ceramides as used in this study, in contrast to the ceramide substances distributed in animal brains, provide superior supplementation that can be expected to be both safe and effective for oral administration. [Emphasis added.]⁶⁹

Confirming this assessment is Lati, who as early as 1995 documented and detailed the safe use—and indeed healthful use-- of ceramides in functional foods:

Ceramide has been highly appraised in the field of functional foods because of its hydration characteristics and vectorization of vitamins (vitamin C and E).

Presently phytoceramides by INOCOSM [Laboratories] are used as ingredients for various functional and cosmeceutical foods.

Because of its hydration characteristics, ceramide is most extensively used in this field, and it is generally used together with vitamins.⁷⁰ . . .

Ceramide has shown numerous benefits as a food additive because of its very unique characteristics, and it is effective not only for the tissues but also for the stabilization of foods in some cases. If a certain amount of phytoceramide is ingested every day, the following benefits could be attained.

- Because ceramide suppresses free radicals, ceramide can enhance the protection of tissues from external harmful effects (pollution, sunshine, and stress).
- Ceramide moisturizes the skin by its hydration capability.
- Ceramide achieves wrinkle prevention by its elastase suppression and collagen protection.

⁶⁹ Kajimoto at p. 12.

⁷⁰ Lati at p. 6.

. . . On the other hand, if ceramide is added to drinks for the benefit of the tissues, ceramide can also stabilize hydrophobic flavoring ingredients and hydrophobic molecules (vitamins) by vectorization.⁷¹

These conclusions are based in part on Lati's 1995 study, which concludes that phyto-ceramides taken orally improve the hydration of the skin. The phyto-ceramides were given at an amount of 20 mg/day for a month. No adverse effects or events were reported.⁷²

Similarly, in a 2004 clinical trial sponsored by SGTI, no serious adverse events were reported in a double-blind, placebo-controlled study. This study began with an ethical review and approval from IRB, similar to those used in drug clinicals. Of the six non-serious adverse events reported over a 16-week study period (with 18 subjects), only three were deemed by the M.D. principal investigator to be related to treatment. Thus, the other three events were from the placebo group, meaning that the AERs of the supplement studied were identical to placebo. All of these few reported mild or moderate adverse events were resolved by the end of the trial. The principal investigator in this study was a medical doctor, who monitored and evaluated all adverse event reports and safety issues. This study used the identical ceramide-containing supplement as the proposed new dietary supplement, in conjunction with a ceramide-containing topical product also formulated by STGI. The Safety Evaluation of the Final Report concluded: "Overall, the Soft Gel oral and topical products combination was found to be safe to use."⁷³

Ceramide Functionality in the Body at the Cellular Level

Sphingolipids (which contain ceramides) "are present in every human cell as a constituent of the biological cell membrane."⁷⁴ The workings of ceramides at the cellular level in the human body has been intensively studied for at least the last ten years. In 1995, it was already shown that "ceramide production is highly compartmentalized at the cell surface," and that ceramide "is an important cellular intermediate in hormone action," in particular in hormone binding.⁷⁵ By 1997, ceramide had

⁷¹ Lati at p. 11.

⁷² Lati.

⁷³ RTL, Inc. (Research Testing Laboratories, Inc.), "A double blind, randomized, placebo controlled, parallel design clinical trial to evaluate the safety and efficacy of dietary formulations in reducing wrinkling in the periorbital area (crow's feet) and increasing skin elasticity." Pages 25-26. Conducted for SGTI. Oct. 29, 2004.

⁷⁴ Possemiers, at p. 1 of 4.

⁷⁵ Liu and Anderson (1995).

been shown to be an important lipid messenger that regulates cell proliferation, differentiation, and apoptosis (or programmed cell death).⁷⁶ This conclusion was a starting point for a 1998 study: “Ceramide is an important lipid messenger involved in mediating a variety of cell functions including apoptosis.”⁷⁷ By 2000, the summary of one published article began: “Ceramide has emerged as a lipid mediator in apoptosis induced by a variety of stresses.” The full article proceeded to extend research results as to the precise involvement of ceramides in apoptosis in human leukemia cells.⁷⁸

