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Final Report on the Safety Assessment of Cocamidopropyl Betaine

Cocamidopropyl Betaine (CAPB) is a zwitterionic ammonium compound that is used primarily as an amphoteric surfactant in shampoos, conditioners, and other cleaning preparations.

The oral LD_{50} of full-strength CAPB was 4.91 g/kg in mice and 7.45 ml/kg in rats. In a 28-day short-term study, CAPB treatment-induced lesions were produced in the nonglandular portion of the stomach in the high-dose group but not in the low-dose group.

A test concentration of 4.5% active CAPB produced slight conjunctival irritation in unrinsed eyes and very slight conjunctival irritation in rinsed eyes. CAPB solutions with 7.5 and 10% activity were not irritating to intact or abraded rabbit skin. When a 15% active solution was tested under occlusive patches for 24 h, well-defined erythema and edema were observed. No evidence of delayed contact hypersensitivity was found in guinea pigs topically administered solutions of 10% active CAPB. No irritation or sensitization was reported in human studies when 3.0% active CAPB was tested.

CAPB was nonmutagenic in four different assay systems. The number of pulmonary adenomas, hepatic hemangiomas, and malignant lymphomas found in mice administered a nonoxidative hair dye formulation containing 0.01% active CAPB for 20 months was similar to the number found in controls.

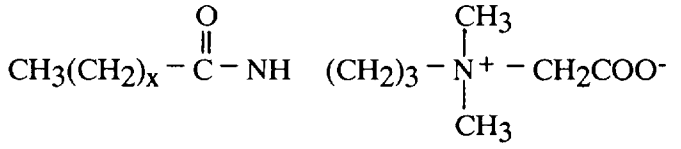
Due to the irritation potential of CAPB, it is concluded that the maximum activity of CAPB used in leave-on cosmetic formulations should not exceed 3.0%. The limitation is expressed as a 10% v/v dilution of a commercial sample that has an activity of 30%. The use of CAPB in rinse-off products is considered to be safe as currently applied.

INTRODUCTION

Cocamidopropyl Betaine, a zwitterionic compound, is used primarily by the cosmetic industry as a pseudoamphoteric surfactant in shampoos, conditioners, and other cleansing preparations. Some of the unpublished data contained in the earlier drafts of this report expressed the concentrations tested either as percent solids or percent activity. The % activity is defined as the % solids (minus) % sodium chloride. To facilitate the review, the CIR Expert Panel requested that all concentrations be expressed as % activity.

CHEMISTRY

Cocamidopropyl Betaine (CAPB) is a zwitterionic ammonium compound conforming to the formula:



Cocamidopropyl Betaine

The R—C(=O)— in amide linkage with the aminopropyl betaine represents fatty acids, ranging in length from 6 to 18 carbons, obtained from the hydrolysis of coconut oil.[1] The predominant fatty acids in two cosmetic grade batches of CAPB are shown in Table 1.

Names for CAPB include 3-(N'-cocoacyl)-amino-N-carboxymethyl-N,N-dimethyl-1-propanaminium hydroxide, N-cocamidopropyl-dimethylglycine, cocoyl amide propylbetaine, and various trade names.[1,3–5]

CAPB is a clear, pale yellow liquid of medium viscosity (300–600 cps) with a slight fatty odor.[3,4,6] CAPB has a boiling point of 212°F, a specific gravity of 1.05 relative to water, and no flash point.[6] CAPB is soluble in water, ethanol, and isopropanol and insoluble in mineral oil.[4]

CAPB is considered a pseudoamphoteric because the quaternary nitrogen of the betaine group cannot donate a proton at pHs above its pK_a, never becoming anionic.[3]

Manufacture of CAPB involves preparation of dimethylaminopropyl cocoamide (3-cocamidopropyldimethylamine) by reacting coconut oil or (hydrolyzed, glyceryl-free) coconut acid with dimethylaminopropylamine in aqueous solution. The dimethylaminopropyl cocoamide, a tertiary amine, is then reacted with sodium chloroacetate to form CAPB and sodium chloride.[3,4,7]

Cosmetic grade CAPB normally is supplied with 35% solids. The concentration of CAPB normally is described by its activity. This is determined by subtracting the percent NaCl from the total percent solids. The characteristics of cosmetic grade CAPB are presented in Table 1.

Impurities

Commercial grades containing concentrations of CAPB greater than 30% may contain solvents, such as propylene glycol.[3] Although most commercial grades contain sodium chloride, low-salt products also are available.[3] The concentration of sodium chloride in cosmetic grade CAPB ranges from 4.0 to 6.0%. Cosmetic grade CAPB may also contain a maximum of 3.0% glycerol.[8]

Although several naturally occurring ammonium compounds analogous to CAPB, such as neurine, carnitine, betaine, choline, and acetylcholine, yield dimethylnitrosamine on reaction with sodium nitrite,[9] no N-nitroso compounds were detected in

TABLE 1. COMPOSITION, CHEMICAL, AND PHYSICAL CHARACTERISTICS OF TWO BATCHES OF COSMETIC GRADE CAPB[2]

Color	Clear pale yellow liquid
Odor	Faint
pH	4.6–5.6
Water content	62–66%
NaCl	4.6–5.6%
Active materials (100(-)H_2O(-)NaCl,%)	29.5–32.5%
Alkalinity	0.725–0.825 Meq/g
Boiling point	230°F
Specific gravity	1.04
Solubility at 25°C	
Water	2g/10 ml
Alcohol	2g/10 ml
Fatty Acids	
C_8	5.6–6.0%
C_{10}	5.4–5.7%
C_{12}	53.1–53.2%
C_{14}	17.4–16.1%
C_{16}	8.3–8.1%
C_{18}	10.2–10.0%

samples of commercially supplied CAPB. CAPB samples with and without internal standards of N-nitroso compounds were analyzed using gas chromatography with a thermal energy analyzer (TEA). CAPB has a secondary amido group that is susceptible to N-nitrosation to an N-nitrosamide. Although a highly sensitive analytical method failed to detect traces of volatile N-nitrosamines in samples of commercial CAPB, this result does not exclude the possibility that in the presence of N-nitrosating agents CAPB gives rise to reactive and unstable nitrosamides. The TEA method does not detect nitrosamides.[10]

