FINAL REPORT ON THE SAFETY ASSESSMENT OF CETRIMONIUM CHLORIDE, CETRIMONIUM BROMIDE, AND STEARTRIMONIUM CHLORIDE[1]

Cetrimonium Bromide, Cetrimonium Chloride, and Steartrimonium Chloride are quaternary ammonium salts used for a variety of purposes in cosmetics at concentrations of up to 10%. Cetrimonium Bromide given orally is poorly absorbed from the intestine and is excreted in feces. Cetrimonium Bromide applied dermally is absorbed into the skin, but not rapidly. Dermal irritation and sensitization and ocular irritation are seen with these quaternary ammonium salts. Cetrimonium Bromide was embryotoxic and teratogenic in mice following intraperitoneal injection of 35 mg/kg; only teratogenic effects were observed with 10 mg/kg. Embryotoxic effects consistent with maternal toxicity were seen in a rat-feeding study using 50 mg/kg/day. Dermal exposure to 2% Cetrimonium Chloride produced no evidence of teratogenicity; nor did 2.5% Steartrimonium Chloride. All mutagenesis assays used were negative. Repeated insult patch tests of concentrations of up to 0.25% Cetrimonium Chloride produced no sensitization reactions, although irritation was observed during induction. Based on the available data Cetrimonium Bromide, Cetrimonium Chloride, and Steartrimonium Chloride are considered safe for use in rinse-off cosmetic products but are safe only at concentrations of up to 0.25% in leave-on products.

Cetrimonium Bromide and Cetrimonium Chloride are quaternary ammonium salts used as cosmetic biocides, antistatic agents, surfactant-cleansing agents, surfactant-emulsifying agents, and surfactant-suspending agents in cosmetic products. Steartrimonium Chloride is also a quaternary ammonium salt that has been used as an antistatic agent and surfactant. This report is a review of the safety data on these ingredients.

CHEMISTRY

Definition and Structure

Cetrimonium Chloride (CAS No. 112-02-7) and Cetrimonium Bromide (CAS No. 57-09-0) are quaternary ammonium salts that conform to the formula shown in Figure 1 (Wenninger and McEwen, 1995a).

[1]Reviewed by the Cosmetic Ingredient Review Expert Panel.

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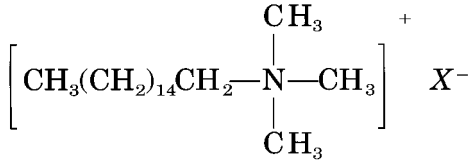

Figure 1. Formula for Cetrimonium Chloride and Cetrimonium Bromide, where X^- is either a chloride or bromide ion.

Other names for Cetrimonium Chloride (Bromide) include 1-Hexadecanaminium, N,N,N-Trimethyl-,Chloride (Bromide); N,N,N-Trimethyl-1-hexadecanaminium Chloride (Bromide); Cetyl Trimethyl Ammonium Chloride (Bromide) (Wenninger and McEwen, 1995a). Cetrimonium Bromide is also known as Alkyltrimethylammonium bromide (Kabara, 1984) and by the trade name Cycloton (Wenninger and McEwen, 1995a). Tradenames for Cetrimonium Chloride include Ammonyx CETAC, Arquad 16-25W, Arquad 16-29W, Barquat CT-29, Carsoquat CT-429, Chemquat 16-29, Chemquat 16-50, CTAC, Cycloton M242C/29, Dehyquart A, Genamin CTAC, Incroquat CTC-50, Varisoft 250, Varisoft 300, and Varisoft 355 (Wenninger and McEwen, 1995a).

Steartrimonium Chloride (CAS No. 112-03-8) is the quaternary ammonium salt that conforms to the formula shown in Figure 2 (Wenninger and McEwen, 1995a).

Other chemical names for this ingredient are N,N,N-Trimethyl-1-Octadecanaminium Chloride; 1-Octadecanaminium, N,N,N-Trimethyl-, Chloride; Stearyl Trimethyl Ammonium Chloride; Stearyltrimethylammonium Chloride; Trimethyl-octadecylammonium Chloride; and Trimethyl-stearylammonium Chloride (Wenninger and McEwen, 1995a; RTECS, 1992). Steartrimonium Chloride is also known by the trade names Hoe S 3615-1, Kemamine Q-9903B, Varisoft TS-50, and Varisoft TSC. Arquad 18-50 is a mixture containing Steartrimonium Chloride (Wenninger and McEwen, 1995a).

Chemical and Physical Properties

Cetrimonium Bromide has a molecular weight of 364.48 and a melting point range of 237°C to 243°C. It is soluble in alcohol and is somewhat soluble in water and acetone but is not soluble in benzene or ether. It is stable at acidic pH (Budavari, 1989), with an optimum biocide pH range of 4 to 10 (Kabara, 1984). It is incompatible with anionics, soap, nitrates, heavy metals, oxidants, rubber, proteins, and blood (Kabara, 1984).

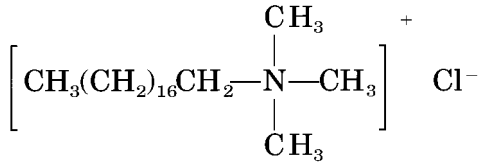

Figure 2. Formula for Steartrimonium Chloride.

Steartrimonium Chloride is an amber liquid that contains approximately 50% active quaternary (Hunting, 1983) and has a molecular weight of 348.13 (RTECS, 1992).

Method of Manufacture

Cetrimonium Bromide is prepared from cetyl bromide and trimethylamine (Budavari, 1989).

Analytical Methods

Cationic surfactants have been determined by colorimetry (Waters and Kupfer, 1976) and by mass spectrometry (Ventura et al., 1989). Steartrimonium Chloride can be identified with gas chromatography (Suzuki et al., 1986), with spectrophotometry using *o*-hydroxyhydroquinonephthalein and manganese (Fujita et al., 1985), and in water with fast atom bombardment mass spectrometry (Ventura et al., 1989).

USE

Cosmetic

Cetrimonium Chloride and Cetrimonium Bromide have the following functions in cosmetics: cosmetic biocide, antistatic agent, surfactant-cleansing agent, surfactant-emulsifying agent, and surfactant-suspending agent. Steartrimonium Chloride functions as an antistatic agent and surfactant (Wenninger and McEwen, 1995b). The product formulation data submitted to the Food and Drug Administration (FDA) in 1994 reported that Cetrimonium Chloride was used in a total of 162 cosmetic formulations, Cetrimonium Bromide was used in 37 cosmetic formulations, and Steartrimonium Chloride was used in 6 formulations (FDA, 1994). These uses are summarized in Table 1.