Through extensive scientific and medical research from 1998 through 2004, ceramides are now known and established as regulating the cell life and death cycle, and its growth, and other processes of the body at the cellular level.⁷⁹ In 1998, one study reported evidence that another role for ceramide is to “act as a second messenger to signal accumulation of the thermoprotectant trehalose,” and thus explaining the “severalfold increase in ceramide observed during heat shock.”⁸⁰ In 1999, it was reported that: “Dietary sphingolipids inhibit chemically induced colon cancer in mice. The most likely mediators of this effect are the metabolites ceramide (Cer) and sphingosine, which induce growth arrest and apoptosis in transformed cells.”⁸¹ In 2002, a short chain analog of ceramide was found to inhibit growth and cause the death of colon cancer cells.⁸² Specifically, ceramide is “a bioactive lipid that plays roles in regulating cell responses to a variety of extracellular signals through the modulation of the activity of down stream effectors, which in turn control basic cellular functions such as cell growth, cell cycle arrest, apoptosis, and senescence.”⁸³ By 2004, it

⁷⁶ Nyberg *et al.* Nutritional Biochem. 1997.

⁷⁷ Zhou, Honglin *et al.* Inhibition of Akt Kinase by Cell-permeable Ceramide and its Implications for Ceramide-induced Apoptosis. J. Biol. Chem., vol. 273, Issue 26, pp. 16568-16575 (June 26, 1998).

⁷⁸ Kondo, Tadakazu *et al.*, Role of c-jun Expression Increased by Heat Shock- and Ceramide-activated Caspase-3 in HL-60 Cell Apoptosis, J. Biol. Chem., Volume 275, Issue 11, pp. 7668-7676 (March 17, 2000).

⁷⁹ Vaena de Avalos, Silvia *et al.* Activation and Localization of Inositol Phosphosphingolipid Phospholipase C, Isp1p, to the Mitochondria during Growth of *Saccharomyces cerevisiae*, Journal of Biological Chemistry, Volume 279, Issue 12, pp. 11537-11545 (March 19, 2004). Abstract and Introduction.

⁸⁰ Wells, Gerald B. *et al.*, Heat-induced Elevation of Ceramide in *Saccharomyces cerevisiae* via de Novo Synthesis, J. Biol. Chem., Volume 273, Issue 13, pp. 7235-7243 (March 27, 1998).

⁸¹ Schmelz, Eva M. *et al.*, Experimental Therapeutics; Ceramide- β -D-Glucuronide; Synthesis, Digestion, and Suppression of Early Markers of Colon Carcinogenesis, Cancer Research 59, pp. 5768-5772 (November 15, 1999).

⁸² Ahn, Eun Hyun and Schroeder, Joseph J., Sphingoid Bases and Ceramide Induce Apoptosis in HT-29 and HCT-116 Human Colon Cancer Cells, Experimental Biology and Medicine 227:345-353 (2002).

⁸³ Vaena de Avalos, Silvia *et al.* (2004). Introduction.

could be stated categorically that “Ceramide and its metabolites . . . are now known to be important mediators of apoptosis and cell survival.”⁸⁴ In an article just accepted in 2005, the authors summarize that earlier models “suggest that ceramide induces cell death through interaction with latent binding sites on the outer or inner mitochondrial membranes, followed by an increase in membranes permeability, as an intermediate step in ceramide signal propagation.”⁸⁵ The results of this research show that ceramides are essential for some of the basic cellular processes in the human body.

Recent Science Shows Disease Mechanisms and Therapeutic Value of Ceramides

The role of ceramides in maintaining good health vs. preventing disease in the human body has also been intensively studied for at least the last ten years. Indeed, a search for “ceramides” in one Biochemical journal alone yielded over 1,000 articles, and a search for ceramides in 2004 yields over 150 articles. One paper hypothesizes about the therapeutic mechanism of ceramides that “it is probable that sphingolipids in food compete for cellular binding sites and facilitate the elimination of pathogenic organisms from the intestine.”⁸⁶ Another 2004 article starts with the well-accepted mechanism that “ceramide is involved in the cellular stress response” to “demonstrate that ceramide controls macroautophagy, a major lysosomal catabolic pathway.”⁸⁷ Because they induce the arrest of cell growth, and apoptosis, ceramide and sphingosine play a role in the prevention of cancer, especially colon cancer, and have effects on patients with Crohn’s disease.⁸⁸ The study of the link between the action of ceramides and the prevention of cancer and other diseases seemed to explode in 2004, producing too many peer-reviewed published articles to summarize here; we have chosen several representative ones.

Specifically, recent findings establish that sphingoid bases ceramide induce apoptosis in colon cancer cells and “implicate them as potential mediators of the protective role . . . in colon

⁸⁴ Kohyama-Koganeya *et al.* (Aug. 2004).

⁸⁵ Novgorodov, Sergei A., *et al.* Positively charged ceramide is a potent inducer of mitochondrial permeabilization, Summary of article accepted for publication in *Journal of Biological Chemistry* (February 18, 2005); un-published as of March 4, 2005, and thus full article not available.

⁸⁶ Pfeuffer and Schrezenmeir at 36.

⁸⁷ Scarlatti, Francesca *et al.*, Ceramide-mediated Macroautophagy Involves Inhibition of Protein Kinase B and Up-regulation of Beclin 1, *J. Biol. Chem.*, Volume 279, Issue 18, pp. 18384-18391 (April 30, 2004).

⁸⁸ Possemiers, at p. 1 of 4.

carcinogenesis.”⁸⁹ Ceramides have also been implicated in the signal transduction leading to apoptosis and thus in celiac disease.⁹⁰ One article linked ceramide metabolism and ceramide as a regulatory of cell fate decisions to “diseases with an imbalance in these biomodulators, such as cancer.”⁹¹ Ceramides have also been studied as a regulator of insulin⁹² and as implicated in the development of atherosclerosis,⁹³ as well as in the proinflammatory cytokines tumor necrosis factor.⁹⁴ Most recently, the fact that ceramide plays a key role in organizing membrane structure, and “because membrane organization is critical for HIV infection,” researchers found that “the accumulation of ceramide in cells renders them resistant to HIV infection because of a block in membrane fusion.”⁹⁵ Our point here is that a substance that is both crucial in cell growth and death, and that is beneficial and therapeutic in preventing many diseases in the human body cannot be unsafe at the small amounts used in SGTI’s new dietary supplement.

Ceramide Use Topically Moves to Ceramide Use Ingested

Ceramides have been traditionally found in topical formulas such as creams and lotions. Possibly the oldest exponent of an emulsifier-free cream is the cold cream. “Cold creams” contain structures which are similar to the bi-layers of the stratum corneum. Therefore Ceramide integrates in the skin barrier layers, and is very resistant against exogenous substances. It is recommended to avoid occlusive components on mineral oil base for this concept as they slow down the formation of the skin’s own protective substances. This can be shown by artificially damaging the skin with adhesive tape strips (stripping). While the skin regenerates under normal conditions within 24 hours, the regeneration process is considerably delayed if the skin is artificially covered.⁹⁶

Internal ingestion of Ceramide is a concept which takes the benefits of Ceramides for skin health and beauty to a new level. While a topical cream can provide only so much of a moisture

⁸⁹ Ahn and Schroeder (2002).

⁹⁰ Di Sabatino *et al.* (2001).

⁹¹ Reiss *et al.* (Jan. 2004).

⁹² Stratford *et al.* (Aug. 2004).

⁹³ London, Megha and Erwin, Ceramide Selectively Displaces Cholesterol from Ordered Lipid Domains (Rafts); Implications for Lipid Raft Structure and Function, *J. Biol. Chem.*, Volume 279, Issue 11, pp. 9997-10004 (March 12, 2004).

⁹⁴ MacKichan, Mary Lee and DeFranco, Anthony L., Role of Ceramide in Lipopolysaccharide (LPS)-induced Signaling, *J. Biol. Chem.*, Volume 274, Issue 3, pp. 1767-1775 (January 15, 1999).

⁹⁵ Finnegan, Catherine M., *et al.*, Ceramide a target for antiretroviral therapy, published online before print October 15, 2004, *Proceedings of the National Academy of Sciences of the United States of America*, Volume 101, Number 43, 15452-15457 (October 26, 2004). Available via the Internet.

⁹⁶ Vesper, Imakawa, Lee, Goldstein, and Chamlin.

layer, an internal formula can carry or transport Ceramides to the cell level, thereby increasing the potential for a Ceramide product to be absorbed into the layers of the skin. One common sense indication that Ceramides are safe when ingested is that they have been included in semi-ingested products such as lipsticks for many years. Lipstick is a cosmetic used by millions of women in the U.S. that is gradually and eventually ingested throughout the day (because of the natural phenomenon of licking one's lips) and is reapplied continuously during the day as well. Although very hard to calculate, some amount of Ceramides in the "ingested" lipstick is consumed every day, assuming at least four re-applications per day for the average day.

A reasonable expectation of safety for ingested ceramides is also indicated by at least three patents, two concerning lipsticks. A patent for a "Long wearing lipstick" includes a wax and "a phytosphingosine type ceramide."⁹⁷ In the detailed description of the invention, we learn that the invention calls for the phytosphingosine-type ceramide to be present in the lipstick, preferably from .20 to .50% by weight. According to the inventors, improved wear of the lipstick, without sacrificing gloss, increased from .1 % of Ceramide 3B to .2 and .5 % respectively. Human clinical trials were performed, in which the experimental ceramide-containing lipsticks were applied twice a day. While lipsticks are semi-ingestible, there are two other patents describing uses of ceramides in fully ingested products, and even pharmaceutical products, as well as topically-applied cosmetics.

In patent 6,136,301, "Lipid mix for lip product," one application is for an OTC drug lip balm.⁹⁸ This invention relates to a mixture of less than about 5% by weight of sphingolipids (including ceramides), for both cosmetic and pharmaceutical formulations for topical use on the lips. One claim is for a method of treating or preventing damage to the lips, and other therapeutic embodiments are described.

Finally, in patent 5,817,646, "Polar lipid composition of plant origin," the composition is comprised of "an injectable, intra-articular, topical or ingestible aqueous emulsion of a polar lipid mixture rich in phospholipids, in glycolipids and in ceramides. . ."⁹⁹ (emphases added).

⁹⁷ Szweda, J.A. *et al.*, United States Patent 5,667,770, "Long wearing lipstick," issued Sept. 16, 1997.

⁹⁸ Pelle, E. *et al.*, United States Patent 6,136,301, "Lipid mix for lip product," issued October 24, 2000 ("Pelle" or '301 patent).

⁹⁹ Gossiaux, P. United States Patent 5,817,646, "Polar lipid composition of plant origin," issued October 6, 1998 ("Gossiaux" or '646 patent).

This mixture is obtained from cereal flour or an extract such as bran or lipids extracted from cereals, and thus is similar to the wheat-derived Ceramides discussed above, which is one type of Ceramide being the subject of this Notification. The mixture in this invention is 90% ceramides by weight, and the concept is to create a polar lipid mixture of plant origin essentially identical to the composition of the constituents of the target cells. The detailed description of the invention explicitly states (at page 7 of 14) that this polar lipid mixture can be used as a dietary supplement, in addition to uses for pharmaceuticals or cosmetics. It also notes that ceramides help with retention of water and thus aid in hydration of the skin. In addition, this invention may be applied to drug treatment: “as a vehicle for the delivery of a vaccine component”; and other tests were performed at the laboratories of the Faculty of Pharmacy of Chatenay Malabry (France).¹⁰⁰ This invention indicates the safety and efficacy of a ceramide product for targeting cells with a specific composition. (Copies of all patents attached.)

A History of Safe Use of Ceramides in Dietary Supplements in Japan

Two dietary supplements containing Ceramides derived from rice (=Oryza sativa) are produced by Oryza Oil & Fat Chemical Co. Ltd. (“Oryza”). The powder form, Oryza Ceramide-P (called a “functional food supplement”) is recommended for moisturizing the skin at 20 mg per day, and for whitening the skin at 30-50 mg per day. Oryza Ceramide-L (the “lotion,” liquid form) is recommended at 200 mg per day for moisturizing—and to be added to “functional foods.” The brochure for a phyto-ceramide (rice based) “nutritional” supplements from Oryza also reports administering to mice 5,000 mg of Oryza Ceramide for 18-24 months with no toxic effect.¹⁰¹ Thus the LD₅₀ (mouse) is more than 5,000 mg/kg. Again, by comparison to all the numbers _____ of Ceramide per day is the maximum recommended use of Ceramide in this Notification. Oryza Ceramide-P and Oryza Ceramide –L have been sold in Japan for approximately 5 years, and to our knowledge no adverse events have been reported.

In several Japanese Internet websites selling nutritional supplements, many different products containing phyto-ceramides are advertised.¹⁰² Among these products is one called “Honen Ceramide

¹⁰⁰ Gossioux, at page 10 of 14.

¹⁰¹ Brochure entitled Oryza Ceramide, Nutritional Supplement, from Oryza Oil & Fat Chemical Co. Ltd, called in the footer: ORYZA CERAMIDE CATALOG ver. 1.0EZ (“Oryza brochure”).

¹⁰² Japanese promotional websites (excerpts), Winter 2003-2004.

