Safety Assessment of Alkyl Sultaines as Used in Cosmetics

Status: Final Report
Release Date: June 6, 2018
Panel Meeting Date: March 5-6, 2018

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ABSTRACT

The Cosmetic Ingredient Review (CIR) Expert Panel (Panel) assessed the safety of 13 alkyl sultaines, which are most frequently reported to function in cosmetics as antistatic agents, surfactants, and skin and hair conditioning agents. The Panel reviewed the available data to determine the safety of these ingredients. The Panel noted gaps in the available safety data for some of the alkyl sultaines in this safety assessment; the available data on some of the ingredients are sufficient, however, and can be read across to support the safety of other members of the group. The Panel concluded that these alkyl sultaines are safe in cosmetics in the present practices of use and concentration described in this safety assessment.

INTRODUCTION

The alkyl sultaines reviewed in this safety assessment are reported to function as antistatic agents, surfactants, and skin and hair conditioning agents in cosmetics, as described by the web-based *International Cosmetic Ingredient Dictionary and Handbook* (wINCI *Dictionary*; Table 1).[1] This report assesses the safety as used in cosmetics of the following 13 alkyl sultaine ingredients:

Capryl Sultaine
Cetyl/Lauryl/Myristyl Hydroxysultaine
Coco-Hydroxysultaine
Coco-Sultaine
Lauryl Hydroxysultaine
Lauryl Sultaine
Myristyl Sultaine
Cocamidopropyl Hydroxysultaine
Erucamidopropyl Hydroxysultaine
Lauramidopropyl Hydroxysultaine
Myristamidopropyl Hydroxysultaine
Oleamidopropyl Hydroxysultaine
Tallowamidopropyl Hydroxysultaine

The sultaines are structurally related to betaines and are sometimes referred to as sulfobetaines. Each of the ingredients named in this report is a sulfopropyl quaternary ammonium salt. The structures of the alkyl sultaines are relatively similar, and certain toxicological data for one ingredient may be informative about the toxicity of one or more of the other ingredients in this report. The Panel has previously reviewed the safety of Cocamidopropyl Betaine and related aminopropyl betaines, and concluded that those ingredients are "safe in cosmetics as long as they are formulated to be non-sensitizing, which may be based on a quantitative risk assessment (QRA)."[2] That caveat was included in the conclusion due, in part, to data indicating sensitization potential of the impurity 3,3-dimethylaminopropylamine (DMAPA), which may exist in final formulations. The aminopropyl betaines are zwitterionic and comprise a quaternary ammonium salt, like the sultaines, but differ structurally as carboxymethyl alkylamidopropyl substituted ammoniums. The Panel also has previously reviewed the safety of alkyl betaines, and concluded that those ingredients are "safe in the present practices of use and concentration, when formulated to be non-irritating."[3] The alkyl betaines are also zwitterionic and comprise ammonium salts, like the sultaines, but differ structurally as carboxymethyl ammonium salts.

This safety assessment includes relevant published and unpublished data that are available for each endpoint that is evaluated. Published data are identified by conducting an exhaustive search of the world's literature. A listing of the search engines and websites that are used and the sources that are typically explored, as well as the endpoints that CIR typically evaluates, is provided on the CIR website (http://www.cir-safety.org/supplementaldoc/preliminary-search-engines-and-websites; http://www.cir-safety.org/supplementaldoc/cir-report-format-outline). Unpublished data are provided by the cosmetics industry, as well as by other interested parties.

Some chemical and toxicological data on Cocamidopropyl Hydroxysultaine and Lauramidopropyl Hydroxysultaine included 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. Additionally, some data on Lauryl Hydroxysultaine was obtained from a hazard assessment by Australia's National Industrial Chemicals Notification and Assessment Scheme (NICNAS). These data summaries are available on the ECHA and NICNAS websites, respectively, and when deemed appropriate, information from the summaries has been included in this report.[4-6]

CHEMISTRY

Definition and Structure

The definition, structures, and functions of the alkyl sultaine ingredients in this safety assessment are provided in Table 1. All of the ingredients in this group comprise a core sultaine structure, as described in Figure 1, and each comprises a sulfopropyl quaternary ammonium salt. Those ingredients with "amidopropyl" in the name vary structurally from the other ingredients in this report at the "R" position. For "amidopropyl" ingredients the R group is alkylamidopropyl, versus simply alkyl for the other ingredients (Figure 2).

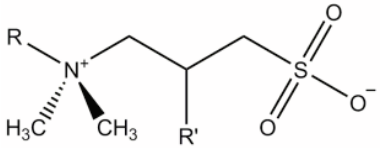
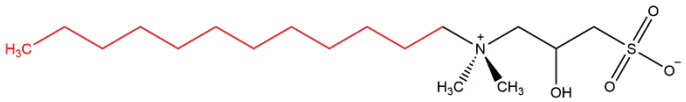
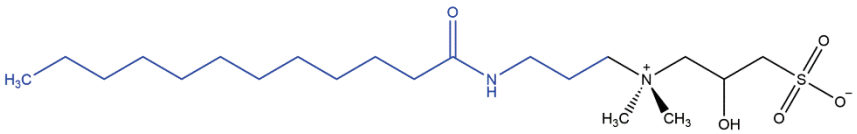


Figure 1. Sultaines, wherein R is alkyl or alkylamidopropyl, and R' is hydrogen or hydroxyl group.



Lauryl Hydroxysultaine



Lauramidopropyl Hydroxysultaine

Figure 2. Examples of alkyl and alkylamidopropyl sultaines.

Physical and Chemical Properties

Available physical and chemical properties for alkyl sultaines are summarized in Table 2. These ingredients are readily soluble in water.[4-6]

Method of Manufacturing

Cocamidopropyl Hydroxysultaine

A supplier has reported that Cocamidopropyl Hydroxysultaine is produced by reacting an inorganic salt with chlorinated epoxide followed by reacting the resulting intermediate with amine.[7] The process undergoes at least 3 checks for quality control with final adjustments made to yield the standard product.

In other submissions, suppliers have reported that Cocamidopropyl Hydroxysultaine is produced through the amidation reaction of coconut oil to form cocamidopropyl dimethylamine.[8,9] This intermediate is then quaternized with 3-chloro-2-hydroxy-1-propanesulfonate to form Cocamidopropyl Hydroxysultaine.

Another supplier reported that Cocamidopropyl Hydroxysultaine is produced by reacting dimethylamino-propylamine with coconut oil.[10] The resulting intermediate is then reacted with a bisulfite solution, a specific chlorine containing petrochemical compound, and water to yield the alkylamidopropyl sultaine ingredient and sodium chloride.

Lauryl Hydroxysultaine

A supplier has reported that Lauryl Hydroxysultaine is produced by quaternizing lauryl dimethylamine *in situ* with sodium oxiran-2-ylmethanesulfonate.[11]

Composition/Impurities

Nitrosamines/Nitrosamides

Although *N*-nitroso-derivative content has not been reported, amidopropyl sultaines comprise secondary amides, and potentially can be nitrosated (alkyl sultaines do not have an amide that is susceptible to nitrosation). Of the approximately 209 nitroso-amines/-amides tested, 85% have been shown to produce cancer in laboratory animals.[12] Nitrosation can occur under physiologic conditions.[13] Depending on the nitrosating agent and the substrate, nitrosation can occur under acidic, neutral, or alkaline conditions. Atmospheric NO_2 may also participate in nitrosation in aqueous solution.[14] Accordingly, amidopropyl sultaines should be formulated to avoid the formation of nitroso-amines/-amides. Additionally, materials used to manufacture these ingredients may include amines susceptible to *N*-nitrosation. Thus, manufacturers should continue to use current good manufacturing practices (cGMPs) to limit residual contamination of these ingredients with such *N*-nitrosatable impurities.

Capryl Sultaine

A manufacturer has reported using a Capryl Sultaine raw material with purity > 98% and levels of propane sultone and *N*,*N*-dimethyl decylamine that are ≤ 100 ppm and ≤ 1000 ppm, respectively.[15,16]

Cocamidopropyl Hydroxysultaine

A supplier has reported that Cocamidopropyl Hydroxysultaine (raw material) contains approximately 50% solids and typically has < 2 ppm DMAPA.[17] Another supplier has reported that unreacted free DMAPA is typically < 10 ppm (< 10 μg/g).[18]

Lauramidopropyl Hydroxysultaine

A supplier has reported that, as a raw material, Lauramidopropyl Hydroxysultaine contains approximately 50% solids and typically has < 3 ppm DMAPA.[17]

Lauryl Hydroxysultaine

Lauryl Hydroxysultaine (28% to 32% active ingredient in an aqueous solution) is reported to contain 20 ppm heavy metals (including lead), 2 ppm arsenic, < 4% quaternary ammonium salts, < 1% free amine, < 14% sodium chloride, and 50% to 57% water.[6]

USE

Cosmetic

The safety of the cosmetic ingredients included in this assessment is evaluated based on data received from the U.S. 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 cosmetics industry in response to surveys, conducted by the Personal Care Products Council (Council), of maximum reported use concentrations by product category.

According to 2018 VCRP data, Cocamidopropyl Hydroxysultaine is used in 280 formulations; the majority of uses are in shampoos, bath soaps and detergents (Table 3).[19] Four other sultaines are in use, with 4 or less uses reported in the VCRP. The results of the concentration of use survey conducted in 2017 by the Council indicate Cocamidopropyl Hydroxysultaine has the highest reported maximum concentration of use; it is used at up to 11.5% in rinse-off products (skin cleansing) and up to 2.5% in leave-on face and neck skin care products.[20] Lauryl Hydroxysultaine is used at up to 5% in rinse-off products (non-coloring shampoos): there were no reported use concentrations in leave-on products. Ingredients with no reported uses in the VCRP or by Council are listed in Table 4.

In some cases, reports of uses were received from the VCRP, but no concentration of use data were provided. For example, Lauryl Sultaine is reported to be used in 2 formulations, but no use concentration data were provided.

Some of the alkyl sultaines may be used in products that can come into contact with mucous membranes. For example, Cocamidopropyl Hydroxysultaine is used in bath soaps and detergents at up to 6.8%.[21] Additionally, some of the alkyl sultaines were reported to be used in hair care products that could possibly be inhaled. For example, Cocamidopropyl Hydroxysultaine was reported to be used in a hair spray at a maximum concentration of 0.05%. 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.[22-25] 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.[22,25]

The alkyl sultaine ingredients described in this safety assessment are not restricted from use in any way under the rules governing cosmetic products in the European Union.[26] An assessment on Lauryl Hydroxysultaine produced by NICNAS concluded that this ingredient was a hazard due to its serious eye irritation potential; however, "when used [at concentrations up to 5% in leave on cosmetic products and up to 10% in rinse off cosmetic products and cleaning products], the notified chemical is not considered to pose an unacceptable risk to public health."[6]

TOXICOKINETICS

No published toxicokinetics studies on alkyl sultaines were discovered and no unpublished data were submitted.

TOXICOLOGICAL STUDIES

Acute Toxicity Studies

Dermal and oral acute toxicity studies for Cocamidopropyl Hydroxysultaine and Lauryl Hydroxysultaine are summarized in Table 5. In an acute dermal study performed in rats, 36.2% Cocamidopropyl Hydroxysultaine in solution has an LD_{50} > 2000 mg active ingredient/kg bw.[5] In acute oral studies, the LD_{50} for 42% Cocamidopropyl Hydroxysultaine was 2950 mg active ingredient/kg bw in rats and 3150 mg active ingredient/kg bw in mice.[5] The LD_{50} for 28% to 32% Lauryl Hydroxysultaine was > 560 - 640 mg/kg bw active ingredient in rats.[6]

Short-Term Toxicity Studies

Cocamidopropyl Hydroxysultaine

The short-term toxicity effects of 36.2% Cocamidopropyl Hydroxysultaine in aqueous solution were assessed in accordance with the Organization for Economic Co-operation and Development (OECD) test guideline 422 (combined repeated dose toxicity study with the reproduction/developmental toxicity screening test) using groups of 10 male and 10 female Sprague-Dawley rats.[5] The test material was administered daily by gavage before mating, during mating, and in females, through day 5 post-partum, at dose levels of 30, 100, or 300 mg/kg/day (exposure duration was 5 weeks in males and 6 to 8 weeks in females). An additional group of 10 males and 10 females received the vehicle control, i.e. drinking water, under the same experimental conditions at a dosing volume of 5 ml/kg/day. The animals were observed daily for clinical signs and mortality. Detailed clinical observations were conducted weekly. Body weights and feed consumption were recorded weekly until mating and then at designated intervals throughout gestation and post-partum. The animals were paired for mating after 2 weeks of treatment and the dams were allowed to litter and care for the pups until day 5 post-partum (see DEVELOPMENTAL AND REPRODUCTIVE TOXICITY (DART) STUDIES Section for reproductive findings).

Prior to killing, blood samples were taken for analysis of blood biochemistry parameters and hematology. The male rats were killed at the end of the mating period and the dams were killed on day 6 post-partum. Body weights and selected organs weights were recorded and a complete macroscopic post-mortem examination including the reproductive organs was performed. The femur of 5 animals in groups 1 to 4 and all group 5 animals were sampled for bone marrow micronucleus analysis (see GENOTOXICITY – In Vivo Section). A microscopic examination was also conducted on selected organs from the first five animals in the control groups and the high-dose groups. Microscopic examination was conducted on all macroscopic lesions from all groups. Based upon the microscopic results of the high-dose group, stomach, forestomach, kidneys, lungs and trachea of the first five animals of the low- and mid-dose groups were also examined.

There were no mortalities before the terminal killings in the 0, 30 and 100 mg/kg/day groups. In the 300 mg/kg/day group, one male was found dead on day 34. At necropsy, there was an enlargement of the lungs (with presence of red discoloration) and white discoloration and an irregular surface of the wall of stomach. The cause of death was moderate subacute bronchoalveolar inflammation, most likely secondary to aspiration of the test material after regurgitation at dosing. This mortality was not considered incidental, but attributed to the test item. Clinical signs of toxicity in the 300 mg/kg/day dose group included loud breathing during days 17 to 19 in one male, during all the pregnancy period in one female, and at the end of the lactation period in another female. Hypersalivation, observed in most animals in the 300 mg/kg/day dose group, was considered to be treatment-related but of minor toxicological importance. No treatment-related effects on mean body weight or mean body weight gain were

observed in the male rats. No treatment-related effects were observed on hematological or blood biochemistry parameters.

No treatment-related effects were observed with organ weight or macroscopic examinations. In the 300 mg/kg/day dose group, microscopic changes were observed in the stomach, lungs, trachea and kidneys. Squamous cell hyperplasia observed in the forestomach was attributed to the irritant properties of the test item. Pulmonary bronchoalveolar inflammation and tracheal epithelial alteration were thought to be related to aspiration of compound after regurgitation at dosing. Minimal to slight degeneration/hypertrophy of the tubular epithelium was observed in the kidneys of the male rats, while minimal tubular vacuolation was observed in some females. In the 100 mg/kg/day dose group, a minimal epithelial alteration in the trachea in a single male rat was not considered an adverse effect because of the low incidence and magnitude. There were no microscopic findings in the stomach, forestomach, kidneys or lungs in this dose group. The authors concluded that the no-observed-adverse-effect-level (NOAEL) for 36.2% Cocamidopropyl Hydroxysultaine was 100 mg/kg/day based on microscopic findings in the forestomach, lungs, trachea and kidneys of animals given 300 mg/kg/day.[5]

DEVELOPMENTAL AND REPRODUCTIVE TOXICITY (DART) STUDIES

Cocamidopropyl Hydroxysultaine

The potential DART effects of 36.2% Cocamidopropyl Hydroxysultaine in aqueous solution were assessed in the short-term toxicity test described above (see TOXICOLOGICAL STUDIES – Short-Term Toxicity Studies Section above), performed in accordance with OECD test guideline 422.[5] The total litter sizes and numbers of pups of each sex were recorded. The pups were observed daily for clinical signs of toxicity. Pup body weights were recorded on days 1 and 3 post-partum. Pups, including those found dead before study termination, were submitted for a macroscopic post-mortem examination.

No treatment-related effects on mating and fertility or unscheduled mortalities were observed. All animals mated within comparable mean number of days. Treatment-related body weight changes in the female rats included a dose-related decrease in mean body weight gain during the premating period and decreases in mean body weights during the pregnancy and lactation periods, which was associated with a non-statistically significant decrease in mean body weight gain during the lactation period. There were no effects on mean feed consumption in the parental animals during any period of the study. There were no relevant differences between control and treatment groups in the following parameters: mean duration of gestation, mean number of corpora lutea, mean number of implantations, mean number of pups delivered, mean pre-implantation loss and mean post-implantation loss. No treatment-related effects were observed in live births, sex-ratio, viability, or lactation indices. No treatment-related clinical signs of toxicity were observed in the pups. There were no significant effects on mean body weight gains in the pups during the post-partum period. No treatment-related findings were observed at necropsy in pups found dead during the observation period or at study end. The authors of this study of 36.2% Cocamidopropyl Hydroxysultaine concluded that the no-observed-effect-level (NOEL) for the reproductive performance of the parental animals was 300 mg/kg/day, which was also the NOEL for toxic effects on the pups.[5]

GENOTOXICITY

In vitro and in vivo genotoxicity studies are summarized in Table 6. Lauryl Hydroxysultaine at 29% was not mutagenic in an Ames test.[6] Cocamidopropyl Hydroxysultaine at up to 50% was not genotoxic in an Ames test, a mouse lymphoma cell mutation assay, or a chromosome aberration study in human lymphocytes.[5] A rat micro-nucleus test of 36.2% Cocamidopropyl Hydroxysultaine found this ingredient did not induce chromosome damage.[5]

CARCINOGENICITY

No published carcinogenicity studies on alkyl sultaines were discovered and no unpublished carcinogenicity data were submitted.

DERMAL IRRITATION AND SENSITIZATION STUDIES

Dermal irritation and sensitization studies are summarized in Table 7. A formulation containing 0.25% Capryl Sultaine was not a skin irritant in rabbits.[27] Cocamidopropyl Hydroxysultaine was not a skin irritant in rabbits when tested at concentration up to 41.5%.[5] Lauryl Hydroxysultaine was slightly irritating to the skin at concentrations up to 32% in rabbits.[6] Cocamidopropyl Hydroxysultaine was not a dermal sensitizer in a guinea pig maximization study in which the test animals were induced via intradermal injection at 10% Cocamidopropyl Hydroxysultaine in deionized water or in Freund's adjuvant and via topical application and at challenge at 42%

Cocamidopropyl Hydroxysultaine.[5] No adverse effects were observed in a clinical in-use study of a formulation containing 0.25% Capryl Sultaine in 24 human subjects for up to 4 weeks.[15,28] Cocamidopropyl Hydroxysultaine was not a dermal sensitizer in a human repeated insult patch tests (HRIPTs) at up to 4% (solids); however, slight to moderate irritation was observed after repeated induction patches in a HRIPT of the ingredient at 2.5%.[5,29] Lauramidopropyl Hydroxysultaine and Lauryl Hydroxysultaine did not cause dermal irritation or sensitization in HRIPTs at 12% solution and 4% solids, respectively.[4,30]

OCULAR IRRITATION STUDIES

Ocular irritation studies are summarized in Table 8.[4-6,31-36] Cocamidopropyl Hydroxysultaine (4% solids), Lauramidopropyl Hydroxysultaine (1.25% and 4% solids), and Lauryl Sultaine (10% and 100%) were predicted to be ocular irritants in in vitro assays.[4,31,32,35,36] In rabbit eyes, Cocamidopropyl Hydroxysultaine (at up to 41.5%) and Lauryl Sultaine (10%) were severe and moderate ocular irritants, respectively.[5,31,33,34] Lauryl Hydroxysultaine at 28% to 32% was irritating to rabbit eyes.[6]

CLINICAL STUDIES

Case Reports

Cocamidopropyl Hydroxysultaine

A 54-year-old man presented with eczema of 2 month duration on the forehead, back of neck, ears, and surrounding areas.[37] He had been using 2 different shampoos that included botanical materials. A similar reaction occurred to a massage product in the past. The patient was patch tested with 40 screening agents and cortico-steroids: ++ reactions to formaldehyde (1% aq.), quaternium-15 (1% pet.); DMDM hydantoin (2% aq.), methyliso-thiazolinone and methylchloroisothiazolinone (0.02% aq.), and Cocamidopropyl Hydroxysultaine (1% aq.) were observed. Tests were read at days 2 and 5, and all were positive by the day 2 reading. Five control subjects tested with Cocamidopropyl Hydroxysultaine were negative. Milder reactions (+ and ?+) were also observed in the patient to cobalt chloride (1% pet.), potassium dichromate (0.5% pet.), and carba mix (1,3-diphenylguanidine, zinc dibutyl-dithiocarbamate, and zinc diethyldithiocarbamate). The patient was negative to cocamidopropyl betaine, as well as to 33 other screening allergens, 5 additional preservatives, and 10 topical corticosteroids.

SUMMARY

The sultaines are structurally related to betaines and are sometimes referred to as sulfobetaines. Each of the ingredients named in this report is a sulfopropyl quaternary ammonium salt. The structures of each of the alkyl sultaines are relatively similar, and certain toxicological data for one ingredient may be informative about the toxicity of one or more of the other ingredients in this report. According to the *Dictionary*, most of the 13 alkyl sultaine ingredients detailed in this report function as antistatic agents, surfactants, and skin and hair conditioning agents in cosmetics.

Cocamidopropyl Hydroxysultaine is reported to be used in 280 formulations; the majority of uses are in shampoos, bath soaps and detergents. Four other sultaines are in use, with 4 or less uses reported in the VCRP. Cocamidopropyl Hydroxysultaine has the highest reported maximum concentration of use; it is used at up to 11.5% in rinse-off products (skin cleansing) and up to 2.5% in leave-on face and neck skin care products. Lauryl Hydroxy-sultaine is used at up to 5% in rinse-off products (non-coloring shampoos); there were no reported use concentrations in leave-on products reported for this ingredient.

In acute dermal studies performed in rats, 36.2% Cocamidopropyl Hydroxysultaine in solution had an LD_{50} > 2000 mg active ingredient/kg bw. In acute oral studies, the LD_{50} for 42% Cocamidopropyl Hydroxysultaine was approximately 3000 mg active ingredient/kg bw in rats and 3150 mg active ingredient/kg bw in mice. The LD_{50} for 28% to 32% Lauryl Hydroxysultaine was > 560-640 mg/kg bw active ingredient in rats.

In a study of 36.2% Cocamidopropyl Hydroxysultaine in rats, the NOAEL for parental toxicity was 100 mg/kg/day based on microscopic findings in the forestomach, lungs, trachea and kidneys of animals given 300 mg/kg/day. This study also evaluated the developmental and reproductive toxicity of this ingredient, and a NOEL of 300 mg/kg/day was determined for both the reproductive performance of the parental animals and for toxic effects on the pups.

Lauryl Hydroxysultaine at 29% was not mutagenic in an Ames test. Cocamidopropyl Hydroxysultaine at up to 50% was not genotoxic in an Ames test, a mouse lymphoma cell mutation assay, or a chromosome aberration study in human lymphocytes. A rat micronucleus test of 36.2% Cocamidopropyl Hydroxysultaine found this

ingredient did not induce chromosome damage. No published carcinogenicity studies on alkyl sultaines were identified and no unpublished carcinogenicity data were submitted.

A formulation containing 0.25% Capryl Sultaine was not a skin irritant in rabbits. No adverse effects such as irritation or pigmentation were observed in a clinical in-use study of a formulation containing 0.25% Capryl Sultaine in 24 human subjects. Cocamidopropyl Hydroxysultaine at up to 41.5% was not a skin irritant in rabbit studies. Lauryl Hydroxysultaine at concentrations of 28% to 32% was a slight dermal irritant in rabbits. Cocamidopropyl Hydroxysultaine was not a dermal sensitizer in a guinea pig maximization study where the test animals were induced via intradermal injection at 10% Cocamidopropyl Hydroxysultaine in deionized water or in Freund's adjuvant and via topical application and at challenge at 42% Cocamidopropyl Hydroxysultaine. Cocamidopropyl Hydroxysultaine was not a dermal sensitizer in a HRIPT at 2.5%; however, slight to moderate irritation was observed after repeated induction patches in the HRIPT. No irritation or sensitization was observed in a HRIPT of 12% Lauramidopropyl Hydroxysultaine. Cocamidopropyl Hydroxysultaine (1%) yielded positive patch tests in a patient that experienced eczema following use of 2 shampoos that contained this ingredient.

Cocamidopropyl Hydroxysultaine (4% solids), Lauramidopropyl Hydroxysultaine (1.25% and 4% solids), and Lauryl Sultaine (10% and 100%) were predicted to be ocular irritants in in vitro assays. In animal studies, Cocamidopropyl Hydroxysultaine (at up to 41.5%) and Lauryl Sultaine (10%) were severe and moderate ocular irritants, respectively, in rabbit eyes. Lauryl Hydroxysultaine at 28% to 32% was irritating to rabbit eyes.

DISCUSSION

The sultaines are structurally related to betaines and are sometimes referred to as sulfobetaines. Each of the ingredients named in this report is a sulfopropyl quaternary ammonium salt. The Panel noted gaps in the available safety data for some of the alkyl sultaines in this safety assessment. Because of structural similarities among the ingredients in the report, data on some of the ingredients can be used to support the safety of ingredients for which no data are available.

The Panel noted the lack of carcinogenicity data for these sultaine ingredients. However, the negative results obtained in both in vitro and in vivo genotoxicity studies alleviated any concerns regarding the need for carcinogenicity data.

The Panel expressed concern that 3,3-dimethylaminopropylamine (DMAPA) and analogous amines that may exist as impurities in the amidopropyl hydroxysultaine ingredients could cause sensitization. Dermal sensitization was not observed in animal or human studies of Cocamidopropyl Hydroxysultaine and Lauramidopropyl Hydroxysultaine, and suppliers have reported that DMAPA impurities are at extremely low levels (< 3 ppm). The Panel noted that the manufacturing processes for each of the amidopropyl hydroxysultaine ingredients are generally similar and are expected to produce the same types of impurities. In quantitative risk assessments (QRAs) submitted to support the CIR safety assessment of Cocamidopropyl Betaine and related fatty acid amidopropyl betaines, conservative weight-of-evidence (WoE) no expected sensitization induction levels (NESILs) were calculated to be 425 $\mu g/cm^2$ for DMAPA. Based on 1) this NESIL, 2) the lack of reported sensitization to the amidopropyl hydroxysultaine ingredients in the literature, and 3) the use concentrations of the these ingredients, the amount of DMAPA present would likely be well below doses expected to induce sensitization; however, to ensure that sensitization does not occur in consumers, the Panel urges manufacturers to minimize the content of DMAPA and related sensitizing agents in cosmetic formulations.

The Panel discussed the issue of incidental inhalation exposure in hair sprays. There were no inhalation toxicity data available. These ingredients are reportedly used at concentrations up to 0.05% in cosmetic products that may be aerosolized. 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.

In past ingredient safety assessments, the Panel had expressed concern over *N*-nitrosation reactions in ingredients containing amine/amide groups. Cocamidopropyl Hydroxysultaine and the other alkylamidopropyl sultaine ingredients in this assessment contain secondary amides that may serves as substrates for *N*-nitrosation. Additionally, these ingredients may contain secondary/tertiary amine impurities which may serves as substrates for

N-nitrosation. Therefore, the Panel recommended that alkylamidopropyl sultaines should not be used in cosmetic products in which *N*-nitroso compounds can be formed, and manufacturers should continue to use cGMPs to limit *N*-nitrosatable impurities in both, alkylamidopropyl sultaines and alkyl sultaines.

The Panel also expressed concern regarding pesticide residues, heavy metals, and other plant species that may be present in botanical ingredients. They stressed that the cosmetics industry should continue to use cGMPs to limit impurities.

The Panel considered the risks inherent in using animal-derived ingredients, namely the transmission of infectious agents. Although tallow may be used in the manufacture of Tallowamidopropyl Hydroxysultaine in this safety assessment and is clearly animal derived, the Panel noted that tallow is highly processed, and tallow derivatives even more so. The Panel agreed with determinations by the U.S. FDA that tallow derivatives are not risk material for transmission of infectious agents.

CONCLUSION

The CIR Expert Panel concluded that the following 13 alkyl sultaines are safe in cosmetics in the present practices of use and concentration described in this safety assessment.

Capryl Sultaine
Cetyl/Lauryl/Myristyl Hydroxysultaine*
Coco-Hydroxysultaine*
Coco-Sultaine*
Lauryl Hydroxysultaine
Lauryl Sultaine
Myristyl Sultaine*
Cocamidopropyl Hydroxysultaine
Erucamidopropyl Hydroxysultaine
Lauramidopropyl Hydroxysultaine*
Myristamidopropyl Hydroxysultaine*
Oleamidopropyl Hydroxysultaine*
Tallowamidopropyl Hydroxysultaine*

**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

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

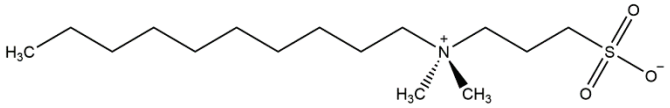
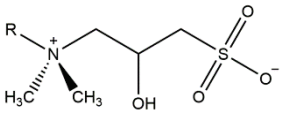
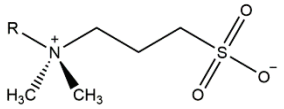
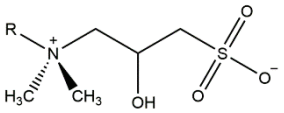
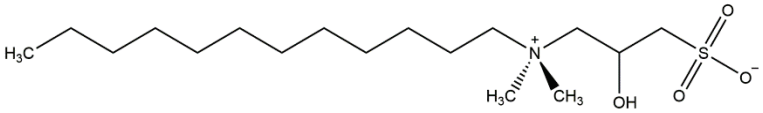
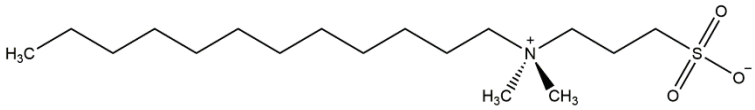
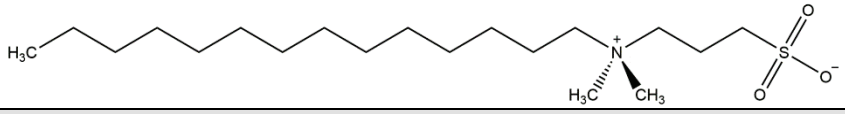
Ingredient CAS No.	Definition & Structure	Function(s)
Alkyl		
Capryl Sultaine 15163-36-7	Capryl Sultaine is the zwitterion (inner salt) that conforms to the formula: 	surfactants – cleansing agents
Cetyl/Lauryl/Myristyl Hydroxysultaine 72869-77-3 (generic)	Cetyl/Lauryl/Myristyl Hydroxysultaine is the zwitterion (inner salt) that conforms generally to the formula:  where R represents a mixture of cetyl, lauryl and myristyl alkyl groups.	hair conditioning agents
Coco-Sultaine	Coco-Sultaine is the zwitterion (inner salt) that conforms generally to the formula:  where R represents the alkyl groups derived from coconut oil.	antistatic agents; hair conditioning agents; skin-conditioning agents – misc.; surfactants – cleansing agents; surfactants – foam boosters; viscosity increasing agents – aq.
Coco-Hydroxysultaine	Coco-Hydroxysultaine is the zwitterion (inner salt) that conforms generally to the formula:  where R represents the alkyl groups derived from coconut oil.	antistatic agents; hair conditioning agents; skin-conditioning agents – misc.; surfacants – cleansing agents; surfactants – foam boosters; viscosity increasing agents – aq.
Lauryl Hydroxysultaine 13197-76-7	Lauryl Hydroxysultaine is the zwitterion (inner salt) that conforms to the formula: 	antistatic agents; hair conditioning agents; skin-conditioning agents – misc.; surfactants – cleansing agents; surfactants – foam boosters; viscosity increasing agents – aq.
Lauryl Sultaine 14933-08-5 52667-78-4	Lauryl Sultaine is the zwitterion (inner salt) that conforms generally to the formula: 	antistatic agents; hair conditioning agents; skin-conditioning agents – misc.; surfactants – cleansing agents; surfactants – foam boosters; viscosity increasing agents – aq.
Myristyl Sultaine 14933-09-6	Myristyl Sultaine is the zwitterion (inner salt) that conforms to the formula: 	exfoliants; surfactants – cleansing agents; surfactants – emulsifying agents
Alkylamidopropyl		

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

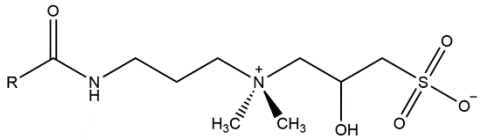
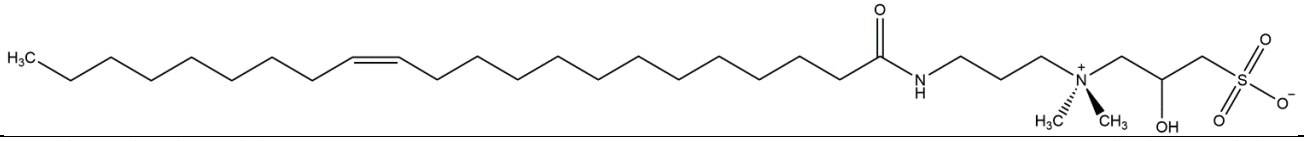
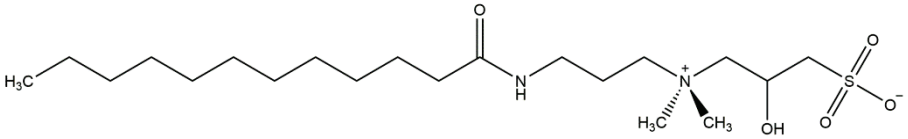
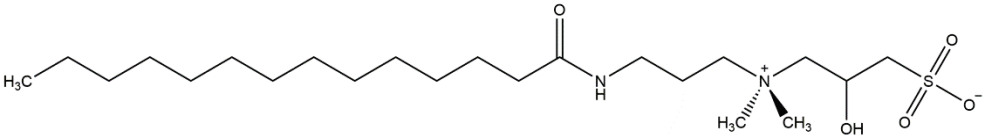
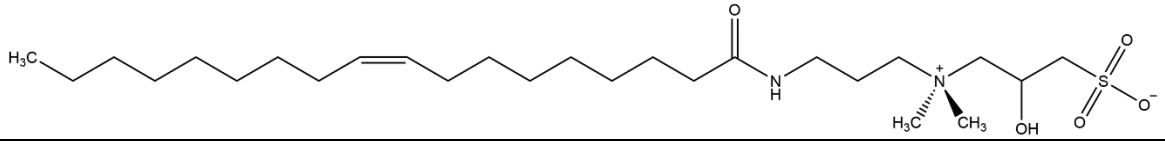
Ingredient CAS No.	Definition & Structure	Function(s)
Cocamidopropyl Hydroxysultaine 70851-08-0 68139-30-0	Cocamidopropyl Hydroxysultaine is the zwitterion (inner salt) that conforms generally to the formula:  where RC(O)- represents the fatty acids derived from coconut oil.	antistatic agents; hair conditioning agents; skin-conditioning agents – misc.; surfactants - cleansing agents; surfactants – foam boosters; viscosity increasing agents – aq.
Erucamidopropyl Hydroxysultaine	Erucamidopropyl Hydroxysultaine is the zwitterion (inner salt) that conforms to the formula: 	antistatic agents; hair conditioning agents; skin-conditioning agents – misc.; surfactants – cleansing agents; surfactants – foam boosters; viscosity increasing agents – aq.
Lauramidopropyl Hydroxysultaine	Lauramidopropyl Hydroxysultaine is the zwitter ion (inner salt) that conforms to the formula: 	antistatic agents; hair conditioning agents; skin-conditioning agents – misc.; surfactants – cleansing agents; surfactants – foam boosters; viscosity increasing agents – aq.
Myristamidopropyl Hydroxysultaine 63663-10-5	Myristamidopropyl Hydroxysultaine is the zwitter ion (inner salt) that conforms to the formula: 	antistatic agents; hair conditioning agents; skin-conditioning agents – misc.; surfactants – cleansing agents; surfactants – foam boosters; viscosity increasing agents – aq.
Oleamidopropyl Hydroxysultaine	Oleamidopropyl Hydroxysultaine is the zwitterion (inner salt) that conforms generally to the formula: 	antistatic agents; hair conditioning agents; skin-conditioning agents – misc.; surfactants – cleansing agents; surfactants – foam boosters; viscosity increasing agents – aq.

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

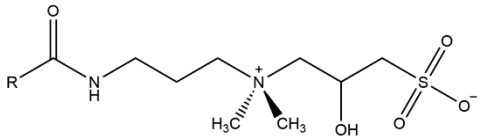
Ingredient CAS No.	Definition & Structure	Function(s)
Tallowamidopropyl Hydroxysultaine	Tallowamidopropyl Hydroxysultaine is the zwitterion (inner salt) that conforms to the formula:  where RC(O)- represents the fatty acids derived from tallow.	antistatic agents; hair conditioning agents; skin-conditioning agents – misc.; surfactants – cleansing agents; surfactants – foam boosters; viscosity increasing agents – aq.

Table 2. Physical and chemical properties

Property	Value	Reference
Cocamidopropyl Hydroxysultaine		
Physical Form	liquid	[5]
Density @20ºC	1.22	[5]
Vapor Pressure mmHg @ 25º C	1.725×10^{-10}	[5]
Boiling point ºC	280.5	[5]
Water Solubility g/l @ 20ºC	556	[5]
Log $P_{oct/wat}$ @ 25ºC	2.1	[5]
Capryl Sultaine		
Physical form	white powder	[16]
Molecular Weight (Da)	307.49	[38]
Water solubility	10% in water gives a clear solution	[16]
Log $P_{oct/wat}$ @ 25ºC	1.26 (estimated)	[39]
Erucamidopropyl Hydroxysultaine		
Molecular Weight (Da)	560.88	[38]
Log $P_{oct/wat}$ @ 25ºC	4.36 (estimated)	[39]
Lauramidopropyl Hydroxysultaine		
Physical form	yellow aqueous solution	[4]
Molecular Weight (Da)	422.63	[38]
Density @20ºC	1.304 – 1.306	[4]
Vapor Pressure mmHg @20ºC	1.725×10^{-10}	[4]
Melting Point ºC	55.0 (mean)	[4]
Boiling Point ºC	311.6 (mean)	[4]
Water Solubility g/l @ 20ºC, pH 8.56-8.64	> 500	[4]
log $P_{oct/wat}$ @ 25ºC	2.1	[4]
Lauryl Hydroxysultaine		
Physical form	colorless to light yellow liquid	[6]
Molecular Weight (Da)	351.55	[38]
Density @ 25ºC	1.108	[6]
Vapor Pressure mmHg @ 25ºC	8.25×10^{-18} (estimated)	[6]
Melting Point ºC	272 (estimated)	[6]
Boiling Point ºC	627 (estimated)	[6]
Lauryl Sultaine		
Molecular Weight (Da)	335.55	[38]
log $P_{oct/wat}$ @ 22ºC	1.65	[6]
Myristamidopropyl Hydroxysultaine		
Molecular Weight (Da)	450.68	[38]
Log $P_{oct/wat}$ @ 25ºC	0.64 (estimated)	[39]
Myristyl Sultaine		
Molecular Weight (Da)	363.60	[38]
Log $P_{oct/wat}$ @ 25ºC	3.22 (estimated)	[39]
Oleamidopropyl Hydroxysultaine		
Molecular Weight (Da)	504.77	[38]
Log $P_{oct/wat}$ @ 25ºC	2.39 (estimated)	[39]

Table 3. Frequency (2018) and concentration of use (2017) according to duration and type of exposure for alkyl sultaines.[19,20]

	# of Uses	*Max Conc of Use (%)*	*# of Uses*	*Max Conc of Use (%)*	*# of Uses*	*Max Conc of Use (%)*	*# of Uses*	*Max Conc of Use (%)*
	Capryl Sultaine		**Cocamidopropyl Hydroxysultaine**		**Erucamidopropyl Hydroxysultaine**		**Lauryl Hydroxysultaine**	
Totals†	**2**	**0.25**	**280**	**0.05-11.5**	**1**	**NR**	**4**	**0.013-5**
Duration of Use								
Leave-On	2	0.25	15	0.05-2.5	NR	NR	NR	NR
Rinse Off	NR	0.25	242	0.1-11.5	1	NR	4	0.013-5
Diluted for (Bath) Use	NR	NR	23	0.97-6	NR	NR	NR	NR
Exposure Type								
Eye Area	NR	NR	NR	NR	NR	NR	NR	NR
Incidental Ingestion	NR	NR	NR	NR	NR	NR	NR	NR
Incidental Inhalation-Spray	NR	0.25[a]	10[a]; 3[b]	0.05; 0.18-0.58[a]	NR	NR	NR	NR
Incidental Inhalation-Powder	NR	0.25[c]	3[b]	2.5[c]	NR	NR	NR	NR
Dermal Contact	2	0.25	180	0.1-11.5	1	NR	2	4.5
Deodorant (underarm)	NR	NR	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	NR	NR	98	0.05-5	NR	NR	2	5
Hair-Coloring	NR	NR	2	1.5	NR	NR	NR	0.013
Nail	NR	NR	NR	NR	NR	NR	NR	NR
Mucous Membrane	NR	NR	119	0.13-6.8	1	NR	NR	NR
Baby Products	NR	NR	3	2.2	NR	NR	NR	NR

	Lauryl Sultaine	
Totals†	**2**	**NR**
Duration of Use		
Leave-On	NR	NR
Rinse Off	2	NR
Diluted for (Bath) Use	NR	NR
Exposure Type		
Eye Area	NR	NR
Incidental Ingestion	NR	NR
Incidental Inhalation-Spray	NR	NR
Incidental Inhalation-Powder	NR	NR
Dermal Contact	2	NR
Deodorant (underarm)	NR	NR
Hair - Non-Coloring	NR	NR
Hair-Coloring	NR	NR
Nail	NR	NR
Mucous Membrane	2	NR
Baby Products	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.] It is possible these products may be powders, but it is not specified whether the reported uses are powders.