Other impurities that are present in coconut oil may be present in CAPB, depending on the degree of refining to which the coconut oil is subjected. Coconut oil may contain free fatty acids and low concentrations of sterols, tocopherol, squalene, and lactones. Concentrations of pigments, phosphatides, gums, and other nonglyceride substances are usually low in coconut oil in contrast to other vegetable oils.[11]

Cosmetic Use

CAPB is contained in 267 of the cosmetic products voluntarily reported to FDA by the cosmetic industry and listed in the product formulation data table (Table 2).[5]

CAPB is primarily used as a pseudoamphoteric surfactant in hair shampoos.[3] Other product categories with formulations containing CAPB are hair conditioners, hair dyes and colors that require patch testing, bath soaps/detergents, skin cleansing preparations, and bubble baths at concentrations ranging from 0.1 to 50% (expressed as a % dilution of commercially supplied Cocoamidopropyl Betaine that is 30% active). CAPB is also an ingredient in one baby shampoo.[3,5,7]

Voluntary filing of product formulation data with FDA by cosmetic manufacturers and formulators conforms to the tabular format listing preset ingredient concentration

ranges and product categories in accordance with Title 21 Section 720.4 of the Code of Federal Regulations.[12] Since certain cosmetic ingredients are supplied by the manufacturer at less than 100% concentration, the value reported by the cosmetic formulator may not necessarily reflect the actual concentration found in the finished product. The actual concentration would be a fraction of that reported to the FDA. Data submitted within the framework of preset concentration ranges provide the opportunity for overestimation of the actual concentration of an ingredient in the particular product. An entry at the lowest end of a concentration range is considered the same as one entered at the highest end of that range, thus introducing the possibility of a two- to ten-fold error in the assumed ingredient concentration.

Products containing CAPB may contact the skin, hair, eyes, and mucous membranes. Use of manicuring and oral hygiene products containing CAPB may result in its ingestion. An approximate final concentration for a bath additive (e.g., bubble baths, bath salts/oils) under normal conditions of use was calculated to be 0.03% (w/w) assuming that 17 g of the bath additive are added to 15 gallons of water under these conditions.[13]

BIOLOGY

No studies were found on the absorption, distribution, metabolism, and excretion of CAPB. It is unclear whether the amide bond of CAPB can be hydrolyzed to yield the fatty acids and 3-aminopropylbetaine. No metabolism data are available on the latter compound.

A 30% active CAPB solution was tested for antibacterial and antimycotic activity using the agar cup plate method.[14] Zones of inhibition were measured for the bacteria and molds around agar cups containing 0.2 ml of the ingredient, which had been diluted with distilled water to 0.5% activity. No inhibition against *Escherichia coli* or *Pseudomonas aeruginosa* was observed. Bacteriostatic activity was detected in cultures of *Staphylococcus aureus, Streptococcus pyogenes,* and *Bacillus subtilis.* Fungicidal activity was observed in cultures of *Candida albicans, Trichophyton mentagrophytes,* and *Pityrosporum ovale.*

ANIMAL TOXICOLOGY

Acute Oral Toxicity Studies

A full-strength CAPB solution, 30% active, was administered by gastric intubation to groups of 10 CFR mice of the Carworth strain weighing 18 to 21 g.[15] Mice were observed for 7 days following the administration. An oral LD_{50} of 6.45 ml/kg within 95% confidence range from 5.66 to 7.35 ml/kg was calculated.[16]

A full-strength solution of CAPB, 30% active, was administered by gavage to groups of 10 (5 female, 5 male) Sprague-Dawley rats weighing approximately 225 g.[17] Single doses of 2.0, 2.71, 3.68, 5.0, or 6.78 g/kg were administered to each of the five groups, and the rats were observed for the following 15 days. Two of 10 rats of the 3.68 g/kg dose group, 6 of 10 rats of the 5.0 g/kg dose group, and 8 of 10 rats of the 6.78 g/kg dose group died 2 to 3 days following CAPB administration. At necropsy, a "bloodlike viscous liquid" was found in the intestines. Surviving rats gained an average of 20 to 130

TABLE 2. PRODUCT FORMULATION DATA FOR COCAMIDOPROPYL BETAINE[5]

Product category	*Total no. of formulations in category*	*Total no. containing ingredient*	*No. of product formulations within each concentration range (%)*						
			>50	*>25–50*	*>10–25*	*>5–10*	*>1–5*	*>0.1–1*	*≤0.1*
Hair shampoos	878	95	—	—	7	24	49	15	—
Hair conditioners and other grooming aids	1118	27	—	—	—	—	13	13	1
Other hair preparations	1223	19	—	—	—	—	6	13	—
Bubble bath products	342	19	—	—	1	4	7	7	—
Other bath preparations	401	39	—	2	—	8	26	3	—
Skin cleansing creams, lotions	751	40	1	—	21	1	10	7	—
Other personal cleanliness preparations	265	12	—	—	—	8	4	—	—
Skin care preparations	2964	9	—	—	—	—	3	6	—
Miscellaneous baby products and other cosmetics	156	7	—	1	—	1	4	1	—
1989 Totals		267	1	3	29	46	122	65	1

g by day 15. Diarrhea was observed in rats of all treatment groups, and decreased motor activity was observed in rats of all treatment groups, except at the lowest dose. Dried blood around the nose and salivation were observed in 3 to 5 male rats of the 5.0 g/kg dosage groups. The acute oral LD_{50} for this full-strength CAPB, 30% active, was 4.91 g/kg within 95% confidence limits of 4.19 to 5.91 g/kg.

Undiluted CAPB, 30% active, of pH 5.5 was administered by gavage to groups of 10 (5 female, 5 male) Wistar rats weighing 150 to 210 g.[18] Dosage groups were 5.00, 6.30, 7.94, and 10.00 ml/kg. The rats were observed for 14 days following CAPB administration. Two rats in the 5.00 ml/kg dosage group died; 1 died within 24 h of administration. Two rats in the 6.30 ml/kg group died within 24 h. Six rats in the 7.94 ml/kg group died; 5 died within 24 h. Eight rats in the 10.00 ml/kg group died; 7 died within 24 h. The oral LD_{50} for the CAPB sample after 14 days of observation was 7.45 ml/kg, with a range of 6.48 to 8.57 ml/kg. The 24-h oral LD_{50} was 8.10 ml/kg, with a range of 6.81 to 9.64 ml/kg. Rats in all dosage groups had decreased motor activity, abnormal body posture, coordination disturbance, cyanosis, diarrhea, and decreased body temperature beginning approximately 20 min after dosage and persisting for 24 h. Surviving rats in all groups had body weight gains of 36 to 45 g and were normal in appearance and behavior. Redness of the stomach and intestinal mucous membrane was observed at necropsy. Mild redness of the intestinal mucous membrane was observed in the animals killed at the termination of the study.

