Article

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Safety Assessment of Alkyl Betaines as Used in Cosmetics

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Abstract

The Cosmetic Ingredient Review Expert Panel (Panel) reviewed the safety of 11 alkyl betaines as used in cosmetics. These ingredients are reported to function as hair and skin conditioning agents, antistatic agents, surfactants-cleansing agents, and viscosity-increasing agents in cosmetic products. Although there are data gaps, the shared chemical core structure, similar functions and concentrations of use in cosmetics, and the expected similarities in physicochemical properties enabled grouping these ingredients and reading across the available toxicological data to support the safety assessment of each individual compound in the entire group. The Panel concluded alkyl betaines were safe as cosmetic ingredients in the present practices of use and concentration, when formulated to be nonirritating.

Keywords

alkyl betaines, cosmetics, safety

Introduction

This safety assessment addresses the safety of 11 alkyl betaines as used in cosmetics. The parent compound, betaine, is a naturally occurring *N*-trimethylated amino acid, also called trimethylglycine, and can be isolated from sugar beets.[1] It is a common component in the human diet. As given in the *International Cosmetic Ingredient Dictionary and Handbook*, these ingredients are reported to function as hair and skin conditioning agents, antistatic agents, surfactants-cleansing agents, and viscosity-increasing agents in cosmetic products. The Cosmetic Ingredient Review (CIR) Expert Panel (Panel) has reviewed the safety of cocamidopropyl betaine and related amidopropyl betaines as used in cosmetics.[2] The Panel concluded that these ingredients "were safe in cosmetics as long as they are formulated to be nonsensitizing, which may be based on a quantitative risk assessment."

The common core chemical structure, similar functions and concentrations in cosmetics, and the predicted physicochemical properties enabled grouping of these ingredients and reading across the available toxicological data to support the safety assessment of each individual compound in this group. Toxicological data on betaine and betaine analogs (synonym: betaines, C12-C14 [even numbered] alkyldimethyl, or C12-C14 alkyldimethyl betaines) in this safety assessment were obtained from robust summaries of data submitted to the European Chemical Agency (ECHA) by companies as part of the REACH chemical registration process. These data are available on the ECHA website.[3,4]

Chemistry

The definitions of the 11 alkyl betaines in this safety assessment and formulas and idealized structures can be found in Table 1. The alkyl betaines are zwitterionic ingredients comprised of tertiary ammonium substituted acetate. These ingredients have a core structure of 2-(alkyldimethylammonio)acetate (ie, *N*,*N*,*N*-trisubstituted glycine; Figure 1).

Therein, the "alkyl" is either methyl, as in the case of betaine itself, or a longer chain alkyl group ranging in length from about 10 (eg, decyl betaine) to about 22 (eg, behenyl betaine) carbons. However, C10 to C22 is an estimate, as the compositions of the ingredients derived from plant and animal

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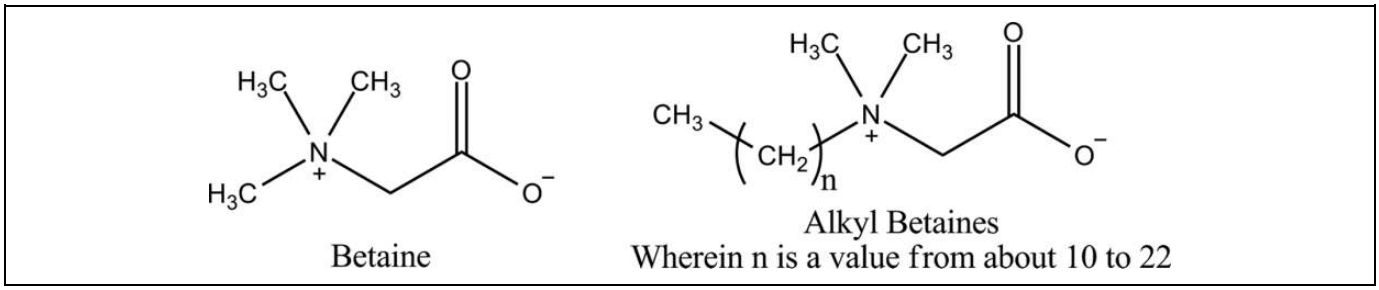


Figure 1. Betaine and the alkyl betaines.

sources (eg, coco-betaine, tallow betaine, and hydrogenated tallow betaine) are variable.

The zwitterionic structures of these ingredients make them amphoteric, a hallmark characteristic of surfactants. The fatty chains, found on most of these ingredients, add a lipophilic tail to these hydrophilic head groups, further imparting surfactant properties. Those ingredients for which chemical property data were available are colorless, crystalline materials with good solubility in water and polar organic solvents. While ingredients that vary from these only by incremental alkyl chain length changes are likely to have similar profiles, chemical and physical properties were neither publicly available nor submitted by other parties for any of the other ingredients.

Physical and Chemical Properties

Available chemical properties can be found in Table 2.

Method of Manufacturing

Betaine. Betaine (food grade) may be extracted from sugar beets via liquid chromatographic separation from sugar beet molasses.[5,6] It is subsequently refined and crystallized. Betaine anhydrous (as animal feed additive) is produced by reacting chloroacetic acid and sodium hydroxide with heat and stirring.[7] Trimethylamine is then added to the mixture, and the resultant solutions are filtered and purified. Betaine hydrochloride (as animal feed additive) follows the same synthesis pathway as betaine anhydrous, except that hydrochloric acid is added and the filtrate is purified.[7]

Coco-betaine. In data supplied by a manufacturer, coco-betaine is produced by reacting fatty dimethyl amines from coconuts with chloroacetic acid in aqueous solution.[8]

Impurities

Betaine. Betaine (food grade) contains very small quantities of chloride, sulfate, and heavy metals.[6] Trace analysis shows very small amounts of polychlorinated biphenyl (PCB), polycyclic aromatic hydrocarbon, and dioxins. No pesticide traces have been detected. Betaine does not contain methanol, ethanol, or isopropanol (limits of detections were 5.0, 2.5, and 0.5 ppm, respectively).

Betaine anhydrous and betaine hydrochloride (as animal feed additives) contained <2.0 mg/kg arsenic and <10 mg/kg heavy metals (expressed as lead).[7] Dioxin content was <0.50 ng/kg and PCB content was <0.35 mg/kg. Betaine content for the anhydrous and hydrochloride forms was $\geq 96\%$ and $\geq 93\%$, respectively.

Coco-betaine. According to information supplied by a manufacturer, coco-betaine is composed of approximately 31% coco-betaine, 7% sodium chloride, and 62% water.[8] There are no solvents, preservatives, or other additives. The product may contain a maximum of 100 ppm dichloroacetic acid, 100 ppm monochloroacetic acid, 0.5% free amines, 2% glycolic acid, 20 ppm heavy metals (copper, lead, tin, platinum, palladium, mercury, arsenic, cadmium, antimony, nickel, chromium, and cobalt), 2 ppm arsenic, 10 ppm iron, and $<3\%$ volatile organic compounds.

C12 to C14 alkyldimethyl betaines. According to information supplied to ECHA, betaines, C12 to 14 alkyldimethyl (a mixture of lauryl betaine and myristyl betaine) consists of betaine, C12-alkyldimethyl; betaine, C14-alkyldimethyl; (carboxylatomethyl) hexadecyldimethylammonium; sodium chloride; sodium glycolate; and unknown impurities.[4] Percentage composition was not provided, and there are no further details.

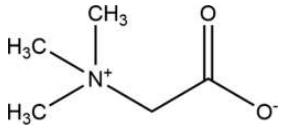
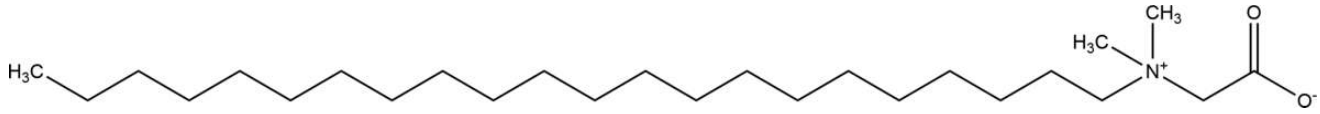
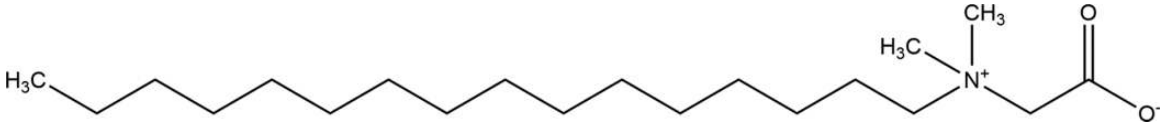
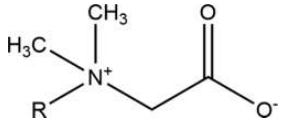
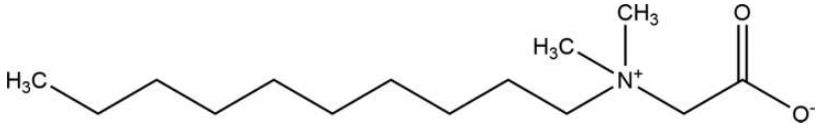
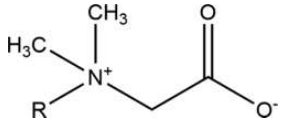
Use

Cosmetic

The safety of the cosmetic ingredients addressed in this safety assessment is evaluated based on data received from the US Food and Drug Administration (FDA) and the cosmetics industry on the expected use of these ingredients in cosmetics. Use frequencies of individual ingredients in cosmetics are collected from manufacturers and reported by cosmetic product category in the FDA Voluntary Cosmetic Registration Program (VCRP) database. Use concentration data are submitted by the cosmetic industry in response to a survey, conducted by the Personal Care Products Council (Council), of maximum reported use concentrations by product category.