& Cysteine,” which contains ceramides extracted from the Konjac tuber, where a 4-capsule serving contains 24 mg of ceramide powder. The ceramide is extracted from Konjac tubers, a food that has been eaten by the Japanese since as far back as the Heian Period (794-1192), and that reportedly contains 7 to 15 times more ceramides than found in rice or wheat. The manufacturer of this particular ceramide nutritional supplement reports that the acute toxicity (LD₅₀) is 5,000mg/kg or more. Also, the advertisement reports a survey in which Konjac ceramides are taken for 4 weeks. Finally, part of the promotion includes that the company’s research has been presented at a Food Science conference:

Unitika Ltd. ranks its Life Health business as one of its most important areas of business. At the end of last year, Unitika succeeded in extracting ceramides contained in the konjac tuber and is expecting to move into new markets with the commercialization of this highly purified, pure plant ceramide as a product with cosmetic and dietary applications.

At the August 2002 Conference of the Japanese Society for Food Science and Technology, [Ceramide’s] function as a new beauty food material was announced, and that it is a beauty ingredient that is attracting attention.

Indeed, the “collaborative campaign” by Honen Corporation and Unitika Ltd. launched its Ceramide product on November 17, 2002. The Unitika Ltd. brochure for the Konjac Ceramide Supplement stresses its safety and seems to imply use as a food additive as well:

“Phyto-Ceramide” which is an extract from Konjac . . . is an edible beauty-care product richly containing Glucosylceramide, a Glycosphingolipid.

Being natural products from Konjac, “Phyto-Ceramide” is a highly safe food, and continuous intake will gain high performance.

As Phyto-Ceramide take on varied appearances, its usage is suitable to any kind of food.¹⁰³

Another Japanese product advertised on the Internet is Morishita Jintan’s “Collagen & Ceramide Concentrate Tablets,” where one daily “pouch” contains 0.6 mg of ceramides. Further, three additional products are advertised on these websites, namely “Cell Este” (Product Number CER-1), “Sup. Ceramide” (Product Number CER-1), and Ozio’s “Ceramide and Cysteine” (Product No. 010000016023). Promotions for these three products state that they contain ceramides and are to be ingested. Ozio’s “Ceramide and Cysteine” advertisement states that it contains 75 mg

¹⁰³ Unitika Ltd., CERAMIDE: Clears the skin as you eat. [Promotional brochure.]

ceramides in a daily dose. Clearly, for the legal and financial reasons concerning customer complaints and product liability, if Ceramide supplements had been or were unsafe at 75 mg of Ceramides per day, then this product would not be able to stay on the market.

Japan does have a counterpart to the Food and Drug Administration in the U.S., and this is called the Ministry of Health, Labor and Welfare. According to a report by International Business Strategies,¹⁰⁴ Japan has very strict limits on the substances allowed in dietary supplements: only 350 synthetic food additives and 490 natural origin additives may be used in food. This appears to require that dietary supplement ingredients, unlike in the U.S., must be GRAS, or at least officially on the “safe” list. Any substance not on the list may not be used in supplements. In addition, additives which have been approved for use in Japan in pharmaceuticals are not permissible for use in supplements unless they are on the list of 350 synthetic food additives. Japan’s regulatory regime has been termed “both restrictive and opaque” by this report (at p. 5), which explains that many ingredients or products that may be sold in other countries may not necessarily be legal in Japan. For example, dietary supplements exported from the U.S. routinely must be both reformulated and relabeled before they may be sold in Japan. Finally, it was not until April 1, 2001 that Japan implemented new regulations permitting health effect claims (*cf.* structure/function claims) on supplement labels. Our main point here is that arguably the Ministry of Health, Labor and Welfare in Japan is much stricter in regard to nutritional supplements than is the FDA under DSHEA, and yet Ceramides are permitted as supplement ingredients in Japan, and have been on the market there for approximately five years. This is yet another factor in SGTI’s determination and conclusion that a ceramide-containing new dietary supplement is reasonably expected to be safe.

