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Safety Assessment of Trimoniums as Used in Cosmetics

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Abstract

Quaternary ammonium salts, including alkyl chain, alkanol, and polymer derivatives (trimoniums) are used in cosmetics mainly as surfactant-cleansing agents, hair-conditioning agents, and antistatic agents. The Cosmetic Ingredient Review Expert Panel reviewed the relevant animal and human data and noted gaps in the available safety data for some of the trimomiums. The available data on many of the trimoniums are sufficient, however, and similar structural activity relationships, functions in cosmetics, and cosmetic product usage supported extending these data to the entire group. These ingredients were determined to be safe in the present practices of use and concentration when formulated to be nonirritating.

Keywords

trimoniums

Introduction

Three quaternary ammonium salts used as cosmetic ingredients, cetrimonium chloride, cetrimonium bromide, and steartrimonium chloride, were found to be safe for use in rinse-off products and safe for use at concentrations of up to 0.25% in leave-on products by the Cosmetic Ingredient Review (CIR) Expert Panel in 1997.[1] These trimethyl ammonium ingredients are part of a larger group of quaternary ammonium salts used in cosmetics. Because the available data on these 3 ingredients are considered relevant to the safety of other quaternary ammonium salts, the safety assessment of cetrimonium chloride, cetrimonium bromide, and steartrimonium chloride was expanded to consider the safety of a larger group of structurally similar ingredients.

The *International Cosmetic Ingredient Dictionary and Handbook* defines many cosmetic ingredients that are structurally similar to cetrimonium chloride, cetrimonium bromide, and steartrimonium chloride, and these additional ingredients have been included in this amended safety assessment.[2] Each of these additional cosmetic ingredients is also a quaternary ammonium salt wherein 3 of the 4 substituents on the nitrogen atom that comprise the quaternary ammonium moiety are methyl groups. This gives rise to the "trimonium" naming convention (a combination of trimethyl and ammonium) used for many of the cosmetic ingredients addressed in this safety assessment. Accordingly, the overall grouping has been given the designation trimoniums and includes 52 ingredients (see Tables 1-3; Figure 1).

The fourth substituent on the nitrogen atom differentiates each of the trimoniums and may be a straight, branched, substituted, or unsubstituted alkyl chain. Having 4 nonhydrogen substituents on the nitrogen atom results in a positively charged ion (cation) which is not pH dependent. The charged nature of these ingredients has a significant, but predictable, impact on their chemical and physical properties.

The ingredients in this safety assessment are segregated according to the function of the fourth substituent into 1 of 3 groups: (1) unsubstituted straight or branched alkyl chain trimoniums; (2) alcohol bearing alkyl chain (alkanol) trimoniums; or (3) an alkyl chain trimonium incorporated into a polymer backbone (Tables 1-3; Figure 1). These trimonium ingredients mostly function as antistatic agents, hair-conditioning agents, cleansing agents, surfactants, and film formers.

While many of these ingredients vary only by hydrocarbon chain length, there are also branched ingredients, alcohols, esters, ethers, and polymers. Nonetheless, these ingredients do comprise a family of chemicals with similar functions in

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Table 1. Straight Chain Alkyl, Trimonium Compound and Their Salts in This Safety Assessment With CAS Numbers, Functions in Cosmetics, Technical Names, and Trade Names.

Ingredient	CAS No.	Functions	Technical Names	Trade Names
Laurtrimonium bromide	1119-94-4	Cosmetic biocide; hair-conditioning agent	Ammonium, dodecyltrimetyl-, bromide; 1-dodecanaminium, N, N, N-trimethyl-, bromide; lauryltrimethylammonium bromide; N, N, N-trimethyl-1-dodecanaminiuim bromide	
Laurtrimonium chloride	112-00-5	Antistatic agents; cosmetic biocide; emulsifying agent	Ammonium, dodecyltrimethyl-, chloride; 1-dodecanaminium, N, N, N-trimethyl-, chloride; dodecyltrimethylammonium chloride; lauryl trimethyl ammonium chloride; N, N, N-trimethyl-1-dodecanaminium chloride	Arquad 12-37 W; Chemquat 12-33; Chemquat 12-50; Empigen 5089; Laurene; Nikkol CA-2150
Myrtrimonium bromide	1119-97-7	Antistatic agents; cosmetic biocide	Ammonium, trimethyltertradecyl-, bromide; myristyl trimethyl ammonium bromide; quaternium-13; 1-tetradecanaminium, N, N, N-trimethyl-, bromide; tetradecytrimethylammonium bromide; tetradonium bromide (INN); N, N, N-trimethyl-1-tetradecanaminium bromide	Mytab; Rhodaquat M-214C/99; Sumquat 6110
Cetrimonium bromide	57-09-0	Antistatic agent; cosmetic biocide; surfactant-emulsifying agent	Cetab; cetrimidum (EP); cetrimonium bromide (INN); cetyl trimethyl ammonium bromide; cetyltrimethylammonium bromide powder; 1-hexadecanaminium, N, N, N-trimethyl-, bromide; hexadecyltrimethylamine bromide; hexadecyltrimethylammonium bromide; N, N, N-trimethyl-1-hexadecanaminium bromide	Acetoquat CTAB; Bromat; Cetrimide; Rhodaquat M-242B/99; RonaCare Cetrimonium Bromide; RonaCare Cetrimonium Bromide; Sumquat 6030
Cetrimonium chloride	112-02-7	Antistatic agent; cosmetic biocide; surfactant-emulsifying agent	Cetyl trimethyl ammonium chloride; 1-hexadecanaminium, N, N, N-trimethyl-, chloride; hexadecyltrimethylammonium chloride; N, N, N-trimethyl-1-hexadecanaminium chloride	AEC Cetrimonium Chloride; Ammonyx CETAC; Ammonyx CETAC-30; Arquad 16-25 W; Arquad 16-29 W; Barquat CT-29; Carsoquat CT-429; CTAC; Dehyquart A-CA; Genamin CTAC; Genamin CTAC 50; Incroquat CTC-30; Jeequat CT-29; Nikkol CA-2330; Nikkol CA-2350; OriStar CMC; Protaquat CT-29; Saboquat CTA; Saboquat CTA 25; Thorquat CTC30; Varisoft 300
Cetrimonium methosulfate	65060-02-8	Antistatic agent; cosmetic biocide; surfactant-emulsifying agent	Cetyltrimethylammonium methyl sulfate; 1-hexadecanaminium, N, N, N-trimethyl-, methyl sulfate; hexadecyltrimethylammonium methosulfate; N, N, N-trimethyl-1-hexadecanaminium methyl sulfate	Crodazosoft DBQ
Steartrimonium bromide	None	Antistatic agent; hair-conditioning agent		
Steartrimonium chloride	112-03-8	Antistatic agent; hair-conditioning agent	1-Octadecanaminium, N, N, N-trimethyl-, chloride; quaternium-10; stearyl trimethyl ammonium chloride; stearyl trimethyl ammonium chloride solution; N, N, N-trimethyl-1-octadecanaminium chloride	Genamin STAC; Nikkol CA-2450; Nikkol CA-2465; OriStar STAC
Steartrimonium methosulfate	18684-11-2	Antistatic agent; hair-conditioning agent	1-Octadecanaminium, N, N, N-trimethyl-, methyl sulfate; stearyl trimethylammonium methyl sulfate; stearyltrimonium methosulfate; N, N, N-trimethyl-1-octadecanaminium methyl sulfate	Empigen CM
Behentrimonium chloride	17301-53-0	Antistatic agent; hair-conditioning agent	1-Docosanaminium, N, N, N-trimethyl-, chloride; N, N, N-trimethyl-1-docosanaminium chloride	Genamin BTLF; Genamin KDMP; Incroquat Behenyl TMC-85; Nikkol CA-2580; Varisoft BT 85 Pellets

(continued)

Table 1. (continued)

Ingredient	CAS No.	Functions	Technical Names	Trade Names
Behentrimonium methosulfate	81646-13-1; 241148-21-0	Antistatic agents; hair-conditioning agents	Behenyl trimethyl ammonium methosulfate; 1-docosanaminium, N, N, N-trimethyl-, methosulfate; N, N, N-trimethyl-1-docosanaminium methosulfate	Global Seven Gloquat BQ; Incroquat Behenyl 18-MEA; Incroquat Behenyl TMS; Incroquat Behenyl TMS; Incroquat Behenyl TMS-50; KeraTint EZ; Varisoft BTMS Pellets
Octacosatrimonium chloride	None	Antistatic agent; hair-conditioning agent	Alkyl (28) Trimethyl Ammonium Chloride	
Straight chain alkyl mixtures				
Ceteartrimonium chloride	None	Antistatic agent; hair-conditioning agent	Alkyl (16,18) trimethylammonium chloride	
Hydrogenated palmtrimonium chloride	None	Antistatic agents; hair-conditioning agent		
Hydrogenated tallowtrimonium chloride	61788-78-1	Antistatic agents; hair-conditioning agent	Quaternary ammonium compounds, (hydrogenated tallow alkyl)trimethyl, chlorides	Arquad HT-50
Soytrimonium chloride	61790-41-8	Antistatic agent; hair-conditioning agent	Quaternary ammonium compounds, trimethylsoy alkyl, chlorides; quaternium-9; N-(soy alkyl)-N, N, N-trimethyl ammonium chloride; soyatrimonium chloride; soy trimethyl ammonium chloride	Arquad S-60 PG; Silab'Capilisse
Tallowtrimonium chloride	8030-78-2	Antistatic agents; hair-conditioning agents	Quaternary ammonium compounds, tallow alkyl trimethyl, chlorides; tallow trimethyl ammonium chloride	ACC AMD-2 Emulsion; Arquad T-50; Dow Corning 1669 Cationic; Emulsion; Emulsil CT-30; Jeesilc 92
Cocotrimonium chloride	61789-18-2	Antistatic agent; hair-conditioning agent	Coconut trimethylammonium chloride; cocóyl trimethyl ammonium chloride; quaternary ammonium compounds, coco alkyl trimethyl, chlorides	AEC Cocotrimonium Chloride; Arquad C-33W
Cocotrimonium methosulfate	None	Antistatic agent; hair-conditioning agent		Luviquat Mono LS; Servamine KAC 458
Branched alkyl				
Octyldodecyltrimonium chloride	None	Antistatic agent; hair-conditioning agent		
Dodecylhexadecyl-trimonium chloride	103807-18-7	Antistatic agent; hair-conditioning agent; surfactant-emulsifying agent	1-Hexadecanaminium, 2-dodecyl-N, N, N-trimethyl-, chloride	Quartamin 280GP

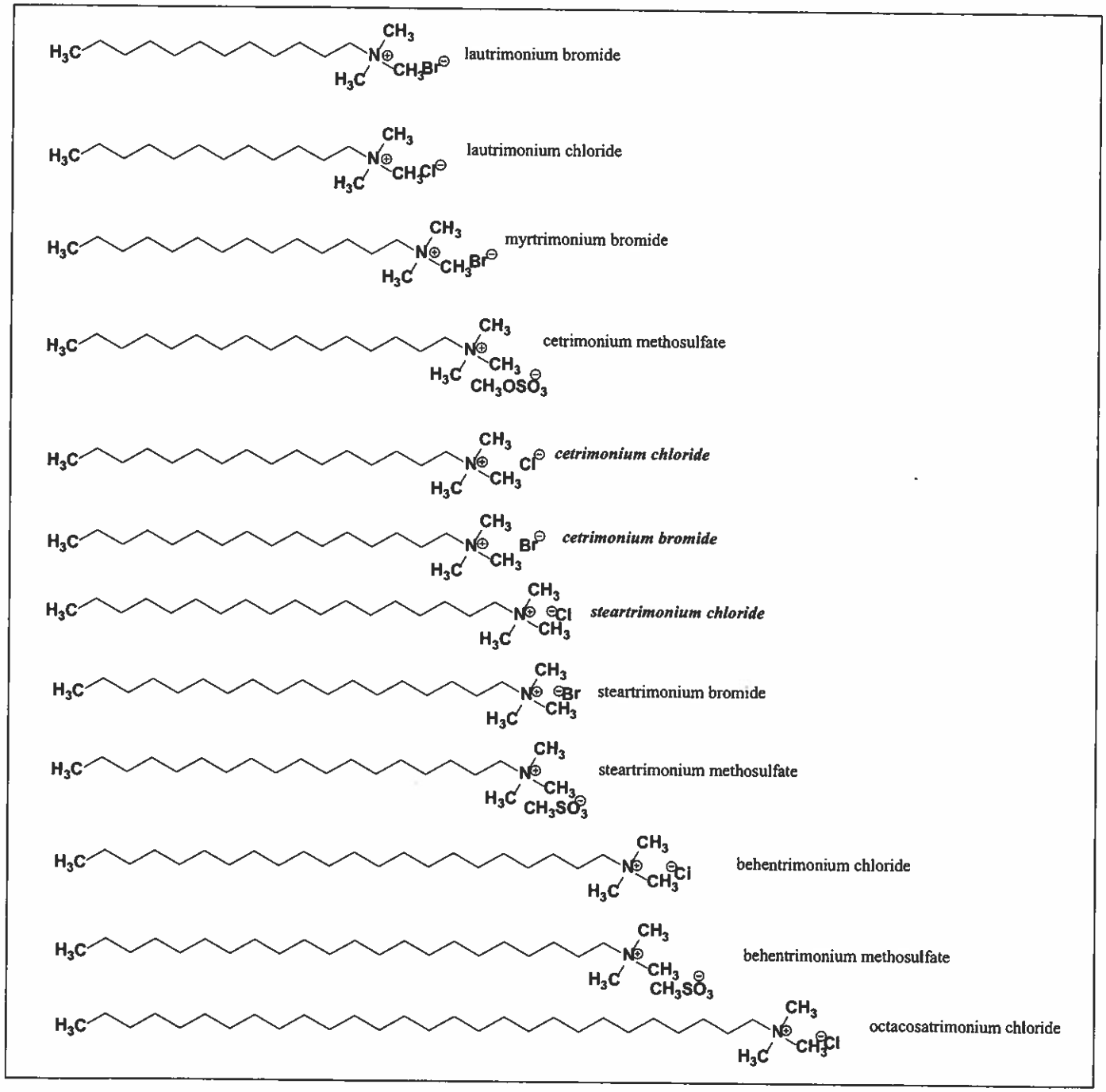


Figure 1. Continued

cosmetics. It is expected that the toxicity of alkanol trimonium compounds would be similar to that of alkyl trimonium compounds and the data on one ingredient may be extrapolated to the other ingredients in this safety assessment—a process usually termed as read-across. To test the read-across reliability, similar toxicity end points were considered across the fourth substituent. Where data were available, the findings were similar. For example, trimoniums dissociate into their ionic components in aqueous cosmetic formulations. Upon incorporation of trimoniums into cosmetic formulations, the cationic chains are attracted to anionic charges in the protein structure of skin and hair, resulting in a conditioning effect. These charged ingredients are unlikely to cross the lipid bilayer but tend to be irritating to the skin and eyes.[3] The lowest molecular weight, and shortest chain, alkyl trimonium in this assessment, laurtrimonium bromide, exhibited percutaneous absorption of <0.1% over 48 hours in rats with no measurable presence in the blood.[4,5] Higher molecular weight, longer chain trimonium ingredients would likely be similarly or less absorbable. Studies of the percutaneous absorption of a trimonium decorated polymer resulted in no evidence of dermal penetration in rats.[5]

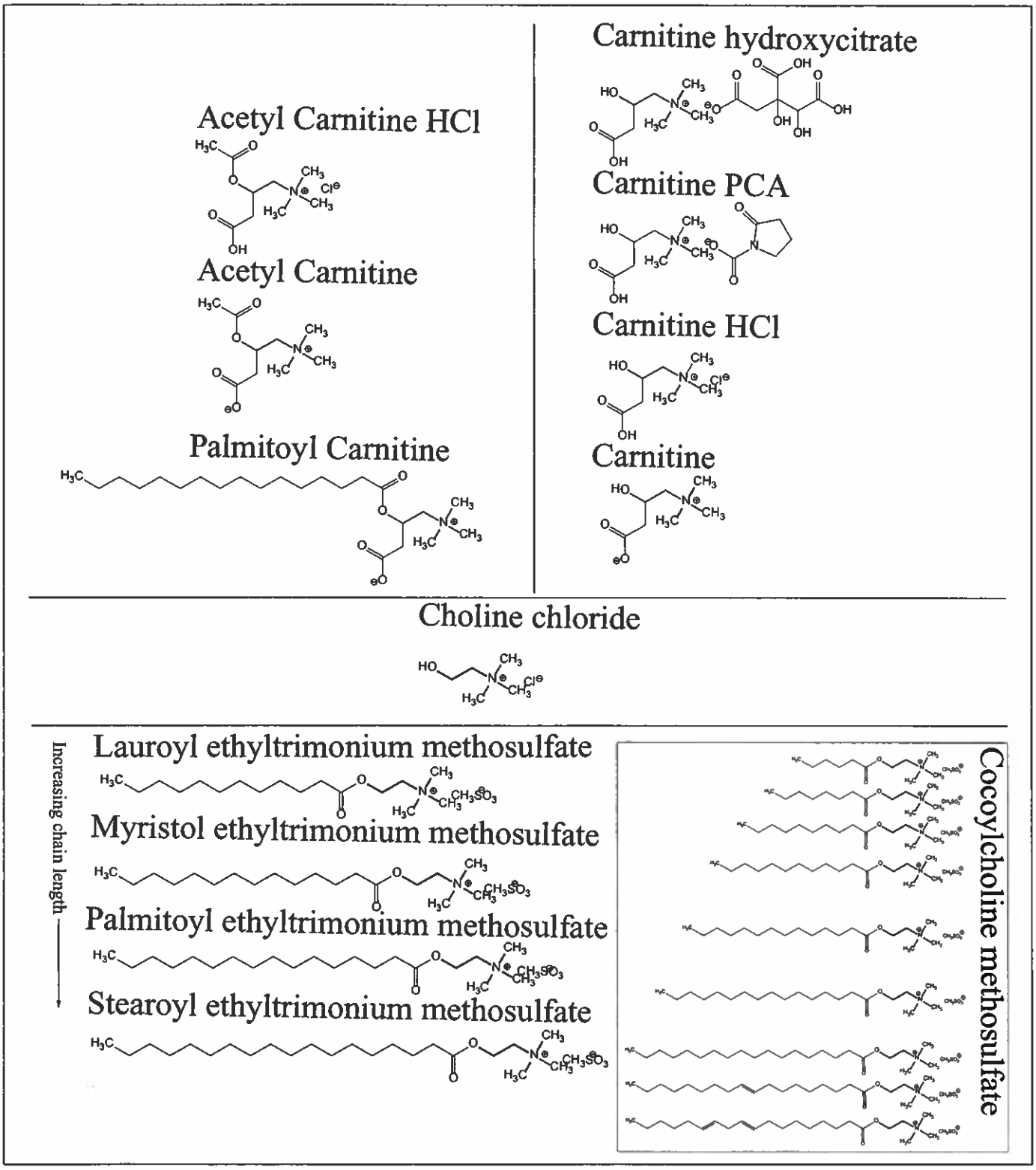


Figure 1. Continued

The polymers represent very large molecular entities, wherein the trimonium groups function to bring a certain charge density to the molecule. Copolymerization or polymerization with nonionic comonomers serves to decrease this charge density to a desired level for applications such as hair and skin conditioning.[6]

Polyquaternium 10, a trimonium functionalized cellulose, is similar to the polymers in this assessment and was previously

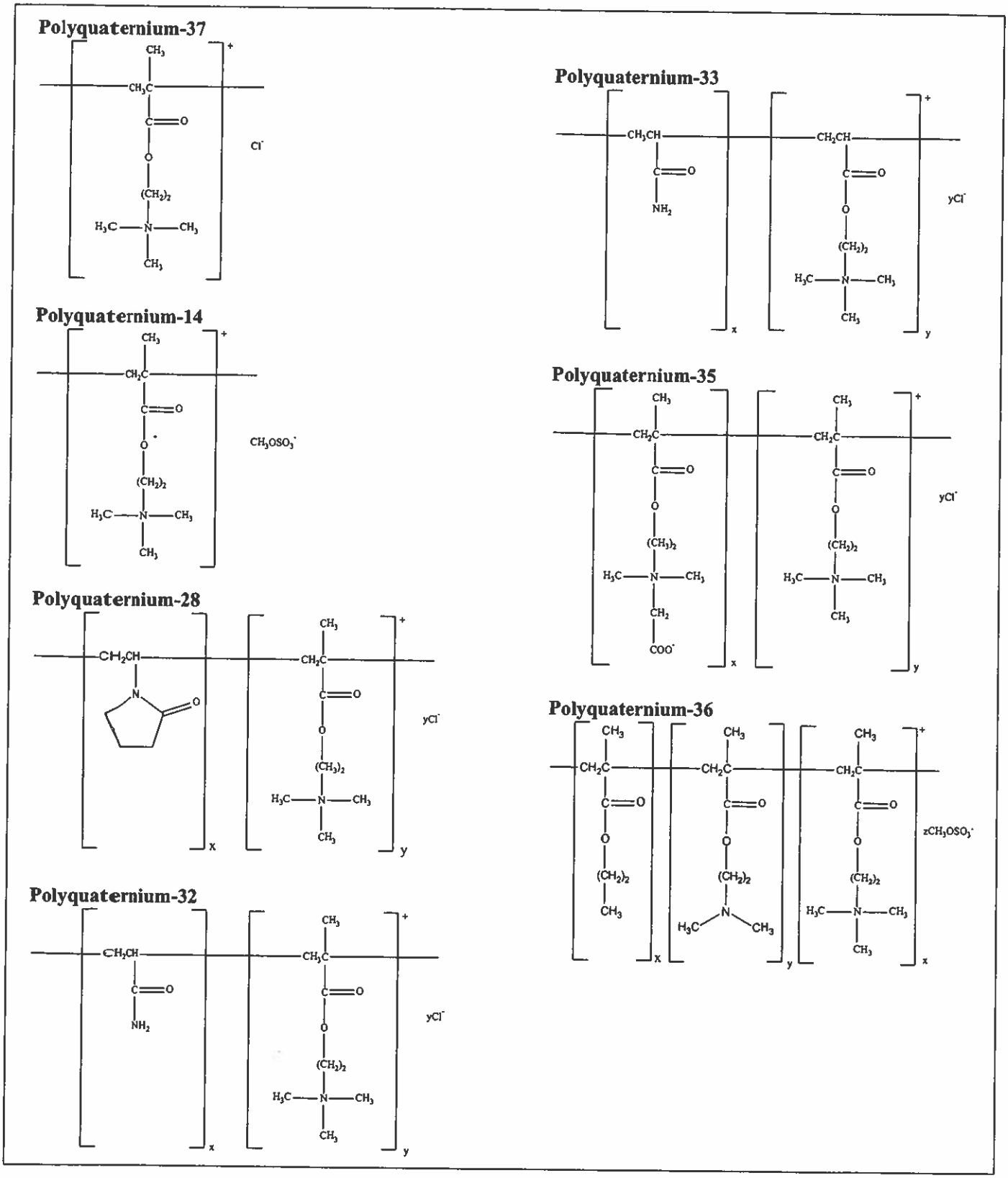
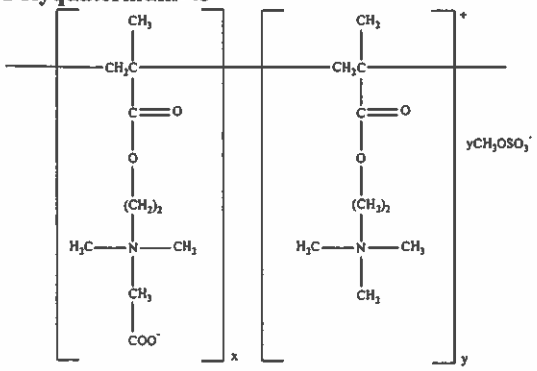


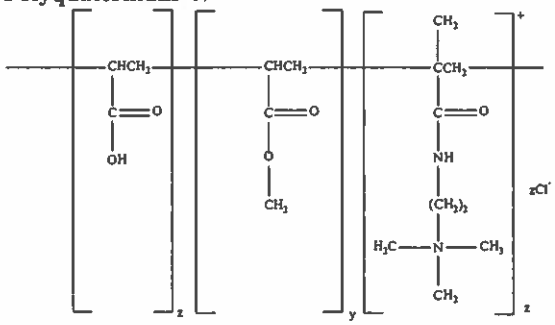
Figure 1. Continued

assessed by the CIR Expert Panel in 1988. It was concluded that "... [p]olyquaternium-10 is safe as a cosmetic ingredient in the present practices of use."[7] This conclusion was confirmed in 2005.[8] While the structure of polyquaternium 10 did not make it a candidate to add to this safety assessment, summaries of the data in that assessment will be included in the

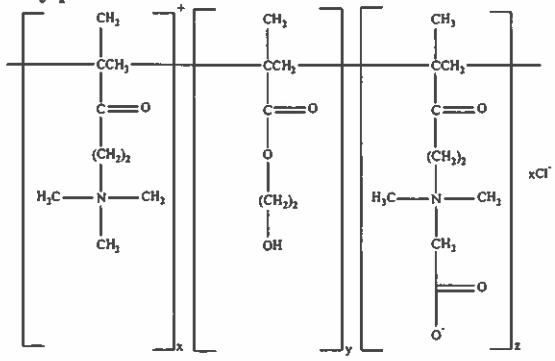
Polyquaternium-45



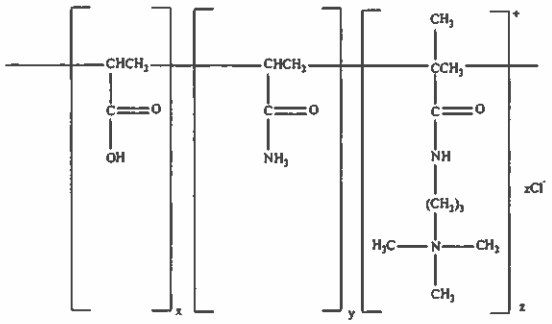
Polyquaternium-47



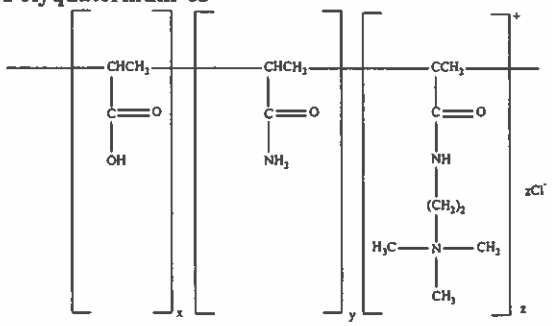
Polyquaternium-48



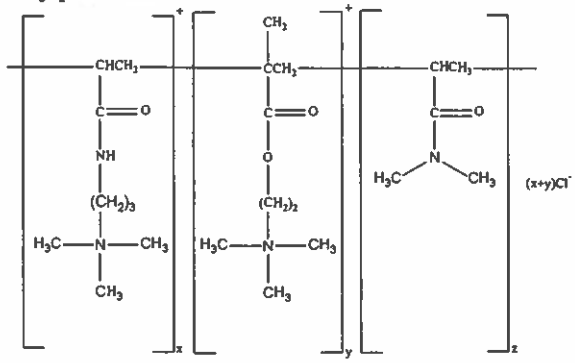
Polyquaternium-53



Polyquaternium-63



Polyquaternium-73



Polyquaternium-91

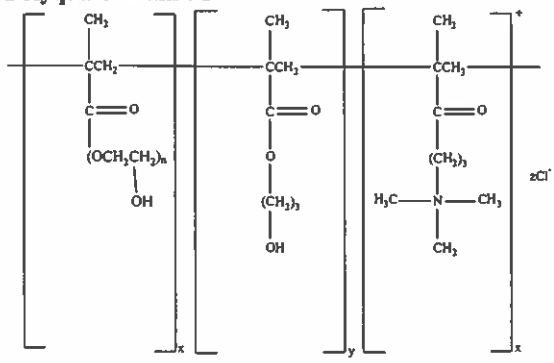


Figure 1. Continued

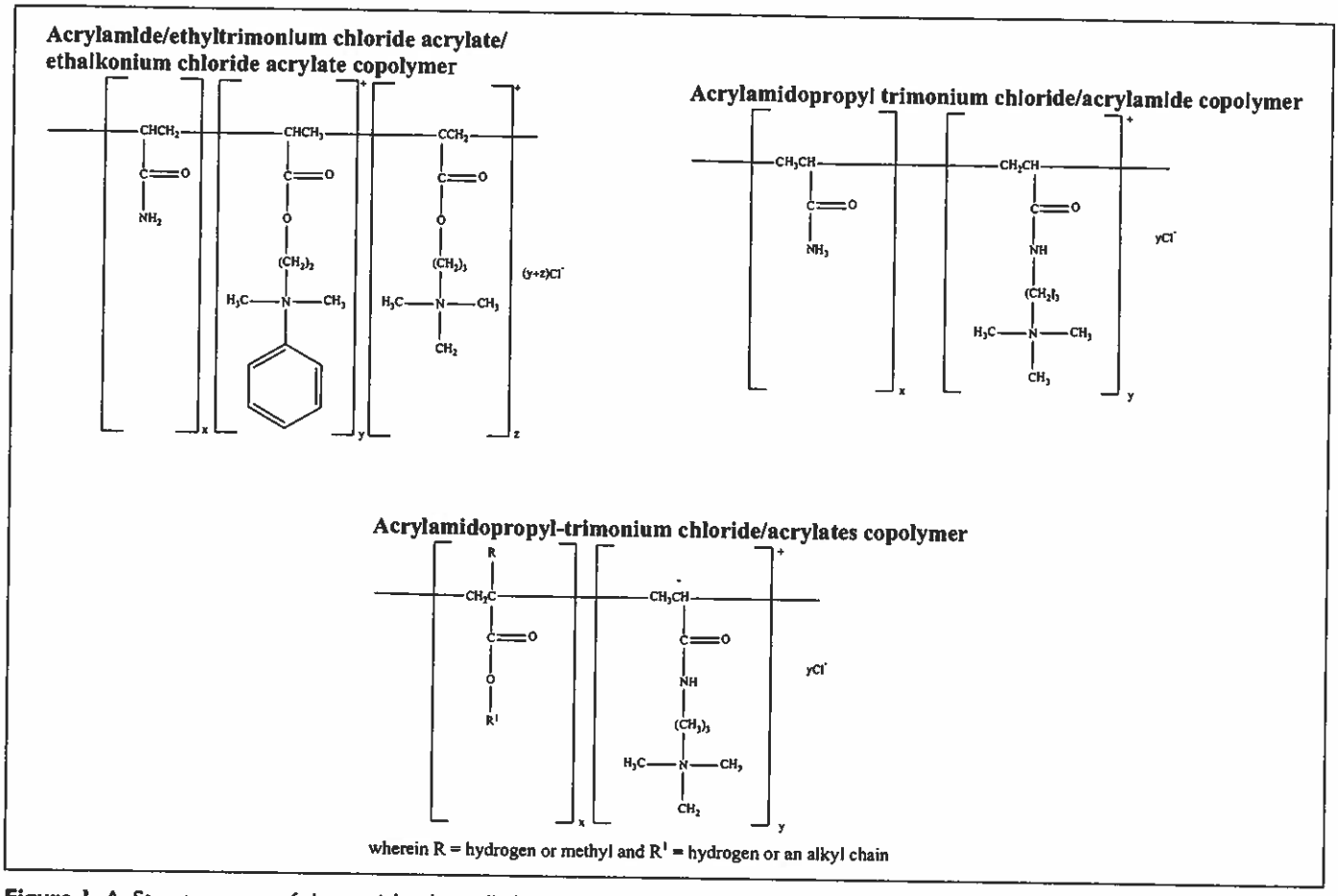


Figure 1. A, Structure map of the straight chain alkyl ingredients in this assessment (including original 3 assessed ingredients: *cetrimonium chloride*, *cetrimonium bromide*, and *steortrimonium bromide*, in boldface).
Cocotrimonium chloride, cocotrimonium methosulfate, tallowtrimonium chloride, soytrimonium chloride, hydrogenated palmtrimonium chloride, hydrogenated tallowtrimonium chloride, and ceteartrimonium chloride are comprised of mixtures of the above ingredients. B, Structure map of the alkanol (choline core structure) ingredients in this assessment. C, Structures of the trimonium polymers. These are idealized structures meant only to demonstrate the identity of the monomer residues. These structures are not meant to propose any suggestion of exact monomer-to-monomer connectivity of the copolymers, as this is unknown and variable.
wherein R = hydrogen or methyl and R^1 = hydrogen or an alkyl chain.

appropriate sections here because those data do support the safety of trimonium polymers.

The data from the original safety assessment of cetrimonium chloride, cetrimonium bromide, and steartrimonium chloride are summarized in the appropriate sections in this safety assessment.

Chemistry

Straight and Branched Chain Alkyl Trimonium Ingredients

- Cetrimonium bromide, cetrimonium chloride, and steartrimonium chloride and the other unsubstituted alkyl trimoniums vary by alkyl chain residue length, the degree of unsaturation, the existence of chain branching, or a combination of these variations. Ingredients in this group are shown in Table 1 with CAS Registry numbers, function in cosmetics, technical names, and trade names. These ingredients are:
  - Laurtrimonium bromide
  - Laurtrimonium chloride
  - Myrtrimonium bromide
  - Cetrimonium chloride
  - Cetrimonium bromide
  - Cetrimonium methosulfate
  - Steartrimonium chloride
  - Steartrimonium bromide
  - Steartrimonium methosulfate
  - Behentrimonium chloride
  - Behentrimonium methosulfate
  - Octacosatrimonium chloride
  - Ceteartrimonium chloride
  - Hydrogenated tallowtrimonium chloride
  - Hydrogenated palmtrimonium chloride
  - Soytrimonium chloride
  - Tallowtrimonium chloride
  - Cocotrimonium chloride
  - Cocotrimonium methosulfate

- Octyldodecyltrimonium chloride and
- Dodecylhexadecyltrimonium chloride

Some of the alkyl residues in this group are derived from botanical sources and are actually mixtures of different chain length salts (eg, soytrimonium chloride). Furthermore, this first group also varies in the identity of the corresponding anions (eg, bromide, chloride, and methosulfate). A mapping of how these molecules vary in length is shown in Figure 1A.

Alkanol Trimonium Ingredients

- The alkanol trimonium ingredients consist of various alcohol-substituted trimoniums and related ethers, esters, and acids. These ingredients can be subdivided into those with a choline function and those with a carnitine function. These ionic compounds also vary by chain length. In one instance, the alkyl residue is derived from botanical ingredients and results in a mixture of salts (cocoylcholine methosulfate). The CAS Registry numbers, functions in cosmetics, technical names, and trade names of the ingredients in this group are shown in Table 2 and the structures are presented in Figure 1B. These ingredients are:
- Choline chloride
- Stearoxypropyltrimonium chloride
- Lauroyl ethyltrimonium methosulfate
- Myristoyl ethyltrimonium methosulfate
- Palmitoyl ethyltrimonium methosulfate
- Stearoyl ethyltrimonium methosulfate
- Cocoylcholine methosulfate
- Carnitine
- Carnitine HCl
- Carnitine hydroxycitrate
- Carnitine PCA
- Palmitoyl carnitine
- Acetyl carnitine and
- Acetyl carnitine HCl

Polymeric Trimonium Ingredients

- The final group of ingredients consists of various quaternized polymers. These ingredients consist of homo- and co-polymers with at least 1 trimonium-containing repeat unit, and range in molecular weight from ~400 000 to 7 000 000.[6] Ingredients in this group are shown in Table 3 with CAS Registry numbers, functions in cosmetics, technical names, and trade names. Idealized structures are provided in Figure 1C. These ingredients are:
- Polyquaternium-37
- Polyquaternium-14
- Polyquaternium-28
- Polyquaternium-32
- Polyquaternium-33
- Polyquaternium-35
- Polyquaternium-36
- Polyquaternium-45
- Polyquaternium-47
- Polyquaternium-48
- Polyquaternium-53
- Polyquaternium-63
- Polyquaternium-73
- Polyquaternium-91
- Acrylamide/ethyltrimonium chloride acrylate/ethalkonium chloride acrylate copolymer
- Acrylamidopropyl trimonium chloride/acrylamide copolymer and
- Acrylamidopropyl-trimonium chloride/acrylates copolymer

Physical and Chemical Properties

The calculated and available experimental chemical properties for the included ingredients are given in Table 4. Surfactants, or surface-active agents, are by definition amphipathic.[9] Typically, one portion of the surfactant will be hydrophilic, while the other end is hydrophobic. One particular category of surfactants is the cationic surfactants. While these ion pairs (or salts) have negatively charged counter ions (anions; eg chloride or bromide), the positively charged cations impart the majority of the surfactant character. The trimoniums are a subcategory of the quaternary ammonium surfactants, wherein 3 of the 4 substituents on the nitrogen atom, comprising the quaternary ammonium, are each methyl group.

Typically, the manufacture of trimoniums occurs by the reaction of a tertiary amine (ie, a nitrogen atom with 2 methyl groups and 1 alkyl group bonded to it) with a classic alkylating agent, such as a methyl halide or dimethyl sulfate.[10] For example, behentrimonium methosulfate can be prepared by the addition of *N*, *N*-dimethyldocosan-1-amine to dimethyl sulfate (Figure 2). Mixtures, like ceteartrimonium chloride (a mixture of cetrimonium chloride and steartrimonium chloride), are often synthesized by the addition of various haloalkanes (ie, a cetyl halide and a stearyl halide) to trimethylamine.[11]

Straight and Branched Chain Alkyl Trimonium Ingredients

The first group of trimoniums in this safety assessment (Table 1) consists of cations, each comprising a nitrogen atom bonded to 3 methyl groups and a simple alkyl chain, which can vary in length from 12 (eg, laurtrimonium bromide) to 28 carbons in length (eg, octacosatrimonium chloride; Figure 1A). All of these straight chain trimoniums are waxy-solids at human physiological temperatures. The branched chain trimoniums, however, are liquids at the same temperatures. Water solubility and volatility decrease and melting/boiling points increase as chain length is increased. For instance, straight chain trimoniums up to 16 carbons in length (eg, lautrimoniums [C12], myrtrimonium bromide [C14], and cetrimoniums [C16] are water soluble, but longer chain trimoniums (eg, steartrimoniums [C18], behentrimoniums [C22], and octacosatrimonium chloride [C28]) are not water soluble.[6]

Table 2. Alkanol Trimonium Ingredients and Related Ethers/Esters/Acids in This Safety Assessment With CAS Numbers, Functions in Cosmetics, Technical Names, and Trade Names.

Ingredient	CAS no.	Function(s)	Technical names	Trade names
Choline chloride	67-48-1	Skin-conditioning agents–humectant	Choline chloride (INN); ethanaminium, 2-hydroxy-N, N, N-trimethyl-, chloride; ethannaminium, 2-hydroxy-N, N, N-trimethyl, chloride; (β-hydroxyethyl)trimethylammonium chloride	Choline chloride aqueous solution
Ether				
Stearoxypropyltrimonium chloride	23328-71-4	Antistatic agent; hair-conditioning agent	Ammonium, trimethyl[3-octadecyloxy)propyl]-, chloride	Quartamin E-80K
Esters				
Lauroyl ethyltrimonium methosulfate	851385-89-2	Surfactant-cleansing agent		Surfactive V 12
Myristoyl ethyltrimonium methosulfate	851385-90-5	Surfactant-cleansing agent		Surfactive V 14
Palmitoyl ethyltrimonium methosulfate	None	Surfactant-emulsifying agent		Surfactive V 16
Stearoyl ethyltrimonium methosulfate	None	Surfactant-emulsifying agent		Surfactive V 18
Ester mixtures				
Cocoylcholine methosulfate	852690-27-8	Surfactants; cleansing agents	Ethanaminium, 2-hydroxy-N, N, N-trimethyl-, esters with coco fatty acids, me sulfates	Surfactive vcc
Alkanol acids				
Carnitine	541-15-1	Antistatic agents; hair-conditioning agents; skin-conditioning agents-miscellaneous; surfactants-cleansing agents; surfactants-foam boosters; viscosity increasing agents-aqueous	Ammonium, (3-carboxy-2-hydroxypropyl)trimethyl-, hydroxide, inner salt, L-3-carboxy-2-hydroxy-N, N, N-trimethyl-1-propanaminiumhydroxide, inner salt; carnitine (INN); levocarnitine (INN); 1-propanaminium, 3-carboxy-2-hydroxy-N, N, N-trimethyl-, hydroxide, inner salt	AEC Carnitine (Acetyl L); Natrulon RC Reparative/Exfoliant; Natrulon RC-50 Reparative/Exfoliant; OriStar LCNT
Carnitine HCl	6645-46-1	Humectants; skin-conditioning agents–miscellaneous	DL-Carnitine HCl; L-carnitine hydrochloride; 1-propanaminium, 3-carboxy-1-, hydroxyl-N, N, N-trimethyl-, chloride; 1-propanaminium, 3-carboxy-2-hydroxy-N, N, N-trimethyl-, chloride	OriStar LCH; Seltzer Chemicals L-Carnitine HCL
Carnitine hydroxycitrate	None	Skin-conditioning agents–miscellaneous		Lipolyse HCC
Carnitine PCA	None	Exfoliants		Vamactive CR
Acid esters				
Acetyl carnitine	14992-62-2	Skin-conditioning agents–miscellaneous	1-Propanaminium, 2-(acetyloxy)-3-carboxy-N, N, N-trimethyl-, inner salt	OriStar ACC
Acetyl carnitine HCl	5080-50-2	Skin-conditioning agents–miscellaneous	1-Propanaminium, 2-(acetyloxy)-3-carboxy-N, N, N-trimethyl-, chloride	Acetyl-L-Carnitine Hydrochloride; OriStar ACH
Palmitoyl carnitine	1935-18-8; 2364-67-2	Skin-conditioning agents–miscellaneous	Ammonium, (3-carboxy-2-hydroxypropyl)trimethyl-, hydroxide, inner salt, palmitate; hexadecanoyl carnitine; palmitic acid, ester with (3-carboxy-2-hydroxypropyl)trimethylammonium hydroxide inner salt; propanaminium, 3-carboxy-N, N, N-trimethyl-2-((1-oxohexadecyl)oxy)-, inner salt	Vexel

Table 3. Polymers Containing Trimonium in This Safety Assessment With CAS Numbers, Functions in Cosmetics, Technical Names, and Trade Names.

Ingredient	CAS No.	Functions	Technical Names	Trade Names
Homopolymers				
Polyquaternium-37	26161-33-1	Antistatic agents; film formers; hair fixatives	Choline, chloride, methacrylate, polymer; ethanaminium, N, N, N-trimethyl-2-[(methyl-1-oxo-2-propenyl)oxy]-, chloride, homopolymer; trimethylaminoethyl methacrylate chloride polymer; N, N, N-trimethyl-2-[(methyl-1-oxo-2-propenyl)oxy]ethanaminium chloride, homopolymer	Kleasol 100XT; OriStar PQ37; Synthalen CN; Synthalen CR; Synthalen CU; Syntran PC 5320; Ultragel 300
Polyquaternium-14	27103-90-8	Antistatic agents; film formers; hair fixatives	Choline, methyl sulfate, methacrylate, polymer; ethanaminium, N, N, N-trimethyl-2-[(2-methyl-1-oxo-2-propenyl)oxy]-, methyl sulfate, homopolymer	
Copolymers				
Polyquaternium-28	131954-48-8	Antistatic agents; film formers; hair fixatives	1-Propanaminium, N, N, N-trimethyl-3-((2-methyl-1-oxo-2-propenyl)amino)-, chloride, polymer with 1-ethenyl-2-pyrrolidinone; vinylpyrrolidone/methacrylamidopropyl-trimethylammonium chloride copolymer	1-Propanaminium, N, N, N-trimethyl-3-((2-methyl-1-oxo-2-propenyl)amino)-, chloride, polymer with 1-ethenyl-2-pyrrolidinone; vinylpyrrolidone/methacrylamidopropyltrimethylammonium chloride copolymer
Polyquaternium-32	35429-19-7	Antistatic agents; film formers; hair fixatives	Acrylamide-dimethylaminoethyl methacrylate methyl chloride copolymer; ethanaminium, N, N, N-trimethyl-2-[(2-methyl-1-oxo-2-propenyl)oxy]-, chloride, polymer with 2-propenamide; acrylamide-dimethylaminoethyl methacrylate methyl chloride copolymer; ethanaminium, N, N, N-trimethyl-2-[(2-methyl-1-oxo-2-propenyl)oxy]-, chloride, polymer with 2-propenamide	
Polyquaternium-33	69418-26-4	Antistatic agents; film formers; hair fixatives	Acrylamide-dimethylaminoethyl; ethanaminium, N, N, N-trimethyl-2-[1-oxo-2-propenyl)oxy]-, chloride, polymer with 2-propenamide.	Ultimer CG-200
Polyquaternium-35	None	Antistatic agents; film formers; hair fixatives		Plex 3074 L
Polyquaternium-36	None	Antistatic agents; film formers; hair fixatives		Plex 4739 L
Polyquaternium-4S	None	Antistatic agents; film formers; hair fixatives		
Polyquaternium-47	None	Film formers; hair fixatives; skin-conditioning agents-miscellaneous	1-Propanaminium, N, N, N-trimethyl-3-((2-methyl-1-oxo-2-propenyl)amino)-, chloride, polymer with methyl 2-propenoate and 2-propenoic acid	Merquat 2001; Merquat 2001N
Polyquaternium-48	None	Antistatic agents; film formers; hair fixatives		Plascize L-4S0
Polyquaternium-53	84647-38-1	Hair-conditioning agents	Acrylic acid/acrylamide/methacrylamidopropyl-trimonium chloride copolymer	Merquat 2003
Polyquaternium-63	None	Hair-conditioning agents; skin-conditioning agents–miscellaneous		Octacare PQ63; OF-308
Polyquaternium-73	None	Antistatic agents; film formers; hair-conditioning agents; hair fixatives		Diaformer C-802; Diaformer C-823; Diasleek C-802; Diasleek C-823
Polyquaternium-91	1020103-28-9	Film formers; hair-conditioning agents; surface modifiers	Ethanaminium, N, N, N-trimethyl-2-[(2-methyl-1-oxo-2-propeny-1-yl)oxy]-, chloride (1:1), polymer with α-(1-oxo-2-propen-1-yl)-θ-hydroxypoly(oxy-1,2-ethanediyl) and 1,2-propanediol mono(2-methyl-2-propenoate)	Film formers; hair-conditioning agents; surface modifiers
Acrylamide/ethyltrimonium chloride acrylate/ethalkonium chloride acrylate copolymer	None	Film former, hair-conditioning agent; skin-conditioning agent–miscellaneous; slip modifier; viscosity increasing agent–aqueous		Ultimer CG-400
Acrylamidopropyl trimonium chloride/acrylamide copolymer	None	Hair conditioner		Salcare SC60
Acrylamidopropyl-trimonium chloride/acrylates copolymer	None	Antistatic agent; film former; hair fixative		Produkt W 37194

Table 4. Physical and chemical properties.

Property	Value
Straight chain alkyl	
Laurtrimonium bromide	
Molecular weight	308.346
Melting point (°C)	246
Log K_{ow}	1.22 (estimated via EPI Suite)[a]
Water solubility	996 mg/L (estimated via EPI Suite)
Vapor pressure	$S \times 10^{-10}$ mm Hg (estimated via EPI Suite)
Laurtrimonium chloride	
Molecular weight	263.895
Melting point (°C)	246, 248-249.S (in ethyl acetate)
Solubility	Carbon tetrachloride, chloroform
Myrtrimonium bromide	
Molecular weight	350.42
Appearance	Amber liquid
Odor	Characteristic
Log K_{ow}	2.2 (estimated via EPI Suite)
Melting point (°C)	247
Water solubility	98 mg/L (estimated via EPI Suite)
Cetrimonium bromide	
Molecular weight	364.48
Melting point (°C)	237-243
Solubility	Water and acetone; Not in benzene and ether
pH	Stable in acidic pH; Optimum biocide range 4-10
Incompatible	Anionics, soap, nitrates, heavy metals, oxidants, rubber, proteins, and blood
Cetrimonium chloride	
Stabilibty (2% in mineral oil/water emulsion)	5°C, 25°C, 37°C, and 60°C for 1 month
Cetrimonium methosulfate	
Molecular weight	395.640
Steartrimonium bromide	
Molecular weight	392.50
Melting point (°C)	233 (estimated via EPI Suite)
Log K_{ow}	4.17 (estimated via EPI Suite)
Water solubility	0.94 m/L (estimated via EPI Suite)
Steartrimonium chloride	
Molecular weight	348.13
Appearance	Amber liquid
Active quaternary (%)	~50
Steartrimonium methosulfate	
Molecular weight	423.69
Behentrimonium chloride	
Molecular weight	404.161
Appearance	Waxy solid (mixed with isopropanol); White solid (mixed with cetearyl alcohol)
pH	4.9 (3% in water; mixed with isopropanol); 6.0-7.5 (10% in water; mixed with cetaryl alcohol)
Density (kg/m^3)	900
Melting Point (°C)	246 (estimated via EPI Suite)
Log K_{ow}	6.13 (estimated via EPI Suite)
Water solubility	0.017 mg/L (estimated via EPI Suite)
Behentrimonium methosulfate	
Molecular weight	479.801
Octacosatrimonium chloride	
Molecular weight	488.33
Log Kow	9.08 (estimated via EPI Suite)
Melting point (°C)	278 (estimated via EPI Suite)
Water solubility	$1.5\ 10^{-3}$ mg/L (estimated via EPI Suite)
Vapor pressure	1.1×10^{-14} mm Hg (estimated via EPI Suite)

(continued)

Table 4. (continued)

Property	Value
Straight chain alkyl mixtures	
Ceteatrimonium chloride	
Melting point (°C)	70
Density	0.88 g/mL
Soytrimonium chloride	
pH	7.5-8.5
Odor	Characteristic
Boiling point	100°
Tallowtrimonium chloride	
Appearance	Clear to pale yellow liquid
Boiling point (°C)	100
Melting point (°C)	20
pH	6-9 (10% aqueous)
Solubility	water
Branched alkyl	
Octyldodecyltrimonium chloride	
Molecular weight	376.108
Dodecylhexadecyl-trimonium chloride	
Molecular weight	488.322
Alkanol	
Choline chloride	
Molecular weight	139.624
Appearance	White crystals
Odor	Slight amine odor
Solubility	Water
pH	Neutral or slightly acidic (aq.)
Melting point	244-247°C
Density	1.1 g/cm^3
Partition coefficient	-3.77 at 25°C
Alkanol ether	
Stearoxypropyltrimonium chloride	
Molecular weight	106.134
Alkonal esters	
Lauroyl ethyltrimonium methosulfate	
Molecular weight	397.S69
Myristoyl ethyltrimonium methosulfate	
Molecular weight	425.623
Palmitoyl ethyltrimonium methosulfate	
Molecular weight	453.676
Stearoyl ethyltrimonium methosulfate	
Molecular weight	481.730
Alkanol acids	
Carnitine	
Molecular weight	161.20
Physical appearance	White solid
Melting point	210.00-212.00°C
Solubility	Practically insoluble in acetone, ethyl acetate
Water	2500 g/L
Carnitine HCL	
Molecular weight	197.66
Physical appearance	Solid
Melting point (decomposes)	142°C
Carnitine hydroxycitrate	
Molecular weight	433.492
Carnitine PCA	
Molecular weight	290.313
Acid esters	

(continued)

Table 4. (continued)

Property	Value
Palmitoyl carnitine	
Molecular weight	399.608
Acetyl Carnitine HCl	
Molecular weight	239.093
Physical appearance	Crystalline powder
Homopolymers	
Polyquaternium-37	
Molecular weight of monomers	225.757
Polyquaternium-14	
Molecular weight of monomers	301.401
Copolymers	
Polyquaternium-28	
Molecular weight of monomers	302.257
Number average molecular weight	>1000
Max low-molecular weight species (%)	<1
Appearance (20°C; 101.3 kPa)	Hazy, highly viscous liquid
Glass transition temperature	177°C
Specific gravity	1050 kg/m^3
Solubility	Freely soluble in water
Partition coefficient (n-octanol/water; 20°C; log $P_{o/w}$)	<−1.2
Hydrolysis after 3 months as a function of pH	
pH 7, room temperature	7.2%
pH 7, 45°C	11.2%
pH 10, 45°C	17%
Adsorption/desorption (K_{OC})	1653
Polyquaternium-32	
Molecular weight of monomers	262.389
Polyquaternium-33	
Molecular weight of monomers	246.346
Physical appearance	Straw colored liquid
Odor	Mild amine odor
Flash point	200°F (93.3°C)
Solubility	Water
Evaporation rate	<1 (butyl acetate = 1)
Polyquaternium-35	
Molecular weight of monomers	407.566
Polyquaternium-36	
Molecular weight of monomers	465.390
Polyquaternium-45	
Molecular weight of monomers	407.313
Polyquaternium-47	
Molecular weight of monomers	349.486
Polyquaternium-48	
Molecular weight of monomers	987.772
Polyquaternium-53	
Molecular weight of monomers	334.475
Polyquaternium-63	
Molecular weight of monomers	317.424
Polyquaternium-73	
Molecular weight of monomers	448.683
Polyquaternium-91	
Molecular weight of monomers	394.566
Acrylamide/ethyltrimonium chloride acrylate/ethalkonium chloride acrylate copolymer	
Molecular weight of monomers	721.39
Acrylamidopropyl trimonium chloride/acrylamide copolymer	
Molecular weight of monomers	259.388
Acrylamidopropyl-trimonium chloride/acrylates copolymer	
Molecular weight of monomers	259.365

[a] Estimation Programs Interface Suite T.[111]

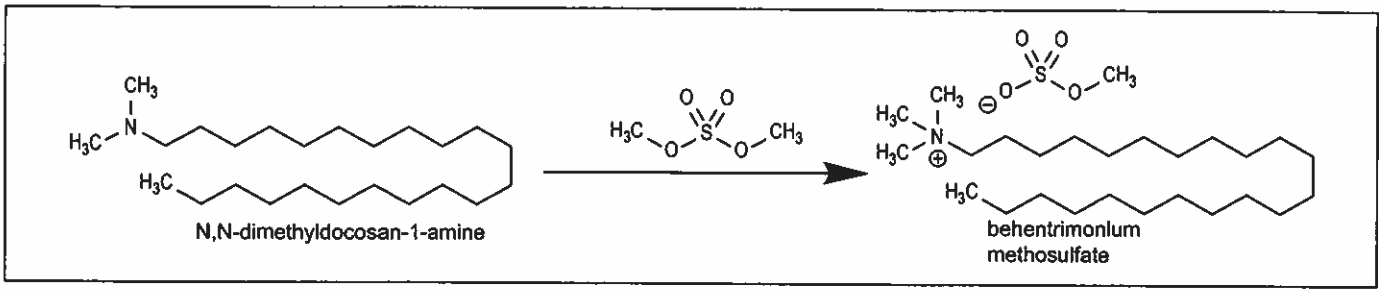


Figure 2. Example synthesis of alkyl trimoniums (behentrimnonium methosulfate).

For example, behentrimonium chloride (Table 1) is a waxy solid and is dispersible in water. Behentrimonium chloride is stable but is incompatible with strong oxidizing agents.[12] When mixed with cetearyl alcohol (concentration not provided) behentrimonium chloride is a white solid with a specific gravity < 1.[13]

Behentrimonium chloride may originate from plant sources that one manufacturer states do not contain any genetically modified organisms.[14] It is made through the process of quaternization of behenyl dimethylamine with methyl chloride, in 30% dipropylene glycol.

Alkanol Trimonium Ingredients

The alkanol trimonium ingredients in this safety assessment (Table 2) differ from the alkyl trimonium ingredients by the addition of an ethoxy functional group attached to the trimonium core nitrogen (Figure 1B). The simplest of these, and a major metabolite of all of the other members in the group, is choline. Choline chloride was reported to not be volatile at 21°C and is stable under ordinary conditions of use and storage.[15] Choline chloride may produce carbon monoxide, carbon dioxide, nitrogen oxides, and hydrogen chloride when heated to decomposition. Choline chloride was reported to be incompatible with strong oxidizers.

Included within this group are the choline ethers and esters, with various chain lengths, which are crystalline solids. Also within this group are the carnitine acids and esters. The carnitines, which are also solids, differ from the cholines by the attachment of an acid or ester functional group at the hydroxyl-bearing carbon of the choline core.

Carnitine is an amino acid derivative found in high energy demanding tissues and was reported to be an essential cofactor for the transport of long-chain fatty acids across the inner mitochondrial membrane into the mitochondrial matrix.[16] Carnitine was reported to be stable under normal temperatures and pressures.[17] It was also reported to be incompatible with strong oxidants and exposure to moist air or water. Carnitine decomposes into nitrogen oxides, carbon monoxide, carbon dioxide, and nitrogen.

Polymeric Trimonium Ingredients

The third group of trimoniums in this safety assessment (Table 3 and Figure 1C) differ from the other groups by their incorporation into a polymer backbone (ie, these are trimonium functionalized/decorated polymers). The molecular weights and various physical properties of the trimonium polymers can vary greatly based on the polymerization conditions utilized in their synthesis. Accordingly, molecular weights of the individual monomer units are provided instead. Typical molecular weights of these polymers, however, are usually above 400 000 g/mol.[6]

One polymer, polyquaternium-28, was reported to be 100% ionized in water and has an infinitely small pK.[18] Polyquaternium-28 is a copolymer comprised of a trimonium monomer and a lactam (ie, a heterocycle containing an amide in the ring structure) monomer. The amide group from the lactam monomer behaves as a weak base. Polyquaternium-28 was reported to be incompatible with strong oxidizing agents and reducing agents. This polymer was reported to be used in an aqueous solution at 20%.

Polyquaternium-10 differs structurally from all of the other trimonium polymers in this safety assessment by having a cellulose backbone. Otherwise, polyquaternium-10 is very closely related to these trimonium polymer ingredients. This large chemical entity (molecular weight 250 000-600 000 g/mol) is substituted with the same trimonium bearing side chains as the other trimonium polymers and provides the same type of cationizing or conditioning effect.

Other

Physical properties were not found for: cocotrimonium methosulfate, cocoylcholine methosulfate, hydrogenated palmtrimonium chloride, and hydrogenated tallowtrimonium chloride and were not calculable since these are mixtures of different chain length trimoniums with unknown ratios.

Methods of Manufacture

The synthesis of behentrimonium methosulfate is representative of ingredients in the alkyl trimonium group and is shown in Figure 2.

The synthesis of polyquaternium-37 is representative of the ingredients in the trimonium polymer group and is shown in Figure 3. The typical synthesis of polyquaternium-10 is via an epoxide and is shown in Figure 4.

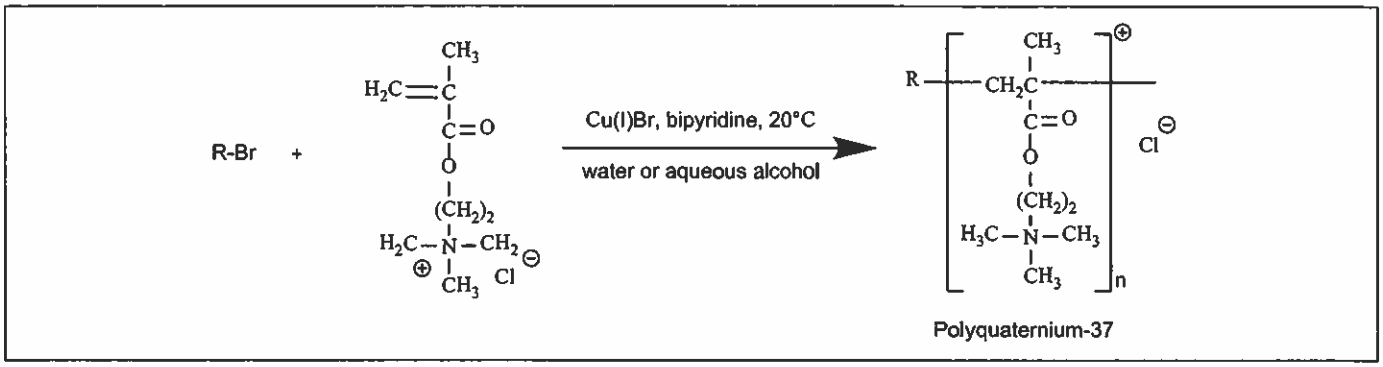


Figure 3. Example synthesis of trimonium decorated polymers (Polyquaternium-37).

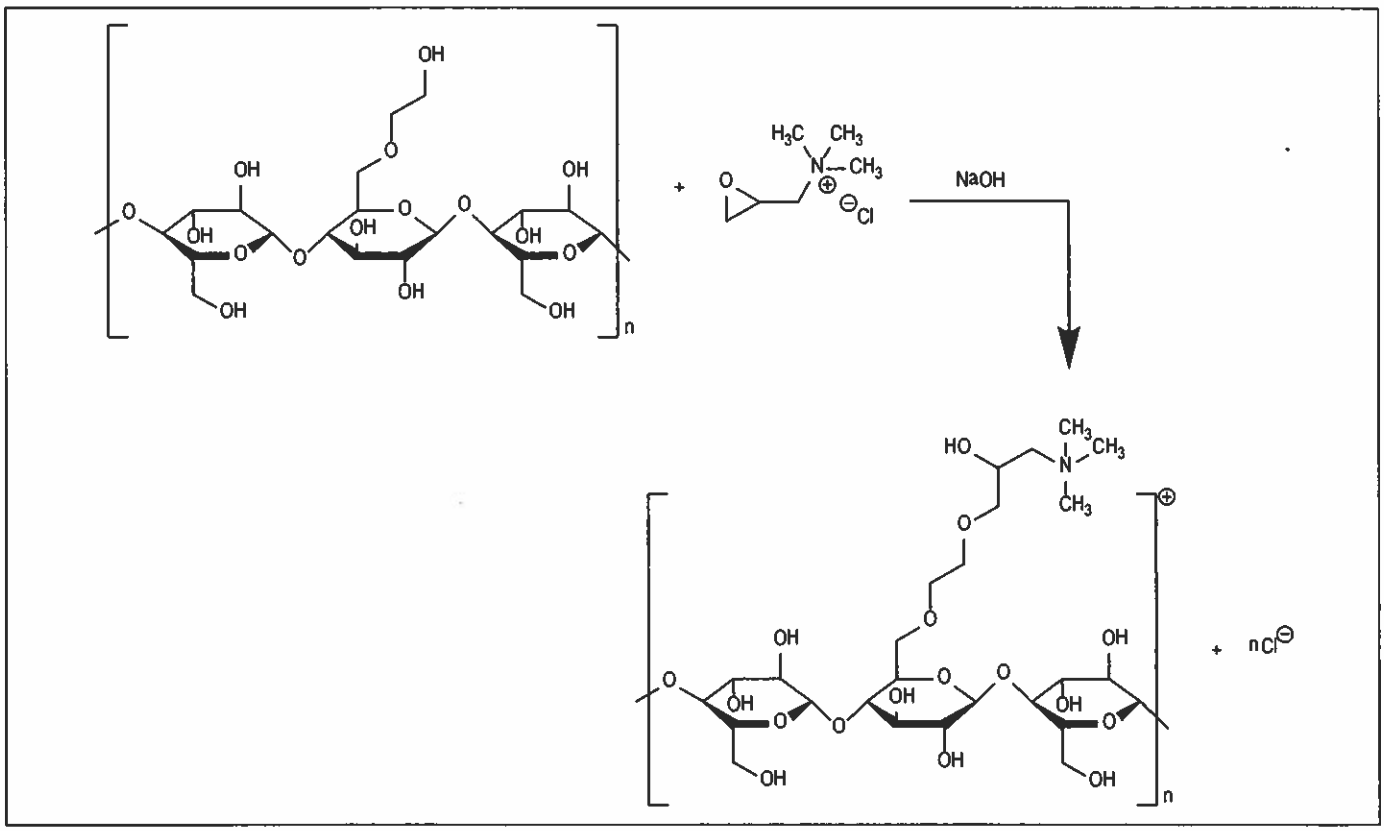


Figure 4. Synthesis of cellulose backbone trimonium decorated polymers (Polyquaternium-10).

Impurities

Straight and Branched Chain Alkyl Trimonium Ingredients

Cetrimonium bromide and myrtrimonium bromide are reported to be 99% pure.[19]

Alkanol Trimonium Ingredients

One supplier reports that the organic impurities of choline chloride to be trimethylamine (max 500ppm), ethylene glycol (500 ppm), organic impurities (ie, trimonium methosulfate, glycol, chloroethanol; max 1500 ppm), color (max 50 hazen), and heavy metals (ie, lead; 20 pm).[20]

Available sources of carnitine are reported to be > 99% pure.[17]

One available source of acetyl carnitine HCl was reported to be 98.5% to 100.0% pure.[21] Lead was reported to be present at <20 ppm and sulfated ash at ≤0.30%.

Polymeric Trimonium Ingredients

Polyquaternium-28 was reported to be >99% pure by one supplier.[18] The maximum content of the residual monomer was reported to be <1%.

Table 5. Historical and Current Cosmetic Product Uses and Concentrations for Cetrimonium Bromide, Cetrimonium Chloride, and Steartrimonium Chloride.[1,22,23]

Product category (total uses)	1994 uses (Andersen 1994)	2010 uses (FDA 2010)	2009 concentrations (Council 2009) (%)
Cetrimonium bromide			
Eye makeup			
Eyeliners (834)	–	–	0.3
Eye shadow (1343)	1	7	0.1
Mascara (528)	–	1	–
Fragrance products			
Colognes and toilet waters (1426)	1	1	–
Powders (237)	3	–	–
Noncoloring hair care products			
Conditioners (1313)	13	27	0.1-3
Sprays/aerosol fixatives (321)	–	–	0.2
Rinses (34)	1	–	–
Shampoos (1487)	1	1	–
Tonics, dressings, etc (1321)	3	7	0.1-0.2
Other (838)	–	–	0.3
Hair coloring products			
Bleaches (147)	–	–	2
Makeup			
Blushers (471)	1	5	–
Face powders (724)	3	4	–
Foundations (624)	1	–	–
Lipsticks (2045)	–	–	0.3
Personal hygiene products			
Underarm deodorants (623)	1	1	0.1
Other (925)	–	1	–
Shaving products			
Aftershave lotions (381)	–	–	0.1
Men's talcum (3)	1	–	–
Skin care products			
Skin cleansing creams, lotions, liquids, and pads (1528)	1	2	–
Face and neck creams, lotions, powder and sprays (1652)	1	–	–
Body and hand creams, lotions, powder and sprays (1875)	–	1	0.1
Moisturizers (2750)	3	3	–
Paste masks/mud packs (462)	–	1	–
Skin fresheners (267)	–	–	0.1
Suntan products			
Other (61)	1	–	–
Total uses/ranges for cetrimonium bromide	37	68	0.1-3
Cetrimonium chloride			
Baby products			
Other (149)	–	5	–
Bath products			
Soaps and detergents (1781)	–	3	0.09
Other (234)	–	1	–
Eye makeup			
Eye lotions (260)	–	–	0.0008
Eye makeup remover (133)	–	1	–
Fragrance products			
Colognes and toilet waters (1426)	–	–	0.1
Noncoloring hair care products			
Conditioners (1313)	86	435	0.1–10
Sprays/aerosol fixatives (321)	1	1	0.08-0.5
Straighteners (181)	–	17	0.2-0.5
Permanent waves (75)	2	6	0.2-0.4
Rinses (34)	3	12	0.08
Shampoos (1487)	1	43	0.004-0.5

(continued)

Table 5. (continued)

Product category (total uses)	1994 uses (Andersen 1994)	2010 uses (FDA 2010)	2009 concentrations (Council 2009) (%)
Tonics, dressings, etc (1321)	17	149	0.1-3
Wave sets (60)	3	2	–
Other (838)	16	141	0.5-2
Hair coloring products			
Dyes and colors (2382)	18	92	0.2-0.3
Tints (6)	–	1	–
Rinses (40)	–	5	–
Shampoos (36)	2	3	–
Color sprays (7)	–	2	–
Bleaches (147)	–	7	–
Other (168)	–	7	0.3
Makeup			
Other (536)	–	–	0.3
Nail care products			
Other (137)	2	1	–
Personal hygiene products			
Underarm deodorants (623)	–	9	0.1
Shaving products			
Aftershave lotions (381)	–	1	–
Skin care products			
Skin cleansing creams, lotions, liquids, and pads (1528)	2	4	0.2
Depilatories (56)	–	–	0.1
Face and neck creams, lotions, powder and sprays (1652)	–	–	0.08-1
Body and hand creams, lotions, powder, and sprays (1875)	2	1	0.2-3
Moisturizers (2750)	3	–	–
Skin fresheners (267)	2	1	–
Other (1446)	3	4	–
Suntan products			
Indoor tanning preparations (247)	–	3	–
Total uses/ranges for cetrimonium chloride	162	959	0.0008-10
Steartrimonium chloride			
Noncoloring hair care products			
Conditioners (1313)	3	19	2
Sprays/aerosol fixatives (321)	–	–	0.2
Permanent waves (75)	1	1	3
Rinses (34)	–	–	2-3
Shampoos (1487)	–	–	3
Tonics, dressings, etc (1321)	–	4	0.3-0.8
Other (838)	2	7	–
Hair coloring products			
Dyes and colors (2382)	–	8	0.4-4
Tints (6)	–	–	0.06
Rinses (34)	–	1	–
Total uses/ranges for Steartrimonium chloride	6	40	0.06-4

Use

Cosmetic

According to information supplied to the Food and Drug Administration (FDA) by industry as part of the Voluntary Cosmetic Registration Program (VCRP), cetrimonium bromide was used in a total of 37 cosmetic formulations at the time of the first safety assessment in 1997 (Table 5).[1] No use concentrations were reported at that time. Currently, VCRP data indicated that cetrimonium bromide was reported to be used in 68 cosmetic products of multiple types.[22] A survey of current use concentrations conducted by the Personal Care Products Council (Council) reported a range from 0.1% to 3%.[23]

Cetrimonium chloride was used in a total of 162 cosmetic formulations at the time of the first safety assessment.[1] Currently, VCRP data indicated that cetrimonium chloride was reported to be used in 959 cosmetic products.[22] A survey of current use concentrations reported a range from 0.0008% to 10%.[23]

Steartrimonium chloride was used in a total of 6 cosmetic formulations at the time of the first safety assessment.[1] Currently, VCRP data indicated that cetrimonium chloride was reported to be used in 40 cosmetic products.[22] A survey

of current use concentrations reported a range from 0.06% to 4%.[23]

The other straight chain alkyl trimonium ingredients (behentrimonium chloride, behentrimonium methosulfate, cetrimonium methosulfate, cocotrimonium chloride, cocotrimonium methosulfate, laurtrimonium chloride, myrtrimonium bromide, soytrimonium chloride, and tallowtrimonium chloride) have reported uses mostly in hair care, skin care, and makeup products at 0.0005% to 10% in rinse-off and at 0.001% to 4% in leave-on products (Table 6).[22,23] These ingredients function as cosmetic biocides, hair-conditioning agents, antistatic agents, emulsifying agents, and surfactant-emulsifying agents. No uses or concentrations of use were reported for ceteartrimonium chloride, cocotrimonium methosulfate, dodecylhexadecyltrimonium chloride, hydrogenated palmtrimonium chloride, hydrogenated tallowtrimonium chloride, laurtrimonium bromide, octacosatrimonium chloride, octyldodecyltrimonium chloride, steartrimonium bromide, and steartrimonium methosulfate.

The alkanol trimonium ingredients and related ethers/esters/acids (acetyl carnitine HCl, carnitine, carnitine HCl, carnitine hydroxycitrate, palmitoyl carnitine) are mostly used in skin care and make-up products at concentrations between 0.001% and 1% in both rinse-off products and leave-on products (Table 7).[22,24] These ingredients function as skin-conditioning agents, antistatic agents, hair-conditioning agents, surfactant-cleaning agents, surfactant-emulsifying agents, surfactants-foam boosters, viscosity increasing agents, humectants, and exfoliants. No uses or concentrations of use were reported for choline chloride, stearoxypropyltrimonium chloride, lauroyl ethyltrimonium methosulfate, myristoyl ethyltrimonium methosulfate, palmitoyl ethyltrimonium methosulfate, stearoyl ethyltrimonium methosulfate, and acetyl carnitine.

Trimonium polymers (acrylamidopropyltrimonium chloride/acrylamide copolymer, polyquaternium-28, polyquaternium-32, polyquaternium-35, polyquaternium-37, and polyquaternium-47) are mostly used in hair care and skin care products at concentrations between 0.04% to 10% in rinse-off products and 0.2% and 3% in leave-on products (Table 8). These ingredients function as antistatic agents, hair fixatives, film formers, skin-conditioning agents–miscellaneous, hair-conditioning agents, slip modifiers, and surface modifiers. No uses or concentrations of use were reported for polyquaternium-14, polyquaternium-33, polyquaternium-36, polyquaternium-45, polyquaternium-48, polyquaternium-53, polyquaternium-63, polyquaternium-73, polyquaternium-91, acrylamide/ethyltrimonium chloride acrylate/ethalkonium chloride acrylate copolymer, and acrylamidopropyl-trimonium chloride/acrylates copolymer.

For ingredients used in cosmetic sprays and aerosols, it is important to consider inhalation safety. Safety of inhaled aerosols depends on the ingredient, the concentration, the duration of the exposure and where they are deposited within the respiratory system.[25] The site of deposition is associated most with the particle size and density of the particle being inhaled.

Absorption of gases and vapors by inhalation is determined by the partitioning of the compound between the blood and the gas phase along with its solubility and tissue reactivity. The important characteristics that affect absorption after exposure to aerosols are the aerosol size and water solubility of any chemical present in the aerosol. In general, the smaller the particle, the further into the respiratory tree the particle will deposit and the greater the impact on the respiratory system.

The parameter most closely associated with this regional deposition is the aerodynamic diameter, d_a, defined as the diameter of a sphere of unit density possessing the same terminal settling velocity as the particle in question. In humans, particles with an aerodynamic diameter of ≤ 10 μm are respirable. Particles with a d_a from 0.1 to 10 μm settle in the upper respiratory tract and particles with a $d_a < 0.1$ μm settle in the lower respiratory tract.[26,27] Nanoparticles have the potential to deliver high amounts of particulates to the lung.[28]

Particle diameters of 60 to 80 μm and ≥ 80 μm have been reported for anhydrous hair sprays and pump hairsprays, respectively.[29] In practice, aerosols should have at least 99% of their particle diameters in the 10 to 110 μm range and the mean particle diameter in a typical aerosol spray has been reported as ~38 μm.[30] Therefore, most aerosol particles are deposited in the nasopharyngeal region and are not respirable.

The Panel has discussed this issue and has decided that in the absence of inhalation toxicity data on a specific ingredient, they will consider the aerosol and spray particle sizes in determining its inhalation safety.

In the European Union (EU), behentrimonium chloride, ceteartrimonium chloride, cetrimonium bromide, cetrimonium chloride, cocotrimonium chloride, hydrogenated palmtrimonium chloride, hydrogenated tallowtrimonium chloride, laurtrimonium bromide, laurtrimonium chloride, myrtrimonium bromide, soytrimonium chloride, steartrimonium bromide, steartrimonium chloride, and tallowtrimonium chloride were restricted to 1% when used as a preservative.[31]

The EU has limited the following ingredients to 1 mg/kg in leave-on body care products and 0.5 mg/kg in other products: polyquaternium-32, polyquaternium -47, polyquaternium-53, acrylamide/ethyltrimonium chloride acrylate/ethalkonium chloride acrylate copolymer, and acrylamidopropyl trimonium chloride/acrylamide copolymer.[32]

Choline chloride is an Annex II substance in the EU and is not to be used in cosmetics in Europe.[33] The Scientific Committee on Cosmetic Products (SCCP)[34] concluded that the available data on choline chloride was not sufficient to address concems about mucous membrane irritation when used in cosmetics.

Noncosmetic

Straight and branched chain alkyl trimonium ingredients. Laurtrimonium bromide was reported to be used for the separation and purification of DNA fragments.[35]

A commercial product containing cetrimonium bromide (and other quaternary ammonium salts) was reported to be used as a topical antiseptic.[1]

Table 6. Frequency of Use and Concentration of the Straight Chain Alkyl Trimonium Ingredients.[22,23]

Product category (Total number of products in each category (FDA 2009))	Frequency of use (FDA 2010)	Concentration of use (%) (Council 2010)
Behentrimonium chloride		
Baby products		
Shampoos (57)	–	0.4
Noncoloring hair care products		
Conditioners (1313)	349	2-5
Sprays/aerosol fixatives (321)	–	0.2-1
Straighteners (181)	1	0.8[a]
Permanent waves (75)	2	2
Rinses (34)	5	3
Shampoos (1487)	2	0.8-2
Tonics, dressings, etc (1321)	41	0.2-3
Wave sets (60)	–	–
Other (838)	112	7
Hair coloring products		
Dyes and colors (2382)	252	2-3
Lighteners with color (22)	1	–
Other (168)	4	–
Personal hygiene products		
Douches (13)	1	–
Shaving products		
Shaving cream (128)	3	–
Skin care products		
Skin cleansing creams, lotions, liquids, and pads (1528)	–	0.9
Face and neck creams, lotions, powder and sprays (1652)	–	–
Moisturizers (2750)	6	–
Other (1446)	2	0.5
Total uses/ranges for behentrimonium chloride	743	0.2-7
Behentrimonium methosulfate		
Baby products		
Other (149)	2	0.3
Bath products		
Soaps and detergents (1781)	–	0.3
Noncoloring hair care products		
Conditioners (1313)	126	0.1-10
Sprays/aerosol fixatives (321)	–	0.8
Straighteners (181)	15	6[b]
Permanent waves (75)	3	–
Shampoos (1487)	3	0.002
Tonics, dressings, etc (1321)	21	0.1-4
Other (838)	24	2
Hair coloring products		
Dyes and colors (2382)	16	3[c]
Tints (6)	1	–
Nail care products		
Cuticle softeners (30)	2	–
Personal hygiene products		
Underarm deodorants (623)	7	–
Other (925)	1	0.3
Shaving products		
Shaving cream (128)	1	
Skin care products		
Skin cleansing creams, lotions, liquids, and pads (1528)	–	0.2
Face and neck creams, lotions, powder and sprays (1652)	1	–
Body and hand creams, lotions, powder and sprays (1875)	18	0.3-0.7
Foot powders and sprays (46)	2	–
Moisturizers (2750)	27	–
Night creams, lotions, powder and sprays (386)	1	–

(continued)

Table 6. (continued)

Product category (Total number of products in each category (FDA 2009))	Frequency of use (FDA 2010)	Concentration of use (%) (Council 2010)
Other (1446)	1	–
Total uses/ranges for behentrimonium methosulfate	273	0.1-10
Cetrimonium methosulfate		
Noncoloring hair care products		
Conditioners (1313)	41	0.09-2
Rinses (34)	2	–
Tonics, dressings, etc (1321)	9	0.6-2
Other (838)	5	–
Hair coloring products		
Other (168)	5	–
Personal hygiene products		
Paste masks (mud packs) (462)	1	1
Total uses/ranges for Cetrimonium methosulfatee	59	0.2-2
Cocotrimonium chloride		
Noncoloring hair care products		
Conditioners (1313)	8	–
Shampoos (1487)	30	3
Tonics, dressings, etc (1321)	33	–
Hair coloring products		
Shampoos (36)	8	–
Total uses/ranges for cocotrimonium chloride	49	3
Cocotrimoium methosulfate		
Noncoloring hair care products		
Tonics, dressings, etc (1321)	8	0.5
Other (838)	2	–
Total uses/ranges for cocotromonium methosulfate	10	0.5
Laurtrimonium chloride		
Eye makeup		
Eyebrow pencils (153)	–	0.001
Eyeliners (834)	2	0.001
Eye shadow (1343)	1	0.001
Mascara (528)	–	0.001
Noncoloring hair care products		
Conditioners (1313)	–	0.008-0.2
Sprays/aerosol fixatives (321)	–	0.003-0.01
Permanent waves (75)	–	0.09
Rinses (34)	1	–
Shampoos (1487)	2	–
Tonics, dressings, etc (1321)	21	0.004
Hair coloring products		
Tints (6)	–	0.0005
Makeup		
Blushers (471)	1	0.001
Foundations (624)	1	0.003
Other (536)	–	0.001
Skin care products		
Body and hand creams, lotions, powder and sprays (1875)	2	
Total uses/ranges for laurtrimonium chloride	35	0.0005-0.2
Myrtrimonium bromide		
Eye makeup		
Eye makeup remover (133)	–	0.03
Shaving products		
Aftershave lotions (381)	–	0.1
Skin care products		
Skin cleansing creams, lotions, liquids, and pads (1528)	3	0.07
Face and neck creams, lotions, powder and sprays (1652)	–	0.03

(continued)

Table 6. (continued)

Product category (Total number of products in each category (FDA 2009))	Frequency of use (FDA 2010)	Concentration of use (%) (Council 2010)
Skin fresheners (267)	2	–
Total uses/ranges for myrtrimonium bromide	5	0.03-0.1
Soytrimonium chloride		
Noncoloring hair care products		
Conditioners (1313)	3	–
Hair coloring products		
Dyes and colors (2382)	206	7
Shampoos (36)	7	–
Lighteners with color (22)	2	–
Other (168)	3	–
Total uses/ranges for soytrimonium chloride	221	7
Tallowtrimonium chloride		
Noncoloring hair care products		
Conditioners (1313)	28	0.03
Straighteners (181)	1	0.02
Permanent waves (75)	2	–
Shampoos (1487)	4	–
Tonics, dressings, etc (1321)	11	0.1
Other (838)	2	
Hair coloring products		
Dyes and colors (2382)	41	–
Lighteners with color (22)		0.03
Total uses/ranges for tallowtrimonium chloride	89	0.03-0.1

[a] 0.4% after dilution.
[b] 3% after dilution.
[c] 1% after dilution.

Alkanol trimonium ingredients. Choline chloride was reported to be considered generally recognized as safe (GRAS) as a nutrient by the FDA.[36]

Choline and carnitine are reported to be used as dietary supplements.[37,38]

Absorption, Distribution, Metabolism, and Excretion

Oral

Straight and branched chain alkyl trimonium ingredients. The original safety assessment reported that orally administered cetrimonium bromide (0.8 mg/kg) was poorly absorbed by the intestinal tract of rats.[1] Greater than 90% of the cetrimonium bromide was recovered 3 days after oral administration in the feces.

Alkanol trimonium ingredients. Choline consumed in the diet is important to the structural integrity of cell membranes, methylation metabolism, cholinergic neurotransmission, transmembrane signaling, and lipid and cholesterol transport and metabolism.[20] Choline was obligatory to human cell survival. It was available in the diet as free choline and as phosphatidylcholines, such as lecithin in egg yolks, vegetables, and animal fat.

Choline consumed in the diet by mammals was reported to be absorbed from the lumen of the small intestine using transporter proteins in the enterocyte.[39-42] In the gut, choline can be liberated from phosphatidylcholine by pancreatic enzymes.[43] Some metabolism by bacteria to form betaine and methylamines was reported to be required before choline can be absorbed from the gut.[44] The free choline enters the portal circulation of the liver, whereas phosphatidylcholine may enter via lymph in chylomicrons.[45]

Choline functions as a precursor for acetylcholine, phospholipids, and the methyl donor betaine.[46,47]

All tissues accumulate choline by diffusion and mediated transport.[48] A specific carrier mechanism transports free choline across the blood–brain barrier at a rate that was reported to be proportional to the serum choline concentration. In the neonates, this choline transporter has a high capacity.[49] The rate at which the liver takes up choline was reported to be sufficient to explain the rapid disappearance of choline injected systemically.[50] The kidney also accumulates choline.[51] Some choline from the kidney appears in the urine unchanged but most was reported to be oxidized within the kidney to form betaine.[52] A significant portion of choline was reported to be oxidized to form betaine in the liver and kidney.[53,54]

Choline was reported to be phosphorylated, converted to cytidine diphosphocholine, and then converted to phosphatidylcholine in the predominant pathway for phosphatidylcholine biosynthesis.[55,56] In an alternative pathway, phosphatidylethanolamine was reported to be sequentially methylated to form phosphatidylcholine by the enzyme phosphatidylethanolamine-N-methyltransferase using S-adenosylmethionine as the methyl

Table 7. Frequency of Use and Concentration of the Alkanol Trimonium Ingredients and Related Ethers/Esters/Acids.[22,24]

Product category (Total Number of Products in Each Category (FDA 2009))	Frequency of use (FDA 2009)	Concentration of Use (%) (Council 2010)
Acetyl carnitine HCl		
Eye makeup		
Lotion (260)	5	0.05
Noncoloring hair care products		
Conditioners (1313)	–	0.01–1
Shampoos (1487)	–	0.01
Other (838)	–	0.01
Make up		
Foundations (624)	–	0.1
Lipstick (2045)	1	0.02
Personal hygiene products		
Underarm deodorants (623)	–	0.1
Other (925)	–	0.05
Skin care products		
Skin cleansing creams, lotions, liquids, and pads (1528)	2	–
Face and neck creams, lotions, powder, and sprays (1652)	1	0.1
Body and hand creams, lotions, powder, and sprays (1875)	2	–
Moisturizers (2750)	16	–
Night creams, lotions, powder, and sprays (386)	1	–
Other (1446)	1	0.05
Suntan products		
Suntan gels, creams, and liquids (106)	–	0.1
Total uses/ranges for acetyl carnitine HCl	30	0.01–1
Carnitine		
Bath products		
Soaps and detergents (1781)	–	1
Eye makeup		
Lotion (260)	2	0.02
Noncoloring hair care products		
Shampoos (1487)	–	0.01
Tonics, dressings, etc (1321)	–	1
Wave sets (60)	1	
Other (838)	1	–
Skin care products		
Skin cleansing creams, lotions, liquids, and pads (1528)	1	–
Face and neck creams, lotions, powder, and sprays (1652)	–	0.01
Body and hand creams, lotions, powder, and sprays (1875)	18	0.02
Moisturizers (2750)	1	0.5
Night creams, lotions, powder, and sprays (836)	2	–
Other (1446)	4	–
Total uses/ranges for carnitine	30	0.01–1
Carnitine HCl		
Skin care products		
Skin cleansing creams, lotions, liquids, and pads (1528)	1	0.0001
Face and neck creams, lotions, powder, and sprays (1652)	–	0.02
Body and hand creams, lotions, powder, and sprays (1875)	–	0.001
Moisturizers (2750)	–	1
Total uses/ranges for carnitine HCl	1	0.001–1
Carnitine hydroxycitrate		
Skin care products		
Body and hand creams, lotions, powder, and sprays (1875)	2	–
Other (1446)	2	–
Total uses/ranges for carnitine hydroxycitrate	4	–
Palmitoyl carnitine		
Skin care products		
Face and neck creams, lotions, powder, and sprays (1652)	1	–
Body and hand creams, lotions, powder, and sprays (1875)	3	–
Moisturizers (2750)	1	–
Total uses/ranges for palmitoyl carnitine	5	–

Table 8. Frequency of Use and Concentration of the Trimonium Polymers.[22,24]

Product category (Total Number of Products in Each Category (FDA 2009))	Frequency of Use (FDA 2009)	Concentration of Use (%) (Council 2010)
Acrylamidopropyltrimonium chloride/acrylamide copolymer		
Noncoloring hair care products		
Rinses (34)	1	–
Shampoos (1487)	2	0.04
Tonics, dressings, etc (1321)	3	–
Other (838)	1	
Total uses/ranges for acrylamidopropyltrimonium chloride/acrylamide copolymer	7	0.04
Polyquaternium–28		
Noncoloring hair care products		
Conditioners (1313)	1	0.3–3
Permanent waves (75)	1	–
Shampoos (1487)	7	–
Tonics, dressings, etc (1321)	7	1–2
Other (838)	5	–
Hair coloring products		
Dyes and colors (2382)	44	0.2
Skin care products		
Other (1446)	8	0.2
Total uses/ranges for polyquaternium–28	73	0.2–3
Polyquaternium–32		
Noncoloring hair care products		
Conditioners (1313)	23	0.8–10
Hair straighteners (181)	–	0.5
Tonics, dressings, etc (1321)	6	1
Other (838)	1	–
Personal hygiene products		
Other (925)	1	–
Skin care products		
Moisturizers (2750)	1	–
Total uses/ranges for polyquaternium–32	32	0.5–10
Polyquaternium–35		
Noncoloring hair care products		
Permanent waves (75)	1	–
Total uses/ranges for polyquaternium–35	1	–
Polyquaternium–37		
Noncoloring hair care products		
Conditioners (1313)	70	0.6–3
Straighteners (181)	1	–
Rinses (34)	2	–
Tonics, dressings, etc (1321))	45	1–3
Other (838)	35	2
Hair coloring products		
Dyes and colors (2382)	1	–
Bleaches (147)	2	3
Other (168)	16	0.2
Make up		
Other (536)	1	–
Nail care products		
Nail creams and lotions (15)	1	2
Other (137)	3	2
Skin care products		
Skin cleansing creams, lotions, liquids, and pads (1528)	4	1–3
Face and neck crams, lotions, powder, and sprays (1652)	2	–
Body and hand crams, lotions, powder, and sprays (1875)	1	1–3
Moisturizers (2750)	23	–
Other (1446)	3	–
Suntan Products		

(continued)

Table 8. (continued)

Product category (Total Number of Products in Each Category (FDA 2009))	Frequency of Use (FDA 2009)	Concentration of Use (%) (Council 2010)
Suntan gels, creams, and liquids (106)	–	0.8
Indoor tanning preparations (247)	2	–
Total uses/ranges for polyquaternium–37	213	0.2–3
Polyquaternium–47		
Baby products		
Baby shampoos (57)	1	–
Noncoloring hair care products		
Conditioners (1313)	3	0.3–1
Shampoos (1487)	4	0.2–2
Tonics, dressings, etc (1321)	3	–
Hair coloring products		
Dyes and colors (2382)	5	0.8
Hair lighteners with colors (22)	1	–
Skin care products		
Skin cleansing creams, lotions, liquids, and pads (1528)	3	0.2
Total uses/ranges for polyquaternium–47	20	0.2–2

donor.[57,58] This was reported to be the major, and possibly only, pathway for synthesis of the choline moiety in adult mammals. It was reported to be most active in the liver but has been identified in many other tissues.[59-61] There are no estimates available as to the relative extent of choline obtained from cell turnover.

Acetyl L-carnitine was reported to be maintained in the human body by dietary intake, some synthesis, and efficient renal reabsorption.[62] Dietary carnitine was reported to be absorbed by active and passive transfer in the intestine. Food provides 54% to 87% of carnitine. The kidneys are an important regulator of carnitine homeostasis. At normal levels, reabsorption of carnitine in the kidneys was reported to be efficient (90%-99% of filtered load). Renal reabsorbtion decreases when the circulating carnitine load increases. Circulating carnitine was reported to be distributing between large and slow turnover (ie, muscle) and small and rapid turnover (ie, liver, kidney, and other tissues). At normal dietary intake levels, whole-body turnover in humans was reported to be 38 to 119 h.

Polymeric trimonium ingredients. No information was discovered on the oral absorption, distribution, metabolism, and excretion of the trimonium polymers.

Dermal

Straight and branched chain alkyl trimonium ingredients. The percutaneous absorptions of [^{14}C] laurtrimonium bromide (0.5%) through nonoccluded rat skin and the resulting blood levels were studied.[4] Percutaneous absorption was low. Application in a cream hair-rinse preparation under user conditions (10 cm^2 area, lathered for 3 minutes, left for 15 minutes, rinsed, dabbed dry) resulted in the absorption of ~0.1% of the administered radioactivity in 48 hours. No measurable radioactivity was present in the blood. Application of the surfactant at a higher concentration (3%) in an aqueous solution resulted in a higher absorption of 0.6% in 72 hours. Some radioactivity, equivalent to < 100 ng of unchanged [^{14}C] laurtrimonium bromide, was measured in the blood after administration to the skin without subsequent rinsing.

The original safety assessment reported that a percutaneous absorption study showed that 0.59% of 1% cetrimonium bromide penetrated rat skin after 15 minutes; 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% cetrimonium bromide in water penetrated after 15 minutes of exposure.[1]

Dehyquart A-CA (25% cetrimonium chloride in a 3.5% emulsion; 25 mg/cm^2 100 mg on 4 cm^2) was applied to the dermatomed, thawed, full thickness skin (1000 μm; n = 6) of the back and flank of castrated male pigs in a diffusion chamber for 30 minutes.[63] The test substance was then washed off and the receptor fluid sampled up to 72 hours. At 72 hours, the amount of test substance was measured by high-pressure liquid chromatography/electron spectroscopic imaging/mass spectrometry (HPLC/ESI/MS) and was below detection limits in the receptor fluid, 0.7 ± 0.6% in the stratum corneum, 0.3% ± 0.3% in the dermis, 1.0% ± 0.9% total in the skin, and 90.2% ± 4.5% in the rinsing solution. The authors concluded that cetrimonium chloride may not be systemically available by a dermal route.

Alkanol trimonium ingredients. Full thickness human skin (n = 12) was used in Franz cells to test the penetration of radiolabeled choline chloride (5%) with and without occlusion.[64] Samples were taken up to 24 hours. At 24 hours, 0.457 μg/cm^2 choline had penetrated into the receptor chamber for occluded skin and 0.383 μg/cm^2 for unoccluded skin, 0.127% and 0.110% of the applied dose, respectively. There was no statistical difference in these results. Total absorption

(epidermis, dermis, and receptor fluid) was 7.42 μg/cm^2 (1.9%) and 13.86 μg/cm^2 (3.43%) for occluded and unoccluded skin. Most of the choline remained in the epidermis and dermis. The authors concluded that choline chloride has a low potential for percutaneous absorption.

Polymeric trimonium ingredients. Less than 1.5% of polyquaternium-10 (100%; 4.0 mL/kg) penetrated the skin of Fisher 344 rats (n = 12) over 24 h.[7]

Intravenous, Subcutaneous, and Intraperitoneal

Straight and branched chain alkyl trimonium ingredients. In the original safety assessment, cetrimonium bromide was rapidly excreted in the urine and feces of rats after subcutaneous and intervenous administration. Greater than 90% of the cetrimonium bromide was recovered 2 days after subcutaneous administration. There were only traces of cetrimonium bromide in the body 24 hours after intravenous (iv) injection.[1]

Alkanol trimonium ingredients. [^{14}C]Carnitine (100 μCi/kg) was injected intraperitoneally (ip) into pregnant CD-1 mice on day 17 of pregnancy.[65] At 1, 2, 3, and 6 h after the injection, the mice were killed and a full body x-ray was performed. The highest concentrations of carnitine were found in the liver, placenta, kidney, myocardium and choroid plexus. No carnitine was observed in the brains of the fetuses or the dam. There was carnitine in the fetuses but less than that in the dam; the distribution was similar and increased with time. Carnitine crossed the placental barrier.

Hepatic Metabolism

Palmitoyl-L-carnitine was reported to be hydrolyzed in the human liver; this process is mainly associated with the mitochondria.[66]

Penetration Enhancement and Inhibition

Straight and branched chain alkyl trimonium ingredients. Cetrimonium bromide (in a mineral oil-water emulsion) enhanced penetration of phenylazoaniline (pH 7.0), benzocaine (pH 7.0), and benzoic acid (pH 7.0) up to 0.5% in a Franz cell using dialysis or hydrophobic polydimethylsiloxane membrane membranes.[67] Concentrations above 0.5% were less effective until penetration was inhibited at ≥1.0%. Cetrimonium bromide inhibited penetration of benzoic acid (pH 3.0) and the penetration of phenol (pH 7.0) was not affected.

Alkanol trimonium ingredients. Palmitoyl carnitine (0.2 mmol/L) was reported to enhance the penetration of Lucifer yellow and Ruthenium red across Caco-2 monolayers by ~10-fold and ~20-fold, respectively.[68] Acetyl carnitine (with a shorter alkyl chain) did not enhance penetration using Caco-2 cell monolayers. Palmitoyl carnitine did not enhance the penetration of PEG 4000. The authors suggest that since there was no damage to the epithelium observed and there was rapid reversal of the effects with the removal of palmitoyl carnitine, the penetration enhancement was not due to cell lysis.

Nasal administration of palmitoyl carnitine (20%) simultaneously with human growth hormone increased the peak penetration by 260%, area under the absorption curve by 64%, and bioavailability by 12.2% in male Wistar rats (n = 5).[69]

Cytotoxicity

Straight and branched chain alkyl trimonium ingredients. The lethal concentration of laurtrimonium chloride for rat primary hepatocytes was >0.048 μL/ mL.[70]

Human K562 erythroleukemic cells were incubated with cetrimonium bromide (0.1 to 10 μmol/L) for 5 days (n = 3).[71] Cell growth decreased in a dose-dependent manner compared to controls. When in a liposome suspension or micellar solution, cell growth decreased in a dose-dependent manner; the IC_{50}s (concentration that inhibits 50% of growth) were 0.88 and 0.62 mmól/L, respectively.

Human keratinocytes from foreskins were incubated in cetrimonium chloride for 3 days.[72] Metabolism was then measured using tetrazolium dye. Concentrations >3 μg/mL completely inhibited dye reduction. At 0.8 μg/mL, there was no inhibition of dye reduction. Concentrations between 0.01 and 0.1 μg/mL enhanced dye reduction. Measurement of DNA content revealed that at these low concentrations, cetrimonium chloride had a stimulatory effect on cell proliferation.

The minimum inhibitory concentration (MIC) of cetrimonium bromide was 16 mg/mL for *Escherichia coli* and 128 μg/mL for *Pseudomonas aeruginosa*.[73] The authors suggested that cetrimonium bromide was able to disrupt membrane function of gram-negative bacteria. In addition to and simultaneously with this disruption, this ingredient can also chelate K^+ ions from the bathing medium.

The EC_{50} (effective concentration) values for cetrimonium bromide and laurtrimonium bromide for *Salmonella typhimurium* were $4.88 \pm 0.08 \times 10^{-6}$ mol/L and $75.00 \pm 3.90 \times 10^{-6}$ mol/L, respectively.[74] The MICs were 1.65×10^{-4} and 4.86×10^{-4} M, respectively.

Toxicology

Acute Oral Toxicity

Straight and branched chain alkyl trimonium ingredients. In 2 separate studies, the oral LD_{50} of laurtrimonium chloride in Sprague-Dawley CD rats (n = 5) was 490 mg/kg (confidence interval [CI] 420 to 570 mg/kg) and 560 mg/kg (500 to 630 mg/kg).[70]

The original safety assessment reported that the oral LD_{50} of 40% w/v cetrimonium chloride was 1000 mg/kg for rats.[1]

The LD_{50} for cetrimonium chloride ranged from ~1550 to 2970 mg/kg for rats in unpublished studies of acute oral toxicity that were reported by the European Union's Committee on Consumer Products (SCCP).[63] The LD_{50} for steartrimonium

chloride was ~700 mg/kg. A mixture of cetrimonium and steartrimonium chloride had an LD_{50} of >2000 mg/kg in rats.

A material safety data sheet (MSDS) reported the LD_{50} for behentrimonium chloride for rats to be >4 g/kg. No further information was provided.[13]

The combined LD_{50} for tallowtrimonium chloride for Sprague-Dawley CFY rats was 1260 (CI 1061-1496) mg/kg, 1289 (CI 1145-1444) mg/kg for males, and between 1000 and 2000 mg/kg for females.[70] In Swiss-Webster mice, the oral LD_{50} of cetrimonium chloride was between 400 and 600 mg/kg.[70] The oral LD_{50} of steartrimonium chloride was 633 mg/kg for male mice and 536 mg/kg for female mice.

Alkanol trimonium ingredients. The oral LD_{50} was reported to be between 3150 and ≥ 5000 mg/kg for choline chloride in rats.[20] Clinical signs after ingestion were restlessness, increased respiration, hypoactivity, convulsions, ruffled coat, staggered gait, and dyspnea. There was some diarrhea. At necropsy, 3 of 10 rats had inflamed lungs.

The oral LD_{50} of choline chloride in mice was reported to be in the range of 3900 and 6000 mg/kg.[20]

The oral LD_{50} of choline chloride was 340 mg/kg for ICR mice and >400 mg/kg for Sprague-Dawley rats.[75]

The oral LD_{50} of choline HCl in Swiss CD-1 holoxenic mice was 3900 mg/kg.[76] All animals showed salivation, lacrimation, respiratory depression, and convulsions prior to death. The maximum cholinergic effects were observed within the first hour, and all the animals died during the first 24 hours of observation. The LD_0 was 2000 mg/kg.

Polymeric trimonium ingredients. The oral LD_{50} of polyquaternium-28 for rats was reported to be >5 g/kg.[18]

Polyquaternium-47 (<30% aqueous; 5000 mg/kg) orally administered to Wistar rats caused no mortality, clinical signs, or body weight changes.[77]

The oral LD_{50} of polyquaternium-10 was >16 g/kg for rats.[7]

Acute Dermal Toxicity

Straight and branched chain alkyl trimonium ingredients. In an acute dermal test of cetrimonium chloride (100%; 4.3 mL/kg) using New Zealand White rabbits (n = 6; 3/sex), 50% of the rabbits died at the only dose level administered.[70] The test substance was applied under occlusion to intact or abraded skin for 24 hours then washed off. All rabbits exhibited normal behavior until day 3 when the rabbits became lethargic, had depressed reflexes, and were cold to the touch. They defecated little or none and had clear fluid coming from their noses and mouths. There was reddening of the nictitating membranes and eyelids. There was substantial weight loss. Skin irritation was noted after 24 hours of exposure, including slight to severe erythema, moderate or severe edema, and whitening of the skin. On day 3, there was moderate or severe atonia and moderate or marked coriaceous skin from day 2. Fissuring was observed in 3 rabbits and desquamation in 1. Necropsy revealed brown, liquid fecal matter; lungs adhered to the chest wall and filled with white granular pockets; enlarged gall bladder; and brownish or clear fluid around the nose and mouth. No visible lesions were observed in the rabbits that survived the 14-day observation period.

Tallowtrimonium chloride (100%; 4.0 mL/kg) applied to the intact and abraded skin of New Zealand White rabbits (3/sex) for 24 hours resulted in 100% mortality between days 3 and 8 of observation.[70] Clinical signs included drooping head and ears, increased respiration rate, increased heart rate, ataxia, depression, excessive salivation, reduced motor reflexes, reduced or lack of feed consumption and defecation. Moderate to severe skin irritation was observed. There was dilation of dermal blood vessels, gastrointestinal tract, and the brain surface. There was enlarged renal blood vessels and posterior vena cava. The pituitary was dark red to purple.

Three of 6 New Zealand White rabbits (3/sex) died during the 24-hour dermal administration of tallowtrimonium chloride (4.7 mg/kg).[70] Two more died during the 14-day observation period. There were no signs of systemic toxicity during exposure. After exposure, the rabbits had depressed reflexes; cold, drooping ears, and intermittent tremors. The 1 surviving rabbit began eating, defecating, and exhibiting normal behavior after 5 days. Slight to moderate erythema, edema, and atonia were observed at the removal of occlusion until death or end of observation period. Necropsy revealed gas-filled, distended large intestines, a thin stomach wall, and red lungs that were adhered to the chest wall.

Polymeric trimonium ingredients. Polyquaternium-10 (4.0 g/kg) was not toxic to rabbits (n = 5).[7] The dermal LD_{50} of a formulation containing polyquaternium-10 (0.5%) was greater than 2 g/kg on the clipped and abraded skin of rabbits.

Acute Intravenous Toxicity

Stroight and branched chain alkyl trimonium ingredients. Intravenous (through tail vein) LD_{50} values for female SPF-bred NMRI mice and female Sprague-Dawley rats for various alkyltrimethylammonium bromides (C10, 12, 14, 16, and 20) ranged from 2.8 to 20 mg/kg for mice and 5.5 to 44 mg/kg in rats.[78] The LD_{50} values increased proportionally with the length of the alkyl group. Most animals that died did so within 1 minute from apparent respiratory failure. The rest died within 20 minutes and had tail necrosis.

Alkanol trimonium ingredients. No data were available on alkanol trimonium ingredients themselves. However, data were found for choline HCl. The LD_{50} of choline HCl in Swiss CD-1 holoxenic mice was 53 mg/kg iv [76] All animals showed salivation, lacrimation, respiratory depression, and convulsions prior to death. The maximum cholinergic effects were observed within the first hour, and all the animals died during the first 24 hours of observation. The LD_0 was 21.5 mg/kg iv

Acute Intraperitoneal Toxicity

Alkanol trimonium ingredients. The ip LD_{50} of choline chloride (50% powder containing 29% colloidal silicic acid and 21% water) was reported to be 225 mg/kg in male and female mice (calculated for pure choline chloride).[64] Mice died within 2 minutes at 1600 mg/kg, at 1 hour at 640 and 800 mg/kg, and at 1 day at 500 mg/kg. Clinical signs were abdominal position, increased respiration rate, convulsions, dyspnea, exophthalmus, and cyanosis immediately after administration. Occasional adhesions in the liver were observed at necropsy.

Male albino rats (n = 14) injected with choline at 45 mg/kg had 29% mortality; guinea pigs (n = 45) had 20% mortality.[79] When injected with 60 mg/kg, rats (n = 20) had 60% mortality and the guinea pigs (n = 39) had 74% mortality. Animals that lived longer than 30 minutes survived in most cases.

Acute Inhalation Toxicity

Polymeric trimonium ingredients. Rats (n = 6) exposed to polyquaternium-10 dust (50 g on a tray stirred up by an intermittent fan) was not toxic over 8 hours.[7]

Short-Term Oral Toxicity

Straight and branched chain alkyl trimonium ingredients. Sprague-Dawley CD rats (n = 10/sex plus 5/sex in high dose group for recovery study) were administered cetrimonium chloride (24% to 26%; 0, 30, 100, and 300 mg/kg in distilled water) by gavage 5 days/week for a total of 23 or 24 applications.[63] The recovery group was allowed to recover for 27 days. There were no effects on survival, feed consumption, or body weight. The males in the high-dose group had increased water consumption compared to controls. No treatment effects were observed through ophthalmological and hematological examination. Clinical chemistry parameters were unaffected by treatment except a small increase (but within the range of historical controls) in serum alanine aminotransferase (ALT) in both sexes in the high-dose group.

There was a slight increase in absolute and relative adrenal weights and a decrease in absolute and relative spleen weights in males. Necropsy revealed a thickening of the forestomach mucosa, associated with edema and sporadic ulceration in males and females in the high-dose group. Inflammatory edema in the forestomach mucosa, sporadic ulceration, and acanthosis up to papillomatous hyperplasia were observed in both sexes in the high-dose group at microscopic examination. No histopathological or microscopic alterations were observed in the mid- and low-dose groups. All treatment-related effects were reversed following the recovery period. The authors concluded that the oral no observed adverse effect level (NOAEL) was 100 mg/kg.

Alkanol trimonium ingredients. Choline (200 mg/kg) was orally administered to Balb/c mice of both sexes (n = 10) daily for 28 days.[47] Saline served as the control. No effects to body weights, organ weights, hematological parameters, splenic cell counts, pathology of the organs, and clinical biochemistry were reported.

Wistar rats were administered L-carnitine and DL-carnitine (1.2 mmol/kg/d) in drinking water for 7 days.[80] Neither enantiomerically pure nor racemic carnitine had an effect on glycemia, food ingestion, or water consumption. Both groups had increased free carnitine in the blood.

Short-Term Dermal Toxicity

Straight and branched chain alkyl trimonium ingredients. A 28-day dermal toxicity test using rabbits of 0.5% cetrimonium chloride dose group showed mild, transient dermal irritation.[81]

Cetrimonium chloride (54.5% in aqueous isopropanol) was dermally administered (0 and 0.5%; 0 and 10 mg/kg) to the clipped skin of New Zealand White rabbits (n = 10; 5/sex) for 5 days/week for 4 weeks.[63] Skin was abraded with a clipper head prior to each application. Treated skin was cleaned with water after 6.5 to 7 hours. Two control rabbits died during the study. There were no treatment-related effects to body weight, hematology, organ weight, gross necropsy findings, or histopathology except that treated areas of the skin had mild to marked acanthosis with active mitosis, hyperkeratosis, and necrosis of the epidermis and hair follicles, with some encrustation and exudates. Slight to moderate erythema was observed in all treated rabbits from days 4 to 8; it disappeared in 4 rabbits by day 17. Very slight to slight edema was observed from days 6 to 12 in 4 rabbits; it subsided by day 17. There was intermittent slight edema during week 4 in 2 rabbits and 1 rabbit developed edema on day 20. There was no desquamation or coriaceousness observed. Three rabbits had slight atonia up to week 4. Slight skin fissuring was observed in most of the treated rabbits that typically disappeared by the end of the study. The SCCP concluded that the skin changes were due to local irritation and not evidence of systemic toxicity. The dermal no observed effects level (NOEL) was 10 mg/kg/d.

Polymeric trimonium ingredients. A 21-day dermal toxicity test of a conditioner containing polyquaternium-10 (1%) demonstrated no toxicity to rabbits (n = 10).[7]

Short-Term Intraparenteneal Toxicity

Alkanol trimonium ingredients. Choline (200 mg/kg) was administered ip to Balb/c mice of both sexes (n = 10) every other day for 28 days.[47] Saline served as the control. No effects on body weights, organ weights, hematological parameters, splenic cell counts, pathology of the organs, and clinical biochemistry, except for increased creatinine levels, were reported.

Male guinea pigs (n = 10) were administered choline (50 mg/kg/d) ip for 5 days/week for 8 weeks (40 doses).[82] The controls (n = 5) were untreated. They were then killed and necropsied. The treated guinea pigs developed lung lesions consisting of peripheral nodules of small cells, neoplastic

bronchiolar epithelium, carcinomatous lesions, and changes in the pleural surface.

Short-Term Inhalation Toxicity

Alkanol trimonium ingredients. Choline (200 mg/kg) was administered by inhalation (described as nasally; no further information was provided) under light anesthesia to Balb/c mice of both sexes (n = 10) every other day for 28 days.[47] Saline served as the control. No effects on body weights, organ weights, hematological parameters, splenic cell counts, pathology of the organs, and clinical biochemistry were observed.

Subchronic Intraperitoneal Toxicity

Alkanol trimonium ingredients. Male albino rats (n = 25) were administered choline chloride (0, 45, 148.5, 225 mg/kg; 0, 0.1, 0.33 or 0.5 × LD_{50} of 450 mg/kg) ip for up to 8 months.[83] The rats were killed at 1, 3, or 8 months and necropsied. After the injections, the rats were initially excited and active and then became dull and sluggish. Weight gains were similar between groups except for the mid dose at 3 months, which was greater. There was a dose-dependent decrease in relative lung weight at 1 month and an increase in the high-dose group at 3 months. There was a decrease in relative weight of the liver and thymus at 1 month, which, in the thymus, continued to 8 months. In the high-dose group, relative weights of the peripheral lymph nodes were increased. There was a dose-dependent decrease in thymocytes at 8 months. Peripheral lymph nodes had increased cell counts in all doses at 1 month and decreased until 8 months. Regional lymph nodes had reduced cell counts at 8 months in the mid- and high-dose groups. At 3 months in the mid-dose group, cuboidal bronchiolar epithelium, collections of lymphoid cells around blood vessels and bronchiols were observed. The high-dose group had collections of plasma cells and lymphocytes around bronchiovascular structures.

Chronic Oral Toxicity

Straight and branched chain alkyl trimonium ingredients. In the original safety assessment, rats administered cetrimonium bromide at 10, 20, and 45 mg/kg/d in their drinking water for 1 year exhibited decreased body weight gain in the high-dose group.[1]

Alkanol trimonium ingredients. Fischer 344 rats (n not provided) were administered choline chloride (500 mg/kg/d) in feed for 72 weeks followed by 30 weeks of observation.[84] Necropsy was performed at 103 weeks. Survival rates, body weights, and relative liver weights were not affected by treatment. The NOAEL was ≥500 mg/kg/d.

Male CD1 mice were fed choline-rich (1.6%; n = 6), choline-deficient (0%; n = 7), or normal choline (0.36%; n = 9) diets for 20 to 24 months.[85] There were no differences in body weight throughout the experiment. There were no differences in mortalities, and these were similar to historical survival for this strain of mice. There were no differences in dendritic spine densities.

CD-1 mice were orally administered choline (0 [n = 23] or 1.6% [n = 17]) in feed for 20 to 24 months.[86] The mice were killed and the brains examined. There were no differences in body weights over the course of the experiment. There were no differences in the concentrations of choline in the striatum, hippocampus, or the cortex between groups.

Baboons (n = 24; *Papio hamadryas*) were orally administered normal or increased levels of choline (100 or 500 mg/1000 calories) in their feed for 3 to 4 years.[87] Weights were steady or slightly increased throughout the study. Serum transaminases and glutamate dehydrogenase activity were increased in the high-dose group. Bilirubin was increased, albumin was decreased, and total protein was similar in the high-dose group.

Chronic Intraperitoneal Toxicity

Alkanol trimonium ingredients. Male albino rats (n = 10) were administered choline chloride (25 mg in distilled water) ip for 5 d/week.[88] Two of the rats were then killed and the lungs necropsied at 90, 180, and 330 days. Controls (n = 5) were handled differently and administered saline intratracheally. Three rats died in the treatment group, none in the control group. At necropsy, the lungs were light pink in color at 90 and 180 days. At 330 days, small white patches appeared on the lobes and the cut surface also revealed clear white mass. At 90 days, sections of lung showed cuboidal type of bronchiolar epithelium along with prominent musculature of the bronchioles and blood vessels. Heavy collections of lymphoid cells around bronchioles were observed together with dilation of lymphatic vessels. A collection of macrophages with pigments as inclusions were observed at the pleural surfaces. Few giant cells were observed in the parenchyrna along with adenomatoid changes. At 180 days, the lungs had hyperreactive ciliated bronchiolar epithelium and mucus adhering on the top of the epithelial lining. The muscles around bronchioles and blood vessels were hypertrophied and found in patches. Heavy collections of plasma cells and lymphocytes were around bronchioles and larger blood vessels. The alveolar macrophages were very prominent and laden with yellowish black pigment. Sporadically, adenomatoid changes of bronchiolar epithelium were found, which were more prominent at 330 days. Examination of the musculature of the bronchioles revealed prominent eosinophilic characteristics and lumen filled with cell debris with slight thickening of pleura.

Ocular Irritation

In the original safety assessment, cetrimonium chloride was classified as a severe ocular irritant as demonstrated by in vitro and in vitro studies.[1] In the original safety assessment, steartrimonium chloride was found to be severely irritating to the eyes of rabbits and guinea pigs.[1]

In in vivo tests, cetrimonium bromide (25%) and cetrimonium chloride (10%) were severe ocular irritants that caused

irreversible damage in rabbits and rats (Table 9). Steartrimonium chloride was rated an irritant in several studies. In in vitro tests, laurtrimonium bromide, cetrimonium chloride, steartrimonium chloride, and behentrimonium chloride were rated mild to severe ocular irritants. Choline chloride was not an irritant. Polyquaternium-28 was rated a minimal irritant. Choline chloride, carnitine, and carnitine HCl may be ocular irritants as demonstrated by in vitro tests. Polyquaternium-33 was reported to be an ocular irritant and polyquaternium-47 was a slight ocular irritant. Polyquaternium-10 was not an ocular irritant to rabbits up to 5% and produced trace irritation in one eye at 10%.

Dermal Irritation

Straight and branched chain alkyl trimonium ingredients—in vitro. A dermal irritation prediction test of cetrimonium bromide using 3T6 mouse fibroblast cells and NCTC 2544 human keratinocyte cells in neutral red uptake (NRU) assay and 3-(4,5-dimethylthiazol-2-Yl)-2,5-diphenyltetrazolium bromide (MTT) assays was conducted.[89] Cetrimonium bromide had IC_{50}s of 165.66 ± 19.75 and 102.6 ± 3.96 μg/mL for the 3T6 fibroblasts and 203.23 ± 16.23 and 117.87 ± 13.70 μg/mL for the NCTC 2544 keratinocytes, respectively. The authors concluded that cetrimonium bromide was more irritating than synthetic lysine-derived anionic surfactants.

The relative irritancy potential of cetrimonium bromide (100%) was 518 by the cell suspension agar diffusion test. The relative irritation potential of sodium lauryl sulfate (> 99%) was 995.[90] In the original safety assessment, cetrimonium chloride (100%) was classified as a skin irritant in an in vitro study using rat skin.[1] Behentrimonium chloride mixed with isopropanol (concentration not provided) was reported in an MSDS to be nonirritating in vitro.[12]

Straight and branched chain alkyl trimonium ingredients—in vivo. Cetrimonium chloride (as Genamin CTAC; 29%; 0.5 mL) was tested on New Zealand albino rabbits (n = 3) under occlusion for 4 hours.[63] There were no mortalities or systemic effects observed. There were slight erythema and edema at 30 minutes. Grades 2 to 3 erythema and 1 to 2 edema were observed up to 72 hours. Dry and brownish patch skin was observed at 48 and 72 hours and at 7 days. There was hardened skin at 7 days, ablation of large scales at 7 and 14 days, and shiny skin at 14 days. Grade 2 erythema was observed at 7 and 14 days. Skin reactions had resolved at day 21. The authors concluded that the test substance was irritating to skin at 29%.

Cetrimonium chloride (as Quartanim 60W25; 25%; 0.5 mL) was tested on New Zealand albino rabbits (n = 3; male).[63] The test substance was placed on shaved skin under semiocclusive dressing for 4 hours. There were no mortalities or systemic effects observed. Grades 2 and 3 erythema were observed up to 14 days. Grades 1 and 2 edema was observed between 60 minutes and 7 days. At day 14, no edema was observed in 2 rabbits and grade 2 edema was observed in the third rabbit. Skin dryness was observed from 24 hours to 14 days. The authors concluded that cetrimonium chloride was irritating to skin at 25%.

In the original safety assessment, steartrimonium chloride (0.75%) was positive in an in vivo skin irritation study using guinea pigs (n = 20).[1]

A single application of steartrimonium chloride (as Genamin STAC; 79.2%; 0.5 g) was tested on New Zealand albino rabbits for 3 minutes (n = 3) and 1 hour (n = 1).[63] The test substance was applied to shaved skin under semiocclusion. There were no mortalities or systemic effects observed. The rabbit exposed for 1 hour had grade 2 erythema up to day 22 and grade 1 edema up to 7 days. At day 22, pink new skin and a scar were noted. No erythema or edema was observed for the rabbits exposed for 3 minutes. The authors concluded that steartrimonium chloride at 79.2% was irritating at 1 hour of exposure but not at 3 minutes.

Steartrimonium chloride and cetrimonium chloride (as Quartamin 86W; 80:20; 28%; 2% and 20%; 0.5 mL) was applied to the shaved skin New Zealand albino rabbits (n = 3) under a semiocclusive patch for 4 hours.[63] There were no mortalities or observed clinical effects. The high-dose group had grade 2 erythema up to 72 hours and followed by crust formation at day 7. Grade 1 edema was observed up to 72 hours. The low dose had grade 1 erythema at 1, 24, and 48 hours in 1 rabbit but no edema was observed. The authors concluded that this mixture of steartrimonium chloride and cetrimonium chloride was irritating to the skin at 20% (4.08% active ingredients) and nonirritating at 2% (0.408% active ingredients).

Behentrimonium chloride (5% in a solution with 0.5% methylcellulose in purified water; 0.5 mL) was applied on the shaved skin of New Zealand albino rabbits (n = 3) under semiocclusion for 3 minutes.[63] There were no mortalities or systemic effects observed. There were no erythema or edema up to 72 hours. The authors concluded that a 5% solution of the test substance was nonirritating after 3 minutes of exposure.

Behentrimonium chloride/cetearyl alcohol mixture (proportion not provided) was reported to be nonirritating to rabbits at 25%.[13] No other information was provided.

Behentrimonium chloride (7.7%-8.3% in 5% methylcellulose in purified water; 0.5 mL) was placed on the shaved skin of New Zealand albino rabbits (n = 3) using a gauze pad for 3 minutes.[91] There were no clinical signs over 5 days. At 1 hour, erythema was observed on all treated rabbits, which resolved by 72 hours. This experiment was repeated.[91] No erythema or edema was observed at any time period up to 72 hours.

Alkanol trimonium ingredients. According to an MSDS, choline chloride may cause irritation with redness and pain.[15] According to an MSDS, carnitine and carnitine HCl may cause skin irritation.[17]

Polymeric trimonium ingredients. According to an MSDS, polyquaternium-33 may be a dermal irritant.[92] Polyquaternium-47 (<30% in aqueous solution; 0.5 mL) was applied to 2 separate patch sites of New Zealand White rabbits (n = 6) for 24 hours.[77] There was no edema during the 48-hour observation period. One rabbit had moderate erythema 30 to 60 minutes after patch removal. All treated rabbits had slight erythema under at least one patch during observation. After

Table 9. In Vitro and In Vivo Ocular Irritation Tests.

Species/Test (n)	Test Substance	Results	Reference
In vitro			
Bovine corneal opacity and permeability (BCOP) assay	Cetrimonium chloride (10% in minimal essential medium)	Mean score of 66.4 over 12 laboratories (0-25, mild irritant; 25.1-55, moderate irritant; ≥ 55.1, severe irritant)	112
Hemoglobin denaturation assay	Cetrimonium bromide (2%; 0.1 mL)	Moderate irritant	113
BCOP	Cetrimonium bromide (10%)	Extremely irritating	19
Silicon microphysiometer test	Cetrimonium bromide and cetrimonium chloride	Maximum average score (MAS) of 44.00 and 25.50, respectively (0 = nonirritant; 110 = severe irritant)	114
Chorioallantoic membrane vascular assay (CAMVA) and BCOP	Hair-conditioning product containing cetrimonium methosulfate (0.09%)	CMVA: Not predicted to be irritating to eye BCOP: Mild ocular irritant	115
Silicon microphysiometer test	Myrtrimonium bromide	MAS=42.70	114
Neutral red uptake (NRU) assay using rabbit epithelial cells (SIRC cell line)	Steartrimonium chloride	IC_{50} = 1.93 ± 0.534, 2.22 ± 2.22, 1.74 ± 0.933, and 1.96 ± 0.552 µg/mL using culture medium, phosphate-buffered saline (PBS), suspension in culture medium, and all 3 combined as solvents, respectively.	116
Crystal violet staining (CVS) assay	Steartrimonium chloride	IC_{50} = 1.27 ± 0.283, 1.17, 2.11 ± 1.12, 1.58 ± 0.752 µg/mL using culture medium, PBS, suspension in culture medium, and all 3 combined as solvents, respectively.	116
Irritation assay using Chinese hamster lung (CHL) cells	Steartrimonium chloride (10%)	MAS = 91.3 with a 24-hour average score of 56.3.	117,118
CHL-CVS test	Steartrimonium chloride (10%)	EC_{50} = 4.20 ± 1.40 µg/mL	117,118
SIRC-CVS assay using SIRC neutral red uptake assay (multilaboratory study)	Steartrimonium chloride	EC_{50} = 1.74 to 2.11 µg/mL	116
EYETEX system (multilaboratory study)	Steartrimonium chloride	Moderate ocular irritant	119
Hen's egg test Chorioallantoic membrane (HET-CAM) assay and chorio-allantoic membrane-trypan blue staining (CAM-TB) assay	Steartrimonium chloride (1%, 10%, and 100%)	MAS = 91.3; 24-hour average score 56.3	120
CAMVA & BCOP	Hair-conditioning product containing steartrimonium chloride (0.75%)	CMVA: Not predicted to be irritating to eye BCOP: Mild ocular irritant	121
CAMVA & BCOP	Hair-conditioning product containing steartrimonium chloride (0.75%)	CMVA: Not predicted to be irritating to eye BCOP: Mild ocular irritant	121
CAMVA & BCOP	Hair-conditioning product containing behentrimonium chloride (5.0%)	CMVA: Not predicted to be irritating to eye BCOP: Mild ocular irritant	122
CAMVA & BCOP	Hair-conditioning product containing behentrimonium chloride (5.0%)	CMVA: Not predicted to be irritating to eye BCOP: Mild ocular irritant	122
BCOP assay	Choline chloride (5 mg/mL; 0.5%; pH 5.3)	Not considered an irritant.	34
In vivo			
Bovine cornea	Laurtrimonium bromide (2×10^{-3} to 10^{-2} mol/L)	Dose-related development of opacity starting at 10^{-3} mol/L.	123
Draize test	Cetrimonium bromide (10%)	Rated as extremely irritating	19
Draize test (multi-laboratory study)	Cetrimonium bromide	HC_{50} = 10.1 ± 12.8 µg/mL	124
Female New Zealand albino rabbits (n = 12).	Cetrimonium chloride (50% active ingredient; 10 µL)	Macroscopic examination and in vivo confocal microscopy revealed increased damage scores compared to controls ($p < .05$). Histological examination revealed almost complete denudation of the corneal epithelium. Keratocyte injury was detected deep within the stroma, extending to the corneal endothelium in some eyes. There was marked death of keratocytes.	125

(continued)

Table 9. (continued)

Species/Test (n)	Test Substance	Results	Reference
Draize eye test (n = 3)	Cetrimonium chloride (10%)	MAS = 69.0. Classified the ocular irritation potential of cetrimonium chloride as severe.	19
Rabbits (n = 3)	Cetrimonium chloride (10%)	MAS score of 69.0. Day 1 49.7. Damage was irreversible.	112
Rabbits (n = 3)	Genamin CTAC (cetrimonium chloride 28%-30%; 0.1 mL)	Swelling of the conjunctivae at 24 hours. At 48 hours, ½ to ¾ corneal surface was affected. Opacity, 1.9; iritis, 1.0; conjunctival redness, 2.3 and conjunctival chemosis, 3.7. Irreversible ocular damage, corneal opacity, and cunjuctival irritation through day 21.	91
New Zealand albino rabbits (n = 3)	Quartamin 60W25 (cetrimonium chloride 24%-26%; 0.1 mL)	Conjuctival irritations as grad 2-3 redness and grad 3-4 swelling up to day 21. Irreversible ocular damage.	91
Rabbit	Cetrimonium chloride 8% of 25% active and 60% of 30% active in an aqueous shampoo matrix	Reversibly irritation at 2.0%	91
Draize test (n not provided)	Steartrimonium chloride	MAS = 91.3 with a 24-hour average score of 56.3	114
New Zealand albino rabbits (n = 3)	Steartrimonium chloride (as Quartamin 86 W; unknown percentage of steartrimonium chloride; 0.1 mL)	No corneal opacity or irititis was observed up to 72 hours. