Safety Assessment of Ceramides as Used in Cosmetics

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All interested persons are provided 60 days from the above release date to comment on this Safety Assessment and to identify additional published data that should be included or provide unpublished data which can be made public and included. Information may be submitted without identifying the source or the trade name of the cosmetic product containing the ingredient. All unpublished data submitted to CIR will be discussed in open meetings, will be available at the CIR office for review by any interested party and may be cited in a peer-reviewed scientific journal. Please submit data, comments, or requests to the CIR Director, Dr. Lillian J. Gill.

The 2014 Cosmetic Ingredient Review Expert Panel members are: Chair, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; Ronald A. Hill, Ph.D.; Curtis D. Klaassen, Ph.D.; Daniel C. Liebler, Ph.D.; James G. Marks, Jr., M.D.; Ronald C. Shank, Ph.D.; Thomas J. Slaga, Ph.D.; and Paul W. Snyder, D.V.M., Ph.D. The CIR Director is Lillian J. Gill, D.P.A. This safety assessment was prepared by Christina L. Burnett, Senior Scientific Analyst/Writer, Ivan J. Boyer, Ph.D., Senior Toxicologist, and Bart Heldreth, Ph.D., Chemist.

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ABSTRACT

The Cosmetic Ingredient Review Expert Panel (the Panel) reviewed the safety of ceramides, which function in cosmetics primarily as hair conditioning agents and skin conditioning agents-miscellaneous. The Panel reviewed relevant animal and human data related to these ingredients. The Panel concluded that ceramides were safe in cosmetics in the present practices of use and concentration described in this safety assessment:

INTRODUCTION

Ceramide ingredients function primarily as hair conditioning agents and skin conditioning agents-miscellaneous in cosmetics.[1] The 23 ingredients reviewed in this safety assessment are listed below:

ceramide 1
ceramide 2
ceramide 3
ceramide 4
ceramide 5
ceramide 1A
ceramide 6 II
ceramide AP
ceramide EOP
ceramide EOS
ceramide NP
ceramide NG
ceramide NS
ceramide AS
ceramide NS dilaurate
caprooyl phytosphingosine
caprooyl sphingosine
hydroxypalmitoyl sphinganine
2-oleamido-1,3-octadecanediol
caproyl sphingosine
hydroxylauroyl phytosphingosine
hydroxycapryloyl phytosphingosine
hydroxycaproyl phytosphingosine

Many of the reports found in the published literature presented efficacy studies on the named cosmetic ingredients, efficacy studies of other cosmetic ingredients or pharmaceuticals in which naturally-occurring ceramide levels in the skin were evaluated, and data on pseudo-ceramides (such as that found in an approved medical device), the chemical structures of which were determined by the Panel to be significantly different from those of the cosmetic ingredients addressed in this report. These studies were not relevant for assessing the safety of the ceramide ingredients included in this assessment. Additionally, a published paper that presents data from toxicology studies of several ceramides (1, 3, 3A, 3B, 6) and a phytosphingosine was reviewed, but the data from this paper were not incorporated into this report because some data points appeared to be merely cumulative to those of unpublished studies that were submitted by the Personal Care Products Council (Council) to the CIR, and the information presented in the paper is too incomplete to advance the development of a proper safety assessment.[2]

Many published reports address the essential nature of extracellular ceramides as components of the epidermal permeability barrier. These ceramides are clearly segregated to the extracellular spaces of the stratum corneum and other upper layers of the epidermis.[3,4] The family of ceramides that serve this function comprise about 50% of the lipid weight, and 5% of the total weight, of the stratum corneum.[4] Many other reports address the central role of ceramides in sphingolipid metabolism and the mediation of antiproliferative and proapoptotic functions inside cells, including keratinocytes. However, the extracellular barrier-forming ceramides are partly O-acylated molecules with long-chain fatty acids, in contrast to the signal-transducing ceramides.[3] Further, naturally-occurring ceramides are nearly cell-impermeant, and metabolic pathways can suppress intracellular ceramide accumulation to protect cells from ceramide-induced apoptosis and other effects. Thus, much of the extensive literature on the signal-transducing properties of ceramides does not appear to be relevant, and was not incorporated into this safety assessment report.

The names of ceramide ingredients have changed recently. For instance the *International Cosmetic Ingredient Dictionary and Handbook* (INCI) name, Ceramide 1, which was originally assigned in 1997, has been retired. For an interim period, trade name assignments formerly published with the INCI name Ceramide 1 will be retained in the retired monograph, and also published with the new name assignment, Ceramide EOP. This means that, during the "interim period," products on the market may be labelled with either name, Ceramide 1 or Ceramide EOP, although both names refer to the same ingredient. Because these name changes are relatively new, current reported use data are associated primarily with the retired names. Likewise, most ingredient-specific data received for these ingredients may be associated with the retired INCI names. Accordingly, throughout this safety assessment report, the retired INCI names are used consistently to refer to the ingredients listed under either nomenclature. However, for future access, the data and the conclusions of the CIR Expert Panel will apply to these ingredients under both the new and the retired nomenclature.

The other name changes include (further explained in Table 1): Ceramide 2 will be replaced by two names, Ceramide NS (limited to sphingosine-based ceramides) and Ceramide NG (limited to sphinganine-based ceramides); Ceramide 3 will be replaced by Ceramide NP; Ceramide 4 and Ceramide 5 will both be replaced by Ceramide AS; and Ceramide 6 II will be replaced by Ceramide AP.

Information on manufacturing methods for several ceramide ingredients submitted to CIR by the Council indicates that these ingredients are produced synthetically. However, at least one reference obtained through a search of the literature suggests that naturally-occurring ceramides are derived primarily from bovine central nervous system tissues, which may raise concerns about the possible transmission of bovine spongiform encephalopathy.[4] It is not clear, based on the information available to date, whether the ceramides used as cosmetic ingredients are produced exclusively by synthetic methods.

CHEMISTRY

Definition and General Characterization

Generally, a ceramide is the amidation reaction product of a sphingoid base and a fatty acid (Figure 1).

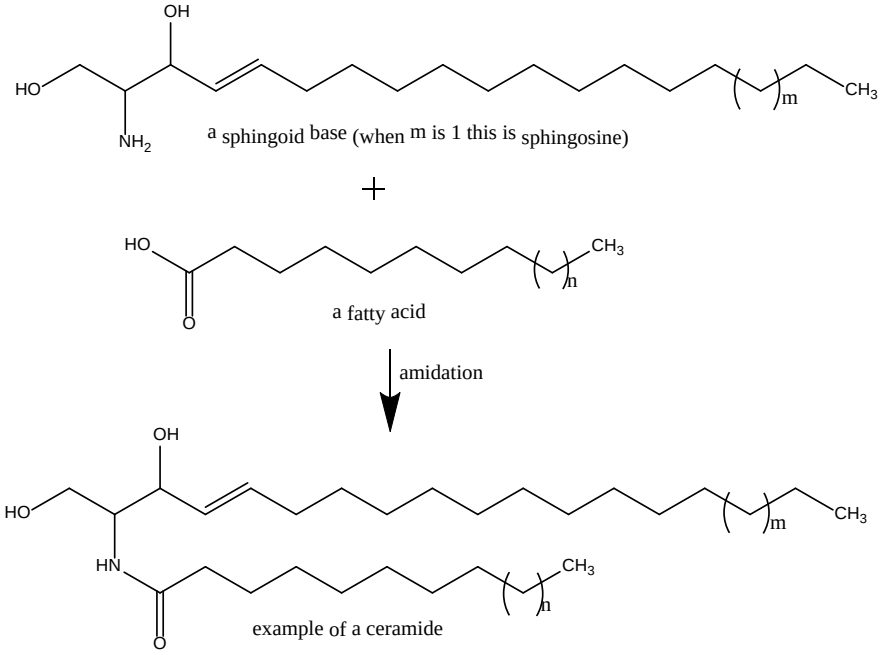

Figure 1. Example of a ceramide structure

The ingredients described herein vary principally in the chain lengths of the sphingoid and fatty acid residues and in the degree of unsaturation in the chains. Additionally, each of these ingredients is a mixture of ceramides, described in more detail in Table 1. Available data on chemical and physical properties are presented in Table 2.

Method of Manufacturing

In biological systems, ceramides are synthesized by de novo synthesis or sphingomyelin hydrolysis or through a salvage pathway.[5] Ceramide manufacture could be accomplished by a variety of synthetic methods, but most methods involve amidation of a fatty acid with a sphingoid base.[6] This can be accomplished by reaction of the sphingoid base with an acyl chloride, but the results are not selective and esterification and amidation occur concurrently. However, mild alkaline hydrolysis can selectively remove the esters. Alternatively, activating the fatty acid with a carbodiimide enables ceramide synthesis without esterification.

Ceramide 2

According to a supplier, ceramide 2 is produced synthetically via amide formation (i.e., reaction of (2S,3R)-sphinganine with methyl stearate to produce an amide), and other constituents/impurities are the isomers[7].

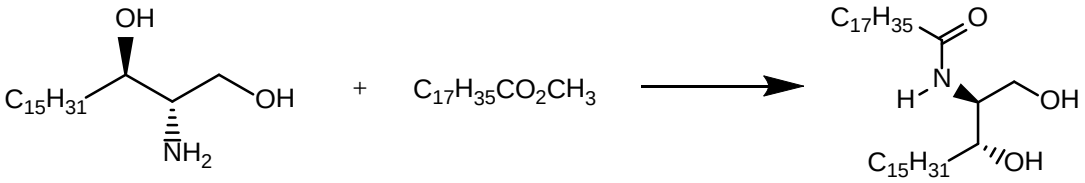

Figure 2. Formation of ceramide 2

Another supplier states that ceramide 2 is a pure substance obtained by reacting a glycine ester derivative and activated palmitic acid before further reacting with stearoyl chloride.[8] This supplier reports that ceramide 2 is a mixture of D,L-erythro and D,L-threo with respective proportions of approximately 75% and 25%.

Ceramide 5

A supplier of ceramide 5 reports that ceramide 5 is produced synthetically via amide formation (i.e., reaction of (2S, 3R)-sphinganine) with methyl 2-hydroxyhexadecanoate to produce an amide. [9]

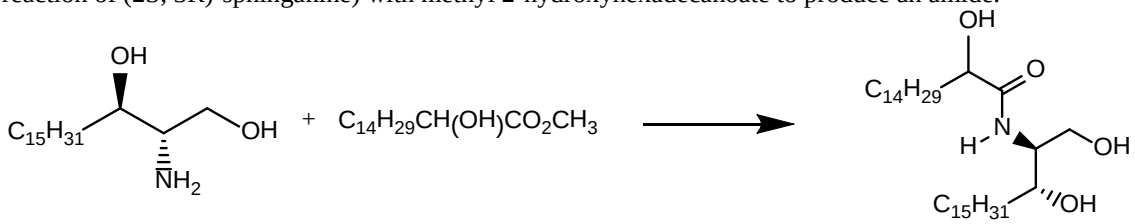

Figure 3. Formation of ceramide 5

2-Oleamido-1,3-Octadecanediol

Industry reported that 2-oleamido-1,3-octadecanediol is obtained by chemical reaction between dihydrosphingosine and an oleic acid derivative.[10]

Hydroxypalmitoyl Sphinganine

Industry has reported that hydroxypalmitoyl sphinganine is obtained by chemical reaction between dihydrosphingosine and a 2-bromohexadecanoic acid derivative, followed by the indirect substitution of bromine by hydroxyl group.[10]

Impurities

Ceramide 2

In a HPLC analysis of ceramide 2, only one peak was detected. No residual solvent was detected and the water content was less than 0.5% (no further details provided).[8]

A heavy metals analysis performed on ceramide 2 (reported under the new name ceramide NG) yielded the following results: lead < 10 ppm, arsenic < 3 ppm, mercury < 1 ppm, cadmium < 1 ppm, nickel < 1 ppm, and palladium < 1 ppm.[11]

Ceramide 5

The residue-on-ignition value for ceramide 5 was reported to be less than 0.5%.[9]

UV/VIS Absorption

Ceramide 2

The ultraviolet (UV) and visible (VIS) absorption spectra of solutions of ceramide 2 were measured with a Perkin Elmer Lambda 15 spectrophotometer.[12] Ceramide 2 was tested at concentrations of 0.001%, 0.01%, 0.1%, and 1% in 95% ethanol or 1,4-dioxane. Negligible absorption was observed [$\boldsymbol{A}$(300 nm) = 0.064 in ethanol and 0.078 in 1,4-dioxane] indicating that significant photochemical reactions in sunlight are unlikely.

USE

Cosmetic

Table 3 presents the current product-formulation use data for ceramide ingredients. These ingredients function primarily as hair conditioning agents and skin conditioning agents-miscellaneous.[1]

According to information supplied to the Food and Drug Administration (FDA) by industry as part of the Voluntary Cosmetic Registration Program (VCRP), ceramide 3 has the most reported uses in cosmetic products, with a total of 359; the majority of the uses are in leave-on skin care preparations.[13] 2-Oleamido-1,3-octadecanediol has the second greatest number of overall uses reported, with a total of 352; the majority of these uses are also in leave-on skin care preparations.

In the Personal Care Products Council's use concentration survey, ceramide 3 had a maximum use concentration of 0.2%, which was reported in lipstick and face and neck skin care preparations.[14] 2-Oleamido-1,3-octadecanediol had a maximum use concentration of 0.7% in hair conditioners.[15] Most of the other use concentrations that were reported had similar use concentrations. Suppliers reported that ceramide 2 and ceramide 5 are used at concentrations up to 4% and 2%, respectively, in cosmetic products (no further details were provided).[7,9]

In some cases, no reported uses were received from the VCRP, but a maximum use concentration was provided in the industry survey. For example, ceramide 1A was not reported in the VCRP, but the industry survey indicated that it is used in leave-on formulations at concentrations up to 0.01%. It should be presumed that ceramide 1A is used in at least one cosmetic formulation.

Table 4 lists the eight ceramide ingredients not indicated to be in use based on the VCRP data and the results of the Council's concentration of use survey.

Some of these ingredients were reported to be used in hair sprays and body and hand or moisturizing sprays and could possibly be inhaled. For example, ceramide 3 was reported to be used in body and hand sprays at a maximum concentration of 0.001%. In practice, 95% to 99% of the droplets/particles released from cosmetic sprays have aerodynamic equivalent diameters >10 µm, with propellant sprays yielding a greater fraction of droplets/particles below 10 µm compared with pump sprays.[16-19] Therefore, most droplets/particles incidentally inhaled from cosmetic sprays would be deposited in the nasopharyngeal and bronchial regions and would not be respirable (i.e., they would not enter the lungs) to any appreciable amount.[17,18]

The ceramide ingredients in this report are not restricted from use in any way under the rules governing cosmetic products in the European Union.[20]

TOXICOKINETICS

Ceramides are among the lipids that make up sphingomyelin, which is a major component of the lipid bilayer of the cell membranes of keratinocytes in the epidermis.[7,9] Ceramides are lipophilic and are likely to be readily absorbed into the skin. However, they are expected to remain in the stratum corneum and not penetrate any deeper.

The absorption, distribution and excretion of an analogous radiolabeled ceramide (palmitoyl D-erythro-sphingosine, [3–^{3}H]) was studied in male HWY rats.[21] The chemical structure of the ceramide tested, and the position of the radiolabel, were given as follows:

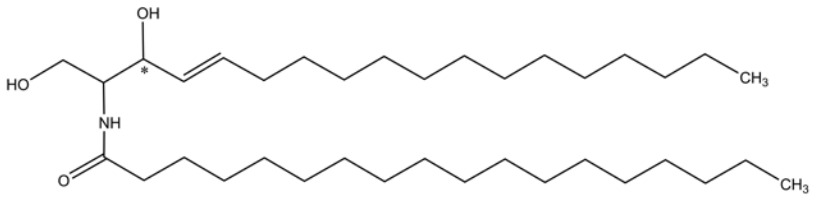

Figure 4. Ceramide analog

The rats received a single oral administration (300 kBq/30 µg/kg) of ^{3}H-ceramide. Blood samples were serially collected from the subclavian vein at 0.5, 1, 2, 4, 8, 12, 24, 48, 72, 96 and 144 h and the concentration of total radioactivity in the plasma was determined. Radioactivity was also measured in urine and feces collected up to 96 h post-treatment and in excised abdominal skin and carcass. Distribution of radioactivity was measured in selected organs and tissues up to 168 h post-treatment. The mean plasma concentration of radioactivity reached a maximum at approximately 10.67 h and then decreased with a half-life of 67.12 h. The mean cumulative excretion of radioactivity in urine and feces was approximately 4.79% and 87.44% of the dose, respectively. At 96 h after dosing, 1.67% and 3.67%, respectively, of the dose were still present in the skin and carcass. The radioactivity in the skin at

12 h was lower than that in plasma and the ratio of skin to plasma concentration was 0.7. However, at 120 h after dosing, the ratio of skin to plasma concentration increased to 4. An analysis of the distribution of radioactivity in a section of skin found radioactivity in the dermis and epidermis. At 72 and 168 h, the radioactivity in the epidermis was 5.6 % and 8.0%, respectively, of the radioactivity in skin, while at these same observation periods, the radioactivity in the dermis was 94.4% and 92.0%, respectively, of the radioactivity in skin. This study found that, following oral exposure, radiolabeled ceramide is distributed gradually in the dermis and then transferred to the epidermis.

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TOXICOLOGICAL STUDIES

Acute Toxicity

Acute oral and dermal toxicity studies are summarized in Table 5.[22-30] The median lethal oral dose (LD_{50}) was greater than 2000 mg/kg in rat studies of ceramide 2, ceramide AP, hydroxypalmitoyl sphinganine, and 2-oleamido-1,3-octadecanediol, and greater than 5000 mg/kg in rat studies of ceramide NP. In dermal studies on rats, the LD_{50}s were greater than 2000 mg/kg for ceramide NP, ceramide AP, and 2-oleamido-1,3-octadecanediol.

Repeated Dose Toxicity

Oral – Non-Human

2-Oleamido-1,3-Octadecanediol

The toxicity of 2-oleamido-1,3-octadecanediol was assessed in a 28-day oral study in groups of 5 male and 5 female Sprague-Dawley rats.[31] The rats received 10, 30, or 100 mg/kg of the test material in carboxymethylcellulose daily via gavage. An additional group of 5 males and 5 females received the vehicle alone as a control. The rats were observed weekly for clinical signs of toxicity and feed and water consumption. The rats were weighed twice weekly. Hematology, blood clinical chemistry, and urinalysis were also performed. Macroscopic and histologic examinations were performed at study end.

No mortalities were observed during the study. With the exception of the 30 mg/kg dose females, behavior, body weight gain, and feed consumption of the treated animals were comparable to those of the control animals. In the 30 mg/kg females, a slight decrease in body weight gain was observed. Decreased water consumption was observed during week 4 in the 10 mg/kg dose males and during week 2 to week 4 in the 30 mg/kg dose males. In the females, increased water consumption was observed in week 2 and week 3 in the 10 mg/kg dose group and during week 1 and week 2 in the 30 and 100 mg/kg dose groups. Mean white cell count in the 10 mg/kg dose females, mean neutrophils count in the 10 and 100 mg/kg dose males, and mean lymphocyte count in the 10 mg/kg dose females were statistically significantly lower than those of the controls, but individual results were within physiological ranges. Alanine transaminase and aspartate transaminase activities were high in one 100 mg/kg dose female, and a histopathological examination of the liver of this animal revealed a moderate single cell necrosis lesion with inflammatory cell infiltration. The mean urea level in the blood in the 30 mg/kg dose females was significantly lower than that of the controls, but individual results were within physiological ranges. No other statistically significant differences were observed in other hematologic and clinical chemistry parameters. Macroscopic examination found mean absolute weight of the heart in the 30 mg/kg dose males and mean relative weight of the heart in the 30 and 100 mg/kg dose males statistically significantly lower than those of the controls, but individual results were within normal physiological ranges. At 100 mg/kg in male rats, an increase in mean absolute and relative weight of the thymus was observed. Based on the findings in the thymus, the no observed effect level (NOEL) for 2-oleamido-1,3-octadecanediol was 30 mg/kg/day in rats.[31]

Dermal – Non-Human

2-Oleamido-1,3-Octadecanediol

The cutaneous tolerance of 2-oleamido-1,3-octadecanediol was tested in 3 male and 3 female Sprague-Dawley rats.[32] The animals received 1 g/kg body weight of test material in powder form applied to the costal cutaneous area once daily for 14 consecutive days. Test sites were occluded for 6 h and then washed. An additional group of 3 male and 3 female rats that did not receive the powder served as control. The rats were observed daily for clinical signs of toxicity, mortality, and cutaneous tolerance. The rats were weighed on days 7 and 14. Macroscopic and histologic examinations were performed at study end. No mortalities were observed during the study. Behavior and mean body weight gains of the treated animals were comparable to those of the control animals. No adverse reactions were observed in the skin. No macroscopic abnormalities of the skin or main abdominal and thoracic organs were observed. No treatment-related skin irritation was noted in the histological evaluation. It was concluded that the cutaneous tolerance of 1 g/kg 2-oleamido-1,3-octadecanediol in rats was good.

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

2-Oleamido-1,3-Octadecanediol

The effects of 2-oleamido-1,3-octadecanediol on reproduction and development were studied in groups of 10 rats/sex/dose by oral gavage.[33] Dose levels tested were 0, 100, 300, and 1000 mg/kg body weight/day at a dose volume of 5 ml/kg body weight. The vehicle was a 0.5% aqueous solution of methylcellulose in purified water. Parental males were exposed to the test material 2 weeks prior to mating, during mating, and about 2 weeks post-mating (approximately 6 weeks total). Parental females were exposed 2 weeks prior to mating, during mating, during gestation, and during at least 4 days of lactation.

In the 1000 mg/kg dose group, one pregnant rat was observed with poor clinical condition and body weight loss toward the end of gestation and did not deliver by day 24 post-mating. Fibrinous and necrotic inflammation of the pericardium was observed microscopically. Another female in the high dose group was also observed with poor clinical condition from the end of gestation until day 2 post-partum. A normal delivery was observed in this rat, though, and no abnormal findings were observed at necropsy. The findings in these 2 high-dose females were not considered treatment-related. No treatment-related clinical effects were noted in the other animals in any dose group. No treatment-related effects were observed with mean body weights, mean feed consumption, or mating or fertility parameters (including mean numbers of corpora lutea, implantation sites per litter, pups delivered, and live pups).

Further, no increased incidences of mortality, adverse effects on body weight, influence on sex ratio, or treatment-related gross malformations were noted in the pups.

In the1000 mg/kg/day dose group, one parental male had several lesions in the urinary tract, including urinary lithiasis, thickened and red urinary bladder mucosa, and dilated right ureter and pelvic cavity of the right kidney. The researchers could not determine whether this finding was treatment-related or a random event. No other treatment-related effects were noted in parental animals at necropsy.

Based on the results of this study on 2-oleamido-1,3-octadecanediol, the researchers determined the maternal no observed adverse effect level (NOAEL) to be 1000 mg/kg body weight/day, and the NOEL for mating and fertility to be 1000 mg/kg body weight/day.[33]

GENOTOXICITY

In vitro genotoxicity studies are summarized in Table 6.[26-28,30,34-39] Ceramide 2, ceramide NP, ceramide 5, ceramide AP, hydroxypalmitoyl sphinganine, and 2-oleamido-1,3-octadecanediol were not mutagenic in Ames assays. Additionally, 2-oleamido-1,3-octadecanediol was not polyploidy-inducing or clastogenic in a metaphase chromosome analysis in Chinese hamster lung cells at a concentration up to 5000 μg/ml with and without metabolic activation.

CARCINOGENICITY

Data on carcinogenicity were not found in the published literature for ceramides, nor were unpublished data provided.

IRRITATION AND SENSITIZATION

Irritation

Ocular

Ocular irritation studies are summarized in Table 7.[26-28,30,40-43] Undiluted ceramide 2 was mildly irritating to rabbit eyes in one study, but not irritating in another. Ceramide NP, ceramide AP, and hydroxypalmitoyl sphinganine were also not irritating in rabbit eye studies when tested neat. 2-Oleamido-1,3-octadecanediol was slightly irritating when tested neat in rabbit eyes.

Dermal

Dermal irritation studies are summarized in Table 8.[26-28,30,44-51] In dermal studies, ceramide 2 (up to 5% in corn oil and undiluted), ceramide 3 (concentration not reported), ceramide 5 (up to 5% in white petrolatum), ceramide AP (undiluted), hydroxypalmitoyl sphinganine (undiluted), and 2-oleamido-1,3-octadecanediol (undiluted) were not irritating to rabbit or guinea pig skin. Slight irritation was observed in a dermal irritation study in rabbits with ceramide NP in propylene glycol, but no irritation was observed in other studies of the undiluted form. No dermal irritation was observed in human patch tests of ceramide 2 (up to 5% in lanolin), ceramide NP (up to 10% dispersion in Vaseline®), ceramide 5 (up to 5% in lanolin), and ceramide AP (5% dispersion in Vaseline®).

Sensitization

Dermal sensitization studies are summarized in Table 9.[26,30,52-54] Ceramide 2 (up to 20%), ceramide NP (up to 10%), ceramide 5 (up to 5%), and hydroxypalmitoyl sphinganine (up to 20%) were not sensitizing in guinea pig maximization tests.

SUMMARY

Ceramides function primarily as hair conditioning agents and skin conditioning agents-miscellaneous in personal care products. Naturally-occurring ceramides are normal constituents of the skin and are essential components of the epidermal permeability barrier.

Ceramide 3 has the most reported uses in cosmetics, with a total of 359; the majority of the uses are in leave-on skin care preparations. 2-Oleamido-1,3-octadecanediol has the second greatest number of overall uses reported, with a total of 352; the majority of those uses are also in leave-on skin care preparations.

In the Council's use-concentration survey, ceramide 3 was reported to have a maximum use concentration of 0.2%, which was reported in lipstick and face and neck skin care preparations. 2-Oleamido-1,3-octadecanediol had a maximum use concentration of 0.7% reported in hair conditioners.

Ceramides are among the lipids that make up sphingomyelin, which is a major component of the lipid bilayer that forms cell membranes of cells in the stratum corneum. Thus, ceramides are lipophilic and likely to be absorbed into the skin. However, they are expected to remain in the epidermis and not penetrate any deeper. An absorption, distribution and excretion study of an analogous radiolabeled ceramide in male rats found that, following oral exposure, the ceramide was distributed gradually to the dermis and then transferred to the epidermis.

The oral LD_{50} was greater than 2000 mg/kg in rat studies of ceramide 2, ceramide AP, hydroxypalmitoyl sphinganine, and 2-oleamido-1,3-octadecanediol, and greater than 5000 mg/kg in rat studies of ceramide NP. In dermal studies, the LD_{50} was greater than 2000 mg/kg in rats exposed to ceramide NP, ceramide AP, or 2-oleamido-1,3-octadecanediol.

In an oral repeated dose toxicity study, the NOEL for 2-oleamido-1,3-octadecanediol was 30 mg/kg/day in rats. At 100 mg/kg in male rats, an increase in mean absolute and relative weights of the thymus was observed. In a dermal repeated dose toxicity study, the cutaneous tolerance of 1 g/kg 2-oleamido-1,3-octadecanediol in rats was good. No skin reactions or systemic effects were observed

The maternal NOAEL and mating and fertility NOEL were both 1000 mg/kg body weight/day, respectively, in a rat reproduction and developmental study of 2-oleamido-1,3-octadecanediol. In the1000 mg/kg/day dose group, one male had several lesions in the urinary tract, including urinary lithiasis, thickened and red urinary bladder mucosa, and dilated right ureter and pelvic cavity of the right kidney. The researchers could not determine whether this finding was treatment-related or a random event. No other treatment-related effects were noted in parental animals. No increased incidences of mortality, adverse effects on body weight, influence on sex ratio, or treatment-related gross malformations were noted in the pups.

Ceramide 2, ceramide NP, ceramide 5, ceramide AP, hydroxypalmitoyl sphinganine, and 2-oleamido-1,3-octadecanediol were not mutagenic in Ames assays. Additionally, 2-oleamido-1,3-octadecanediol was not polyploidy-inducing or clastogenic in a metaphase chromosome analysis in Chinese hamster lung cells at concentrations up to 5000 μg/ml with and without metabolic activation.

Data on carcinogenicity were not found for ceramides in the published literature, nor were unpublished data provided.

Undiluted ceramide 2 was mildly irritating to rabbit eyes in one study, but not irritating in another. Ceramide NP, ceramide AP, and hydroxypalmitoyl sphinganine were also not irritating in rabbit eye studies when tested neat. 2-Oleamido-1,3-octadecanediol was slightly irritating when tested neat in rabbit eyes. In dermal studies, ceramide 2 (up to 5% in corn oil and undiluted), ceramide 3 (concentration not reported), ceramide 5 (up to 5% in white petrolatum), ceramide AP (undiluted), hydroxypalmitoyl sphinganine (undiluted), and 2-oleamido-1,3-octadecanediol (undiluted) were not irritating to rabbit or guinea pig skin. Slight irritation was observed in a dermal irritation study in rabbits with ceramide NP in propylene glycol, but no irritation was observed in other dermal studies of the undiluted ingredient. No dermal irritation was observed in human patch tests of ceramide 2 (up to 5% in lanolin), ceramide NP (up to 10% Vaseline dispersion), ceramide 5 (up to 5% in lanolin), and ceramide AP (5% Vaseline dispersion).

Ceramide 2 (up to 20%), ceramide NP (up to 10%), ceramide 5 (up to 5%), and hydroxypalmitoyl sphinganine (up to 20%) were not sensitizing in guinea pig maximization tests.

DISCUSSION

The Panel considered the available data on ceramides and noted that there were no substantive data on reproductive and developmental toxicity or carcinogenicity. However, the Panel's concerns were reduced after reviewing the negative results of a reproductive and developmental toxicity study in rats and of *in vitro* genotoxicity assays, as well as the findings of no systemic toxicity at high doses in single and repeated oral dose animal studies, little to no irritation in ocular and dermal animal studies, no dermal irritation in human studies, and no dermal sensitization in multiple animal studies. The Panel noted that ceramides with structures that were identical or very similar to the structures of these cosmetic ingredients exist naturally in the stratum corneum, and commented that the ceramides that are cosmetic ingredients would not be readily absorbed through the skin.

The Panel determined that these ceramide ingredients are safe as used, assuming that the ingredients are not derived from bovine central nervous system tissues.

The Panel discussed the issue of incidental inhalation exposure from hair sprays and body and hand or moisturizing sprays. There were no inhalation toxicity data available. The Panel considered pertinent data indicating that incidental inhalation exposures to ceramides in such cosmetic products would not cause adverse health effects, including data characterizing the potential for ceramides to cause systemic toxicity, ocular or dermal irritation or sensitization, and other effects. The Panel noted that 95% – 99% of droplets/particles produced in cosmetic aerosols would not be respirable to any appreciable amount. The potential for inhalation toxicity is not limited to respirable droplets/particles deposited in the lungs. In principle, inhaled droplets/particles deposited in the nasopharyngeal and thoracic regions of the respiratory tract may cause toxic effects depending on their chemical and other properties. However, coupled with the small actual exposure in the breathing zone and the concentrations at which the ingredients are used, the available information indicates that incidental inhalation would not be a significant route of exposure that might lead to local respiratory or systemic effects. A detailed discussion and summary of the Panel's approach to evaluating incidental inhalation exposures to ingredients in cosmetic products is available at http://www.cir-safety.org/cir-findings.

CONCLUSION

The CIR Expert Panel concluded that the following ceramide ingredients are safe in cosmetics in the present practices of use and concentration described in this safety assessment:

ceramide 1
ceramide 2
ceramide 3
ceramide 4*
ceramide 5*
ceramide 1A
ceramide 6 II
ceramide AP
ceramide EOP
ceramide EOS
ceramide NP
ceramide NG*
ceramide NS
ceramide AS*
ceramide NS dilaurate*
caprooyl phytosphingosine
caprooyl sphingosine
hydroxypalmitoyl sphinganine
2-oleamido-1,3-octadecanediol
caproyl sphingosine*
hydroxylauroyl phytosphingosine*
hydroxycapryloyl phytosphingosine*
hydroxycaproyl phytosphingosine*

**Not reported to be in current use. Were ingredients in this group not in current use to be used in the future, the expectation is that they would be used in product categories and at concentrations comparable to others in this group.*

TABLES AND FIGURES

Table 1. Definitions and idealized structures of the ingredients in this safety assessment.[1]

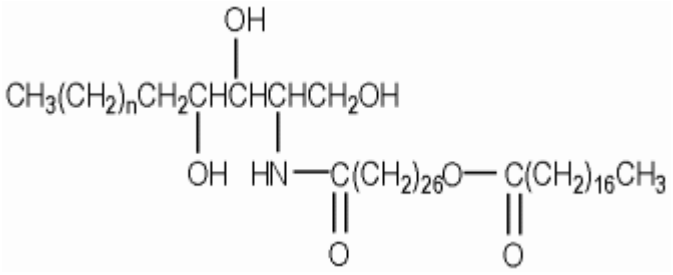
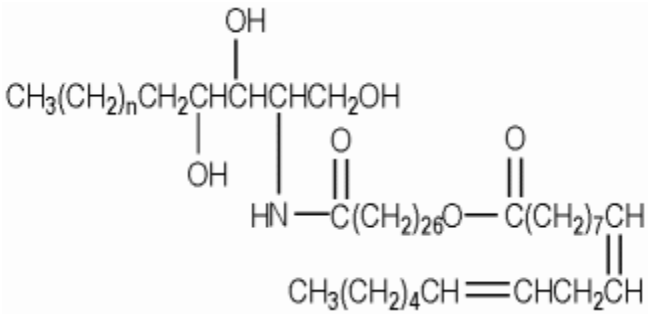
Ingredient CAS No.	Definition / Structure
Ceramide 1 (Retired) 100403-19-8	Ceramide 1 (Retired) is the *N*-acylated phytosphingosine having the erythro structure that conforms generally to the formula:  where n has a value ranging from 10 to 20. The INCI Name, Ceramide 1, originally developed in 1997, was designated with a retired status in 2014. For an interim period of time, trade name assignments formerly published with the INCI Name Ceramide 1 will be retained in the retired monograph, and also published with the new name assignment, **Ceramide EOP**. For further information, consult the Introduction, Retired INCI Names.
Ceramide 1A (Retired) 100403-19-8	Ceramide 1 A (Retired) is the *N*-acylated phytosphingosine having the erythro structure that conforms generally to the formula:  where n has a value ranging from 10 to 20. The INCI Name, Ceramide 1 A, originally developed in 1997, was designated with a retired status in 2014. For an interim period of time, trade name assignments formerly published with the INCI Name Ceramide 1 A will be retained in the retired monograph, and also published with the new name assignment, **Ceramide EOP**. For further information, consult the Introduction, Retired INCI Names.
Ceramide EOP	Ceramide EOP, formerly known under either of the INCI Names, Ceramide 1 or Ceramide 1 A, is the *N*-acylated sphingolipid consisting of Phytosphingosine having the D-erythro structure linked to an omega-hydroxy acid which is esterified with a saturated or unsaturated fatty acid.

Table 1. Definitions and idealized structures of the ingredients in this safety assessment.[1]

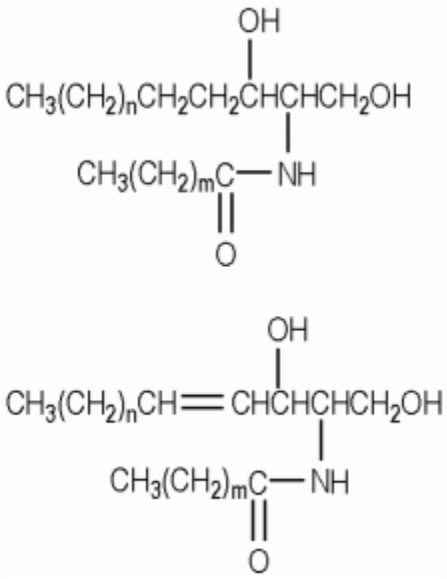
Ingredient CAS No.	Definition / Structure
Ceramide 2 (Retired) 100403-19-8	Ceramide 2 (Retired) is the *N*-acylated sphingolipid having the erythro structure that conforms generally to the formula:  where m has a value ranging from 14 to 28 and n has a value ranging from 10 to 16. The INCI Name, Ceramide 2, originally developed in 1997, was designated with a retired status in 2014. For an interim period of time, trade name assignments formerly published with the INCI Name Ceramide 2 will be retained in the retired monograph, and also published with the new name assignment as either **Ceramide NS or Ceramide NG**. For further information, consult the Introduction, Retired INCI Names.
Ceramide NS	Ceramide NS, formerly known under the INCI Name Ceramide 2, is the *N*-acylated sphingolipid consisting of sphingosine having the D-erythro structure linked to a normal saturated or unsaturated fatty acid. As opposed to the broader definition for Ceramide 2 which includes sphingolipids consisting of either sphingosine or dihydrosphingosine (sphinganine), Ceramide NS is limited to sphingosine-based ceramides.
Ceramide NG	Ceramide NG, formerly known under the INCI Name Ceramide 2, is the *N*-acylated sphingolipid consisting of Sphinganine having the D-erythro structure linked to a normal saturated or unsaturated fatty acid. As opposed to the broader definition for Ceramide 2 which includes sphingolipids consisting of either sphingosine or dihydrosphingosine (sphinganine), Ceramide NG is limited to sphinganine-based ceramides.