A full-strength solution of CAPB, 30% active, was administered by gavage to groups of five albino rats weighing 200 to 300 g.[19] Single doses of 2.0, 4.0, 5.0, 6.3, 8.0, and 16.0 g/kg were given to each of the six groups, and the rats were observed for 14 days. One of the 5 rats in the 4.0 g/kg group died on day 14; 2 of the 5 rats in the 5.0 g/kg group died, 1 each on days 5 and 11; 3 of the 5 rats in the 6.3 g/kg group died, 1 each on days 4, 6, and 10; all 5 rats in the 8.0 g/kg group died, with deaths occurring on days 1, 4 and 14; all 5 rats in the 16.0 g/kg group died on day 1. Sluggishness, nasal hemorrhaging, diarrhea, and wetness around the hindquarters were observed, increasing in severity with dosage. The oral LD_{50} for this full-strength, 30% active CAPB solution was estimated at 4.9 g/kg, with a 95% confidence limit of 3.7 to 6.5 g/kg.

Short-term Toxicity

A short-term study was done using male and female Sprague-Dawley rats (8/sex/group) and a full strength (30.6% active) solution Cocamidopropyl Betaine. Three dose groups (100, 500, and 1000 mg/kg body weight) were treated daily by gavage for at least 28 days. A control group of 16 animals was given deionized water. Rats dying during the study and those killed on completion of dosing were necropsied, and tissues were collected for histopathological evaluation.[20]

The results included the observation of increased number of deaths in the treated groups as compared to controls, but the mortality did not follow a dose–response relationship.

The principal necropsy findings in these dead rats were those of congestive changes in several tissues, with additional alterations in the lungs of some rats.

One death, that of the high-dose female (B80380), can be ascribed to a dosing accident. It is possible that the one death of a male of the low-dose group and one

female of the mid-dose group can be attributed to accidental placing of the compound into the lungs. The other deaths were related to compound administration and not to the lesions induced. This conclusion is supported by the observation that deaths occurred later (3–4 weeks of study in the mid-dose group, as compared to the high-dose groups: deaths at 1–2 weeks of study). However, doubling of the dose of compound (from 500 to 1000 mg/kg) did not increase mortality, so a dose–response relationship with the mortality is not evident.

The only organ with apparent treatment-induced lesions was the nonglandular portion of the stomach. The lesions were suggestive of irritation by the compound and included subacute inflammation and epithelial hyperplasia (all 7 stomachs examined from the high-dose females). These lesions were found in only 1 of 5 stomachs examined from the high-dose males that survived the 28 days of dosing. The loss of 3 males during the first 2 weeks of dosing did not prevent adequate evaluation of the response of male rats to the compound.

The 28-day study allowed for the determination that the target organ for Cocamidopropyl Betaine is the nonglandular portion of the stomach, and the lesions probably are related to an irritant effect of the compound. Why the incidence was great only in the high-dose females is not readily explained. The study provides information on the short-term effects of high doses at 500 and 1000 mg/kg of full-strength 30.6% active CAPB. Both males and females of the 100 mg/kg dose group were comparable to concurrent controls.

Skin Irritation Studies

A full-strength CAPB solution, 30% active, was tested for skin irritation using 6 adult New Zealand White (NZW) rabbits weighing 2 to 4 kg.[21] Volumes of 0.5 ml of the undiluted CAPB (pH not stated) were applied to intact and abraded sites on the clipped backs of the rabbits. Sites were covered by occlusive patches for 24 h. Scoring was done ½ h after patch removal and 72 h later. Scores for intact and abraded sites were similar. With a mean primary irritation index (PII) of 3.75 (scale of 0–8), CAPB was moderately irritating. CAPB was corrosive to the skin of rabbits because eschar formation was observed at both sites in all rabbits after 72 h (Table 3).

A full-strength CAPB solution, 30% active, was tested for skin irritation using 6 albino rabbits.[19] The pH of the solution was not stated. Volumes of 0.5 ml of the CAPB solution were applied to intact and abraded sites on the backs using occlusive patches. After 4 h, sites were rinsed and scored. Treatment sites also were evaluated 24 and 48 h following the application. Very slight to well-defined erythema (scores of 1–2 on scale of 0–4) was observed at intact and abraded sites. No edema was observed. The mean PII was 0.5, and the CAPB solution was considered a "mild primary irritant."

A 15% active solution of CAPB was tested for skin irritation using 3 male albino rabbits.[22] A volume of 0.5 ml was applied under a 24-h occlusive patch to intact and abraded sites on the clipped abdomen of each rabbit. The pH of the test material was not stated. Sites were scored 24 and 72 h after CAPB application. Well-defined erythema (score of 2) was observed at intact and abraded sites after 24 and 72 h. Slight edema (score of 2, max = 4) was observed at both sites after 24 h. Edema was barely perceptible after 72 h. With a PII of 3.50, CAPB was not considered a primary skin irritant ("a primary skin irritant has a PII $\geq$ 5").

TABLE 3. SKIN IRRITATION STUDIES ON COCAMIDOPROPYL BETAINE (CAPB)

Description of CAPB	*Quantity applied*	*Species*	*Method*	*Results*	*Reference*
CAPB, 30% active[a]	0.5 ml	6 NZW rabbits	SIOPT (single insult occlusive patch test to intact and abraded sites)	PII (Primary Irritation Index) = 3.75 (scale 0–8). Eschar formation	21
CAPB, 30% active[a]	0.5 ml	6 albino rabbits	SIOPT	PII = 0.5. Very slight to well-defined erythema and no edema	19
15% active[a] solution	0.5 ml	3 albino rabbits	SIOPT	PII = 3.50. Well-defined erythema, slight edema	22
10% active[a] solution, pH 4.5	0.5 ml	6 NZW rabbits	SIOPT	PII = 0.3. Very slight erythema, no edema	23
10% active[a] solution, pH 6.1	0.5 ml	1 albino rabbit	SIOPT	PII = 0.25	24
7.5% active[a] solution	0.5 ml	3 albino rabbits	SIOPT	No irritation	25

[a]Reference cited as full strength.