Table 4. Ingredients not reported in use[19,20]

Cetyl/Lauryl/Myristyl Hydroxysultaine
Coco-Hydroxysultaine
Coco-Sultaine
Lauramidopropyl Hydroxysultaine
Myristamidopropyl Hydroxysultaine
Myristyl Sultaine
Oleamidopropyl Hydroxysultaine
Tallowamidopropyl Hydroxysultaine

Table 5. Acute toxicity studies

Ingredient and Concentration	Dose/Study Protocol	Results	LD_{50}	Reference
		Dermal		
36.2% Cocamidopropyl Hydroxysultaine in solution	2000 mg active ingredient/kg bw under semi-occlusive patch for 24 h in 1 group of 5 Sprague-Dawley rats per sex	No mortalities or clinical signs of toxicity; very slight or well-defined erythema noted at dose site in 2 females on day 2; mean body weight gains slightly lower than historic controls in females only	> 2000 mg active ingredient/kg bw	[5]
		Oral		
42% Cocamidopropyl Hydroxysultaine in aqueous solution	1000, 2000, or 3000 mg active ingredient/kg bw via gavage in 3 groups of 5 Wistar rats per sex	Mortalities in 2/5 females in the 2000 mg/kg and 3000 mg/kg dose groups and in 3/5 males in the 3000 mg/kg dose group; hemorrhagic and lytic mucous membrane alterations in the gastrointestinal tract were considered treatment-related in rats that died post-dosing; clinical signs in the 3000 mg/kg dose group included reduced activity, diarrhea, squatting, piloerection and/or reduced skin turgor; body weight gains were normal and no test material-related findings at necropsy in surviving animals.	Female rats = 3020 mg active ingredient/kg bw; both sexes = 2950 mg active ingredient/kg bw; male rats = not determined because deaths only in high-dose group.	[5]
41.5% Cocamidopropyl Hydroxysultaine in aqueous solution	830 mg active ingredient/kg bw via gavage in 1 group of 5 Wistar rats per sex	No mortalities; slightly soft feces observed on dosing day; no other clinical signs; no effects in body weight gains and no relevant findings at necropsy.	> 830 mg active ingredient/kg bw for both sexes	[5]
42% Cocamidopropyl Hydroxysultaine in aqueous solution	6.0, 7.5, or 10 ml/kg bw via gavage in 3 groups of 10 CFW mice	2 mice in the low dose group, 5 mice in the mid-dose group, and 8 mice in the high dose group died during the 5-day observation period; clinical signs of toxicity not reported; necropsy not performed	7.8 ml/kg bw; equivalent to 3150 mg active ingredient/kg bw	[5]
28%-32% Lauryl Hydroxysultaine in aqueous solution	2000 mg/kg bw in 5 Wistar Crl: (WI) BR rats per sex	No mortalities observed; no signs of systemic toxicity; no abnormalities at necropsy; body weight gains were as expected	> 2000 mg/kg bw for aqueous solution; 560-640 mg/kg bw for the active ingredient	[6]

Table 6. Genotoxicity studies

Ingredient	Concentration/Dose	Method	Results	Reference
		In Vitro		
Lauryl Hydroxysultaine	29% aqueous solution at up to 1000 µg/plate with metabolic activation and up to 100 µg/plate without metabolic activation	Ames test in *Salmonella typhimurium* strains TA1538, TA1535, TA1537, TA98, TA100 and *Escherichia coli* strain WP2uvrA	Not mutagenic	[6]
Cocamidopropyl Hydroxysultaine	50% aqueous solution at up to 20 µl/plate, with and without metabolic activation	Ames test in *S. typhimurium* strains TA1535, TA1537, TA1538, TA98, and TA100	Not mutagenic	[5]
Cocamidopropyl Hydroxysultaine	36.2% aqueous solution at up to 200 µg/ml without metabolic activation and up to 400 µg/ml with metabolic activation	Mouse lymphoma cell mutation assay in accordance with OECD test guideline 476; 2 independent experiments performed using L5178Y TK +/- mouse lymphoma cells; in first experiment, cells were exposed to test material at concentrations up to 200 µg/ml for 3 h without metabolic activation and up to 400 µg/ml with metabolic activation; in second experiment, the cells were exposed to test material at up to 100 µg/ml without metabolic activation for 24 h and up to 200 µg/ml with metabolic activation for 3 h	Not mutagenic; cytotoxicity observed at higher concentrations	[5]
Cocamidopropyl Hydroxysultaine	36.2% aqueous solution at up to 600 µg/ml without metabolic activation and up to 300 µg/ml with metabolic activation	Chromosome aberrations study with cultured human lymphocytes in accordance with OECD test guideline 473; study conducted as 2 independent experiments; without metabolic activation, cells were exposed to the test substance for 3 (experiment 1), 20 or 44 h (experiment 2), whereas with metabolic activation the treatment period was of 3 h in both experiments; in experiment 1 without metabolic activation and in both experiments with metabolic activation, cells were rinsed after the 3 h of treatment with the test substance and placed in fresh medium culture until the harvest time; cells were harvested 20 or 44 h after the beginning of the experiment	Cocamidopropyl Hydroxysultaine did not induce structural chromosome aberrations with and without metabolic activation at any treatment time; however, in the second experiment, increases in the numerical aberrations were noted when compared to the vehicle control cultures; the numerical aberrations exclusively consisted of polyploidy; no dose-response relationship or consistency between cell cultures; treatment-related cytotoxicity was observed	[5]
		In Vivo		
Cocamidopropyl Hydroxysultaine	36.2% aqueous solution at 0, 30, 100, or 300 mg/kg/day	Micronucleus assay conducted as part of the Short-Term Toxicity/DART study described above (see TOXICOLOGICAL STUDIES and DEVELOPMENTAL AND REPRODUCTIVE TOXICITY (DART) STUDIES) in groups of 10 male and 10 female Sprague-Dawley rats; another group of 5 males and 5 females received a single dose of 30 mg/kg cyclophosphamide (positive control) on the day prior to the scheduled killing of the other test animals.	The test material did not induce damage to the chromosomes or the mitotic apparatus of rat bone marrow cells at doses up to 300 mg/kg/day	[5]