Concentration of use values are no longer reported to the FDA by the cosmetic industry (Federal Register, 1992). Data submitted to the FDA

Table 1. Cosmetic product formulation data

Ingredient	Product category	Total no. of formulations in category	Total no. of formulations containing ingredient
Cetrimonium chloride	Hair conditions	614	86
	Hair sprays (aerosol fixatives)	306	1
	Permanent waves	387	2
	Hair rinses (noncoloring)	58	3
	Shampoos (noncoloring)	852	1
	Tonics, dressings, and other grooming aids	563	17
	Wave set	98	3
	Other hair preparations	376	16
	Hair dyes and colors (requiring caution statement and patch-test instructions)	1458	18
	Hair shampoos (coloring)	15	2
	Other manicuring preparations	75	1
	Cleansing	746	2
	Body and hand preparations (excluding shaving preparations)	984	2
	Moisturizing products	839	3
	Skin fresheners	222	2
	Other skin-care preparations	790	3
	1994 Total		***162***
Cetrimonium bromide	Eye shadows	571	1
	Colognes and toilet waters	804	1
	Powders	294	3
	Hair conditioners	614	13
	Rinses (noncoloring)	58	1
	Shampoos (noncoloring)	852	1
	Tonics, dressings, and other hair-grooming aids	563	3
	Blushers (all types)	279	1
	Face powders	295	3
	Foundations	345	1
	Deodorants (underarm)	273	1
	Men's talcum	9	1
	Cleansing products	746	1
	Face and neck preparations (excluding shaving preparations)	232	1
	Moisturizing preparations	839	3
	Other skin-care preparations	790	1
	Other suntan preparations	61	1
	1994 Total		***37***
Steartrimonium chloride	Hair conditioners	614	3
	Permanent waves	387	1
	Other hair preparations	376	2
	1994 Total		***6***

Source. FDA (1994).

in 1984, however, indicated that Cetrimonium Chloride was used at concentrations of up to 10% in hair conditioners, tonics, dressing, grooming aids, and wave sets; up to 5% in hair rinses (noncoloring) and moisturizing products; and up to 1% in permanent waves and face and neck preparations (excluding shaving preparations). Cetrimonium Bromide was used at concentrations of up to 5% in rinses; and up to 1% in hair conditioners, tonics, dressings, grooming aids, face and neck preparations (excluding shaving preparations), and moisturizing preparations. Steartrimonium Chloride was reported to be used at concentrations of up to 1% in foundations in 1984, but no listing existed for Steartrimonium Chloride in any hair-care products (FDA, 1984). For shampoos, isopropyl alcohol is usually incorporated into Steartrimonium Chloride to provide fluidity and clarity (Hunting, 1983).

International

Cetrimonium Bromide, Cetrimonium Chloride, and Steartrimonium Chloride, as alkyl (C12-C22) trimethyl ammonium bromide and chlorides, are on the list of allowed preservatives in cosmetics of the European Economic Community (EEC). They have a concentration maximum of 0.1% (EEC, 1991). Cetrimonium Chloride, Cetrimonium Bromide, Cetrimonium Bromide Powder, and Steartrimonium Chloride are approved for cosmetic use in Japan. Some concentration limits are set for various uses (Nikko Chemicals Co., Ltd., 1992).

Noncosmetic

A commercial product containing Cetrimonium Bromide (and other quaternary ammonium salts) is used as a topical antiseptic (Budavari, 1989). The use of Cetrimonium Bromide has been suggested for the removal of hydatid cysts (Frayha et al., 1981) and in the treatment of colorectal carcinoma (Umpleby and Williamson, 1984).

GENERAL BIOLOGY

Biochemistry

An aqueous (aq) solution of 5.0×10^{-4} or 5.0×10^{-3} M Cetrimonium Chloride was incubated with adenosine triphosphate (ATP) and various short-chain carboxylic acids at pH 3.0, 5.0, and 8.0. Samples were ether extracted and analyzed by high-pressure liquid chromatography. Cetrimonium Chloride significantly accelerated the hydrolysis of ATP at pH 5.0 and 8.0 but not at 3.0 (Tabushi et al., 1981).

Freshly prepared liver microsomes were incubated with 0.1 to 5 mM Cetrimonium Bromide, along with nicotinamide-adenine dinucleotide phosphate (NADP), glucose 6-phosphate, glucose 6-phosphate dehydrogenase, semicarbazide hydrochloride, $MgCl_2$, KCl, and phosphate buffer, for 30 min. *N*-Demethylation of Cetrimonium Bromide was determined by formaldehyde concentration. Concentrations over 1 mM Cetrimonium Bromide significantly inhibited demethylase activities (Maduagwu, 1985).

Absorption, Distribution, and Metabolism

Isomaa (1975) investigated the absorption, distribution, and excretion of Cetrimonium Bromide following oral administration in rats. Groups of female Sprague-Dawley rats were administered 0.8 mg/kg [^{14}C]Cetrimonium Bromide by gastric intubation. Animals were killed 2, 4, 8, 24, 72, and 96 h after administration, and tissue samples were taken for radioassay. Similarly treated animals were kept in metabolism cages. Urine and feces were collected at 4-h intervals for 3 days, expired CO_2 was collected at 4-h intervals for 24 h, and bile samples were taken at 2-h intervals for 12 h.

Approximately 80% of the administered radioactivity was found in the gastrointestinal tract at 8 h, 2% of the administered dose was excreted in bile during the first 12 h following treatment, and only very small amounts of radioactivity were detected in the blood plasma. The investigators concluded that Cetrimonium Bromide was poorly absorbed by the intestines. Small amounts of radioactivity were detected in the liver, kidneys, spleen, heart, lungs, and skeletal muscles, with only traces detected in the tissues by day 4. By day 3, 92% of the administered radioactivity was excreted in the feces, and 1% was eliminated in the urine. No radioactivity was detected in expired CO_2. The results of thin-layer chromatography indicated that Cetrimonium Bromide was metabolized to some extent.

The rate and route of excretion of Cetrimonium Bromide following intravenous administration was determined using two male Wistar rats. Each rat was injected with 0.023% ^{14}C-Cetrimonium Bromide in 0.9% aq. NaCl and was placed in a metabolism cage for 24 h. Urine, feces, and expired air were analyzed for radioactivity, the animals were killed after 24 h, and their organs were analyzed for radioactivity. A total of 85.8% of the radioactivity was recovered: 58.9% in the urine, 11.6% in the feces, and 15.3% in the tissues. The investigators noted that unidentified metabolites were detected in the urine and that unchanged Cetrimonium Bromide was found in the feces. No radioactivity was found in expired air (Bartnik and Wingem, 1979).

The investigators also conducted a study to monitor tissue distribution of Cetrimonium Bromide in rats. Three male Wistar rats were administered 0.135 to 0.174% ^{14}C-Cetrimonium Bromide via a jugular cannula. Two of the rats were killed after 15 min and the organs were removed for analysis. The blood of the third rat was analyzed for radioactivity 3, 9, 15, 30, 60, 120, and 300 min following administration. In the two rats killed after 15 min, radioactivity concentrations were greater in the liver (24.8%) and kidneys (5.54%). In the other rat, radioactivity in the blood decreased sharply in less than 30 min, and very little radioactivity could be determined 5 h following administration. After 24 h, low concentrations of radioactivity were found in the liver (2.08%) and kidneys (0.36%) (Bartnik and Wingen, 1979).

In another study, three Wistar rats were administered 0.29% ^{14}C-Cetrimonium Bromide in 0.9% aq. NaCl subcutaneously. Each rat was placed in a metabolism cage for 48 h, urine and fecal specimens were collected, and the rats were killed at the end of the study and their bodies homogenized. A total of 96.2% of the administered radioactivity was recovered: 68.1% in the urine, 14.1% in the feces, and 13.9% in the tissues. Most of the radioactivity was eliminated in the urine within the first 24 h (Bartnik and Wingen, 1979).

Albino Wistar rats (number not specified) were given a single aqueous dose of 2.9×10^{-4} mol/kg Cetrimonium Bromide, 1.5×10^{-3} mol/kg sodium nitrite, or a combination of these two compounds by gavage. Animals were killed and blood samples were taken 2 h after treatment. Serum alkaline phosphatase, glutamic-pyruvic transaminase, and total bilirubin concentration were analyzed from treated and control animals. No significant changes in these parameters were observed (Maduagwu, 1985).

The common bile duct of albino Wistar rats were cannulated, and the animals were injected intraperitoneally with 1.7×10^{-4} mol/kg Cetrimonium Bromide. The bile of both treated and control animals was collected over 4 h and was analyzed by thin-layer and paper chromatography. The metabolite of Cetrimonium Bromide was a secondary amine conjugate (Maduagwu, 1985).

The absorption of Cetrimonium Bromide through the skin of rats was studied. Three groups of five male Wistar rats had ^{14}C-Cetrimonium Bromide applied to the clipped skin of their backs using nonocclusive methods. Group 1 had 1% Cetrimonium Bromide in water applied for 15 min followed by rinsing, and radioactivity was monitored for up to 72 h. Group 2 had 0.5% Cetrimonium Bromide in a hair-rinse formulation applied for 5 min followed by rinsing, and radioactivity was monitored for 48 h. Group 3 had 3% Cetrimonium Bromide applied in water with no rinsing, and radioactivity was monitored for 48 h. The

percutaneous absorption for groups 1, 2, and 3 was 0.59%, 0.093%, and 3.15%, respectively (Bartnik and Wingen, 1979). The calculated total daily absorption for this study was determined by the European Cosmetic, Toiletry, and Perfumery Association (COLIPA) (1984) and is presented in Table 2.

Permeability Enhancement

The lingual frena from New Zealand rabbits were removed and placed in a culture dish with appropriate media. The tissue was cut into four pieces and mounted in modified Ussing chambers and allowed to equilibrate. One side of the chamber was filled with oxygenated medium; 1 μCi of radioactive solute; and 0.025%, 0.1%, or 1.0% Cetrimonium Bromide. The radioactive solute contained [*methoxy*-^{3}H]dextrans, [1,2-^{14}C]ethylene glycol, [U-^{14}C]glucose, [*carboxyl*-^{14}C]-inulin, [^{14}C]urea, [1,7-^{14}C]heptanediol, and [1-^{14}C]*n*-propanol. The other side was filled with oxygenated medium only. Every 30 min, a sample was taken from each chamber and the radioactive content analyzed. The Fick formula was used to obtain permeability constants. Cetrimonium Bromide significantly increased the permeability of the oral mucosa in a concentration-dependent manner (Siegel and Gordon, 1986).

Hematology

Erythrocytes were isolated from blood collected from Sprague-Dawley rats. Concentrations of 5 to 50 μM Cetrimonium Bromide were incubated with the erythrocytes at 37° for 1 h. At 1, 20, 40, and 60 min, aliquots of cells were removed from incubation and immediately examined by phase-contrast microscopy for morphologic changes and classification. Potassium release and cell volume also were measured during the course of incubation.

Starting almost immediately and continuing for the first 20 min, the predominate cell shape was sphero-echinocyte. During the next 40 min, the number of discocytes and then sphero-stomatocyte shapes began to increase. The concentrations at which this occurred was 7.5 to 10 μM Cetrimonium Bromide. The higher concentrations of Cetrimonium Bromide had a greater ratio of stomatocytes to echinocytes, and echinocytes were seen earlier in the incubation period. A concentration-dependent increase in the modal volume of erythrocytes was observed. Over the range of concentrations, this increase preceded potassium release and continued until 100% hemolysis occurred. Concurrently, another sample of erythrocytes was incubated with 5 μM [trimethyl-^{14}C]Cetrimonium Bromide. Over a period of 90 min, aliquots of cells

Table 2. Calculated percutaneous absorption

Group	Percutaneous absorption rate[a]	Quantity of Cetrimonium Bromide absorbed (mg)	Daily absorption (mg/kg/day)
1	0.59%	0.12	0.002
2	0.093%	0.02	0.0004
3	3.15%	0.63	0.012

[a] Bartnik and Wingem, 1979.

Source. European Cosmetic, Toiletry, and Perfumery Association (1984).

were iced, centrifuged, and counted. The adsorption of Cetrimonium Bromide to the erythrocytes reached equilibrium after approximately 1 min and was equivalent to approximately 1.6×10^7 molecules of Cetrimonium Bromide per cell (Isomaa and Paatero, 1981).