A volume of 0.5 ml of a 10% active solution of CAPB was applied to intact and abraded skin of 6 NZW rabbits for 24 h under occlusive patches.[23] The CAPB sample was a clear liquid with a pH of 4.5. Slight erythema (score of 1) was observed at two intact sites (one after 24 h and the other after 72 h). Four abraded sites had very slight erythema after 24 h, which subsided at two sites 48 h later. No edema was observed. The PII for the 10% active CAPB sample was 0.3; the sample was considered a "nonirritant."

A 10% active solution of CAPB with a pH of 6.1 was tested for skin irritation using 1 albino rabbit.[24] A 0.5 ml volume of the CAPB sample was applied under occlusive patch to intact and abraded skin sites. After scoring 24 and 72 h later, a PII of 0.25 was calculated (a nonirritant).

A 7.5% active solution of CAPB was applied topically to intact and abraded sites on the clipped backs of 3 albino rabbits.[25] The pH was not stated. Treatment sites received applications of 0.5 ml CAPB and were covered with occlusive patches for 24 h. Sites were scored for irritation at patch removal and 48 h later. No irritation was observed.

Skin Sensitization Studies

Delayed contact hypersensitivity of 15 male Pirbright white guinea pigs weighing 400 ± 50 g to a commercial 10% active sample of CAPB was examined[26] using the maximization test.[27] In preliminary studies, maximum concentrations of intradermal injections and topical induction and challenge applications were determined. Three pairs of intradermal injections were made in the dorso-scapular region, which had been clipped free of hair. Test animals were administered 0.1 ml of a 50% aqueous solution of Freund's complete adjuvant at the first pair of sites, 0.1 ml of 0.5% (v/v) dilution of the CAPB sample in sterile isotonic saline at the second pair of sites, and 0.1 ml of 0.5% (v/v) dilution of the CAPB sample in a 1:1 mixture of isotonic saline and Freund's complete adjuvant at the third pair of sites. One week following the injections, a single occlusive 48-h induction patch of 60% (v/v) dilution of the CAPB sample in distilled water was applied to the same shaved interscapular area. Five control animals received intradermal injections and induction patches without the CAPB solution. All animals received a single occlusive 24-h challenge patch of 10% (v/v) dilution of the CAPB sample in distilled water on the left flank 2 weeks after the induction. Well-defined irritation was observed at all sites receiving intradermal injections of Freund's adjuvant. Temporary slight irritation was observed following injections of the 0.5% CAPB sample dilution in all test animals. Topical application of the 60% CAPB sample dilution resulted in slight dermal reactions. The slight (barely perceptible) erythema observed on the skin of 2 test animals after 24 h was considered unrelated to CAPB treatment, but was attributed to slight reactions to the elastic adhesive bandages used for site occlusion. With the exception of slight reactions to the bandages, no reactions were observed in controls throughout the 72-h observation period. No evidence of delayed contact hypersensitivity was found.

A full-strength, 30% active CAPB sample was tested for skin sensitization using the Magnusson-Kligman maximization test and a modified Draize test.[28] In the maximization test, 20 albino guinea pigs (mean body weight: 300–400 g) received intradermal injections of (1) Freund's complete adjuvant alone, (2) 0.1% aqueous dilution of the CAPB sample, and (3) 0.1% aqueous dilution of the CAPB sample plus the adjuvant.

One week later, the topical 48-h occlusive induction patch containing the 10% aqueous dilution of the CAPB sample was applied. The 20 animals in the control group received intradermal injections and topical application of water alone. After 3 weeks, single 24-h occlusive patches were applied to the clipped flanks of all animals. A 10% aqueous dilution of the CAPB sample was applied to the left flank, and water was applied to the right. The lesions at necropsy were erythema and edema in 8 of the 20 test animals after the challenge application. Microscopic findings included epidermal acanthosis, inter- and intracellular edema, and massive infiltration of the superficial layers of the dermis with lymphocytes, monocytes, and a few eosinophils with a tendency to invade the epidermis in two of the animals. Less prominent microscopic lesions of "acanthosis, mild intracellular edema and a moderate lymphomononuclear infiltrate in the superficial dermis" were found in 4 additional animals. Slight acanthosis was observed in the remaining 2 animals.

In the modified Draize test, 0.1 ml of an aqueous 0.15% active solution of CAPB was injected intradermally into the shaved back of each of 20 albino guinea pigs once daily for 5 days.[28] Injections of 0.1 ml of the complete adjuvant were administered intradermally to an adjacent area with the third and fifth CAPB injections. Seven days after the last injection, an intradermal injection of 0.015% active CAPB was administered to the left flank, and a control injection of water was administered to the right. Slight erythema and edema were observed macroscopically in 6 of the 20 test animals. Slight acanthosis was observed microscopically. Control animals in the maximization and modified Draize tests had no "dermatitis-type clinical or histological" alterations. A few controls had "moderate acanthosis with edema and vasodilation in the subjacent papillary layer of the dermis." Investigators concluded that the commercially supplied CAPB tested by these methods, the maximization and modified Draize tests, is capable, "as indicated by histopathological appearances of treated skin," of producing a delayed-type contact sensitization.

A formulation containing 0.75% active CAPB was tested in a delayed contact hypersensitivity test. Closed patches containing 0.4 ml of the test solution were applied to the shaved area on the left shoulder of 20 albino guinea pigs. After 6 h, the patch was removed, and the area was rinsed with warm water. This procedure was repeated at the same site for the following 2 weeks. The animals were left untreated for 2 weeks before the primary challenge test in which a 2.5% solution of the 0.75% active CAPB was applied to a freshly clipped skin site not previously treated, for a 6-h period. Responses were graded after 24 and 48 h. There was no evidence of sensitization following the exposure to the three dermal treatments or challenge dose.[29]

Ocular Irritation Studies

A full-strength sample of CAPB (30% active) was tested for ocular irritation using 9 NZW rabbits.[30] A volume of 0.1 ml was instilled into the conjunctival sac of one eye of each rabbit. The treated eyes of 6 rabbits were left unrinsed, and those of 3 rabbits were rinsed with saline approximately 4 sec after instillation. Mean eye irritation scores for treated, unrinsed eyes were 32.5 ± 4.4 after 24 h, 31.7 ± 3.3 after 48 h, 41.7 ± 11.7 after 72 h, and 27.2 ± 11.4 after 7 days (scale 0–110). Corneal opacity, slight iritis, and conjunctival irritation and necrosis were noted in treated, unrinsed eyes. Under these conditions, the sample was considered corrosive. Minimal irritation (mean score =

10.0 ± 2.0 after 24 h), subsiding after 48 h, was noted in treated eyes that had been rinsed after sample instillation (Table 4).

A volume of 0.1 ml of a 30% active CAPB solution was instilled into the conjunctival sac of one of the eyes of 3 albino rabbits using the Draize method.[31] Diffuse corneal opacity was observed by day 3 following instillation. Slight iritis was observed by day 4. Mild conjunctival erythema, chemosis, and discharge were noted from day 1.

In a Draize test for ocular irritation, two groups of 3 albino rabbits received 0.1 ml instillations of 4.5% active solution of CAPB into the conjunctival sac of one eye.[34] Four seconds after instillation, treated eyes of one group were rinsed. Slight conjunctival erythema and chemosis were noted in all treated, unrinsed eyes by day 2 following instillation and subsided by day 7. Slight conjunctival irritation was observed in two of three treated, rinsed eyes on the first 2 days of observation. There was "no corneal involvement or iris congestion."

An instillation of 0.1 ml of a sample of 10% active CAPB was made into the conjunctival sac of one of the eyes of 9 NZW rabbits.[32] Treated eyes of 6 of the rabbits were not rinsed, and those of 3 of the rabbits were flushed with saline 4 sec after instillation of the CAPB sample. Mean eye irritation scores for treated, unrinsed eyes were 25.7 ± 8.3 after 24 h, 16.7 ± 10.9 after 48 h, and 9.3 after 72 h. No irritation was observed on day 7. Treated, rinsed eyes had a mean score of 2.0 ± 2.0 after 24 h, returning to normal after 48 h. The CAPB sample was considered moderately irritating to treated, unrinsed eyes and practically nonirritating to treated, rinsed eyes under these conditions.

One albino rabbit receiving a 0.1 ml administration of a 10% active CAPB solution (pH 6.1) had Draize scores of 28 after day 1, 25 after day 2, 30 after day 3, 14 after day 4, and 7 after day 7 of the observation period.[24]

Six NZW rabbits (body weight range 2.4–2.6 kg) received an instillation of 0.1 ml of 7.5% active CAPB with a pH of 8.3 into the conjunctival sac of the left eye.[33] Mild to moderate conjunctival irritation was observed in all treated eyes after 24 h. The treated eye of 1 rabbit had moderate corneal opacity after the second day. These alterations disappeared by the sixth day after instillation.

Three albino rabbits received a 0.1 ml instillation of a 6% active CAPB solution into the conjunctival sac of the right eye.[38] Mild conjunctival erythema and slight discharge were observed in all treated eyes for the first 2 days after instillation, clearing by the third day.

In a Draize test for ocular irritation, two 3.0% active CAPB samples were instilled into the conjunctival sac of 6 albino rabbits.[39] Scores for corneal irritation were 0 for the first 2 observation days, 1.66 for the third and fourth days, and 4.16 on the seventh day (max score = 80) for one of the CAPB samples.[40,41] No corneal irritation was observed in eyes treated with the other sample. Both samples produced iritis by the first day (scores of 8.33 and 5 on a scale of 0 to 10), which decreased in severity by the seventh day (scores of 4.16 and 0). Both samples produced conjunctival irritation (scores of 15.37 and 14.33 on a scale of 0 to 20), which decreased in severity by the seventh day (scores of 6 and 0).

A 3.0% active CAPB sample was tested for ocular irritation using 6 male albino rabbits.[42,43] The "average ocular index" was 41.6 (max = 110) 24 h after instillation of 0.1 ml of the sample. The sample was considered an ocular irritant.

A single instillation of 0.1 ml of a product formulation containing 6.0% active CAPB was made into the conjunctival sac of each of 6 albino rabbits in a Draize Eye test.[35] Conjunctival irritation (mean score of 4; max = 20) was observed in all treated eyes on the first day following instillation, decreasing in severity on the second day. No corneal irritation or iritis was observed.

A volume of 0.1 ml of a liquid soap formulation containing 2.3% active CAPB was instilled into the conjunctival sac of each of 9 NZW rabbits.[36] Three of the nine treated eyes were rinsed with 20 ml deionized water 30 sec after treatment. A maximum average irritation score of 18.7 (max 110) was calculated for unrinsed, treated eyes. This score was 20.0 for rinsed, treated eyes. Irritation was observed primarily in the iris and conjunctiva. Under both sets of conditions, the product was considered "moderately irritating" to the eyes of rabbits.

Another liquid formulation containing 2.3% active CAPB was tested for ocular irritation using 9 NZW rabbits. The conjunctival sac of the eyes of 3 rabbits received the same 20 ml rinse with deionized water 30 sec after treatment.[37] The maximum average irritation score for the six treated, unrinsed eyes was 1.7 (max 110). Slight conjunctival erythema and chemosis were observed in 1 rabbit 2 days after treatment and in the eye of another for the entire 7-day observation period. Slight discharge also was observed in the treated eye of the latter from 72 h to 7 days following treatment. The formulation was considered "minimally irritating" to treated, unrinsed eyes of rabbits. The maximum average irritation score for the three treated, rinsed eyes was 3.3. Mild conjunctival erythema and chemosis were observed in all tested eyes 1 to 2 days following the instillation. The formulation was considered "mildly irritating" to treated, rinsed eyes of rabbits.