Table 1. Definitions and idealized structures of the ingredients in this safety assessment.[1]

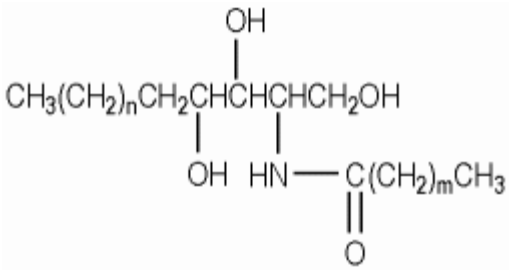
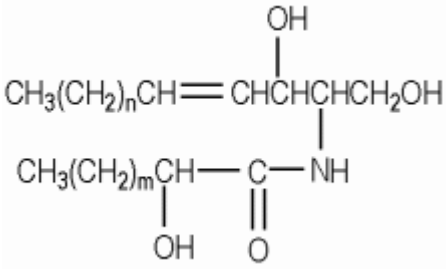
Ingredient CAS No.	Definition / Structure
Ceramide 3 (Retired) 100403-19-8 72968-43-5	Ceramide 3 (Retired) is the *N*-acylated phytosphingosine having the erythro structure that conforms generally to the formula:  where m has a value ranging from 12 to 28 in which the acyl moiety may be saturated, mono-unsaturated, or di-unsaturated and n has a value ranging from 10 to 20. The INCI Name, Ceramide 3, originally developed in 1997, was designated with a retired status in 2014. For an interim period of time, trade name assignments formerly published with the INCI Name Ceramide 3 will be retained in the retired monograph, and also published with the new name assignment, **Ceramide NP**. For further information, consult the Introduction, Retired INCI Names.
Ceramide NP	Ceramide NP is the *N*-acylated sphingolipid consisting of Phytosphingosine having the D-erythro structure linked to normal saturated or unsaturated fatty acid.
Ceramide 4 (Retired) 100403-19-8	Ceramide 4 (Retired) is the *N*-acylated sphingolipid having the erythro structure that conforms generally to the formula:  where m has a value ranging from 13 to 27 in which the acyl moiety may be saturated or mono-unsaturated and n has a value ranging from 10 to 16. Ceramide 4 is similar to Ceramide 5, however, the acylating hydroxy acids are generally shorter in Ceramide 4 than in Ceramide 5. The INCI Name, Ceramide 4, originally developed in 1997, was designated with a retired status in 2014. For an interim period of time, trade name assignments formerly published with the INCI Name Ceramide 4 will be retained in the retired monograph, and also published with the new name assignment, **Ceramide AS**. For further information, consult the Introduction, Retired INCI Names.

Table 1. Definitions and idealized structures of the ingredients in this safety assessment.[1]

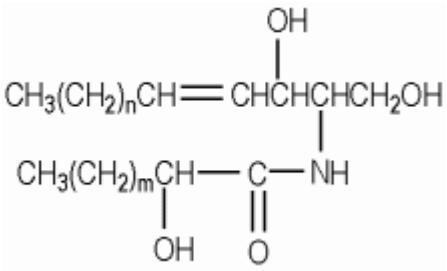
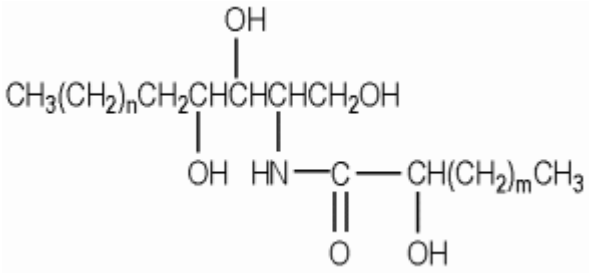
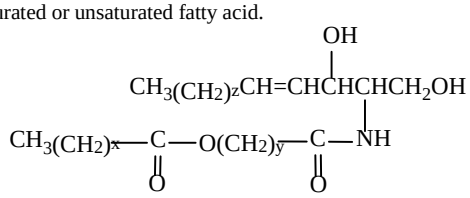
Ingredient CAS No.	Definition / Structure
Ceramide 5 (Retired) 100403-19-8	Ceramide 5 (Retired) is the *N*-acylated sphingolipid having the erythro structure that conforms generally to the formula:  where m has a value ranging from 13 to 27 in which the acyl moiety may be saturated or mono-unsaturated and n has a value ranging from 10 to 16. Ceramide 5 is similar to Ceramide 4, however, the acylating hydroxy acids are generally longer in Ceramide 5 than in Ceramide 4. The INCI Name, Ceramide 5, originally developed in 1997, was designated with a retired status in 2014. For an interim period of time, trade name assignments formerly published with the INCI Name Ceramide 5 will be retained in the retired monograph, and also published with the new name assignment, **Ceramide AS**. For further information, consult the Introduction, Retired INCI Names.
Ceramide AS	Ceramide AS, formerly known under either of the INCI Names, Ceramide 4 or Ceramide 5, is the *N*-acylated sphingolipid consisting of sphingosine having the D-erythro structure linked to an alpha-hydroxy saturated or unsaturated fatty acid.
Ceramide 6 II (Retired) 100403-19-8	Ceramide 6 II (Retired) is the *N*-acylated phytosphingosine having the erythro structure that conforms generally to the formula:  where m has a value ranging from 13 to 27 and n has a value ranging from 12 to 20. The INCI Name, Ceramide 6 II, originally developed in 1997, was designated with a retired status in 2014. For an interim period of time, trade name assignments formerly published with the INCI Name Ceramide 6 II will be retained in the retired monograph, and also published with the new name assignment, Ceramide AP. For further information, consult the Introduction, Retired INCI Names.
Ceramide AP	Ceramide AP, formerly known under the INCI name Ceramide 6 II, is the *N*-acylated sphingolipid consisting of Phytosphingosine having the D-erythro structure linked to an alpha-hydroxy saturated or unsaturated fatty acid.
Ceramide EOS	Ceramide EOS is the *N*-acylated sphingolipid consisting of sphingosine having the D-erythro structure linked to an esterified omega-hydroxy saturated or unsaturated fatty acid. 

Table 1. Definitions and idealized structures of the ingredients in this safety assessment.[1]

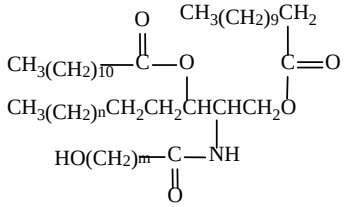
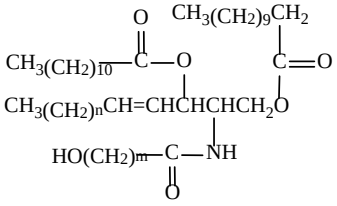
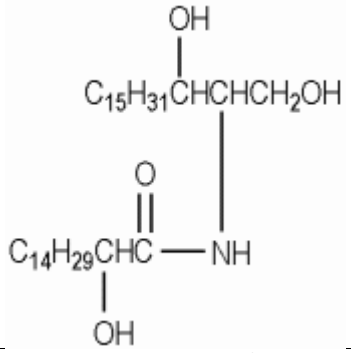
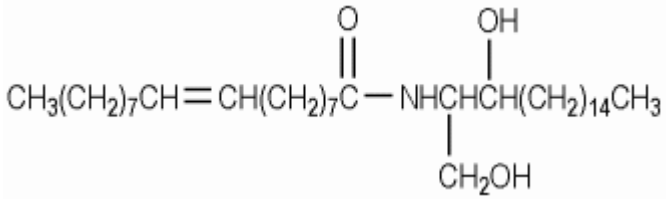
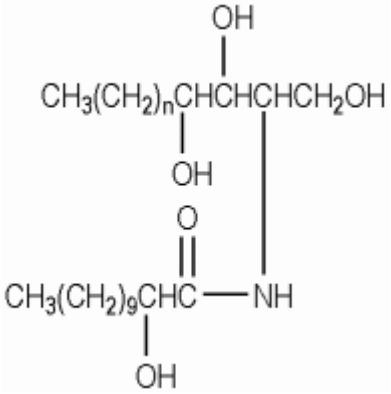
Ingredient CAS No.	Definition / Structure
Ceramide NS Dilaurate	Ceramide NS Dilaurate is the diester of Ceramide NS and lauric acid.  
Hydroxypalmitoyl Sphinganine	Hydroxypalmitoyl Sphinganine is the organic compound that conforms to the formula: 
2-Oleamido-1,3-Octadecanediol 54422-45-6	2-Oleamido-1,3-Octadecanediol is the organic compound that conforms to the formula: 
Hydroxylauroyl Phytosphingosine	Hydroxylauroyl Phytosphingosine is a synthetic *N*-acylated sphingolipid that conforms generally to the formula:  where n has a value ranging from 10 to 20.

Table 1. Definitions and idealized structures of the ingredients in this safety assessment.[1]

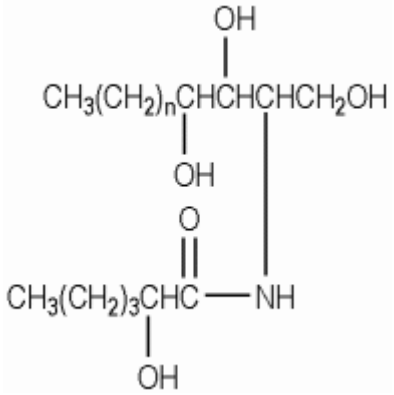
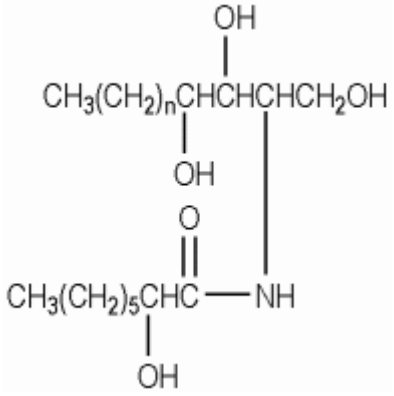
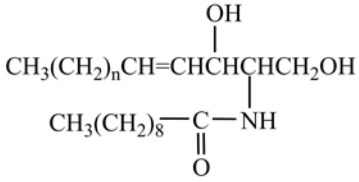
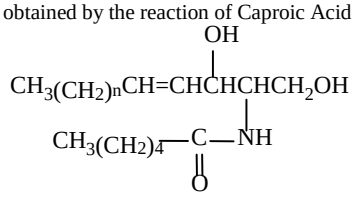
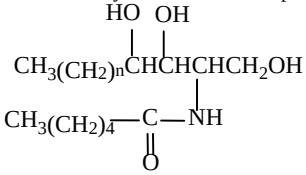
Ingredient CAS No.	Definition / Structure
Hydroxycaproyl Phytosphingosine	Hydroxycaproyl Phytosphingosine is a synthetic *N*-acylated sphingolipid that conforms generally to the formula:  where n has a value ranging from 10 to 20.
Hydroxycapryloyl Phytosphingosine	Hydroxycapryloyl Phytosphingosine is a synthetic *N*-acylated sphingolipid that conforms generally to the formula:  where n has a value from 10 to 20.
Caproyl Sphingosine 100403-19-8 (generic)	Caproyl Sphingosine is a synthetic *N*-acylated sphingolipid that conforms generally to the formula:  where n has a value of 10 to 16.
Caprooyl Sphingosine 100403-19-8 (generic)	Caprooyl Sphingosine is the product obtained by the reaction of Caproic Acid and sphingosine. 
Caprooyl Phytosphingosine	Caprooyl Phytosphingosine is the product obtained by the reaction of Caproic Acid and Phytosphingosine. 

Table 2. Chemical properties of ceramides

Property	**Value**	**Reference**
Ceramide 2		
Physical Form	Creamy white crystals	[55]
Molecular Weight g/mol	511.90	[55]
Melting Point ^{o}C	90.0-100.0	[55]
Flash Point ^{o}C	> 100	[55]

Table 3. Frequency (2014) and concentration of use (2013 and 2014) according to duration and type of exposure for ceramide ingredients.[13-15]

	# of Uses	*Max Conc of Use (%)*	*# of Uses*	*Max Conc of Use (%)*	*# of Uses*	*Max Conc of Use (%)*	*# of Uses*	*Max Conc of Use (%)*
	Ceramide 1		**Ceramide 2**		**Ceramide 3**		**Ceramide 1A**	
Totals[†]	**54**	**0.0000005-0.1**	**110**	**0.000005-0.2**	**359**	**0.00000001-0.2**	**NR**	**0.01**
Duration of Use								
Leave-On	48	0.0000005-0.1	99	*0.000005-0.2*	298	0.00000001-0.2	NR	0.01
Rinse Off	6	0.00001-0.00065	11	*0.0001-0.1*[c]	61	0.000001-0.013	NR	NR
Diluted for (Bath) Use	NR	NR	NR	*NR*	NR	NR	NR	NR
Exposure Type								
Eye Area	8	0.00002-0.00005	30	0.000005-0.2	29	0.002-0.05	NR	0.01
Incidental Ingestion	4	0.001	9	0.018-0.064	48	0.01-0.2	NR	NR
Incidental Inhalation-Spray	13[a]; 12[b]	0.00065	30[a]; 12[b]	0.0001; 0.01[a]	2; 109[a]; 63[b]	0.00000001-0.001	NR	NR
Incidental Inhalation-Powder	12[b]	0.0001	1; 12[b]	0.01	2; 63[b]; 2[d]	0.0001-0.01	NR	NR
Dermal Contact	47	0.0000005-0.045	86	0.000005-0.2	259	0.00005-0.2	NR	0.01
Deodorant (underarm)	NR	NR	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	NR	0.000325-0.00065	10	0.0001-0.01	48	0.00000001-0.013	NR	NR
Hair-Coloring	NR	NR	NR	NR	NR	NR	NR	NR
Nail	NR	0.1	NR	0.0004	NR	NR	NR	NR
Mucous Membrane	4	0.001	9	0.018-0.064	55	0.01-0.2	NR	NR
Baby Products	NR	NR	NR	NR	3	NR	NR	NR

	Ceramide AP		**Ceramide EOP**		**Ceramide EOS**		**Ceramide 6 II**	
Totals[†]	**14**	**0.00005-0.2**	**14**	**0.000001-0.01**	**14**	**0.01**	**52**	**0.00003-0.2**
Duration of Use								
Leave-On	14	0.00005-0.2	14	0.000001-0.01	14	0.01	48	0.00003-0.2
Rinse Off	NR	0.0005	NR	0.00001	NR	NR	4	0.0001-0.00065
Diluted for (Bath) Use	NR	NR	NR	NR	NR	NR	NR	NR
Exposure Type								
Eye Area	1	0.0025-0.01	1	0.00005-0.01	1	NR	8	0.0025-0.01
Incidental Ingestion	NR	0.01	NR	0.001	NR	NR	4	0.01
Incidental Inhalation-Spray	2[a]; 11[b]	NR	2[a]; 11[b]	0.01	2[a]; 11[b]	0.01	12[a];12[b]	0.00003-0.00065
Incidental Inhalation-Powder	11[b]	0.01	11[b]	0.0001	11[b]	NR	12[b]	0.01
Dermal Contact	14	0.00005-0.2	14	0.000001-0.01	14	0.01	46	0.00003-0.2
Deodorant (underarm)	NR	NR	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	NR	NR	NR	NR	NR	NR	NR	0.00033-0.00065
Hair-Coloring	NR	NR	NR	NR	NR	NR	NR	NR
Nail	NR	NR	NR	NR	NR	NR	NR	NR
Mucous Membrane	NR	0.01	NR	0.001	NR	NR	4	0.01
Baby Products	NR	NR	NR	NR	NR	NR	NR	NR

Table 3. Frequency (2014) and concentration of use (2013 and 2014) according to duration and type of exposure for ceramide ingredients.[13-15]

	# of Uses	*Max Conc of Use (%)*	*# of Uses*	*Max Conc of Use (%)*	*# of Uses*	*Max Conc of Use (%)*	*# of Uses*	*Max Conc of Use (%)*
	Ceramide NP		**Ceramide NS**		**Caprooyl Phytosphingosine**		**Caprooyl Sphingosine**	
Totals†	**16**	**0.00005-0.2**	**14**	**0.001-0.006**	**24**	**0.001**	**14**	**0.00033-0.00065**
Duration of Use								
Leave-On	16	0.00005-0.2	14	0.001-0.006	24	0.001	14	0.00065
Rinse Off	NR	0.0005-0.01	NR	0.001	NR	NR	NR	0.00033-0.00065
Diluted for (Bath) Use	NR	0.001	NR	NR	NR	NR	NR	NR
Exposure Type								
Eye Area	2	0.0025-0.005	1	NR	2	NR	1	NR
Incidental Ingestion	NR	0.2	NR	NR	NR	NR	NR	NR
Incidental Inhalation-Spray	2[a]; 11[b]	NR	2[a]; 11[b]	NR	2[a]; 12[b]	NR	2[a]; 11[b]	0.00065
Incidental Inhalation-Powder	11[b]	0.01	11[b]	NR	5; 12[b]	NR	11[b]	NR
Dermal Contact	16	0.00005-0.1	14	0.001-0.006	24	0.001	14	NR
Deodorant (underarm)	NR	NR	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	NR	NR	NR	NR	NR	NR	NR	0.00033-0.00065
Hair-Coloring	NR	NR	NR	NR	NR	NR	NR	NR
Nail	NR	NR	NR	NR	NR	NR	NR	NR
Mucous Membrane	NR	0.001-0.2	NR	NR	NR	NR	NR	NR
Baby Products	1	NR	NR	NR	NR	NR	NR	NR

	Hydroxypalmitoyl Sphinganine		**2-Oleamido-1,3-Octadecanediol**	
Totals†	**57**	**0.0025-0.062**	**352**	**0.01-0.7**
Duration of Use				
Leave-On	53	0.0025-0.062	241	0.01-0.2
Rinse Off	4	0.0025-0.0049	111	0.01-0.7
Diluted for (Bath) Use	NR	NR	NR	NR
Exposure Type				
Eye Area	5	0.0049	46	0.01-0.2
Incidental Ingestion	10	0.025	2	0.01
Incidental Inhalation-Spray	24[a]; 10[b]	NR	2; 43[a]; 7[b]	0.012-0.05; 0.05-0.1
Incidental Inhalation-Powder	10[b]	0.0025	7[b]	NR
Dermal Contact	47	0.0025-0.062	51	0.01-0.2
Deodorant (underarm)	NR	NR	NR	NR
Hair - Non-Coloring	NR	NR	250	0.011-0.7
Hair-Coloring	NR	NR	6	0.01-0.1
Nail	NR	NR	NR	0.01-0.014
Mucous Membrane	10	0.025	2	0.01
Baby Products	NR	NR	NR	NR

NR = Not reported.