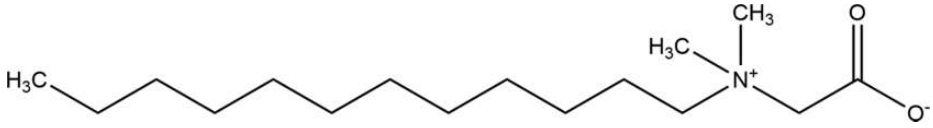
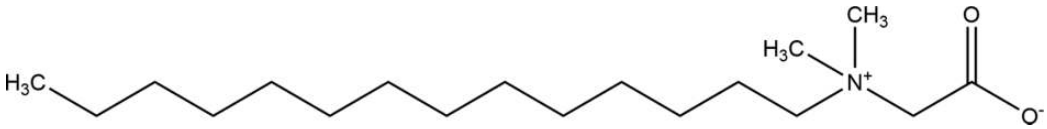
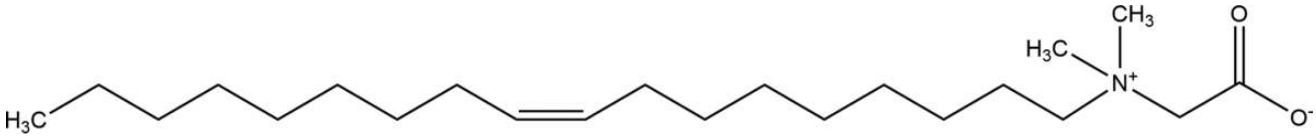
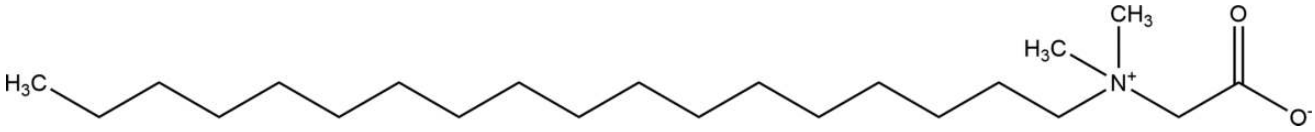
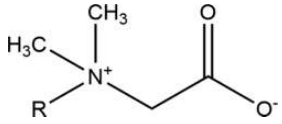
Table 3 presents the current product formulation data for alkyl betaines. Betaine mainly functions as a hair conditioning agent, humectant, and skin conditioning agent—humectant in cosmetic products.[9] The remaining alkyl betaines additionally

Table 1. Definitions and Functions of the Ingredients in This Safety Assessment.[9,a]

Ingredient/CAS number	Definition and structure	Function
Betaine/107-43-7	Betaine is the zwitterion (inner salt) that conforms to the formula. *Betaine is the N,N,N-trimethylammonium zwitterion of glycine* 	Hair conditioning agents, humectants, skin conditioning agents (humectants)
Behenyl betaine	Behenyl betaine is the zwitterion (inner salt) that conforms to the formula. *Behenyl betaine is the N-behenyl-N,N-dimethylammonium zwitterion of glycine* 	Antistatic agents, hair conditioning agents, skin conditioning agents (misc), surfactants-cleansing agents, surfactants-foam boosters, viscosity-increasing agents (aqueous)
Cetyl betaine/ 693-33-4	Cetyl betaine is the zwitterion (inner salt) that conforms to the formula. *Cetyl betaine is the N-cetyl-N,N-dimethylammonium zwitterion of glycine* 	Antistatic agents, hair conditioning agents, skin conditioning agents (misc), surfactants-cleansing agents, surfactants-foam boosters, viscosity-increasing agents (aqueous)
Coco-betaine/ 68424-94-2	Coco-betaine is the zwitterion (inner salt) that conforms generally to the formula. *Coco-betaine is the N-cocyl-N,N-dimethylammonium zwitterion of glycine.*  wherein R represents the alkyl groups derived from coconut oil, *wherein coconut is primarily comprised of capric (6%-10%), lauric (44%-52%), myristic (13%-19%), and palmitic (8%-11%) acids*[37]	Antistatic agents, hair conditioning agents, skin conditioning agents (misc), surfactants-cleansing agents, surfactants-foam boosters, viscosity-increasing agents (aqueous)
Decyl betaine/ 2644-45-3	Decyl betaine is the zwitterion (inner salt) that conforms generally to the formula. *Decyl betaine is the N-decyl-N,N-dimethylammonium zwitterion of glycine* 	Antistatic agents, hair conditioning agents, skin conditioning agents (misc), surfactants-cleansing agents, surfactants-foam boosters, viscosity-increasing agents (aqueous)
Hydrogenated tallow betaine	Hydrogenated tallow betaine is the zwitterion (inner salt) that conforms generally to the formula. *Hydrogenated tallow betaine is the ammonium zwitterion of glycine, wherein nitrogen is substituted with 2 methyl groups and a fatty chain derived from hydrogenated tallow*  wherein R represents the alkyl groups derived from hydrogenated tallow, *wherein tallow is primarily comprised of oleic (37%-43%), palmitic (24%-32%), stearic (20%-25%), myristic (3%-6%), and linoleic (2%-3%) acids; and*	Antistatic agents, hair conditioning agents, skin conditioning agents (misc), surfactants-cleansing agents, surfactants-foam boosters, viscosity-increasing agents (aqueous)

(continued)

Table 1. (continued)

Ingredient/CAS number	Definition and structure	Function
	hydrogenation of tallow would result in the reduction of some of the unsaturated acids to saturated acids such as increased stearic acid and decreased linoleic and oleic acids[38]	
Lauryl betaine/ 683-10-3	Lauryl betaine is the zwitterion (inner salt) that conforms generally to the formula. *Lauryl betaine is the N-lauryl-N,N-dimethylammonium zwitterion of glycine*	Antistatic agents, hair conditioning agents, skin conditioning agents (misc), surfactants-cleansing agents, surfactants-foam boosters, viscosity-increasing agents (aqueous)
		
Myristyl betaine/ 2601-33-4	Myristyl betaine is the zwitterion (inner salt) that conforms generally to the formula. *Myristyl betaine is the N-myristyl-N,N-dimethylammonium zwitterion of glycine*	Abrasives, antistatic agents, hair conditioning agents, skin conditioning agents (misc), surfactants-cleansing agents, surfactants-foam boosters, viscosity-increasing agents (aqueous)
		
Oleyl betaine/ 871-37-4	Oleyl betaine is the zwitterion (inner salt) that conforms generally to the formula. *Oleyl betaine is the N-oleyl-N,N-dimethylammonium zwitterion of glycine*	Antistatic agents, hair conditioning agents, skin conditioning agents (misc), surfactants-cleansing agents, surfactants-foam boosters, viscosity-increasing agents (aqueous)
		
Stearyl betaine/ 820-66-6	Stearyl betaine is the zwitterion (inner salt) that conforms to the formula. *Stearyl betaine is the N-stearyl-N,N-dimethylammonium zwitterion of glycine*	Antistatic agents, hair conditioning agents, skin conditioning agents (misc), surfactants-cleansing agents, surfactants-foam boosters, viscosity-increasing agents (aqueous)
		
Tallow betaine	Tallow betaine is the zwitterion (inner salt) that conforms generally to the formula. *Tallow betaine is the ammonium zwitterion of glycine, wherein nitrogen is substituted with 2 methyl groups and a fatty chain derived from tallow*	Antistatic agents, hair conditioning agents, skin conditioning agents (misc), surfactants-cleansing agents, surfactants-foam boosters, viscosity-increasing agents (aqueous)
		
	wherein R represents the alkyl groups derived from tallow, *wherein tallow is primarily comprised of oleic (37%-43%), palmitic (24%-32%), stearic (20%-25%), myristic (3%-6%), and linoleic (2%-3%) acids*[38]	

Abbreviations: CAS, Chemical Abstracts Service; misc, miscellaneous.
[a]The italicized text represents additions made by Cosmetic Ingredient Review (CIR) staff.

function as antistatic agents, skin conditioning agents—miscellaneous, surfactants-cleansing agents and foam boosters, and viscosity-increasing agents. According to 2013 VCRP data, betaine has the most reported uses in cosmetic and personal care products, with a total of 459; the majority of the uses are in leave-on skin care preparations.[10] Lauryl betaine has the

Table 2. Physical and Chemical Properties.

	Property	Reference
Betaine		
Physical form	Deliquescent scales or prisms	39
Molecular weight, g/mol	117.15	5,39
Melting point, °C	293 (decomposes)	5
Water solubility, g/L	160	39
Other solubility, g/L	55 in methanol, 8.7 in ethanol, and sparingly soluble in ether	39
Cetyl betaine		
Vapor pressure, mm Hg at 25°C	2.4×10^{-12}	28
Melting point, °C	243	28
Boiling point, °C at 760 mm Hg	566	28
Water solubility, mg/L at 25°C	171	28
Log K_{ow}	2.44	28
Lauryl betaine		
Physical form	Crystals or colorless needles	39
Molecular weight, g/mol	271.44	39
Melting point, °C	183-185	39
Water solubility	Easily soluble in water	39
Other solubility	Easily soluble in methanol, ethanol, and benzene; moderately soluble in acetone	39
Dissociation constant (pKa)	1.8	39

second greatest number of overall uses reported, with a total of 338; the majority of those uses are in rinse-off personal cleanliness products.