A liquid soap formulation containing 2.0% active CAPB was tested for ocular irritation by instilling 0.1 ml into the conjunctival sac of one eye of each of 4 NZW rabbits.[35] Treated eyes were rinsed 30 sec later with 40 ml distilled water. Mean corneal irritation scores were 13.8 after 1 h, 18.8 after 24 h, 11.3 after 48 h, 5 after 72 h, and 1.3 after 7 days (max 80). Mean iridial irritation scores were 3.8 after 1 h and 24 h, decreasing to zero after 7 days. Mean conjunctival irritation scores were 11 after 1 h, 7.5 after 24 h, 4 after 48 h, 3.5 after 72 h, and 2 after 7 days. No irritation was observed 14 days after the instillation. With a total mean irritation score of 30.0 (max. total = 110.0), the formulation was considered "moderately irritating."

MUTAGENICITY

A 31.0% active commercial sample of Cocamidopropyl Betaine was tested in the *Salmonella*/mammalian-microsome mutagenicity assay using the five strains, TA98, TA100, TA1535, TA1537, and TA1538, both with and without metabolic activation by Aroclor-induced rat liver microsomes. The experimental protocol is a modification of Ames et al.[44] The amounts of CAPB solution tested were 0.004, 0.02, 0.1, 0.2, and 0.4 µl per plate. CAPB is toxic above 0.3 µl per plate. The test material did not cause a significant increase in the number of revertants per plate in any of the tester strains with or without metabolic activation.[45]

The mutagenic potential of a 30.9% active sample of Cocamidopropyl Betaine was tested in a L5178Y TK ± mouse lymphoma mutagenesis assay with and without exogenous metabolic activation by Aroclor-induced rat liver microsomes. The experi-

TABLE 4. EYE IRRITATION STUDIES ON COCAMIDOPROPYL BETAINE (CAPB)

Concentration of CAPB tested	*No./strain of rabbit*	*Results*	*Reference*
30% active[a]	9/NZW	Max. mean score (unrinsed, $n = 6$) = 41.7 after 72 h, decreased to 27.2 after 7 days (scale 0–110). Minimal irritation in rinsed eyes ($n = 3$)	30
30% active[b]	3/albino	Defused corneal opacity at day 3. Mild conjunctival erythema, chemosis, and discharge on day 1	31
10% active,[b] pH 6.1	1/albino	Max. unrinsed score = 28 after 24 h, 7 by day 7	24
8.6% active[a]	9/NZW	Max. unrinsed score, 25.7 after 24 h, zero by day 7. Mean score rinsed ($n = 3$) = 2.0 after 24 h, zero by 48 h	32
7.5% active, pH 8.3	6/NZW	Mild to moderate conjunctival irritation after 24 h, disappearing by day 6	33
4.5% active[b]	6/albino	Slight conjunctival irritation in 3 unrinsed eyes. Very slight conjunctival irritation in 2 of 3 rinsed eyes	34
Formulation containing 6.5% active[b] CAPB	6/albino	Conjunctival irritation after day 1	35
Soap formulation containing 2.3% active[b] CAPB	9/NZW	Max. mean score (unrinsed, $n = 6$) = 18.7, primarily irritation of iris and conjunctiva. Max. mean score (rinsed, $n = 3$) = 20.0	36
Soap formulation containing 2.3% active[b] CAPB	9/NZW	Max. mean score (unrinsed, $n = 6$) = 1.7. Max. mean score (rinsed, $n = 3$) = 3.3. Primarily conjunctival irritation	37
Soap formulation containing 2.0% active[b] CAPB	4/NZW	Max. total score = 30.0 (max. 110). Irritation of cornea, iris, and conjunctiva	35

[a]Reference cited as % solids.

[b]Referenced as full strength.

mental protocol was based on that described by Clive and Spector.[46] The test substance was solubilized in water and diluted for testing at concentrations of 0.001, 0.01, 0.1, 1.0, 10, and 100 μl/ml. Test material toxicity was determined by comparing the cell population growth at each dose with that of the solvent controls. Cell population density was determined 24 and 48 h after the initial exposure to the test material by removing 1 ml samples from each centrifuge tube, making 1:10 dilutions in 0.1% trypsin, incubating at 37°C for 10 min, and counting the cells with an electric cell counter. None of the treated cultures that were cloned had a significant increase in mutation frequency over the average mutant frequency of the solvent controls. The test substance was negative in this assay.[47]

In an Ames *Salmonella*/microsome reverse mutation assay using *Salmonella typhimurium* strains TA1535, TA1537, TA1538, TA98, and TA100, CABP was examined for mutagenic activity. The full-strength, 30% active test material was evaluated for the ability to cause base pair changes or frameshift mutations in the genome of *S. typhimurium*, using three plates per dose, as measured by *his*− to *his*+ reversion with and without S9 metabolic activation. Concentrations for the mutagenicity assay were selected from a preliminary range-finding test in which 14 concentrations of the test material from 0.018 μl per plate to 150 μl per plate were tested using the strain TA100. Eight concentrations between 0.001 and 0.300 μl per plate were selected based on solubility properties of the test material. The number of revertants were counted following an incubation period at 37°C for 48 to 72 h. The test material did not produce increased numbers of *his*+ revertent colonies with or without metabolic activation. Cocamidopropyl Betaine was not mutagenic to the *S. typhimurium* indicator organisms under these test conditions.[48]

CARCINOGENICITY STUDIES

An aqueous preparation of a nonoxidative hair dye formulation containing an unspecified grade of CAPB at a concentration of 0.09% active CAPB was tested for carcinogenicity using groups of 60 male and female random-bred Swiss Webster mice from the Eppley colony.[49] The formulation also contained 5% propylene glycol, 4% benzyl alcohol, 0.6% Kelzan, 0.9% lactic acid, 0.04% fragrance, and less than 0.1% each of the disperse brown, red, yellow, and blue dyes. A dose of 0.05 ml per mouse was applied three times weekly for 20 months to interscapular skin that was clipped free of hair and shaved. Mortality, behavior, and physical appearance of the mice were observed daily. Dermal changes in particular were noted. Body weights were recorded weekly. Ten males and 10 females from each group were killed at 9 months for a hematological study and necropsy. Urinalysis also was performed. Mean absolute and relative weights of kidney and liver per group were calculated. At termination, all mice were necropsied, and the tissues were examined microscopically. No adverse effects were noted on average body weight gains, survival, hematological or urinalysis values in any group. Varying degrees of chronic inflammation of the skin were seen in all groups, including controls. Other lesions occurred, but were considered unrelated to hair dye treatment. A total of 7 pulmonary adenomas, 4 hepatic hemangiomas, and 14 malignant lymphomas were observed in the 60 treated female mice. Thirteen pulmonary adenomas, 10 hepatic hemangiomas, and 4 malignant lymphomas were observed in the 59 treated male mice. These incidences of neoplasms did not differ significantly from those in the two control groups containing mice that were shaved and received no topical treatment.