Table 7. Dermal irritation and sensitization studies

Ingredient	Concentration/Dose	Method	Results	Reference
		Irritation - Animal		
Capryl Sultaine	Formulation containing 0.25%	Dermal irritation study in 6 rabbits (strain not specified); undiluted test material applied as single 0.5 ml dose to intact and abraded 1 in^2 areas for 24 h	Not a skin irritant; primary irritation score = 0; minimal inflammation observed on test sites	[27]
Cocamidopropyl Hydroxysultaine	41.5% aqueous solution	Dermal irritation study in 3 male New Zealand White rabbits in accordance with OECD TG 404; test material applied as a single 0.5 ml dose to a shaved 6 cm^2 area of intact skin for 4 h with a semi-occlusive patch	Not a skin irritant; very slight erythema (grade 1) observed at 1h post-patch removal in all animals that remained in 1 animals up until 48 h; mean scores for erythema and edema were 0.22 and 0.00, respectively	[5]
Cocamidopropyl Hydroxysultaine	16% solids aqueous solution	Dermal irritation study in 2 male and 1 female New Zealand White rabbit in accordance with 16 CFR § 1500.41; test material applied to clipped abraded and non-abraded skin (~10% skin surface) for 24 h with occlusive patch	Not a skin irritant; very slight erythema observed in 2 males at the abraded and non-abraded sites and well-defined erythema (score 2) observed in the female at both skin sites; very slight edema observed in the female only; no reactions at 72 h	[5]
Lauryl Hydroxysultaine	28% to 32% aqueous solution	Dermal irritation study in 3 male New Zealand White rabbits in accordance with OECD TG 404; test material applied to intact skin for 4 h with a semi-occlusive patch	Slight dermal irritant; mean score of 1 (very slight erythema) was reported for 2/3 animals; duration of the reaction was less than 7 days; mean score for edema in all 3 animals was 0	[6]
Lauryl Hydroxysultaine	0.4% aqueous solution in intradermal exposures; 1% aqueous solution in topical exposures	Guinea pig maximization study; study not validated	No irritation reported; no adequate reporting of the induction scores, positive controls or challenge scores; no conclusion made on sensitization	[6]
		Sensitization - Animal		
Cocamidopropyl Hydroxysultaine	42% aqueous solution; intradermal injection was at 10% in deionized water or in Freund's adjuvant; topical induction and challenge was undiluted	Guinea pig maximization test in accordance with OECD TG 406 in 20 Pirbright guinea pigs; occlusive patch	Not sensitizing; no skin reactions observed	[5]
		Sensitization - Human		
Capryl Sultaine	Formulation containing 0.25%	In-use study in human subjects with "rough skin conditions"; material was applied either a single time (n=15) or twice daily for 4 weeks (n=9) on intact and/or abraded skin; subjects observed for 4-8 weeks following exposure cessation	No adverse events were observed	[15,28]
Cocamidopropyl Hydroxysultaine	4% solids	HRIPT in 51healthy volunteers; 0.2 ml applied with 1 inch square semi-occluded patches on the upper back	No irritation or sensitization observed	[29]
Cocamidopropyl Hydroxysultaine	2.5% aqueous solution	HRIPT in 44 healthy volunteers; 0.3 ml applied with a 20 mm^2 occluded patch to the upper arm	Not sensitizing; slight to moderate irritation observed in 45% of subjects after repeat induction patches; strong irritation reactions observed in 2 subjects	[5]
Lauramidopropyl Hydroxysultaine	Formulation containing 42% test material, diluted to a 12% solution in distilled water; pH adjusted to 6.03	HRIPT in 54 healthy volunteers; 0.2 ml applied with 1 inch square semi-occluded patches on the upper back	No irritation or sensitization observed	[4]
Lauryl Hydroxysultaine	4% solids	HRIPT in 51healthy volunteers; 0.2 ml applied with 1 inch square semi-occluded patches on the upper back	No irritation or sensitization observed	[30]

Table 8. Ocular irritation studies

Ingredient	Concentration/Dose	Method	Results	Reference
		In Vitro		
Cocamidopropyl Hydroxysultaine	4% solids in distilled water; test dosage = 0.3 ml or 0.3 g	HET-CAM assay	Predicted to be moderately irritating	[35]
Lauramidopropyl Hydroxysultaine	4% solids; test article at 100% was diluted to 20% in distilled water; test volume = 100 µl	MatTek EpiOcular™ tissue model; test article at 100% was diluted to 20% in distilled water	Predicted to be mildly irritating	[36]
Lauramidopropyl Hydroxysultaine	42% in a formulation that was diluted to 1.25%	HET-CAM assay	Predicted to be moderately irritating as diluted solution; predicted to be severely irritating undiluted	[4]
Lauryl Sultaine	diluted at 10% in minimum essential medium; test volume = 0.75 ml	validation of the BCOP assay in 12 separate laboratories	Predicted to be severely irritating; mean score = 80.6	[31]
Lauryl Sultaine	100%	validation of the HET-CAM assay in 3 independent assays	Predicted to be irritating; mean score = 8.3	[32]
		Animal		
Cocamidopropyl Hydroxysultaine	41.5% aqueous solution; test volume = 0.1 ml	Eye irritation/corrosion study in accordance with OECD TG 405 in 3 male New Zealand White rabbits; test material instilled in the conjunctival sac of the right eye of each and untreated eye was the control	Severe eye irritant; grade 2 to grade 3 hyperemia and grade 2 to grade 3 edema, redness of the bulbar conjunctivae, lacrimation, and congestion and injection of the iris observed within 1 h; reactions observed up to 72 h post-dosing; some corneal and conjunctival abnormalities persisted up to 14 days post-dosing, with conjunctival chemosis observed in 1 rabbit up until 21 days post-dosing	[5]
Cocamidopropyl Hydroxysultaine	Aqueous 10% solids solution of pH 7.0; test volume = 0.1 ml	Eye irritation/corrosion study in accordance with 16 CFR § 1500.42 in 3 New Zealand White rabbits; test material instilled in the conjunctival sac of the right eye of each and the untreated eye was the control; eyes were not rinsed	Severe eye irritant; corneal opacity (score 2) observed at 24 h in all rabbits and persisted up to day 7 in 1 rabbit; iridial changes observed at 24 h and persisted up to day 4 in rabbit; conjunctival irritation observed through day 7 in 2 rabbits with decreasing intensity; conjunctival discharge observed in all animals	[5]
Cocamidopropyl Hydroxysultaine	Aqueous 16% solids solution of pH 7.0; test volume = 0.1 ml	Eye irritation/corrosion study in accordance with 16 CFR § 1500.42 in 3 New Zealand White rabbits; test material instilled in the conjunctival sac of the right eye of each and the untreated eye was the control; eyes were not rinsed	Severe eye irritant; corneal opacity (score 2) observed at 24 h in all rabbits and persisted up to day 7 in 1 rabbit; iridial changes observed in 2 animals and persisted up to day 7 in 1 rabbit; conjunctival redness (score 2 to 3) observed at 24 h and persisted until day 7 in 1 rabbit and day 4 in 2 rabbits; chemosis observed with varying intensity in each animal through day 7; conjunctival discharge observed in all animals at 24 and 48 h, decreasing in intensity thereafter	[5,33]

Table 8. Ocular irritation studies

Ingredient	Concentration/Dose	Method	Results	Reference
Lauryl Hydroxysultaine	28% to 32% in an aqueous solution	Eye irritation/corrosion study in accordance with OECD TG 405 in 3 male New Zealand White rabbits; eyes were not rinsed	Irritant; test material produced conjunctival irritation, corneal opacity, and iridial inflammation in all rabbits; one treated eye appeared normal at day 14 observation while a second appeared normal at day 21 observation; opacity was still observed in the third eye on day 21.	[6]
Lauryl Sultaine	diluted at 10% in minimum essential medium	Draize method in rabbits (no further details reported	Moderate irritant; maximal average score (MAS) and day 1 score each = 39.7; reversibility of damage after 21 days	[31]
Lauryl Sultaine	10% w/v in phosphate buffered saline; 100 µl	Modified Draize method in 3 albino rabbits, sex not reported; test material instilled in conjunctival sac of 1 eye each	Irritant; MAS = 43.7	[34]

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