According to the VCRP data, decyl betaine, tallow betaine, and hydrogenated tallow betaine have no reported uses in cosmetics in the United States. In the Council's use concentration survey, betaine had a maximum use concentration range of 0.0001% to 8.7%, with the 8.7% reported in rinse-off noncoloring hair conditioners.[11] Lauryl betaine had a maximum use concentration range of 0.015% to 8.8%, with 8.8% reported in rinse-off noncoloring hair shampoos. The Council reports that they do not have any suppliers listed for decyl betaine, hydrogenated tallow betaine, stearyl betaine, or tallow betaine.[12]

Betaine and lauryl betaine were reported to be used in hair sprays, body and hand products, noncoloring hair powders, and indoor tanning preparations that may be aerosolized or become airborne and could possibly be inhaled. For example, betaine is used in hair sprays at up to 3% and in noncoloring powders at up to 0.0001%. 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 as compared with pump sprays.[13-16] Therefore, most droplets/particles incidentally inhaled from cosmetic sprays would be deposited in the nasopharyngeal and bronchial regions and would not be respirable (ie, they would not enter the lungs) to any appreciable amount.[14,15] Conservative estimates of inhalation exposures to respirable particles during the use of loose powder cosmetic products are 400-fold to 1,000-fold less than protective regulatory and guidance limits for inert airborne respirable particles in the workplace.[17-19] Alkyl betaines are not restricted from use in any way under the rules governing cosmetic products in the European Union.[20]

Noncosmetic

A biocide that contains cetyl betaine was concurrently being studied, during the preparation of this report, as a preventative treatment of human immunodeficiency virus type 1 and other sexually transmitted diseases in vaginal microbicides and contraceptives.[21,22] Betaine hydrochloride has been approved by the FDA to treat homocystinuria (by reducing homocysteine levels).[23] It is also present as an active ingredient in over-the-counter digestive aids; however, the FDA has determined that there are inadequate data to establish general recognition for the safety and effectiveness of the ingredient for this specified use (21 CFR §310.545).

Toxicokinetics

Betaine

Low percutaneous permeation for betaine was observed in a percutaneous absorption study using Franz chambers with freshly isolated human epidermis.[3] The study followed Organization for Economic Cooperation and Development Guideline 428, but was not Good Laboratory Practice compliant. Betaine at 5% in saline or emulsion was applied to the epidermis samples. The exposure was observed for 24 hours. Approximately 0.1% of the applied dose in both vehicle types permeated through the epidermis samples.

The pharmacokinetics and acute effects on plasma total homocysteine of orally administered betaine (see "use of betaine hydrochloride in treating homocystinuria") were assessed in healthy human volunteers (3 men and 7 women).[24] Information on the absorption and elimination of betaine was also developed. In a double-blind crossover study, each patient ingested the betaine in doses of 1, 3, and 6 g mixed with 150 mL orange juice. The doses were ingested 7 days apart following a 12-hour overnight fast. Blood samples for serum betaine concentration measurement were drawn just before receiving the betaine dose, at 20-minute intervals during the first 3 hours and then at 4, 7, and 24 hours postdosing. Urine samples were collected before dosing and during the 24-hour follow-up period. Within 2 hours, a dose-dependent increase in serum betaine concentration was observed. Absorption and elimination of betaine were dose dependent, with urinary excretion of betaine increasing with betaine dose. Only a very small proportion of the ingested betaine was excreted in urine, however, with 3.2%, 4.3%, and 7.4% of the 1, 3, and 6 g doses accounted for, respectively.

Table 3. Frequency and Concentration of Use (2013) According to Duration and Type of Exposure for Alkyl Betaines.[10,11]

	Number of uses	Maximum concentration of use (%)	Number of uses	Maximum concentration of use (%)	Number of uses	Maximum concentration of use (%)
	Behenyl Betaine		Betaine		Cetyl Betaine	
Totals[a]	4	8.4	459	0.0001-8.7	14	0.36-7.4
Duration of use						
Leave-on	1	NR	326	0.0001-8	NR	NR
Rinse-off	3	8.4	130	0.09-8.7	14	0.36-7.4
Diluted for (bath) use	NR	NR	3	0.01	NR	NR
Exposure type						
Eye area	NR	NR	31	0.1-3	NR	NR
Incidental ingestion	NR	NR	6	0.05-3	NR	NR
Incidental inhalation: spray	NR	NR	4	0.2-3[b]	NR	NR
Incidental inhalation: powder	NR	NR	4	0.0001[c]	NR	NR
Dermal contact	4	8.4	379	0.01-6.5	12	0.36-7.4
Deodorant (underarm)	NR	NR	NR	NR	NR	NR
Hair: noncoloring	NR	NR	50	0.0001-8.7	2	NR
Hair: coloring	NR	NR	22	0.44	NR	NR
Nail	NR	NR	NR	3	NR	NR
Mucous membrane	NR	NR	19	0.01-3	6	0.36-7.4
Baby products	NR	NR	4	NR	NR	NR
	Coco-Betaine		Lauryl Betaine		Myristyl Betaine	
Totals[a]	227	0.53-9.8	338	0.015-8.8	6	0.84
Duration of use						
Leave-on	4	1.8-2	29	0.016-1.2	NR	NR
Rinse-off	213	0.53-9.8	281	0.015-8.8	6	0.84
Diluted for (bath) use	10	3.1-5.1	28	1	NR	NR
Exposure type						
Eye area	1	NR	2	0.016	NR	NR
Incidental ingestion	NR	2	NR	NR	NR	NR
Incidental inhalation: spray	NR	NR	1	NR	NR	NR
Incidental inhalation: powder	NR	NR	NR	NR	NR	NR
Dermal contact	141	0.53-9.8	308	0.016-8	6	0.84
Deodorant (underarm)	NR	NR	NR	NR	NR	NR
Hair: noncoloring	70	0.63-8	29	0.015-8.8	NR	NR
Hair: coloring	16	1.5-2.3	1	0.19-3	NR	NR
Nail	NR	NR	NR	NR	NR	NR
Mucous membrane	87	2-5.1	262	0.75-4.2	6	0.84
Baby products	3	NR	1	NR	NR	NR
	Oleyl Betaine		Stearyl Betaine			
Totals[a]	5	23.7	1	NR		
Duration of use						
Leave-on	NR	NR	1	NR		
Rinse-off	5	NR	NR	NR		
Diluted for (bath) use	NR	23.7	NR	NR		
Exposure type						
Eye area	NR	NR	NR	NR		
Incidental ingestion	NR	NR	NR	NR		
Incidental inhalation: spray	NR	NR	NR	NR		
Incidental inhalation: powder	NR	NR	NR	NR		
Dermal contact	3	23.7	NR	NR		
Deodorant (underarm)	NR	NR	NR	NR		
Hair: noncoloring	2	NR	1	NR		
Hair: coloring	NR	NR	NR	NR		
Nail	NR	NR	NR	NR		
Mucous membrane	1	23.7	NR	NR		
Baby products	NR	NR	NR	NR		

Abbreviation: NR, none reported.

[a]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.

[b] 0.5% in an aerosol hair spray, 3% in a pump hair spray, 0.2% in a body and hand spray.

[c] 0.0001% in a powder noncoloring hair preparation.

Cetyl Betaine and Lauryl Betaine

The absorption of radiolabeled cetyl betaine (5.4 mM) and lauryl betaine (16 mM) was determined using diffusion cells containing excised hairless mouse skin.[25] Lauryl betaine was well absorbed into the receptor phase (approximately 50% of the applied dose within 24 hours), while cetyl betaine partitioned into the skin but slowed transfer to the receptor phase (approximately 1.3% of the applied dose absorbed within 24 hours). Skin digests at the end of the 24 hours found that 15% of the applied dose of cetyl betaine and 25% of the applied dose of lauryl betaine were associated with the tissue. This study also examined the effects of cetyl betaine and lauryl betaine (same concentrations as used in the absorption study) on skin barrier function in hairless mouse skin in vitro. Excised skin was pretreated with each test material for 16 hours. After pretreatment, the permeation of the model compound, nicotinamide, across membranes was measured and the results were compared to the flux across control membranes that were exposed to buffer alone. All surfactants decreased skin barrier function to some extent. The degree of nicotinamide penetration enhancement was correlated with the ratio of the surfactant pretreatment concentration to the surfactant critical micelle concentration. The authors of the study suggested that solubilization of stratum corneum lipids may be an important mechanism explaining the effects observed.

The dermal uptake of cetyl betaine and lauryl betaine was measured in vivo with human skin.[26] Male volunteers received ^{14}C-radiolabeled test materials in aqueous solution on the dorsal upper arm for 30 minutes. The concentrations of cetyl betaine and lauryl betaine applied were 0.14, 1.0, and 5.4 mM and 16, 100, and 800 mM, respectively. The positive control was 50 mM caffeine. At the end of the exposure period, the remaining test materials were rinsed from the skin, and the skin was washed. The stratum corneum at the test sites was removed with repeated tape stripping. Dermal uptake was assessed by measuring the recovered radioactivity from the tape strips and compared to predicted penetration values. The measured uptake of cetyl betaine and lauryl betaine was 28 to 160 nmol/cm2 and 2.3 to 19.5 nmol/cm^2, respectively. The predicted penetration values were 51 to 292 nmol/cm^2 for cetyl betaine and 3.7 to 35 nmol/cm^2 for lauryl betaine. Caffeine penetrated at expected amounts. The tape stripping indicated that the radiolabel was mostly found in the outer layers of the stratum corneum.

The same study also assessed skin barrier function using the same test concentrations for both test materials.[26] Nonradiolabeled cetyl betaine and lauryl betaine were applied to the skin for 30 minutes. The transepidermal water loss (TEWL) was assessed. No changes in TEWL values were observed after treatment of the skin with the betaines or with saline controls.

Toxicological Studies

Acute Toxicity

Acute toxicity studies are presented in Table 4. The oral LD_{50} values of betaine, cetyl betaine, lauryl betaine, and C12 to C14 alkyldimethyl betaines were 11.1 g/kg, 1.62 g/kg, 0.071 g/kg, and 3 mL/kg, respectively, in rats, and 2.64 g/kg for coco-betaine (30%) in a mouse study. Also in rats, the dermal LD_{50} values were greater than 16 g/kg for cetyl betaine and 1.3 g/kg for lauryl betaine. The intravenous LD_{50} of betaine in mice has been reported to be 0.83 g/kg body weight (bw). The LD_{50} values were 0.15 g/kg for cetyl betaine and 0.053 g/kg for lauryl betaine in an intraperitoneal study in rats.[3-5,25]

Repeated Dose Toxicity

Repeated dose toxicity data are summarized in Table 5. No effects were observed at the highest dose tested for betaine in rats. No significant toxic effects were observed in rats that received up to 0.35 g/kg/d cetyl betaine in a 91-day oral study. The no observed effect level (NOEL) for coco-betaine was 250 mg/kg/d and the lowest observed effect level (LOEL) was 500 mg/kg/d in a 90-day oral study in rats when tested up to 500 mg/kg/d. In a study of C12 to C14 alkyldimethyl betaines, systemic no observed adverse effects levels (NOAELs) were 50 and 100 mg/kg bw/d in oral rat studies that tested the material up to 300 and 1,000 mg/kg/d, respectively. The systemic lowest observed adverse effects levels (LOAELs) for these 2 studies were 150 (due to increased salivation, increased urea, and nonneoplastic histopathologic changes in the kidney and bladder) and 300 mg/kg bw/d (due to decreased food consumption, bw gain, and absolute bw), respectively.[3,4,27]

Reproductive and Developmental Toxicity

Reproductive and developmental toxicity studies are summarized in Table 6. Dermal reproductive and developmental toxicity studies of cetyl betaine in rabbits determined the maternal LOAEL to be 10 mg/kg/d due to decreased bw gain and a maternal NOAEL could not be established. The developmental LOAEL was 40 mg/kg/d and the developmental NOAEL was 20 mg/kg/d. In oral reproductive and developmental toxicity studies of cetyl betaine in rats, the LOAEL for the dams was 50 mg/kg due to decreased bw gain and a maternal NOAEL could not be calculated. The developmental LOAEL was 250 mg/kg and the developmental NOAEL was 150 mg/kg. In an oral C12 to C14 alkyldimethyl betaines study, the reproductive NOEL was 150 mg/kg bw/d and the reproductive LOAEL was 300 mg/kg bw/d due to decreased pup weight and litter size and increased postimplantation loss and postnatal loss. Another oral C12 to C14 alkyldimethyl betaines study determined the reproductive NOAEL to be 300 mg/kg/d when the test material was tested up to 1,000 mg/kg/d.[4,28,29]

Carcinogenicity

Repeated dose toxicity results are presented in Table 5. Betaine was not carcinogenic when tested up to 5% in a 104-week dietary rat study.[3]

Table 4. Acute Toxicity Studies in Animals.

Ingredient and concentration/dose	Method	Results/conclusions	References
Oral			
Betaine anhydrous, >97% pure; doses = 5, 10, 12.5, 15, 20 g/kg bw in water	OECD Guideline 401 for acute oral toxicity in Crj:CD (SD) rats; 5 rats of each sex per dose; GLP compliant	LD_{50} (calculated) = 11.1 g/kg bw in males and females; symptoms observed in each dose group included lethargy, decreased motor activity, prone posture, ataxia, musculature tremor, bradypnea, hyperpnea, piloerection, ungroomed appearance, hunched posture, and death	3,5
Cetyl betaine (94.9% pure) and lauryl betaine (98.9% pure) in 25% (wt/vol) solutions; doses not reported	Groups of 5 male Sprague Dawley rats received either test material via oral gavage	LD_{50} = 1,620 mg/kg for cetyl betaine and 71 mg/kg for lauryl betaine; symptoms in some animals for either test material were sluggishness, diarrhea, and lacrimation; weight gains were within normal parameters in surviving animals; gross necropsy of the animals that died during the study found the gastrointestinal tract distended with red fluid and lungs mottled and red; no significant differences in the pharmacotoxic signs or gross necropsy findings between the 2 test materials	25
Coco-betaine tested at 30% active ingredient and at 10% in water; doses = 6,670, 8,350, 10,000 mg/kg bw	OECD Guideline 401 for acute oral toxicity in CF-1 mice; 10 mice per dose; not GLP compliant	LD_{50} (calculated) = 2,640 mg/kg bw for 30% active ingredient and 8,800 mg/kg bw for 10% in water	4
Betaines, C12-C14-alkyldimethyl (a mixture of lauryl betaine and myristyl betaine) in aqueous solution tested at 0, 1.6, 2.5, 3.2, 4.0, 5.0, and 8.0 mL/kg bw	OECD Guideline 401 for acute oral toxicity in CFY rats; 5 rats of each sex per dose; not GLP compliant	LD_{50} (calculated) = 3 mL/kg bw in males and females; lethargy and diuresis observed at 3.2 mL/kg and greater; death preceded by ataxia and coma and occurred within 5-27 hours postdosing; total recovery in survivors 5 days postdosing	4
Dermal			
Cetyl betaine (94.9% pure) and lauryl betaine (98.9% pure); doses not reported	Groups of 5 male Sprague Dawley rats received either test material dermally on clipped trunks; test sites were occluded for 24 hours, after which the test sites were wiped clean of the test materials	LD_{50} = >16 g/kg for cetyl betaine and 1.3 g/kg for lauryl betaine; erythema, edema, desquamation, necrosis, and scab formation observed on test sites for both test materials, as was sluggishness and reddish nasal and ocular discharges; bw gains within normal parameters; no treatment-related changes due to either test material observed at gross necropsy	25
Intravenous/intraperitoneal			
Betaine; no further details provided	Intravenous acute study in mice; no further details provided	LD_{50} = 830 mg/kg bw; no further details provided	5
Cetyl betaine (94.9% pure) and lauryl betaine (98.9% pure) in 5% and 25% (wt/vol) solutions in distilled water; doses not reported	Groups of 5 male Sprague Dawley rats received either test material intraperitoneally	LD_{50} = 150 mg/kg for cetyl betaine and 53 mg/kg for lauryl betaine; sluggishness, diarrhea, lacrimation, and distended abdomen observed in animals that received either test material; bw gains within normal parameters; no treatment-related changes due to either test material observed at gross necropsy	25

Abbreviations: bw, body weight; OECD, Organization for Economic Cooperation and Development; GLP, Good Laboratory Practice.

Table 5. Repeated Dose Toxicity in Animals.

Ingredient and concentration/dose	Method	Results/conclusions	References
Betaine >95% pure; doses = 0%, 1%, 2%, and 5% in animal feed	OECD Guideline 407 for repeated dose 28-day oral toxicity in female Sprague Dawley rats; number per dose not provided; GLP compliant	NOAEL >5,771 mg/kg bw/d; NOEL could not be derived due to high tolerance and reversibility of slight to moderate hepatocellular vacuolation effects in rats	3
Betaine >95% pure; tested up to 5% in animal feed	OECD Guideline 408 for repeated dose 90-day oral toxicity in male and female Sprague Dawley rats; 20 rats of each sex per dose; GLP compliant	NOAEL and NOEL could not be determined due to high tolerance, slight hematology, and hepatic changes that included increased liver weights, hepatocellular vacuolation, but no microscopic evidence of hepatotoxicity; no significant systemic signs of toxicity were observed in any treatment group during dosing	3
Betaine >99.9% pure; tested up to 5% in animal feed for both chronic and carcinogenicity studies	OECD Guideline 453 for combined chronic toxicity/carcinogenicity studies in male and female Fischer 344 rats; 52-week study had 10 rats of each sex per dose; 104-week study had 25 rats of each sex per dose	NOEL determined for up to 5%, betaine was not carcinogenic; increased liver and kidney weights observed in both sexes in the 5% dose group; decrease in mean corpuscular volume and mean corpuscular hemoglobin observed; increased platelet count observed; minor effects in blood biochemistry for chronic study	3
Cetyl betaine tested at 32% active adjusted to be delivered at doses of 0, 0.05, 0.15, and 0.35 g/kg/d	91-Day subchronic oral toxicity study in Sprague Dawley rats; 10 rats of each sex per dose group; test material administered in feed; GLP compliant	All animals survived until end of treatment period; no treatment-related clinical observations; mean bw and bw gains significantly decreased in high-dose males which was accompanied by significantly decreased total feed consumption—these observations were attributed to palatability problems of diet than toxic effects of test material; slight clinical chemistry changes observed in high-dose animals; no gross or histologic alterations, including reproductive organs, attributed to test material observed	27
Coco-betaine tested at 29%-33% active material in water at 0, 125, 250, and 500 mg/kg bw/d	OECD Guideline 408 for repeated dose 90-day oral toxicity in male and female Sprague Dawley rats; 10 rats of each sex per dose; GLP compliant	NOEL = 250 mg/kg bw/d; LOEL = 500 mg/kg bw/d due to increased water consumption. Irritative effects observed in the forestomach of mid- and high-dose group, possibly due to gavage dosing. No adverse effects on reproductive organs noted in microscopic or macroscopic examinations	4
Betaines, C12-C14-alkyldimethyl (a mixture of lauryl betaine and myristyl betaine) in water at 0, 50, 150, and 300 mg/kg bw/d	OECD Guideline 422 for combined repeated dose oral toxicity study with reproduction/developmental toxicity screening test in male and female Wistar rats; 10 rats of each sex per dose; GLP compliant	NOAEL (systemic) = 50 mg/kg bw/d; LOAEL (systemic) = 150 mg/kg bw/d due to increased salivation, increased urea, and nonneoplastic histopathologic changes in the kidney and bladder; NOEL (reproduction) = 150 mg/kg bw/d; LOAEL (reproduction) = 300 mg/kg bw/d due to pup weight, litter size, postimplantation loss, and postnatal loss	4
Betaines, C12-C14-alkyldimethyl (a mixture of lauryl betaine and myristyl betaine) in water at 0, 33, 100, 300, and 1,000 mg/kg bw/d	OECD Guideline 422 for combined repeated dose oral toxicity study with reproduction/developmental toxicity screening test in male and female Wistar rats; 3 rats of each sex per dose; not GLP compliant	NOAEL (systemic) = 100 mg/kg bw/d; LOAEL (systemic) = 300 mg/kg bw/d due to decreased food consumption, bw gain, and absolute bw; no reproductive results reported due to small group sizes	4

Abbreviations: bw, body weight; GLP, Good Laboratory Practice; LOAEL, lowest observed adverse effects level; LOEL, lowest observed effect level; OECD, Organization for Economic Cooperation and Development; NOAEL, no observed adverse effects level; NOEL, no observed effect level.

Table 6. Reproductive and Developmental Toxicity.

Ingredient and concentration/dose	Method	Results/conclusions	References
Betaines, C12-C14-alkyldimethyl (a mixture of lauryl betaine and myristyl betaine); 0, 50, 150, or 300 mg/kg bw/d active ingredient in water	OECD Guideline 422 for combined repeated dose toxicity study with the reproduction/developmental toxicity screening test in male and female Wistar rats by oral gavage; 10 animals per sex per dose; males dosed 4 weeks, females dosed 7 weeks; microscopic examination of the parental reproductive organs was performed; GLP compliant	In parental animals, sedation, salivation, and irritation effects in the stomach and bladder due to the irritating nature of the test material were observed in the high-dose group. Additionally, reduced bw gain and reduced absolute bws were observed in the high-dose group and in the mid-dose group, but in a milder form. Reduced pup weight, liter size, and increased postimplantation and postnatal loss were observed in the high-dose group and were considered secondary to maternal toxicity. No adverse effects were observed in the low-dose group and their offspring	[4]
Betaines, C12-C14-alkyldimethyl (a mixture of lauryl betaine and myristyl betaine); 0, 33, 100, 300, or 1,000 mg/kg bw/d in water	OECD Guideline 422 for combined repeated dose toxicity study with the reproduction/developmental toxicity screening test in male and female Wistar rats by oral gavage; 3 animals per sex per dose; males dosed 4 weeks, females dosed 6 weeks; microscopic examination of the parental reproductive organs not described; not GLP compliant	All parental animals in the 1,000 mg/kg/d dose group died within 24 hours of the first dose. At 300 mg/kg/d, no adverse effects on reproduction were observed. The NOAEL of the test substance for reproduction was 300 mg/kg/d	[4]
Cetyl betaine; 0, 10, 20, 40, 100, or 200 mg/kg/d in 5% isopropanol in dosages of 2 mL/kg	Dermal development toxicity/ teratogenicity study in female New Zealand white rabbits. Groups of 8 artificially inseminated rabbits received the test material for 4 hours daily on approximately days 6 through 18 of gestation. Test substance-related mortality and severe topical effects occurred in the 100 and 200 mg/kg dose groups after the eighth and sixth dosages, respectively, and administration of these dose levels was discontinued. Two additional test groups (n = 8 each) of noninseminated rabbits were added: one received a new vehicle control (not reported) and the other received 2 mg/kg/d of the test material in the new vehicle control. All animals were observed daily for signs of toxicity, skin irritation, abortion (inseminated rabbits), death, bw, and feed consumption. Rabbits that died during the study were examined for pregnancy (inseminated rabbits) and cause of death. The inseminated rabbits were killed on day 19 of gestation and the noninseminated rabbits were killed 24 hours after the 13th daily dosage was administered. Inseminated rabbits underwent a complete gross necropsy, including examination of the brain, uterus, and fetuses. Microscopic examination of the parental reproductive organs not described. GLP compliant	Maternal LOAEL = 10 mg/kg/d; maternal NOAEL could not be established; developmental LOAEL = 40 mg/kg/d; developmental NOAEL = 20 mg/kg/d. In the 100 and 200 mg/kg dose groups, 3 rabbits each died or were killed during the course of the study. Clinical observations in these groups and the 40 mg/kg dose group included uncoordinated movement, partial paralysis, red exudate of vaginal origin present in the cage pan, green matted fur, ataxia, and alopecia. All skin reactions, including erythema, desquamation, atonia, fissuring, eschar, and exfoliation were dose dependent. All rabbits in each dose group had a minimum of grade 1 erythema observed at least once. No rabbits in any dose group had edema. When compared to the control group, average bw gain was inhibited in rabbits of the 2 through 200 mg/kg dose groups and was considered to be dose dependent. The severity of the effect was slight in the 2 and 10 mg/kg dose groups and marked in the 100 and 200 mg/kg dose groups. Decreased average daily feed consumption was noted in the 2 through 200 mg/kg dose groups and was also considered to be dose dependent. It was considered biologically significant in the 40-200 mg/kg dose groups. Pregnancy was observed in 6 or 7 of the 8 rabbits in each dose group. An increased incidence of resorptions was observed in the maternally toxic doses of 40, 100, and 200	[28,29]

(continued)

Table 6. (continued)

Ingredient and concentration/dose	Method	Results/conclusions	References
		mg/kg/d. A decrease in average litter size was observed in the 100 and 200 mg/kg dose groups. All fetuses were alive at cesarean delivery, but were not examined, and no further data about the fetuses are available. The results determined that doses of 0, 2, 10, and 20 mg/kg would be used in a definitive rabbit teratology study (results of this study have not been found)	
Cetyl betaine; received 0, 50, 150, and 250 mg/kg/d of 30.4% active cetyl betaine in 10% ethanol (correction factor of 3.2895 was utilized to achieve proper amount of active ingredient)	Oral developmental toxicity/ teratogenicity study, female Sprague Dawley rats, n/group not stated. The control group received ethanol in deionized water at a volume of 5 mL/kg, which was the same amount of ethanol that the 250 mg/kg cetyl betaine dose group received. The rats received the test material daily for 10 days starting on gestation day 6. The animals were observed twice daily for signs of toxicity and bws and feed consumption were recorded on days 0, 6, 9, 12, 16, and 20 of gestation. On gestation day 20, all surviving rats were killed, and the uterus and the fetuses were examined and measured for number and location of viable and nonviable fetuses, early and late resorptions, number of total implantations, and corpora lutea, fetal bws, sex, external malformations or developmental variations, and skeletal abnormalities. Microscopic examination of the parental reproductive organs not described. GLP compliant	Maternal LOAEL = 50 mg/kg based on the inhibited bw gain; maternal NOAEL could not be established; developmental LOAEL = 250 mg/kg; developmental NOAEL = 150 mg/kg. No mortalities observed in any of the dams in the control or treatment groups. In the 250 mg/kg dose group, clinical observations included stained and matted fur primarily on the limbs, neck, ventral thorax, and facial area, excessive salivation, respiratory rales, diarrhea, decreased activity, hypothermia, lacrimation, labored breathing, and wheezing. Similar observations were made in the 150 mg/kg dose group, with the stained and matted fur and respiratory rales the predominant signs of toxicity. Inhibition of maternal bw gain was observed as a dose-related trend during overall gestation and the treatment periods at all dose levels. Weight loss was observed during the first treatment interval in the 150 and 250 mg/kg dose groups. Decreased feed consumption was also observed in all treated groups during the treatment period in a dose-dependent manner. Feed consumption was noted to be inhibited at 250 mg/kg during the overall gestation period, but the mean values for the 50 and 150 mg/kg dose groups were comparable to controls. In the fetuses, no significant differences between the control and treated groups were evident with respect to the number of corpora lutea, total implantations, postimplantation loss, viable fetuses, and fetal bws. Fetal malformation in the treated groups was not significantly different from that of the controls. Reduced or absent ossification of the skull, sternebrae #5 and/or #6, and other sternebrae occurred more frequently in the 250 mg/kg dose group. These effects were considered to be biologically significant as they were observed in conjunction with reduced maternal bw gains. No other developmental variations were noted	[28,29]

Abbreviations: bw, body weight; GLP, Good Laboratory Practice; LOAEL, lowest observed adverse effects level; NOAEL, no observed adverse effects level; OECD, Organization for Economic Cooperation and Development.

Table 7. Genotoxicity.

Ingredient and concentration/dose	Method	Results/conclusions	References
In vitro			
Betaine monohydrate >95% pure; concentrations up to 10 mg/mL with and without S9 metabolic activation	Chromosome aberration study using human lymphocytes in whole blood cultures with and without metabolic activation; GLP compliant	Not clastogenic	3,5
Betaine monohydrate >97% pure; concentrations plated = 8-5,000 μg/plate with and without S9 activation	Bacterial reverse mutation assay using *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 with and without S9 metabolic activation; GLP compliant	Not genotoxic	3,5
Betaines, C12-C14-alkyldimethyl (a mixture of lauryl betaine and myristyl betaine); up to 5.0 mg/plate with and without S9 metabolic activation	OECD Guideline 471 for bacterial reverse mutation (Ames) assay in *S typhimurium* strains TA97a, TA98, TA100, TA1535, and TA102 with and without metabolic activation; GLP compliant	Not genotoxic with or without metabolic activation	4
Alkyl dimethyl betaine (no further description) 30.2% active ingredient; up to 100 μg/mL with S9 and up to 75 μg/mL without S9	OECD Guideline 476 for in vitro mammalian cell gene mutation test in Chinese hamster ovary (CHO) cells—HGPRT locus with and without metabolic activation; GLP compliant	Not genotoxic with or without metabolic activation	4
Alkyl dimethyl betaine (no further description) 30% active ingredient; up to 200 μg/mL without S9 with 4-hour exposure, up to 100 μg/mL without S9 with 20-hour exposure, and up to 150 μg/mL with S9	OECD Guideline 473 for in vitro mammalian chromosome aberration test in CHO cells with and without metabolic activation; GLP compliant	Not genotoxic with or without metabolic activation	4
In vivo			
Betaine monohydrate >98% pure; doses = 0, 0.5, 1, or 2 g/kg in saline	OECD Guideline 474 for mammalian erythrocyte micronucleus test using male and female CD-1 mice; test material or the positive control cyclophosphamide administered by gavage; exposure periods were 24, 48, or 72 hours; GLP compliant	Micronuclei were not induced in the bone marrow of mice dosed up to 2 g/kg	3,5

Abbreviations: GLP, Good Laboratory Practice; HGPRT, hypoxanthine-guanine phosphoribosyltransferase; OECD, Organization for Economic Cooperation and Development.

Genotoxicity

In vitro and in vivo genotoxicity studies are presented in Table 7. Betaine and C12 to C14 alkyldimethyl betaines were not genotoxic in in vitro and in vivo studies.[3-5]

Irritation and Sensitization

Irritation and Anti-Irritation

Nonhuman and human irritation and anti-irritation studies are presented in Table 8. Betaine had anti-irritating effects on the skin in several efficacy studies in humans. In dermal studies, coco-betaine was not irritating in a rabbit study when tested at 16% and was less irritating than sodium lauryl sulfate (SLS) in a human study at an unknown concentration. No dermal irritation reactions were observed in human studies of lauryl betaine at 0.1%, but were observed at concentrations of 1% and 10%. Dermal irritation results were mixed in rabbit studies of C12 to C14 alkyldimethyl betaines, with irritation observed at 30% and at an unknown concentration in 2 studies, and no irritation was observed in 2 other studies at unknown concentrations. Betaine at 10% was not an ocular irritant in rabbits nor were C12 to C14 alkyldimethyl betaines at unknown concentrations in several rabbit studies; however, coco-betaine at 16% and 30% and lauryl betaine at 10% were ocular irritants. In human mucosal studies testing the efficacy of toothpaste, betaine did not produce adverse effects.[1,3-5,30-33]

Sensitization

Nonhuman and human sensitization studies are presented in Table 9. Betaine (up to 50%), coco-betaine (up to 5%), lauryl betaine (0.1%), and C12 to C14 alkyldimethyl betaines (up to 100%) were not sensitizing in nonhuman and human dermal studies.[3-5,34,35]

Phototoxicity

No relevant published phototoxicity studies on alkyl betaines were discovered and no unpublished data were submitted.

Table 8. Irritation and Anti-Irritation Studies.

Ingredient and concentration/dose	Method	Results/conclusions	References
Dermal—nonhuman			
Coco-betaine tested at 16% in solution	OECD Guideline 404 for acute dermal irritation/corrosion in 3 albino rabbits; occlusive on shaved and abraded skin	Mean erythema score = 0.5/4, fully reversible within 24 hours; mean edema score = 0.5/4, fully reversible within 24 hours. Not irritating	4
Betaines, C12-C14-alkyldimethyl (a mixture of lauryl betaine and myristyl betaine) tested as 30% active material neat	OECD Guideline 404 for acute dermal irritation/corrosion in 3 New Zealand white rabbits; semiocclusive on shaved skin; GLP compliant	Very slight to slight edema between 30 minutes and 72 hours postdosing. Very slight to moderate erythema up to day 7. Skin was dry, rough and had fine-to-coarse scales with desquamation. Effects fully reversible within 14 days. Classified as irritating	4
Betaines, C12-C14-alkyldimethyl (a mixture of lauryl betaine and myristyl betaine), concentration not reported	OECD Guideline 404 for acute dermal irritation/corrosion in 6 New Zealand White rabbits; occlusive on shaved skin; not GLP compliant	Mean erythema score = 1.83/4, not fully reversible within 72 hours; mean edema score = 0.83/4, not fully reversible within 72 hours; not irritating	4
Betaines, C12-C14-alkyldimethyl (a mixture of lauryl betaine and myristyl betaine), concentration not reported	OECD Guideline 404 for acute dermal irritation/corrosion in 6 New Zealand white rabbits; occlusive on shaved skin; GLP compliant	Mean erythema score = 1.17/4, not fully reversible within 7 days; mean edema score = 0.72/4, not fully reversible within 7 days; not irritating	4
Betaines, C12-C14-alkyldimethyl (a mixture of lauryl betaine and myristyl betaine), concentration not reported	OECD Guideline 404 for acute dermal irritation/corrosion in 6 New Zealand white rabbits; occlusive on shaved skin; not GLP compliant	Mean erythema score = 1.83/4, not fully reversible within 72 hours; mean edema score = 1.08/4, not fully reversible within 72 hours; moderate irritant	4
Dermal—human			
Betaine; tested up to 10%	Efficacy study of test material in reducing irritation in soap was assessed in 2 studies with healthy patients (n = 28 and n = 21)	Soap containing betaine found to be less irritating than those without the test material, but not in a dose-dependent manner	1,30
Betaine; tested up to 5% in several vehicles	Acute dermal irritation/corrosion study in 26 healthy volunteers; 2 occlusive Finn patches for 2 consecutive 24-hour periods; test site area = 50 mm^2	Not irritating; some anti-irritancy observed with some of the vehicles	3
Betaine; >95% pure tested at 3.5% with 2% sodium lauryl sulfate (SLS) in water	20 male and 20 female test patients with test products applied on permutated test areas on different arms inside the crook of the elbow for 4 weeks; test areas washed and measurement of transepidermal water loss (TEWL) was measured after 4 weeks and 6 hours after the last washing; signs of irritation were observed at the end of the test and test areas were assessed for erythema, flaking, roughness, pruritus, and formation of papules	Betaine found to lessen irritation effects of SLS and considered anti-irritating	3
Coco-betaine in distilled water	Potential of 4 surfactants, including coco-betaine, to cause dermal irritation was assessed in a TEWL study with Finn chambers in 27 healthy volunteers; 24-hour exposure	SLS had greatest mean TEWL (15.5 $g/m^2 \cdot h$), followed by coco-betaine (12.6 $g/m^2 \cdot h$), sodium laurate (10.6 $g/m^2 \cdot h$), and polysorbate-60 (9.8 $g/m^2 \cdot h$); no severe irritation (3+ or 4+) was observed following the exposure to 2 g/100 mL of the test substances (the mean overall scores for coco-betaine and SLS were 1.03 and 1.833, respectively); coco-betaine had less irritation potential than SLS	31
Lauryl betaine at 0.1% active ingredient	Acute dermal irritation in 19 human patients; not occluded; 30-hour exposure	No reactions observed.	4
Lauryl betaine at 1% and 10% active ingredient	Acute dermal irritation in 7 human patients; occluded; 24-hour exposure	10% solution had 1 strong erythema, 4 moderate, and 2 mild; 1% solution had 5 strong erythema, 1 moderate, and 1 mild	4

(continued)

Table 8. (continued)

Ingredient and concentration/dose	Method	Results/conclusions	References
Ocular—nonhuman			
Betaine monohydrate >95% pure; 10% (wt/vol) in distilled water	OECD Guideline 405 for acute ocular irritation/corrosion in albino rabbits; GLP compliant; no further details were provided	Not irritating	3,5
Coco-betaine tested at 16% solids with no vehicle	OECD Guideline 405 for acute ocular irritation/corrosion in 3 albino rabbits; unwashed eyes; not GLP compliant	Test material caused corneal involvement and conjunctival irritation that did not clear by day 7 postdosing. Irritating	4
Coco-betaine tested at 30% active material with no vehicle	OECD Guideline 405 for acute ocular irritation/corrosion in 3 albino rabbits; unwashed eyes; not GLP compliant	Test material caused corneal and iris involvement and conjunctival irritation that did not clear by day 7 postdosing. Irritating	4
Lauryl betaine tested at 10% (vol/vol) solution in distilled water	OECD Guideline 405 for acute ocular irritation/corrosion in 3 New Zealand white rabbits; unwashed eyes; GLP compliant	Irritating	4
Betaines, C12-C14-alkyldimethyl (a mixture of lauryl betaine and myristyl betaine), concentration not reported	OECD Guideline 405 for acute ocular irritation/corrosion in 9 New Zealand white rabbits; washed and unwashed eyes; not GLP compliant	Mean cornea score = 0/4; mean iris score = 0.11/2, fully reversible within 72 hours; mean conjunctivae score = 0.78/4, not fully reversible within 72 hours; mean chemosis score = 0.17/4, fully reversible within 72 hours. Not irritating	4
Betaines, C12-C14-alkyldimethyl (a mixture of lauryl betaine and myristyl betaine), concentration not reported	OECD Guideline 405 for acute ocular irritation/corrosion in 9 New Zealand white rabbits; washed and unwashed eyes; not GLP compliant	Mean cornea score = 0.83/4, not fully reversible within 72 hours; mean iris score = 0.55/2, not fully reversible within 72 hours; mean conjunctivae score = 1.33/3, not fully reversible within 72 hours; mean chemosis score = 0.72/4, not fully reversible within 72 hours. Not irritating	4
Betaines, C12-C14-alkyldimethyl (a mixture of lauryl betaine and myristyl betaine), concentration not reported	OECD Guideline 405 for acute ocular irritation/corrosion in 6 New Zealand white rabbits; unwashed eyes; GLP compliant	Mean cornea score = 0.22/4, fully reversible within 72 hours; mean iris score = 0.55/2, not fully reversible within 72 hours; mean conjunctivae score = 1.33/3, not fully reversible within 72 hours; mean chemosis score = 0.83/4, not fully reversible within 72 hours. Not irritating	4
Betaines, C12-C14-alkyldimethyl (a mixture of lauryl betaine and myristyl betaine), concentration not reported	OECD Guideline 405 for acute ocular irritation/corrosion in 6 New Zealand white rabbits; unwashed eyes; GLP compliant	Mean cornea score = 0/4; mean iris score = 0.11/2, fully reversible within 48 hours; mean conjunctivae score = 0.78/3, fully reversible within 72 hours; mean chemosis score = 0.28/4, fully reversible within 72 hours. Not irritating	4
Betaines, C12-C14-alkyldimethyl (a mixture of lauryl betaine and myristyl betaine), concentration not reported	OECD Guideline 405 for acute ocular irritation/corrosion in 9 New Zealand white rabbits; washed and unwashed eyes; not GLP compliant	Mean cornea score = 0.22/4, fully reversible within 72 hours; mean iris score = 0.22/2, fully reversible within 72 hours; mean conjunctivae score = 1.16/3, not fully reversible within 72 hours; mean chemosis score = 0.33/4, not fully reversible within 72 hours. Not irritating	4
Betaines, C12-C14-alkyldimethyl (a mixture of lauryl betaine and myristyl betaine), concentration not reported	OECD Guideline 405 for acute ocular irritation/corrosion in 9 New Zealand white rabbits; washed and unwashed eyes; not GLP compliant	Mean cornea score = 0.61/4, not fully reversible within 72 hours; mean iris score = 0.22/2, not fully reversible within 72 hours; mean conjunctivae score = 1.44/3, not fully reversible within 72 hours; mean chemosis score = 0.72/4, not fully reversible within 72 hours. Not irritating	4

(continued)

Table 8. (continued)

Ingredient and concentration/dose	Method	Results/conclusions	References
Betaines, C12-C14-alkyldimethyl (a mixture of lauryl betaine and myristyl betaine), concentration not reported	OECD Guideline 405 for acute ocular irritation/corrosion in 6 New Zealand white rabbits; unwashed eyes; GLP compliant	Mean cornea score = 0/4; mean iris score = 0.28/2, fully reversible within 72 hours; mean conjunctivae score = 1.05/3, not fully reversible within 72 hours; mean chemosis score = 0.72/4, fully reversible within 72 hours. Not irritating	4
Betaines, C12-C14-alkyldimethyl (a mixture of lauryl betaine and myristyl betaine), concentration not reported	OECD Guideline 405 for acute ocular irritation/corrosion in 9 New Zealand white rabbits; washed and unwashed eyes; GLP compliant	Mean cornea score = 0.06/4, fully reversible in 48 hours; mean iris score = 0.16/2, fully reversible within 48 hours; mean conjunctivae score = 0.67/3, fully reversible within 72 hours; mean chemosis score = 0.22/4, fully reversible within 72 hours. Not irritating	4
Mucosal—human			
Betaine at 4% in a toothpaste	Study of the effects of betaine to reduce mucosal irritation in toothpastes containing SLS in 20 patients; patients exposed to the test materials on buccal mucosa with a test chamber kept in place for 15 minutes. Irritation was assessed visually and with electrical impedance for up to 45 minutes	Toothpaste containing 4% betaine alone did not irritate the mucosa in vivo; toothpastes that contained SLS, including those with betaine, were observed to have irritating effects on the oral mucosa	32
Betaine at 4% in a toothpaste	Study testing the efficacy of betaine to reduce "dry mouth" in toothpaste with SLS using 13 patients	No adverse effects to the toothpaste containing 4% betaine	33

Abbreviations: GLP, Good Laboratory Practice; OECD, Organization for Economic Cooperation and Development; .

Clinical Use

Case Reports

Coco-betaine. Two cases of eczematous lesions were reported following exposure to shampoos containing coco-betaine.[36] In the first case, a 44-year-old woman presented with acute eczematous lesions with erythema, edema, and vesiculation on the backs and palms of her hands a few days after using a shampoo with chestnut leaf extract. Her scalp also itched and was slightly red. Previous patch tests showed positive reactions to paraphenylenediamine, benzocaine, wool alcohols, parabens, chinoform, perfumes, nickel sulfate, and cobalt chloride. Patch tests with the shampoo and individual components showed a ++ reaction to the shampoo in open test as is, and in patch test at 2% aqueous (aq), ++ reaction to parahydroxybenzoic acid esters (5% pet.) and +++ reaction to coco-betaine (2% aq). No reactions were observed to the perfume component. The dermatitis cleared when the patient changed shampoos.

In the second case, a 22-year-old woman presented with red, swollen face and weeping eczematous lesions. Red, oozing, and crusted acute lesions were also observed on her shoulders and scalp. The symptoms occurred after using a new shampoo. Patch tests with the shampoo and the individual components showed a +++ reaction to the shampoo in open test as is, and in patch test at 2% aq, ++ reaction to coco-betaine (2% aq) and ++ reaction to sodium lauryl ether sulfate (2% aq). The symptoms cleared when the patient changed shampoos.[36]

Summary

The alkyl betaines are zwitterionic ingredients comprised of tertiary ammonium substituted acetate. These cosmetic ingredients mainly function as hair and skin conditioning agents. With the exception of betaine, alkyl betaines may also function as antistatic agents, surfactants-cleansing agents, and viscosity-increasing agents. The common core chemical structure, similar functions and concentrations in cosmetics, and the expected absorption, distribution, and metabolism enabled grouping these ingredients and reading across the available toxicological data to support the safety assessment of each individual compound in the entire group.

According to information supplied to FDA's VCRP in 2013, betaine has the most reported uses in cosmetic and personal care products, with a total of 459; the majority of the uses are in leave-on skin care preparations. Lauryl betaine has the second greatest number of overall uses reported, with a total of 338; the majority of those uses are in rinse-off personal cleanliness products. In an industry survey, betaine was reported to have a maximum use concentration range of 0.0001% to 8.7%, with the 8.7% reported in rinse-off noncoloring hair conditioner. Lauryl betaine was reported to have a maximum use concentration range of 0.015% to 8.8%, with 8.8% reported in rinse-off noncoloring hair shampoos.

Aside from use in cosmetics, betaine is a common food component and is used to treat homocystinuria. Absorption and

Table 9. Dermal Sensitization Studies.