CLINICAL ASSESSMENT OF SAFETY

Skin Irritation

A 1.0% aqueous dilution of a product formulation containing 6.0% active CAPB was tested for skin irritation using a single insult occlusive patch test and 19 panelists.[35] Fifteen panelists had no irritation, and a ± score was recorded for 4 panelists. The formulation was considered "practically nonirritating."

Daily doses of 0.2 ml of an 8% aqueous dilution of a liquid soap formulation containing 6.5% active CAPB were applied via occlusive patches to the forearms of 12 human subjects for 5 days.[50] An erythema score of 0.48 (scale 0–4) was calculated.

In a study of cumulative irritation, 0.3 ml of two soap formulations were applied to skin sites on the backs of 10 panelists using occlusive patches.[51] Each formulation contained 1.9% active CAPB and was described as a "cream colored" or "white liquid". Daily 23 h patches were applied for 21 consecutive days. The total irritation scores for all subjects for all 21 applications of the two formulations were 588 and 581. The maximum possible score is 630. The average irritation times for the formulations were 1.48 and 1.69 days, and the median irritation time was 2 days.

Skin Sensitization

A repeated open application procedure was performed with a 10% w/v aqueous dilution of a shampoo containing 18.7% active CAPB, using 30 human volunteers to determine skin sensitization. Filter paper disks, 3/8 inch in diameter, containing 0.1 ml of the test solution, were placed lengthwise about 1 inch apart on the inner left forearm of each subject. The disk was removed after 10 min. The application site was air-dried, covered with a dry gauze, and scored 48 h later. Induction applications were made on Monday, Wednesday, and Friday of the first 3 weeks. Challenge patch strips were applied simultaneously to both the induction arm and the alternate arm, positioned between the shoulder and elbow, 18 days after the last induction application. The areas were scored 24, 48, and 72 h following the removal of the patch after a 6-h period. The same procedures were performed with another test substance containing an identical concentration of CAPB. No sensitization was seen in any of the 88 subjects exposed to the test materials in a shampoo base under any open patching conditions in both the induction and challenge applications.[52]

Other skin sensitization potential studies similar to the above study were performed. Induction applications were made to the same site unless reactions became so strong that a first or second adjacent site had to be used for complete induction, and the sites were scored following a 48-h period. An alternate site was used for the challenge test and was scored after 48 and 96 h. In one study, a 0.9% active aqueous solution of CAPB was tested on 93 human volunteers who had slight responses to the test material. These responses were attributed to primary irritation, rather than sensitization, during both the induction and challenge tests.[8]

In another similar study, the skin sensitization potential of a formulation containing 10% active CAPB was tested on 100 human volunteers. No evidence of sensitization was observed with the test material.[53]

An investigation of the potential of CAPB to induce contact skin sensitization was conducted using 141 human subjects. All applications contained a concentration of 1.5% active CAPB in distilled water, until a protocol modification changed the concentration to 3.0% active CAPB. Subjects who began the study a week earlier

received two applications at a concentration of 1.5%, and all other applications of the test material at a concentration of 3.0%. Induction applications were made to the same, previously untreated site on the back three times per week for 3 successive weeks. The material was applied directly to the skin surface using a template to measure the area and a glass rod to spread the sample over the area. The test sites were covered with a 2 × 2 gauze pad and held in place with surgical tape. The patches were removed after 24 h. Following a 10 to 15-day nontreatment period, the challenge application was applied to a previously untreated site for 24 h, and the site was scored 24 and 72 h after patch removal. No responses were observed during either the induction or challenge tests.[54]

Photosensitization

As an addition to the above protocol, an investigation of the potential of a 3.0% active aqueous solution of CAPB to induce contact photoallergy was tested using 30 human subjects. Duplicate applications of the test material were made to previously untreated sites on the back, and one set of the sites was exposed to UVA, with an average irradiance of approximately 0.180 W/cm^2. In addition, 13 panelists were irradiated with 2× minimal erythemic dose (MED) of UVB, with an average irradiance of 0.300 W/cm^2. Three repetitive applications of each test material were performed on a single site for 3 successive weeks. Within 10 min after removal of the patch on Tuesday and Saturday, the patch sites were exposed to UVA and/or UVB radiation. Both sets of test sites were scored 48 h after sample application nos. 1, 2, 4, 5, 7, and 8 and 72 h after sample application nos. 3, 6, and 9. After a 2-week nontreatment period, a single challenge application of duplicate sets of patches of each test material was made to naive sites. One set of patches was removed after 24 h and exposed to UVA within 10 min. At the conclusion of the light exposure, the other set of patches was removed. All sites were scored 24, 48, and 72 h following the removal of the patches. No response was exhibited by any of the 30 subjects during the challenge application. The 11 subjects who had mild to moderate erythemic responses at the irradiated sites during the induction testing received both UVA and UVB irradiation. These responses were not uncommon and were said to have resulted from the sunburn derived from UVB exposure.[54]

SUMMARY

Cocamidopropyl Betaine (CAPB) is a zwitterionic ammonium compound containing a moiety of either a saturated or unsaturated fatty acid ranging in length from 6 to 18 carbons in amide linkage with aminopropyl betaine. The source for these fatty acids, predominantly lauric acid, is coconut oil. Cosmetic grade CAPB, an aqueous solution, normally contains 35% solids. The NaCl content of these solids ranges from 4.5 to 5.6%. The concentration, when expressed as activity, is determined by subtracting the % NaCl from the % total solids. No N-nitroso compounds were detected in samples of commercially supplied CAPB analyzed by gas chromatography–thermal energy analysis.

CABP is used primarily as a amphoteric surfactant in shampoos, conditioners, and other cleansing preparations. It was listed as an ingredient in 152 of the cosmetic product formulations voluntarily reported to FDA.[5] Reported concentrations of full-strength active CAPB in these products range from 0.01 to 50%.

The oral LD_{50} of full-strength commercial samples of 30% active CAPB was 4.91 g/kg in CFR mice and 7.45 ml/kg in Wistar rats. The oral LD_{50} of a 30% active CAPB in albino rats of unspecified strain was 4.9 g/kg.

In a 28-day short-term study in which groups of 8 male and female animals received 0, 100, 500, and 1000 mg/kg of 30% active CAPB, treatment induced lesions were produced in the nonglandular position of the stomach in the high-dose groups. Both males and females of the low-dose (100 mg/kg) group were comparable to concurrent controls.

Topical administration of varying commercial grades of CAPB (7.5%–30% activity) in single insult occlusive patch tests involving rabbits resulted in PIIs ranging from 0 to 3.75 (max = 8). Slight edema was observed with CAPB with a 10% activity, but not with CAPB with a 7.5% activity.

No evidence of delayed contact hypersensitivity was found in Pirbright white guinea pigs topically administered solutions of 10% active CAPB in a Magnusson-Kligman maximization test. Microscopic changes in the treated skin of albino guinea pigs indicated slight delayed-type contact sensitization by a 3.0% active CAPB solution in a maximization test and a modified Draize test.

Maximum mean irritation scores for eyes of rabbits treated with 30% active CAPB and left unrinsed ranged from 26 to 42 (max = 110). Scores for rinsed eyes ranged from 2 to 10. Irritation was observed primarily in the conjunctivae of treated eyes. At 4.5% active CAPB, there was slight conjunctival irritation in unrinsed eyes and very slight irritation in rinsed eyes. Scores for product formulations containing 2.2 to 6.3% active CAPB ranged from 4 to 30 in unrinsed, treated eyes of rabbits and were 3.3 and 20.0 in rinsed, treated eyes of rabbits.

The mutagenic potential of 30.9% and 31.0% active CAPB formulations was tested in the *Salmonella*/mammalian microsome mutagenicity assay and the L5178Y TK +/− mouse lymphoma assay. CAPB was nonmutagenic in these assays. CAPB was not mutagenic to the *S. typhimurium* indicator organisms in the Ames *Salmonella*/microsome reverse mutation assay.

In a single insult occlusive patch test of a 1.0% aqueous dilution of a product formulation containing 6.3% active CAPB, no skin irritation was observed in 15 of 19 human subjects; 4 of the subjects had slight irritation. Slight erythema was observed after occlusive patching of 12 subjects with an 8% aqueous dilution of a soap formulation containing 2.0% active CAPB daily for 5 days. Two soap formulations containing 2.25% active CAPB were considered primary irritants after a 21-day consecutive occlusive patch study. Skin sensitization potential of CAPB was tested in a number of studies using dilutions of 1.0, 1.5, and 3.0% active CAPB. No evidence of skin sensitization was observed during the induction or challenge tests. An additional study investigated the potential of a 3.0% active solution of CAPB to induce contact photoallergy. There was no response to the challenge tests except for those exposed to both UVA and UVB radiation, who had mild to moderate erythemic responses that were not uncommon and were said to have resulted from the sunburn derived from UVB exposure.

DISCUSSION

The referenced toxicity test data cited in this report expressed the concentration of Cocamidopropyl Betaine (CAPB) as either dilutions of full-strength CAPB expressed as total % solids or by % activity (% activity = % total solids − % NaCl). The concentration of use data voluntarily reported to FDA by cosmetic manufacturers is expressed as a percentage of full-strength CAPB. All test data that were expressed as CAPB, % solids, were changed and expressed as the calculated % activity. When the concentration was expressed as "fullstrength," it is assumed that it had an activity of 30% (FDA use concentrations of 50% = 15% active, 25% = 7.5% active, 3.0% = 1.0% active).

Comparison of toxicity test data from different studies, but similar protocols, in which the concentrations were expressed in terms of either full-strength or 35% solids, or dilutions thereof, and full-strength 30% activity or dilutions thereof, are in essential agreement.

The Expert Panel is aware that nitrosamides may be an impurity in CAPB. No N-nitrosamines were detected in samples of commercially supplied CAPB analyzed by gas chromatography—thermal energy analysis. However, CAPB has the potential to form N-nitroso compounds in cosmetic formulations in the presence of N-nitrosating agents. CAPB was nonmutagenic in the assays included in this report.

The number of pulmonary adenomas, hepatic hemangiomas, and malignant lymphomas found in mice administered a nonoxidative hair dye formulation containing 0.01% active CAPB for 20 months was similar to the number found in controls. However, due to the low test concentrations and low number of animals used, the test results are applicable only to the test conditions used.

A test concentration of 4.5% active CAPB produced slight conjunctival irritation in unrinsed eyes and very slight conjunctival irritation in rinsed eyes. (This concentration is equivalent to an FDA reported use concentration of 15%.)

In two studies, 30% active CAPB was moderately irritating to intact rabbit skin. CAPB solutions with 7.5 and 10% activity were not irritating to intact or abraded rabbit skin. When a 15% active solution was tested under occlusive patches for 24 h, well-defined erythema and edema were observed. Under the conditions of this test, CAPB was a primary irritant. No irritation or sensitization was reported in human studies when 3.0% active CAPB was tested. This is equivalent to an FDA reported use concentration of 10%.

Due to the irritation potential of CAPB, the Expert Panel believes that the maximum activity of CAPB used in leave-on cosmetic formulations should not exceed 3.0%. The limitation is expressed as a 10% v/v dilution of a commercial sample that has an activity of 30%. Rinse-off products are considered to be safe as currently used.

CONCLUSION

Based on the available data included in this report, the Expert Panel concludes that Cocamidopropyl Betaine is safe for use in rinse-off cosmetic products at the current levels of use. The concentration of use for products designed to remain on the skin for prolonged periods of time should not exceed 3.0%. The latter is expressed as a 10% dilution of a full-strength Cocamidopropyl Betaine solution that has an activity of 30%.

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