The effects of Cetrimonium Bromide on platelet cytoskeletal assembly was investigated. Platelets were isolated from the blood of volunteers and incubated with either ^{14}C-serotonin or ^{32}P-orthophosphate. Aliquots of equal amounts of cells with radioactive serotonin then were incubated with concentrations of 2.5 or 5.0 μM Cetrimonium Bromide for up to 1 h. At regular intervals, samples were taken, challenged with arachidonate or thrombin, and analyzed for serotonin release. The cytoskeletal core of a sample of ^{32}P-orthophosphate–treated platelets incubated with 15, 25, or 30 μM Cetrimonium Bromide and challenged with arachidonate or thrombin was isolated. These samples were run on an electrophoresis gel to determine phosphorylation of certain platelet proteins.

At first, Cetrimonium Bromide inhibited thrombin-induced platelet release of serotonin. After approximately 12 min, however, platelets recovered and release was eventually stimulated to more than 100% of controls. No recovery from the inhibition of arachidonate-induced platelet serotonin release was observed. Cetrimonium Bromide did not significantly inhibit the incorporation of actin-binding protein or myosin heavy chain proteins in the inner cytoskeletal cores in thrombin-induced cells. Inhibition by Cetrimonium Bromide of arachidonate-induced protein incorporation into the cytoskeletal core was observed (Carroll and Cox, 1984).

The adjuvant activities of Cetrimonium Chloride and Cetrimonium Bromide were studied. Groups of five albino guinea pigs were given a subcutaneous injection of 1 Lf purified diphtheria toxoid in 0.2 mL borate-succinate buffer (pH 7.5). This was administered with a dose of 100 μg of an aliphatic nitrogenous base in suspension, including Cetrimonium Chloride and Cetrimonium Bromide to determine adju-

vant potentials of these compounds. A second injection of toxoid was administered 28 days following the first injection. Guinea pigs were bled 10 days after the second injection, and antitoxin titers were measured by the guinea-pig intracutaneous method. Two separate commercial preparations of Cetrimonium Chloride had strong adjuvant activity. A commercial preparation of Cetrimonium Bromide had weak adjuvant activity (Gall, 1966).

Perfused mast cells exposed to 37.5 μM Cetrimonium Chloride were observed by time-lapse video photography for a period of 30 min. After 3 min, histamine was released. After 6.5 min, 60% of the cells swelled and then degranulated. After 13 min, a second histamine and lactate dehydrogenase releases occurred. After 23.5 min, the remaining 40% of the cells started to swell, and the intracellular perigranular membrane started to rupture (Parsons et al., 1986).

Splenic lymphocytes prepared from Balb/c and NMRI mice were incubated with sublytic concentrations of Cetrimonium Bromide in medium for 30 min. Some cultures were washed (some with Triton X-100) and incubated with fluorescein-conjugated goat (or rabbit) anti-mouse IgG. Cells were plated on coverslips, allowed to incubate, washed, and mounted. Other cultures were incubated with monoclonal antitubulin and then stained with fluorescein-conjugated rabbit anti-mouse IgG. Cultures for electron microscopy were incubated with ferritin-conjugated goat anti-mouse IgG. Pretreatment with Cetrimonium Bromide significantly decreased the amount of surface immunoglobin capping in a dose-dependent manner. No structural changes were observed by electron microscopy. Cetrimonium Bromide neither affected tubulin fibers seen by fluorescence nor affected the interaction of surface immunoglobulin with elements of the cytoskeleton. The authors theorize that Cetrimonium Bromide inhibited cap formation by altering the association between the surface of the cell and the cytoskeleton (Paatero et al., 1986).

Virucidal, Bactericidal, and Parasiticidal Effects

Cetrimonium Chloride has inhibitory effects on activated sludge microorganisms, *Photobacterium phosphoreum*, and *Spirillum volutans* (Dutka et al., 1983). Cetrimonium Bromide has virucidal effects on human rotavirus (Rodgers et al., 1985) and bactericidal effects on *Pseudomonas aeroginosa* (Evans et al., 1985; el-Nima, 1984), *Streptococcus mutans* (Yotis et al., 1983), *Escherichia coli*, and a variety of gram-negative bacteria (Hammond et al., 1987).

Parasiticidal effects on *Echinococcus granulosus* also were observed with Cetrimonium Bromide (Frayha et al., 1981).

TOXICOLOGY

Acute Toxicity

Oral

The reported oral LD_{50} of Cetrimonium Chloride for rats was 250 to 300 mg/kg (Armour Industrial Chemicals Company, 1967), and a 40% solution of Cetrimonium Bromide in water had an oral LD_{50} of 1000 mg/kg for rats (Scientific & Technical Information Network [STN] International, 1989).

The oral LD_{50} for Steartrimonium Chloride for 10 ddY mice over a 14-d observation period was 633 mg/kg for male mice and 536 mg/kg for female mice. Signs of toxicity observed during the study were hypoactivity and diarrhea. Congestion of the brain and kidneys were found at necropsy (Hasegawa et al., 1989).

Intraperitoneal

The intraperitoneal LD_{50} of 40% (w/v) Cetrimonium Bromide was 56.2 mg/kg for rats (STN International, 1989).

Subchronic Dermal Toxicity

A 28-day percutaneous toxicity test of Cetrimonium Chloride was conducted using New Zealand White rabbits. A suspension of 54.5% Cetrimonium Chloride was diluted with water to form a 0.5% (w/v) solution. Doses of 2 mL/kg/day of this solution were applied to the clipped and abraded skin of five male and five female rabbits for 6.5 to 7.0 h, 5 days a week for 4 weeks. A control group of rabbits was treated using the same schedule with distilled water. Observations for signs of toxicity and for mortality were made twice a day, dermal irritation was scored daily, and body weights were measured weekly. Blood samples were taken prior to dosing and then at week 4 for hematologic analyses. Necropsy was performed on the animals either at the time of death or when they were killed at the end of the study.

No deaths occurred in the experimental group, and no evidence of toxicity or hematological changes was present. Slight to moderate erythema was observed in all of the treated rabbits between days 4 and 8 but disappeared in four rabbits by day 17. Very slight to slight edema was observed between days 6 and 12 in four rabbits and subsided by day 17. Two rabbits had intermittent evidence of slight edema during week 4, and one rabbit developed edema on day 20. No evidence of desquamation or coriaceousness was present in three rabbits. In the other rabbits, slight atonia was observed but persisted into week 4 in only three animals. Similarly, very slight to slight desquamation and

coriaceousness was noted in most of the rabbits, but desquamation was found only in three animals and coriaceousness in two animals by week 4. Slight fissuring was observed in most of the rabbits but typically disappeared by the end of the study.

At necropsy, treatment-related changes were found only in the skin. The application sites of two rabbits were reddened, and one rabbit had scabbing. Findings from microscopic examination included mild to marked acanthosis with active mitosis, hyperkeratosis, and partial to extensive necrosis of the epidermis and hair follicles with or without encrustation with exudate. No statistically significant changes were observed for the hepatic or renal weights between the experimental and control animals (International Research and Development Corporation, 1979).

Chronic Oral Toxicity

Cetrimonium Bromide, at concentrations of 10, 20, and 45 mg/kg/day, was administered via the drinking water to rats for 1 year. The only effect noted was a decrease in body weight gain in the 45-mg/kg/day–dose group (Kabara, 1984).

Dermal Irritation

In Vitro

Cetrimonium Chloride was tested in a proposed in vitro model for identifying skin-irritating chemicals. The method was based on the correlation between irritation potential and the ability to reduce the penetration barrier of the skin by lysis of the stratum corneum. The clipped hair coat of male Wistar rats was removed as a single pelt and subjected to tape stripping, abrasion, or immersion in water. Epidermal slices were taken from the pelt and were treated with Cetrimonium Chloride (as supplied). Slices were evaluated after 1, 4, and 24 h of exposure. The reduction in electrical resistance of the treated epidermal slices after contact with Cetrimonium Chloride was taken as a measure of irritating potential. The threshold resistance value for a positive effect was 4kΩskin disc. Cetrimonium Chloride was classified as a skin corrosive. No response was observed at the 1-h reading, but Cetrimonium Chloride caused resistance values ranging from 2.4 to 1.4 kΩskin disc between 4 and 24 h (Oliver et al., 1988).

In Vivo

The primary skin irritation of 0.1%, 0.5%, 1.2%, and 2.5% Cetrimonium Chloride was evaluated using groups of six albino male rabbits of the

SPF-Russian strain. Cetrimonium Chloride (0.5 g) was applied to intact and abraded sites on the backs of the rabbits for 24 h. Comparable sites were left untreated. All of the sites were scored at 24 h, rinsed, and then scored again at 48 h. The results are shown in Table 3.

Cetrimonium Chloride (0.2 mL), at concentrations of 1% and 10%, was applied to the penile mucosa of one rabbit, and observations were made at 1, 2, and 24 h. The overall irritant score was 0 for the 1% solution and 1.08 for the 10% solution, of a possible maximum score of 4 (Armour Industrial Chemicals Company, 1967).

Steartrimonium Chloride was reported to the Environmental Protection Agency as severely irritating to the skin, having a primary irritation index of 5.4. The test animal, dose, and maximum possible irritation score were not specified, but it was noted that clinical evidence of irritation included necrosis, sloughing of skin, slight to moderate erythema and very slight edema at 24 h, and severe erythema and edema at 72 h (Sherex Chemical Company, Inc., 1986).

Dermal Sensitization

Twenty guinea pigs were tested in the Buehler assay for dermal sensitization. The induction dose was 0.75% Steartrimonium Chloride in 80% ethanol, and the challenge dose was 0.5% Steartrimonium Chloride in 80% ethanol. Fifteen animals had positive reactions, and the average irritation score was 1.5 (maximum possible score was not reported) (Sherex Chemical Company, Inc., 1986).

Ocular Irritation

In Vitro

Bovine red blood cells (RBC) were collected and isolated weekly. Aliquots of 2×10^8 RBC/mL were made. Samples, which had a 0.125 mmol/L fixed oxyhemoglobin concentration, then were incubated for 10 min with different concentrations of surfactants, including Cetrimonium Chloride. Aliquots were then centrifuged for 1 min, and the supernatant was photometrically analyzed for hemolysis at 530 or 560 nm. From this, the concentration at which 50% of the RBC were lysed was determined (H_{50}). Another aliquot of RBC was prepared with 1 mg/mL of surfactant, centrifuged, and measured at 540 and 575 nm. The denaturation index (DI) was then computed using the following formula:

$$DI = 100\ (R_1 - R_i)/(R_1 - R_2)(\%)$$

R_1 is the extinction at 575 nm divided by the extinction at 540 nm for oxyhemoglobin; R_2 is the ratio of the two extinctions for 3.47 mmol sodium dodecyl sulfate in an RBC aliquot; and R_i is the ratio of the two

Table 3. Primary Irritation Scores for Cetrimonium Chloride

	Erythema and eschar index (Max. possible score: 4)		Edema index (Max. possible score: 4)	
Concentration	24 h	48 h	24 h	48 h
0.1% (w/w)	0.25	0.00	0.00	0.00
0.5% (w/w)	1.75	1.75	0.50	0.00
1.2% (w/w)	2.75	2.75	2.00	1.75
2.5% (w/w)	3.75	3.75	2.25	2.00

Source: From Danochemo (1983a).

extinctions for the assayed material. The lysis/denaturation (L/D) quotient was determined (H_{50}/DI) and compared with literature values for ocular irritancy. The H_{50} for Cetrimonium Chloride was 7 ppm, the DI was approximately 65%, and the L/D ratio was 0.1. Under the researchers' classification for ocular irritation, Cetrimonium Chloride was "irritating to very irritating." The Draize ocular irritancy score referenced was classified "very irritant" (Pape and Hoppe, 1990).

When this study was repeated, the H_{50} without added bovine serum albumin (BSA) was 8 ppm and the DI was 70%. The H_{50} with BSA was 60 ppm and the DI was 13%. Cetrimonium Chloride without BSA was considered "very irritant" (Pape and Hoppe, 1991).

Cetrimonium Chloride was studied using the chick chorioallantoic membrane test as described by Kalweit et al. (1987). A stereomicroscope was used to observe the reactions. Irritation indices were calculated from the first appearance of hemorrhage, vascular lysis, and coagulation. Cetrimonium Chloride (concentration not given) produced hemorrhages and vascular lysis but no coagulation (Pape and Hoppe, 1991). A Neutral red uptake assay as described by Kalweit et al. (1987) was performed. The NR_{50}/KB_{50} (concentration inducing 50% cell death in BALB/c 3T3 fibroblasts) of Cetrimonium Chloride was 3 ppm (Pape and Hoppe, 1991).

In Vivo

Groups of six albino male rabbits of the SPF-Russian strain were used to assess the ocular irritation potential of 0.1%, 0.5%, 1.2%, and 2.5% (w/w) Cetrimonium Chloride. The right conjunctival sac of each rabbit was instilled with 0.1 mL of Cetrimonium Chloride, and the left eye served as an untreated control. The eyes were examined 24 h later both with and without fluorescein staining. All of the eyes were rinsed with 0.9% sodium chloride and examined at 48 and 72 h. The results are reported in Table 4. The investigators reported that after 6, 14, and 21 days, all of the eyes were restored to normal conditions (Danochemo, 1983b).

Table 4. Average ocular irritation scores for Cetrimonium Chloride

Concentration	Type of lesion (Max. possible score)	Grading period 24 h	48 h	72 h
0.1% (w/w)	Cornea (4)	0.00	0.00	0.00
	Iris (2)	0.00	0.00	0.00
	Conjunctival chemosis (4)	1.00	0.05	0.00
	Conjunctival redness (3)	0.50	0.00	0.00
0.5% (w/w)	Cornea (4)	0.05	0.00	0.00
	Iris (2)	0.25	0.00	0.00
	Conjunctival chemosis (4)	1.50	1.25	0.00
	Conjunctival redness (3)	1.25	0.50	0.00
1.2% (w/w)	Cornea (4)	1.25	0.75	0.00
	Iris (2)	1.50	1.00	0.50
	Conjunctival chemosis (4)	2.75	1.75	1.25
	Conjunctival redness (3)	1.00	0.75	0.25
2.5% (w/w)	Cornea (4)	2.75	1.50	0.75
	Iris (2)	2.25	1.25	1.00
	Conjunctival chemosis (4)	4.00	2.75	1.75
	Conjunctival redness (3)	3.50	2.25	1.75

Source. Danochemo (1983b).

The ocular irritation potential of 1% and 10% solutions of Cetrimonium Chloride was investigated by instilling one conjunctival sac of each of two rabbits with 0.2 mL of either solution. Observations were made after 1, 24, and 48 h. The overall scores for the 1% and 10% Cetrimonium Chloride solutions were 3.6 and 47.5, respectively, of a possible maximum score of 110 (Armour Industrial Chemicals Company, 1967).

Steartrimonium Chloride (dose not stated) was severely irritating to the eyes of nine rabbits. Both rinsed and unrinsed eyes had irritation for up to 13 days after administration. One rabbit had red watery discharge, and six had hemorrhagic peripheral areas (Sherex Chemical Company, Inc., 1986).

Itagaki et al. (1991) investigated the ocular irritation potential of Steartrimonium Chloride. The conjunctival sac of one eye of each of three rabbits was instilled with 100 μL of 10% aq. Steartrimonium Chloride. Steartrimonium Chloride was highly irritating; the total irritation score (maximum possible score, 110) was 60.3, and the maximum corneal score was 36.7.

In another study, 100 μL of a 5% (w/v) solution of Steartrimonium Chloride was instilled into the conjunctival sac of guinea pigs. The eyes were scored for signs of irritation after 0.5, 1, 2, 3, 4, 5, 6, and 24 h.

Steartrimonium Chloride was extremely irritating, having a total irritation score of 96 (maximum possible score, 110) (Bracher et al., 1988).

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Groups of 20 to 22 pregnant Wistar rats were administered 8, 25, or 50 mg/kg/day Cetrimonium Bromide on days 5 to 14 of gestation by gastric intubation. A negative control group of pregnant rats was administered corn oil and a positive control group of rats was given aspirin on day 10 of gestation. All of the dams were killed on day 20 of gestation and uterine contents examined. Fetal survival was reduced, and the incidence of resorption sites was increased in the dams from the high-dose group only. No evidence of adverse effects on litter size, viability, or litter weight was present in any of the experimental groups, and no tissue or skeletal anomalies were found in the fetuses (Lewis 1977).

In an intraperitoneal study, the teratogenic potential of Cetrimonium Bromide was assessed using NMRI mice. Groups of eight pregnant mice were given a single intraperitoneal injection of 10.5 or 35.0 mg/kg Cetrimonium Bromide on day 8, 10, 12, or 14 of gestation. A control group of mice was injected with water. The number of fetal deaths in the 10.5 mg/kg Cetrimonium Bromide group was not significantly different from that of the control group. There was an increase in fetal deaths in the 35.0 mg/kg Cetrimonium Bromide group, however. An increased incidence of cleft palate and of skeletal defects of the skull and sternum was observed in the fetuses from both treatment groups (Isomaa and Ekman, 1975).

In a dermal study, groups of 20 pregnant New Zealand White rabbits were given topical applications (2 mL/kg) of 0.5%, 1.0%, and 2.0% (v/v) Cetrimonium Chloride (100% active) for 2 h on days 7 to 18 of gestation. A control group of pregnant rabbits was treated with deionized water. The does were observed regularly for signs of toxicity and mortality, and the application sites were scored daily. All of the does were killed on day 29 and necropsy was performed. The following parameters were studied: number of does with resorptions, number of does with viable fetuses, number of viable fetuses, postimplantation loss, total implantations, and number of corpora lutea. All of the fetuses were examined for teratological effects.

One doe from each of the mid- and high-dose groups and two does in the control group died during the study. The cause of these deaths could not be determined by postmortem examination. One mid-dose doe and one control doe aborted prior to death. Of the surviving does, one doe from each of the mid- and high-dose groups aborted. None of the does of the low-dose group aborted or died during the study. No statistically significant changes in maternal body weight, mean body

weight gain, nor feed consumption were observed in any of the treatment groups.

Dermal irritation, in the form of erythema, edema, atonia, desquamation, fissuring, and coriaceousness, was observed in all of the treated does and increased in a dose-related fashion. Marked to moderate irritation was observed primarily in the mid- and high-dose groups. At necropsy, there was a slight increase in the incidence of congested lungs in does of the high-dose group. Overall, the reproductive parameters and the incidence of fetal malformations and developmental variations were not significantly different from those of the control group (International Research and Development Corporation, 1985).

The embryotoxic and teratogenic potential of Steartrimonium Chloride was tested using CFY Sprague-Dawley rats. Groups of 10 to 20 rats had 0.9%, 1.5%, and 2.5% Steartrimonium Chloride (0.5 mL/rat) applied to the shaved skin of the scapula region on days 6 to 15 of gestation. The material was neither rinsed nor placed under occlusive patches. The dams were killed on day 20, the litter values were determined, and the fetuses were examined for skeletal and visceral abnormalities.

No treatment-related deaths occurred, and other than marginally lower weight gain during the dosing period, there were no systemic signs of toxicity or macroscopic changes in the internal organs of the dams. The authors noted significant dermal irritation at the sites of application, but these effects were not detailed. The litter sizes, postimplantation loss, and litter and mean fetal weights were similar to those of control animals. There was no increase in the incidence of fetal malformations, and the types of malformations and anomalies that were found were similar to those seen in the concurrent control group and historical controls (Palmer et al., 1983).

MUTAGENICITY

Cetrimonium Chloride, at concentrations of 0.05 to 1 μg/plate, was negative in an Ames test using *Salmonella typhimurium* strains TA98 and TA100 with and without metabolic activation. Negative results also were obtained with 5 to 10 μg/plate Cetrimonium Chloride in the presence of S9, but these concentrations were toxic to the bacteria without metabolic activation (Inoue et al., 1980).

In another Ames test, Cetrimonium Chloride was tested with *S. typhimurium* strains TA98, TA100, TA1535, TA1537, and TA1538 at concentrations of 1, 5, 25, 125, and 625 μg/plate. 4-Nitro-o-phenylenediamine was used as the positive control for TA1537, TA1538, and TA98, and sodium azide was used for TA1535 and TA100. Cetrimonium Chloride was negative both with and without metabolic activation for each of the strains tested (COLIPA, 1979).

Another study tested 50% Cetrimonium Chloride using *S. typhimurium* strains TA1535, TA1537, and TA1538. Concentrations of 0.005 to 5.0 μg/plate Cetrimonium Chloride were used. 2-Acetylaminofluorene, neutral red, and §naphthylamine were used as positive controls. Cetrimonium Chloride was not mutagenic in any of the strains tested both with and without metabolic activation (Huntingdon Research Centre, 1977). Cetrimonium Bromide was also negative in an Ames test (no details given) (Kabara, 1984).

In forward-mutation and reverse-mutation tests using *E. coli* strain 343/113, concentrations of 1, 10, and 50 μg/mL Cetrimonium Chloride did not increase the number of mutant colonies from cell cultures incubated in different mediums for 20, 40, or 72 h (Müller, 1976).

Cetrimonium Chloride (24–26% active ingredient) was also tested in a chromosome aberration assay using Chinese hamster V79 cells. Cells were treated for 4 h with 0.3, 1.0, and 3.0 μg/mL Cetrimonium Chloride and then were cultured for 18 h. Cells treated with 1.0 and 3.07 μg/mL Cetrimonium Chloride also were cultured for 7 and 18 h, respectively. Tests also were conducted using metabolic activation; 1.0, 3.0, and 10.0 μg/mL Cetrimonium Chloride were tested for a culture interval of 18 h, and 10 μg/mL Cetrimonium Chloride was tested for intervals of 7 and 28 h. Ethylmethanesulfonate and cyclophosphamide were used as the positive controls for the tests with and without metabolic activation, respectively. Cetrimonium Chloride was not mutagenic in tests both with and without metabolic activation (Heidemann, 1989).

An in vitro cell transformation assay was used to assess the carcinogenic potential of Cetrimonium Chloride. Cryopreserved primary cultures of Syrian golden hamster embryo cells were used as the source of target and feeder cells. Cetrimonium Chloride, at doses of 0.05, 0.1, 0.5, and 1.0 μg/mL, did not cause transformation (Inoue et al., 1980).

CARCINOGENICITY

Published data on the cacinogencity of Cetrimonium Chloride, Cetrimonium Bromide, and Steartrimonium Chloride were not found.

CLINICAL STUDIES

Dermal Irritation and Sensitization

A repeated insult patch test of Cetrimonium Chloride was conducted using 114 volunteers. Occlusive patches containing 0.3 mL of 0.25% (w/v) Cetrimonium Chloride (100% active) were applied to the upper arm of each of the subjects for 24 h on Mondays, Wednesdays, and Fridays for 3 weeks. Seventeen days following the last application, a challenge patch of 0.25% Cetrimonium Chloride was applied to a previ-

ously untreated site. Mild irritation was observed in several subjects during induction, but no sensitization was observed. One case of contact urticaria occurred, but the authors could not determine whether it was treatment related because the subject refused to participate in a follow-up diagnostic patch test (Hill Top Research, Inc., 1983).

Using the same testing procedures, 2.0% (w/v) aq. Cetrimonium Chloride (25% active) in a hair-conditioning product was tested as a 20% aq. solution. A total of 106 volunteers completed the study. Slight to moderate erythema was observed in several subjects during the induction phase of the experiment. Following challenge, six individuals had mild erythematous reactions. In five of these subjects, the reactions were transient and were attributed to primary irritation. The sixth subject tested negatively when retested with a 0.25% (w/w) solution (Hill Top Research, Inc., 1984a).

In another study, 1.6% Cetrimonium Chloride (25% active) in a hair conditioner was tested as a 10.0% (w/v) aq. solution. Of the 101 subjects who completed the study, only one individual responded to the challenge patch. The response was a mild and transient erythematous reaction. Three subjects had mild erythema with papules and/or edema during induction, but these reactions subsided quickly, and none of the individuals responded to the challenge patch (Hill Top Research, Inc., 1984b).

When 0.8% Cetrimonium Chloride (25% active) in a hair conditioner was tested as a 10.0% (w/v) aq. solution, a few individuals had mild erythematous reactions during induction but no sensitization reactions were observed in the 101 subjects who completed the study (Hill Top Research, Inc., 1984c).

Negative results were also obtained when 1.6% Cetrimonium Chloride (25% active) in a hair conditioner was tested as a 10.0% (w/v) aq. solution. None of the 107 subjects that completed the study had reactions to the challenge patch, and only one subject had slight irritation during induction (Hill Top Research, Inc., 1984d).

Broeckx et al. (1987) studied cosmetic intolerance in 5202 patients. Almost all patients completed a Belgian Tri-Contact Patch-test series or similar patch test series. Of the original population, 33 (0.6%) had an allergic reaction to a product containing Cetrimonium Bromide. Of the 156 patients with allergies to cosmetics, only one had a positive reaction to that product.

Case Studies

A 20-month-old child was treated for erythema and blistering with bullae formation on a 14 × 8–cm area of skin on his chest. The causative agent was a shampoo containing a 12% solution of Cetrimonium Bromide (Inman, 1982).

A 5-year-old child had a sensitization reaction (erythema and edema) to an above-elbow plaster cast on his left arm. At patch testing, reactions to 0.5% Cetrimonium Bromide and 0.25% benzalkonium chloride were observed. The authors postulated the child had previously been sensitized to Cetrimonium Bromide and had an allergic reaction to the benzalkonium chloride in the cast preparation (Staniforth, 1980).

Thirty minutes after an operation to excise hydatid cysts, which included a liberal irrigation of the affected area of the liver with 1% Cetrimonium Bromide, a 45-year-old woman began to have signs of methemoglobinemia. A sample of the patient's blood was analyzed spectrophotometrically after adding 0.25% Cetrimonium Bromide for 30 min. The methemoglobin content increased from 4.4% to 19.3%. The patient had no history of cyanotic attack in a previous surgery without Cetrimonium Bromide (Baraka et al., 1980).

Within 15 min of a rectal lavage using 1% Cetrimonium Bromide, a patient suffered from a loss of vision, convulsions, and coma. Thirteen hours after treatment, despite intensive therapy, the patient died. Another patient suffered instability of blood pressure and cardiac ischemia during and immediately after a rectal lavage with a solution containing Cetrimonium Bromide (concentration not stated) (Saeed et al., 1991).

SUMMARY

Cetrimonium Bromide and Cetrimonium Chloride are quaternary ammonium salts used as cosmetic biocides, antistatic agents, surfactant-cleansing agents, surfactant-emulsifying agents, and surfactant-suspending agents in cosmetic products. Steartrimonium Chloride is also a quaternary ammonium salt that is used as an antistatic agent and surfactant.

Cetrimonium Bromide was poorly absorbed by the intestinal tract of rats following oral administration. The majority of the administered dose was eliminated in the feces, and evidence that metabolism occurred to some extent was present.

In intravenous and subcutaneous studies, Cetrimonium Bromide was rapidly excreted in the urine and feces. Unidentified metabolites were detected in the urine following intravenous exposure, but only unchanged Cetrimonium Bromide was present in the feces.

In a percutaneous absorption study, 0.59% of 1% Cetrimonium Bromide in water penetrated the skin of rats after 15 min of exposure followed by rinsing; 0.93% of 0.5% Cetrimonium Bromide in a hair-rinse formulation penetrated after 5 min of exposure followed by rinsing; and 3.15% of 3.0% of Cetrimonium Bromide in water penetrated after 15 min of exposure without rinsing.

The oral LD_{50} of 40% (w/v) Cetrimonium Bromide was 1000 mg/kg for rats. In acute studies with mice, the LD_{50} for Steartrimonium Chloride were 633 mg/kg for male mice and 536 mg/kg for female mice. In a 28-day dermal toxicity test of 0.5% Cetrimonium Chloride, the only adverse finding in rabbits was mild, transient dermal irritation.

When rats were administered 10, 20, and 45 mg/kg/day Cetrimonium Bromide in their drinking water for 1 year, the only sign of toxicity was reduced body weight gain in the 45 mg/kg/day group.

Cetrimonium Chloride was classified as a skin irritant in an in vitro study, and Steartrimonium Chloride was positive in in vivo skin irritation and sensitization studies.

Cetrimonium Chloride was classified as a severe ocular irritant in in vitro studies, and Steartrimonium Chloride was severely irritating to the eyes of rabbits and guinea pigs. Cetrimonium Bromide was not teratogenic in an oral study with rats. Mild embryonic effects were observed with 50 mg/kg/day Cetrimonium Bromide, but these were attributed to maternal toxicity rather than a teratogenic effect. At lower doses, Cetrimonium Bromide had no embryotoxic or teratogenic effects.

In an intraperitoneal study, Cetrimonium Bromide interfered with the embryonic development of mice at a dose of 10 mg/kg and was lethal to developing embryos at 35.0 mg/kg. Teratogenic effects were observed in both treatment groups.

There was no evidence of teratogenicity by 2.0% Cetrimonium Chloride in a dermal study with rabbits. The only adverse effect observed was dermal irritation at the sites application. When tested in a dermal teratogenicity study, 2.5% Steartrimonium Chloride was not maternally toxic, embryotoxic, or teratogenic.

Cetrimonium Chloride was negative both with and without metabolic activation in an Ames test at concentrations of up to 625 μg/plate, in forward-mutation and reverse-mutation tests at concentrations of up to 50 μg/ml, and in a cell transformation assay up to 1.0 μg/mL. Negative results were also obtained in a chromosome aberration test at concentrations of up to 3.0 μg/mL without metabolic activation and up to 10.0 μg/mL in tests with exogenous metabolic activation. Cetrimonium Chloride and Cetrimonium Bromide were both negative in Ames tests, and Cetrimonium Chloride also was negative in an in vitro cell transformation assay.

Cetrimonium Chloride was negative in human repeated insult patch tests at concentrations of up to 0.25% for 100% active solutions and up to 0.4% for 25% active solutions. Slight dermal irritation was observed during the induction phases of these experiments, but no evidence of sensitization was observed. A few case reports of adverse reactions to Cetrimonium Bromide have been reported in the literature.

DISCUSSION

The Cosmetic Ingredient Review Expert Panel considered the clinical data on dermal irritation and sensitization and noted that 0.25% (the highest concentration tested) Cetrimonium Chloride was a mild irritant but not a sensitizer. It was agreed that a concentration of 0.25% would be an appropriate limit for leave-on products. In support of this decision, the expert panel also noted that in ocular studies, a concentration of 0.1% Cetrimonium Chloride was nonirritating to the eyes of rabbits and that 0.5% caused mild irritation which cleared by 72 h.

Given the negative reproductive and developmental toxicity data from studies using dermal application, the uniformly negative mutagenicity test data, and the percutaneous absorbtion of these ingredients, the concentration limits imposed above were considered more than adequate to preclude any potential developmental toxicity or carcinogenic effects.

Because Cetrimonium Chloride is chemically similar to Cetrimonium Bromide and Steartrimonium Chloride, it is expected that the concentration limit placed on Cetrimonium Chloride also would apply to these compounds. It was agreed that no concentration limit was necessary for rinse-off products.

CONCLUSION

On the basis of the animal and clinical data presented in this report, the Cosmetic Ingredient Review Expert Panel concludes that Cetrimonium Chloride, Cetrimonium Bromide, and Steartrimonium Chloride are safe for use in rinse-off products and are safe for use at concentrations of up to 0.25% in leave-on products.

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