Ingredient and concentration/dose	Method	Results/conclusions	References
Nonhuman			
Betaine monohydrate >97% pure; up to 50% tested for induction and challenge	OECD Guideline 406 for skin sensitization—guinea pig maximization test in female Dunkin-Hartley guinea pigs; groups of 10 animals; GLP compliant	Not sensitizing	3,5
Betaines, C12-C14-alkyldimethyl (a mixture of lauryl betaine and myristyl betaine); 5%, 10%, 25%, 50% (wt/vol) in ethanol/water (7/3, vol/vol), and 100%	OECD Guideline 429 for LLNA in CBA mice; 4 mice per dose; GLP compliant	Stimulation indices (SI) = 2.4 (5%), 6.2 (10%), 14.7 (25%), 19.0 (50%), and 26.0 (100%). EC3 = 5.8% (wt/vol). Slight (at 10%) to severe (at 100%) erythema observed upon second application. Not sensitizing	4
Betaines, C12-C14-alkyldimethyl (a mixture of lauryl betaine and myristyl betaine); 0.1% of 30% active material	Draize test in 6 male Dunkin-Hartley guinea pigs	Not sensitizing	4
Betaines, C12-C14-alkyldimethyl (a mixture of lauryl betaine and myristyl betaine); 5% for induction, 1% for challenge; water vehicle	OECD Guideline 406 for skin sensitization—Buehler test in 20 female Himalayan spotted guinea pigs with 10 control animals; GLP compliant	No mortalities or signs of systemic toxicity. No skin effects observed in the first and second week of induction. Discrete/patchy to moderate confluent erythema (grades 1 and 2) observed in 12/20 animals in the third week of induction. Not sensitizing	4
Betaines, C12-C14-alkyldimethyl (a mixture of lauryl betaine and myristyl betaine); 0.5% (wt/wt) intradermal induction, 25% (wt/wt) epicutaneous induction, 1% (wt/wt) challenge	OECD Guideline 406 for skin sensitization—Magnusson and Kligman guinea pig maximization test in 10 male and 10 female Hartley guinea pigs; 5 of each sex for control; GLP compliant	Irritation reactions observed. Discrete erythema (grade 1) in 5/20 at 24 hours postchallenge. Moderate erythema (grade 2) in 2/20 at 48 hours postchallenge. Not sensitizing	4
Coco-betaine; 0.5% intradermal induction, 5% epicutaneous induction, 1% challenge	OECD Guideline 406 for skin sensitization—guinea pig maximization test in female Dunkin-Hartley guinea pigs; 10 animals/dose; GLP compliant	Not sensitizing	4
Human			
Betaine at 8.7% in a fragrance-free white lotion/moisturizer; tested neat	Human repeat insult patch test (HRIPT) in 102 patients; semioccluded patches consisted of 2 cm^2 Webril pads with 0.2 mL of the test material and were applied to the infrascapular area of the back or to the upper arm	Not sensitizing	34
Betaine at 5% in a leave-on product	HRIPT in 51 patients; occlusive; patients received on their backs 0.2 mL of the test material with Parke-Davis Readi bandage (approximately 0.05 mL/cm^2)	No skin irritation or allergic contact dermatitis	35
Lauryl betaine at 0.1% active ingredient	HRIPT in 20 volunteers; occlusive	One strong reaction in volunteer after day 6 of induction and a mild reaction in another volunteer after day 7. No reactions observed immediately after challenge. Four delayed reaction observed during the next 4 days with 1 strong, 1 moderate, and 2 mild in form. Reactions were considered due to primary irritation and not due to sensitization	4

Abbreviations: GLP, Good Laboratory Practice; LLNA, local lymph node assay; OECD, Organization for Economic Cooperation and Development.

elimination of betaine in humans were dose dependent, with urinary excretion of betaine increasing with betaine dose.

Cetyl betaine and lauryl betaine were observed to decrease skin barrier function in hairless mouse skin in vitro. Cetyl betaine and lauryl betaine absorbed into mouse skin in vitro, with lauryl betaine absorbing at a faster rate. Dermal penetration rates, measured from the stratum corneum collected from tape stripping, for cetyl betaine and lauryl betaine were 51 to

292 nmol/cm^2 and 3.7 to 35 nmol/cm^2, respectively, in 30-minute exposures to human skin in vivo.

The oral LD_{50} values of betaine, cetyl betaine, lauryl betaine, and C12 to C14 alkyldimethyl betaines were 11.1 g/kg, 1.62 g/kg, 0.071 g/kg, and 3 mL/kg, respectively, in rats, and 2.64 g/kg for coco-betaine (30%) in a mouse study. Also in rats, the dermal LD_{50} values were greater than 16 g/kg for cetyl betaine and 1.3 g/kg for lauryl betaine. The intravenous LD_{50} of betaine in mice has been reported to be 0.83 g/kg bw. The LD_{50} values were 0.15 g/kg for cetyl betaine and 0.053 g/kg for lauryl betaine in an intraperitoneal study in rats.

In repeated dose studies, no effects were observed at the highest dose tested (5%) for betaine in rats. No significant toxic effects were observed in rats that received up to 0.35 g/kg/d cetyl betaine in a 91-day oral study. The NOEL for coco-betaine was 250 mg/kg/d and the LOEL was 500 mg/kg/d in a 90-day oral study in rats when tested up to 500 mg/kg/d. In C12 to C14 alkyldimethyl betaines, systemic NOAELs were 50 and 100 mg/kg bw/d in oral rat studies that tested the material up to 300 and 1,000 mg/kg/d, respectively. The systemic LOAEL for these 2 studies were 150 (due to increased salivation, increased urea, and nonneoplastic histopathologic changes in the kidney and bladder) and 300 mg/kg bw/d (due to decreased food consumption, bw gain, and absolute bw), respectively.

Dermal reproductive and developmental toxicity studies of cetyl betaine in rabbits determined the maternal LOAEL to be 10 mg/kg/d due to decreased bw gain and a maternal NOAEL could not be established. The developmental LOAEL was 40 mg/kg/d and the developmental NOAEL was 20 mg/kg/d. In oral reproductive and developmental toxicity studies of cetyl betaine in rats, the LOAEL for the dams was 50 mg/kg due to decreased bw gain and a maternal NOAEL could not be calculated. The developmental LOAEL was 250 mg/kg and the developmental NOAEL was 150 mg/kg. In an oral C12 to C14 alkyldimethyl betaines study, the reproductive NOEL was 150 mg/kg bw/d and the reproductive LOAEL was 300 mg/kg bw/d due to decreased pup weight and litter size and increased postimplantation loss and postnatal loss. Another oral C12 to C14 alkyldimethyl betaines study determined the reproductive NOAEL to be 300 mg/kg/d when the test material was tested up to 1,000 mg/kg/d.

Betaine was not carcinogenic when tested up to 5% in a 104-week dietary rat study. Betaine and C12 to C14 alkyldimethyl betaines were not genotoxic in in vitro and in vivo studies.

Betaine had anti-irritating effects on the skin in several efficacy studies in humans. In dermal studies, coco-betaine was not irritating in a rabbit study when tested at 16% and was less irritating than SLS in a human study at an unknown concentration. No dermal irritation reactions were observed in human studies of lauryl betaine at 0.1% but were observed at concentrations of 1% and 10%. Dermal irritation results were mixed in rabbit studies of C12 to C14 alkyldimethyl betaines, with irritation observed at 30% and at an unknown concentration in 2 studies and no irritation observed in 2 other studies at unknown concentrations. Betaine at 10% was not an ocular irritant in rabbits nor were C12 to C14 alkyldimethyl betaines at unknown concentrations in several rabbit studies; however, coco-betaine at 16% and 30% and lauryl betaine at 10% were ocular irritants. In human mucosal studies testing the efficacy of toothpaste, betaine at 4% did not produce adverse effects.

Betaine (up to 50%), coco-betaine (up to 5%), lauryl betaine (0.1%), and C12 to C14 alkyldimethyl betaines (up to 100%) were not sensitizing in nonhuman and human dermal studies. Allergic reactions to coco-betaine have been reported in case reports.

Discussion

The Panel considered the available data on alkyl betaines and noted the low systemic toxicity at high doses in single dose and repeated dose oral animal studies, no reproductive/developmental toxic effects in animal studies, no genotoxicity in in vitro and in vivo studies, and no sensitization in multiple tests. A dermal uptake study of cetyl betaine and lauryl betaine that included tape stripping indicated that these ingredients were mostly found in the outer layers of the stratum corneum. The Panel noted that most surfactants exhibit some irritancy, as was noted in dermal and ocular studies of coco-betaine, lauryl betaine, and C12 to C14 alkyldimethyl betaines. Thus, the Panel stated that products that include these ingredients should be formulated to be nonirritating.

Although there are data gaps, the shared core chemical structure, similar functions and concentrations in cosmetics, and the expected similarities in physicochemical properties enabled grouping these ingredients and reading across the available toxicological data to support the safety assessment of each individual compound in the entire group.

The Panel noted that there were no data available on the UV absorption or phototoxicity of alkyl betaines; however, because none of the structures that comprise these ingredients are chromophores, the Panel felt that there was no concern that these ingredients would cause adverse effects from UV exposure.

The Panel expressed concern about animal-derived ingredients, namely, the transmission of infectious agents. They stressed that these ingredients must be free of detectible pathogenic viruses or infectious agents (eg, bovine spongiform encephalopathy prions). These ingredients should be produced according to current good manufacturing procedures (cGMPs) and should conform to regulations for producing substances from animal-derived materials.

The Panel also expressed concern about pesticide residues and heavy metals that may be present in botanical ingredients. The Panel stated that the cosmetics industry should continue to use cGMPs to limit impurities.

The Panel discussed the issue of incidental inhalation exposure from hair sprays, body and hand products, noncoloring hair powders, and indoor tanning preparations. There were no inhalation toxicity data available. Betaine is reportedly used at concentrations up to 3% in cosmetic products that may be aerosolized and up to 0.0001% in cosmetic products that may become airborne. Although the Panel noted that droplets/

particles from spray and loose powder cosmetic products 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 Panel concluded that the following 11 alkyl betaines are safe in cosmetics in the present practices of use and concentration described in the safety assessment, when formulated to be nonirritating.

Behenyl betaine
Betaine
Cetyl betaine
Coco-betaine
Decyl betaine*
Hydrogenated tallow betaine*
Lauryl betaine
Myristyl betaine
Oleyl betaine
Stearyl betaine
Tallow betaine*

*Indicates not 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.

Authors' Note

Unpublished sources cited in this report are available from the Executive Director Bart Heldreth, Cosmetic Ingredient Review, 1620 L Street, NW, Suite 1200, Washington, DC 20036, USA.

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C. Burnett contributed to conception and design, contributed to acquisition, analysis, and interpretation, and drafted the manuscript. B. Heldreth, W. Bergfeld, D. Belsito, R. Hill, C. Klaassen, D. Liebler, J. Marks, R. Shank, T. Slaga, P. Snyder, and F. A. Andersen contributed to conception and design, contributed to analysis and interpretation, and critically revised the manuscript. All authors gave final approval and agree to be accountable for all aspects of work ensuring integrity and accuracy.

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