Conclusion of Reasonable Expectation of Safety

Under 21 C.F.R. § 190.6 (a), the procedure for a new dietary ingredient premarket Notification is that the manufacturer presents the basis on which it has concluded that “a dietary supplement containing such dietary ingredient will reasonably be expected to be safe.” Again, we observe that this statutory and regulatory standard: a reasonable expectation of safety is a lower standard than either GRAS or for an approved food additive. Webster’s dictionary had defined reasonable expectation as a believe “not conflicting with reason,” but SGTI’s scientific and market evidence of safety is stronger than that. In this Notification, we have documented a reasonable

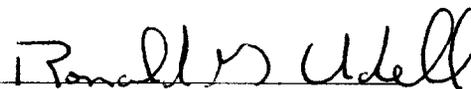
¹⁰⁴ International Business Strategies, Dietary Supplements in Japan, December 2001.

expectation of safety for a new dietary supplement containing wheat germ oil-derived Ceramides, at the amount _____ in at least five ways: 1) the presence of Ceramides in much larger amounts in ordinary foods that make up the human diet and that are consumed daily; 2) ceramides are found throughout the human body and the skin; 3) two toxicity studies showing that the LD 50 is more than 5,000 mg/kg; 4) clinical trials on rats and humans demonstrating no mortality or toxicity, safety, and no adverse events; 5) several scientific articles published in peer-reviewed journals showing that ceramides are essential to cellular processes in the human body, and may have therapeutic and disease-fighting value; and 6) dietary supplements marketed and consumed in Japan containing Ceramides at the same or higher levels than for the proposed SGTI product, in a country that regulates supplements more rigorously than in the U.S. In addition, as stated above, Ceramides are naturally present in human skin, the central nervous system, and spinal marrow. Copies of all articles and reports cited herein are attached, pursuant to 21 C.F.R. § 190.6 (b)(4). Together, these articles and reports, in conjunction with the facts, data, reasoning, and analysis above, demonstrate that a dietary supplement containing wheat-derived Ceramides at _____ and under the conditions of use above, is reasonably expected to be safe. We believe that this conclusion for our proposed new dietary supplement containing wheat germ-derived Ceramides is rational, amenable to reason, and agreeable to reason, as defined by Black's Law Dictionary®.

A Note on Confidentiality

In order to present full background on this NDI, this Notification must include proprietary information and trade secrets of SGTI, and thus is the Confidential version of the submission. In particular, the Mass Spectrometry of Ceramide done by SGTI's supplier, Ennagram, and the 2004 clinical trial of Soft Gel's supplement and topical products, both containing Ceramides, are attached in their entirety. Pursuant to 21 C.F.R. sec. 190.6 (e), SGTI, through its counsel, Susan Brienza, Esq. of Patton Boggs LLP (Denver Office), reserves the right to redact all commercial confidential, proprietary and trade secret information within 90 days of this filing, and to provide that redacted version to this office, before the Notification is publicly displayed or added to the FDA's public Docket. This will include redacted versions of the two SGTI attachments above, and any others containing proprietary information.

SOFT GEL TECHNOLOGIES, INC.

By: 
Ronald G. Udell, President

Date: March 4, 2005

References

Ahn, Eun Hyun and Schroeder, Joseph J., Sphingoid Bases and Ceramide Induce Apoptosis in HT-29 and HCT-116 Human Colon Cancer Cells, *Experimental Biology and Medicine* 227:345-353 (2002).

Applegate, Elizabeth and Grivetti, Louis, Search for the Competitive Edge: A History of Dietary Fades and Supplements, *The Journal of Nutrition*, 77:869S-873S (1997). Available at <http://www.nutrition.org/cgi/content/full/127/5/869S>.

Berra, B. *et al.*, Dietary Sphingolipids in Colorectal Cancer Prevention, *Eur. J. of Cancer Prev.*, Volume 11, pp. 193-197 (2002).

Bouwstra, J.A. *et al.*, Role of ceramide 1 in the molecular organization of the stratum corneum lipids. *J. Lipid Res.*, 39(1), pp. 186-96, Jan. 1998 (Abstract from PubMed).

Chamlin, S.L. *et al.*, Ceramide-Dominant Barrier Repair Lipids Alleviate Childhood Atopic Dermatitis: Changes in Barrier Function Provide a Sensitive Indicator of Disease Activity, *Journal of Am. Acad. Dermatol.*, Volume 47, pp. 198-208, Number 2, PR 198-208 (2002).

Di Sabatino, Antonio, *et al.*, Ceramide involvement in the signal transduction leading to increased enterocyte apoptosis in Celiac Disease, *Communications des Digestive Disease Week* (May 2001).

Finnegan, Catherine M., *et al.*, Ceramide a target for antiretroviral therapy, published online before print October 15, 2004, *Proceedings of the National Academy of Sciences of the United States of America*, Volume 101, Number 43, 15452-15457 (October 26, 2004).

Futerman, A., Ceramide Metabolism Compartmentalized in the Endoplasmic Reticulum and Golgi apparatus, Current Topics in Membranes, Volume 40, p. 93 (1994).

Goldstein, A., Ceramides in the Stratum Corneum: Structure, Function, and New Methods to Promote Repair, Int. J. of Derma., Volume 42, pp. 256-259 (2003).

Gossioux, P. United States Patent 5,817,646, "Polar lipid composition of plant origin," issued October 6, 1998.

Hoang, T., Sphingolipids (a Powerpoint presentation), March 28, 2002, p. 12; also citing to Vesper. Tinyee Hoang is a Graduate Student in Food Science at Washington State University.

Horibata, Yasuhiro *et al.*, Unique Catabolic Pathway of Glycosphingolipids in a Hydrozoan, Hydra magnipapillata, Involving Endoglycoceramidase, J. Biol. Chem., Volume 279, Issue 32, pp. 33379-33389 (August 6, 2004).

Imokawa, G., Structures and Functions of Stratum Corneum Lipids in the Skin, Journal Japan Oil Chem. Society, Volume 44, Number 10, pp. 1-23 (1995).

International Business Strategies, Dietary Supplements in Japan, December 2001.

Kajimoto, O. *et al.*, Clinical Investigation of the Skin Beautifying Effects of a Beauty Supplement Containing Rice Derived Ceramides-Objective Assessment of Dry Skin through Analysis of Three Dimensional Microscopy Images. Authors are affiliated with the Health Management Center, Osaka University of Foreign Studies; Dermatology Department, Faculty of Medicine, Osaka City University; and the Institute of Comprehensive Medical Science (undated publication).

Kohyama-Koganeya, Ayako, *et al.*, Drosophila Glucosylceramide Synthase (A Negative Regulator of Cell Death Mediated by Proapoptotic Factors), Journal of Biological Chemistry, Volume 279, Issue 34, 35995-36002 (August 20, 2004).

Kondo, Tadakazu *et al.*, Role of c-jun Expression Increased by Heat Shock- and Ceramide-activated Caspase-3 in HL-60 Cell Apoptosis, J. Biol. Chem., Volume 275, Issue 11, pp. 7668-7676 (March 17, 2000).

Kretchmer (a subsidiary of Quaker Oats), current label for Wheat Germ Oil.

Lati, E., New Research and Development in Moisture Retention Mechanism and Moisturizing Agents, Fragrance Journal, Volume 23, Number 81 (1995).

Lee, M., Analysis of Ceramides in Cosmetics by Revised-Phase Liquid Chromatography/Electrospray Ionization Mass Spectrometry with Collision-Induced Dissociation, Rapid Commun. Mass Spectrom., 17:64-75 (2003).

Liu, Pingsheng and Anderson, Richard G.W., Compartmentalized Production of Ceramide at the Cell Surface, The American Society for Biochemistry and Molecular Biology, Inc., Volume 270, Number 45, pp. 27179-27185 (November 10, 1995).

London, Megha and Erwin, Ceramide Selectively Displaces Cholesterol from Ordered Lipid Domains (Rafts); Implications for Lipid Raft Structure and Function, J. Biol. Chem., Volume 279, Issue 11, pp. 9997-10004 (March 12, 2004).

MacKichan, Mary Lee and DeFranco, Anthony L., Role of Ceramide in Lipopolysaccharide (LPS)-induced Signaling, J. Biol. Chem., Volume 274, Issue 3, pp. 1767-1775 (January 15, 1999).

Merrill, A.H., Jr. *et al.*, Sphingolipid Uptake by Isolated Segments of the Rat Intestine, FASEB Journal, P. 3A, 469 (1989).

Merrill, A.H., Jr. *et al.*, Importance of Sphingolipids and Inhibitors of Sphingolipid Metabolism as Components of Animal Diets, Journal of Nutrition, Volume 127, pp. 830S-833S (1997).

Motta, S. *et al.*, Ceramide Compositions of the Psoratic Scale, Biochimica et Biophysica Acta, 1182: pp. 147-151 (1993).

Novgorodov, Sergei A., *et al.* Positively charged ceramide is a potent inducer of mitochondrial permeabilization, Summary of article accepted for publication in Journal of Biological Chemistry (February 18, 2005); un-published as of March 4, 2005, and thus full article not available.

Nyberg, Lena *et al.*, Localization and capacity of spingomyelin digestion in the rat intestinal tract, Journal of Nutritional Biochemistry, Volume 8, pp. 112-118, March 1997.

Orgretmen, B., Biochemical Mechanisms of the Generation of Endogenous Long Chain Ceramide to Exogenous Short Chain Ceramide in A549 Human Lung Adenocarcinoma Cell Line, J. of Bio. Chem., Volume 227, Number 15 (2002).

Pelle, E. *et al.*, United States Patent 6,136,301, "Lipid mix for lip product," issued October 24, 2000.

Pfeuffer, M. and Schrezenmeir, J., Dietary sphingolipids: Metabolism and potential health implications, Institute of Physiology and Biochemistry of Nutrition, Federal Dairy Research Centre, Volume 53, pp. 31-42 (January 23, 2003).

Possemiers, Sam, Research: 1. Microbial metabolism of dietary sphingolipids and its implications for human health. [no date] Report of Lab MET, the Laboratory of Microbial Ecology and Technology. Ghent University, Belgium. Available via the Internet.

Reiss, Ulrike, *et al.*, Sphingosine-phosphate Lyase Enhances Stress-induced Ceramide Generation and Apoptosis, Journal of Biological Chemistry, Volume 279, Issue 2, pp. 1281-1290 (January 9, 2004).

Richeux, F., BioHC Appendix 2, Report to the Investigator TA0423-PH-03/0307 (1996); Appendix 2 (1997). [See cover letter of Report.]

Scarlatti, Francesca *et al.*, Ceramide-mediated Macroautophagy Involves Inhibition of Protein Kinase B and Up-regulation of Beclin 1, J. Biol. Chem., Volume 279, Issue 18, pp. 18384-18391 (April 30, 2004).

Schmelz, E.V., Uptake and Metabolism of Sphingolipids in Isolated Intestinal Loops of Mice, J. of Nutrition, 124 (5): 702-712 (1994).

Schmelz, Eva M. *et al.*, Experimental Therapeutics; Ceramide- β -D-Glucuronide; Synthesis, Digestion, and Suppression of Early Markers of Colon Carcinogenesis, Cancer Research 59, pp. 5768-5772 (November 15, 1999).

Stratford, Suzanne, *et al.*, Regulation of Insulin Action by Ceramide (Dual Mechanisms Linking Ceramide Accumulation to the Inhibition of Akt/Protein Kinase B), Journal of Biological Chemistry, Volume 279, Issue 35, pp. 36608-36615 (August 27, 2004).

Sullards, M.C., *et al.*, Structure determination of soybean and wheat glucosylceramides by tandem mass spectrometry, Journal of Mass Spectrometry, Volume 35, pp. 347-353 (2000).

Szweda, J.A. *et al.*, United States Patent 5,667,770, "Long wearing lipstick," issued Sept. 16, 1997.

Unitika Ltd. Health & Amenity Business Dept., CERAMIDE: Clears the skin as you eat, a promotional brochure on a supplement with Phyto-Ceramide extracted from Konjac (no date).

Vaena de Avalos, Silvia, *et al.*, Activation and Localization of Inositol Phosphosphingolipid Phospholipase C, Isp1p, to the Mitochondria during Growth of *Saccharomyces cerevisiae*, Journal of Biological Chemistry, Volume 279, Issue 12, pp. 11537-11545 (March 19, 2004).

Vesper, H. *et al.*, Sphingolipids in Food and the Emerging Importance of Sphingolipids to Nutrition, J. of Nutrition, 129: 1239-1250 (1999).

Wells, Gerald B. *et al.*, Heat-induced Elevation of Ceramide in *Saccharomyces cerevisiae* via de Novo Synthesis, J. Biol. Chem., Volume 273, Issue 13, pp. 7235-7243 (March 27, 1998).

Yamamura, T. and Tekezuka, T., Change in sphingomyelinase activity in human epidermis during aging, Journal of Dermatological Science, 1 (2):79-84 (1990).

YBS Corporation, Wheat Germ Oil, Containing Vegetable Ceramides, including "Characteristics of 'Wheat Germ Oil-S'" (at page 3 of 4). Available on the Internet at www.ybsweb.co.jp/exhf5.html.

Zettersten, E., Optimal Ratios of Topical Stratum Corneum Lipids Improve Barrier Recovery in Chronologically Aged Skin, Journal of American Academy of Dermatology, Volume 37, Number 3, Part 1 (1997).

Zhou, Honglin *et al.* Inhibition of Akt Kinase by Cell-permeable Ceramide and its Implications for Ceramide-induced Apoptosis. J. Biol. Chem., vol. 273, Issue 26, pp. 16568-16575 (June 26, 1998).