† Because each ingredient may be used in cosmetics with multiple exposure types, the sum of all exposure types may not equal the sum of total uses.

[a]. It is possible these products may be sprays, but it is not specified whether the reported uses are sprays.

[b]. Not specified whether a powder or a spray, so this information is captured for both categories of incidental inhalation.

[c]. 0.1% in a rinse-off "other" skin care preparation.

[d]. It is possible these products may be powders, but it is not specified whether the reported uses are powders.

Table 4. Ingredients that are not reported to be in use

Ceramide 4
Ceramide 5
Ceramide AS
Ceramide NG
Ceramide NS Dilaurate
Caproyl Sphingosine
Hydroxylauroyl Phytosphingosine
Hydroxycapryloyl Phytosphingosine
Hydroxycaproyl Phytosphingosine

Table 5. Acute toxicity studies.

Ingredient	Concentration/Dose	Animal System	Method	Results	Reference
		Oral			
Ceramide 2	0%, 5%, 10%, or 20% (w/v) (0, 500, 1000, or 2000 mg/kg) in olive oil; dose volume10 ml/kg.	5 male and 5 female Sprague-Dawley rats	Oral gavage	LD_{50} > 20% (2000 mg/kg) for both sexes; no mortalities; non-significant dose-dependent depression in body weight gain was observed in both males and females from 7 days post-administration; diarrhea was observed in some male and female rats in the 5% and 10% dose groups, but diarrhea was also observed in the control group and thus was attributed to the vehicle and not the test material; no other adverse effects observed	[29]
Ceramide 2	2000 mg/kg (no further details provided)	rats (no further details provided)	Oral	LD_{50} > 2000 mg/kg; no signs of toxicity observed	[30]
Ceramide NP	5000 mg/kg (no further details provided)	5 male and 5 female rats (strain not reported)	Oral gavage	LD_{50} > 5000 mg/kg; ataxia noted after each dosing; no other adverse effects observed	[22]
Ceramide NP	5000 mg/kg body weight in propylene glycol given as 2 dosages of 2500 mg/kg body weight within 24 h	5 male and 5 female Wistar rats	Oral gavage	LD_{50} > 5000 mg/kg; ataxia noted after each dosing; no other adverse effects observed	[26]
Ceramide NP	2000 mg/kg body weight in propylene glycol; dose volume 10 ml/kg body weight	2 male and 2 female Wistar rats	Oral gavage	LD_{50} > 2000 mg/kg; uncoordinated movement 2 and 4 h post-treatment in all animals; piloerection in 1 female 2 h post-treatment	[27]
Ceramide AP	2000 mg/kg body weight in propylene glycol; dose volume 10 ml/kg body weight	2 male and 2 female Wistar rats	Oral gavage	LD_{50} > 2000 mg/kg; uncoordinated movement 2 and 4 h post-treatment in all animals; hunched posture in 1 female 2 and 4 h post-treatment	[28]
Hydroxypalmitoyl Sphinganine	2000 mg/kg in methylcellulose; dose volume 10 ml/kg	5 male and 5 female Sprague-Dawley rats	Oral gavage	LD_{50} > 2000 mg/kg; body weight gain not affected by treatment; no mortalities; no abnormalities at necropsy	[24]
2-Oleamido-1,3-Octadecanediol	2000 mg/kg in 0.5% methylcellulose, dose volume 10 ml/kg	5 female Sprague-Dawley rats	Oral gavage	Maximal non-lethal dose was 2000 mg/kg; body weight gain slightly decreased in 2/5 animals during week 1 and 1/5 animals during week 2 but overall comparable to historical controls; no abnormalities at necropsy	[25]
		Dermal			
Ceramide NP	2000 mg/kg body weight in propylene glycol; dose volume 10 ml/kg body weight	2 male and 2 female Wistar rats	Dermal patch (no further details provided)	LD_{50} > 2000 mg/kg; body weight gain not affected by treatment; no mortalities; no adverse effects observed	[27]
Ceramide AP	2000 mg/kg body weight in propylene glycol	2 male and 2 female Wistar rats	Dermal patch for 24 h	LD_{50} > 2000 mg/kg; body weight gain not affected by treatment; no mortalities; no adverse effects observed	[28]
Ceramide AP	2000 mg/kg body weight in propylene glycol	2 male and 2 female Wistar rats	Dermal patch for 24 h	LD_{50} > 2000 mg/kg; body weight gain not affected by treatment; no mortalities; no adverse effects observed	[28]
2-Oleamido-1,3-Octadecanediol	2000 mg/kg	5 male and 5 female Sprague-Dawley rats	Dermal patch semi-occluded for 24 h	LD_{50} > 2000 mg/kg; body weight gain not affected by treatment; no mortalities; no abnormalities at necropsy	[23]

Table 6. Genotoxicity studies

Ingredient	Concentration/Dose	Method	Results	Reference
In Vitro				
Ceramide 2	0, 4.88, 19.5, 78.1, 313, 1250, or 5000 µg/plate with and without metabolic activation	Ames test in *Salmonella typhimurium* strains TA 98, TA 100, TA 1535, TA and 1537 and *Escherichia coli* strain wP2uvrA	Not mutagenic	[34]
Ceramide 2	50, 150, 500, or 1500 µg/plate with and without metabolic activation	Ames test in *S. typhimurium strains* TA 98, TA 100, TA 1535, and TA 1537	Not mutagenic	[35]
Ceramide 2	4.98-79.75 µg/plate (no further details provided)	Ames test in *S. typhimurium* (no further details provided)	Not mutagenic	[30]
Ceramide NP	100, 333, 1000, 3330, or 5000 µg/plate with and without metabolic activation	Ames test in *S. typhimurium* strains TA 98, TA 100, TA 1535, and TA 1537	Not mutagenic	[26]
Ceramide NP	3, 10, 33, 100, 333, 1000, 3330, or 5000 µg/plate with and without metabolic activation	Ames test in *S. typhimurium* strains TA 98 and TA 100	Not mutagenic	[27]
Ceramide 5	Up to 5000 µg/plate with and without metabolic activation	Ames test in *S. typhimurium* strains TA 98, TA 100, TA 1535, and TA 1537 and in *E. coli* strain wP2uvrA	Not mutagenic	[36]
Ceramide AP	3, 10, 33, 100, 333, 1000, 3330, or 5000 µg/plate with and without metabolic activation	Ames test in *S. typhimurium* strains TA 98 and TA 100	Not mutagenic	[28]
Ceramide AP	3, 10, 33, 100, 333, 1000, 3330, or 5000 µg/plate with and without metabolic activation	Ames test in *S. typhimurium* strains TA 98 and TA 100	Not mutagenic	[28]
Hydroxypalmitoyl Sphinganine	25, 50, 100, 200, or 400 µg/plate with and without metabolic activation	Ames test in *S. typhimurium* strains TA 98, TA 100, TA 1535, and TA 1537 and in *E. coli* strain wP2uvrA	Not mutagenic	[37]
2-Oleamido-1,3-Octadecanediol	312.5, 625, 1250, 2500 or 5000 µg/plate with and without metabolic activation	Ames test in *S. typhimurium* strains TA 98, TA 100, TA 1535, and TA 1537 and in *E. coli* strain wP2uvrA	Not mutagenic	[38]
2-Oleamido-1,3-Octadecanediol	1250, 2500, or 5000 µg/ml with and without metabolic activation	Metaphase chromosome analysis in Chinese hamster lung cells; 3 treatment periods without metabolic activation consisted of a 6 h treatment with cell harvest 18 h later and 24 and 48 h treatments with immediate cell harvest after; 1 treatment period with metabolic activation consisted of a 6 h treatment with cell harvest 18 h later	No evidence of either polyploidy-inducing or clastogenic activity	[39]

Table 7. Non-human ocular irritation studies.

Ingredient	Concentration/Dose	Method	Results	Reference
Ceramide 2	100 mg undiluted	Eye irritation study in 3 rabbits (no further details provided)	No irritation	[30]
Ceramide 2	0.1 g undiluted	Eye irritation study in 6 male Japanese white rabbits; eyes washed in 3 rabbits 30 sec after treatment	Mildly irritating; all 3 rabbits in the non-washed group had mild discharge and moderate redness in the conjunctivae 1 h post-treatment, with slight chemosis in 1 animal, adverse effects disappeared by day 3 in two of the rabbits and by day 4 in the third; mild discharge in 2 rabbits and mild redness in 3 rabbits observed in conjunctivae of the washed group 1 h post-treatment, the discharge disappeared by day 1 post-treatment and all 3 animals were recovered by day 2; no other adverse effects were observed.	[43]
Ceramide NP	100 mg	Eye irritation study in New Zealand White rabbits; eyes assessed daily for 1 week post-treatment (no further details provided)	No irritation	[40]
Ceramide NP	27 mg (~ 0.1 ml) undiluted powder	Eye irritation study in 3 New Zealand White rabbits; test material instilled into conjunctival sac of one eye and rinsed after 24 h; reactions observed 1, 24, 48, and 72 h post-treatment	Not irritating; conjunctiva score of 1 in one rabbit at 1 and 48 h and 2 at 24 h; iris score of 1 in all rabbits at 1 h; chemosis score of 1 in one rabbit at 1 h; all effects reversed by 72 h (no further details provided)	[26]
Ceramide NP	51 mg (~ 0.1 ml) undiluted powder	Eye irritation study in 1 male New Zealand White rabbit; test material instilled into conjunctival sac of one eye for 24 h; reactions assessed 1, 24, 48, and 72 h post-treatment	Not irritating; conjunctiva score of 2 at 1 and 24 h and score of 1 at 48 h; iris score of 1 at 1 h; chemosis score of 2 at 1 h and score of 1 at 24 h; all effects reversed by 72 h (no further details provided)	[27]
Ceramide AP	51 mg (~ 0.1 ml) undiluted powder	Eye irritation study in 1 male New Zealand White rabbit; test material instilled into conjunctival sac of one eye for 24 h; reactions assessed 1, 24, 48, and 72 h post-treatment	Not irritating; conjunctiva score of 2 at 1 h and score of 1 at 24 h; chemosis score of 1 at 1 h; all effects reversed by 48 h (no further details provided)	[28]
Ceramide AP	64.7 mg (~ 0.1 ml) undiluted powder	Eye irritation study in 1 male New Zealand White rabbit; test material instilled into conjunctival sac of one eye for 24 h; reactions assessed 1, 24, 48, and 72 h post-treatment	Not irritating; conjunctiva score of 2 at 1 h and score of 1 at 24 and 48 h; chemosis score of 1 at 1 h; all effects reversed by 72 h (no further details provided)	[28]
Hydroxypalmitoyl Sphinganine	100 mg in powder form	Eye irritation study in 3 male New Zealand White rabbits; test material instilled into conjunctival sac of left eye; eyes not rinsed; reactions observed 1, 24, 48, and 72 h post-treatment	Not irritating; very slight or slight conjunctival inflammation observed 1 h post-treatment in 2/3 animals and for 48 h in the remaining animal; very slight corneal opacity noted 24 h post-treatment in the last animal; no reactions after 48 h in any animal	[41]
2-Oleamido-1,3-Octadecanediol	100 mg in powder form	Eye irritation study in 3 male New Zealand White rabbits; test material instilled into conjunctival sac of left eye; eyes not rinsed; reactions observed 1, 24, 48, and 72 h post-treatment	Slightly irritating; very slight to moderate conjunctival reactions (very slight to moderate chemosis, very slight or slight redness of the conjunctiva and discharge) were observed in all animals from day 1, with some reactions persisting up to day 8; iritis was noted in one animal on day 1	[42]

Table 8. Dermal irritation studies.

Ingredient	Concentration/Dose	Method	Results	Reference
		Non-Human		
Ceramide 2	2% or 5% in corn oil	Skin irritation study in 5 female Hartley/Dunkin albino guinea pigs; non-occluded patches (dose volume 0.02 ml, dose area 1.5 cm^2) on clipped, intact skin; patch sites assessed at 24 and 48 h post-application	Not irritating	[50]
Ceramide 2	undiluted	Acute dermal irritation study in 3 rabbits (no further details provided)	Not irritating	[30]
Ceramide 3	not reported	Skin irritation study in 6 male New Zealand White rabbits; semi-occluded patches on clipped skin for 24 h; patch sites assessed at 1, 24, 48, and 72 h post-patch removal	Not irritating	[44]
Ceramide NP	not reported	Skin irritation study in 6 male New Zealand White rabbits; semi-occluded patch on clipped intact skin for 24 h; patch sites assessed at 1, 24, 48, and 72 h post-patch removal	Not irritating	[44]
Ceramide NP	0.5 g, undiluted, powder form	Skin irritation study in 3 New Zealand White rabbits (sex not reported); semi-occluded patch (dose area 6 cm^2) on clipped intact skin for 4 h; patch sites assessed at 50 min, 24, 48, and 72 h post-patch removal	Not irritating	[26]
Ceramide NP	0.5 g in 0.8 ml of propylene glycol	Skin irritation study in 6 New Zealand White rabbits (sex not reported): semi-occluded patch (dose area 6 cm^2) on clipped intact skin for 4 h that was repeated on the same application sites for a total of 10 applications; patch sites assessed for up to 44 h after last patch	Slightly irritating; very slight or well defined erythema with or without very slight edema; no indication of enhancement of skin irritation after daily, repeated exposure to same skin-area and no signs of irreversible effects during the observation period	[26]
Ceramide NP	0.5 g, undiluted powder form	Skin irritation study in 1 male New Zealand White rabbit; semi-occluded patch on clipped, intact skin for 4 h; patch sites assessed at 1, 24, 48, and 72 h post-patch removal	Not irritating	[27]
Ceramide 5	2% or 5% in white petrolatum	Skin irritation study in 5 female Std:Hartley series albino guinea pigs; non-occluded patches (dose volume 0.02 ml, dose area 1.5 cm^2) on clipped, intact skin; patch sites assessed at 24 and 48 h post-application	Not irritating	[51]
Ceramide AP	0.5 g, undiluted powder form	Skin irritation study in 1 male New Zealand White rabbit; semi-occluded patch on clipped, intact skin for 4 h; patch sites assessed at 1, 24, 48, and 72 h post-patch removal	Not irritating	[28]
Hydroxypalmitoyl Sphinganine	undiluted powder form moistened with 0.5 ml distilled water	Skin irritation study in 3 male New Zealand White rabbits; semi-occluded (500 mg dose; dose area 6 cm^2) on clipped skin; patch sites assessed at 1, 24, 48, and 72 h post-patch removal	Very slight erythema in 2 animals on days 2 and 3; not irritating	[45]
2-Oleamido-1,3-Octadecanediol	undiluted powder form	Skin irritation study in 3 male New Zealand White rabbits; semi-occluded (500 mg dose) for up to 4 h on clipped skin; patch sites assessed at 1, 24, 48, and 72 h post-patch removal	Very slight erythema noted in 1 animal with a mean score of 0.7; not irritating	[46]

Table 8. Dermal irritation studies.

Ingredient	Concentration/Dose	Method	Results	Reference
		Human		
Ceramide 2	5% in lanolin	Human patch test in 43subjects; test material applied on upper arms with "TORII test plaster"; sites occluded for 24 h; sites assessed 2 and 24 h post-plaster removal	No dermal irritation	[49]
Ceramide 2	concentration not reported	Human patch test in 40 subjects; sites occluded for 24 h; 0.1 g on 17 cm diameter patch	No dermal irritation	[47]
Ceramide NP	10% Vaseline dispersion	Human patch test in 33 subjects; sites occluded for 24 h; sites assessed 1 and 24 h post-patch removal; 0.01 g in a Finn chamber	No dermal irritation	[26]
Ceramide NP	5% Vaseline solution	Human patch test in 44 subjects; sites occluded for 24 h; sites assessed 1 and 24 h post-patch removal; 0.01 g in a Finn chamber	No dermal irritation	[27]
Ceramide 5	3 % and 5% in lanolin	Human patch test in 43subjects; test material applied on upper arms with "TORII test plaster"; sites occluded for 24 h; sites assessed 2 and 24 h post-plaster removal	No dermal irritation	[48]
Ceramide AP	5% Vaseline solution	Human patch test in 44 subjects; sites occluded for 24 h; sites assessed 1 and 24 h post-patch removal; 0.01 g in a Finn chamber	No dermal irritation	[28]

Table 9. Non-human dermal sensitization studies.

Ingredient	Concentration	Method	Results	Reference
Ceramide 2	20% w/w	Skin sensitization test (Buehler test) in 10 male and 10 female guinea pigs with 5 male and 5 female control guinea pigs (no further details provided)	Not sensitizing	[30]
Ceramide 2	5% (w/w) for dermal induction; 2% and 5% for challenge; vehicle was corn oil	Guinea pig maximization test in 10 female Hartley/Dunkin albino guinea pigs; additional group of 10 received distilled water as control	Not sensitizing	[52]
Ceramide NP	5% (w/w) for intradermal induction; 25% (w/w) for dermal induction; and 2%, 5%, and 10% (w/w) for challenge; vehicle was propylene glycol	Guinea pig maximization test using 10 male and 10 female Himalayan albino guinea pigs for the test material	Not sensitizing	[26]
Ceramide 5	5% (w/w) for dermal induction; 2% and 5% for challenge; vehicle was white petrolatum	Guinea pig maximization test using 5 female Std: Hartley series albino guinea pigs; additional group of 2 guinea pigs were negative control	Not sensitizing	[53]
Hydroxypalmitoyl Sphinganine	2% (w/w)for intradermal induction, 20% (w/w) for dermal induction, 20% in challenge; vehicle was paraffin oil	Guinea pig maximization test using 10 male and 10 female Dunkin Hartley guinea pigs for the test material	Not sensitizing	[54]

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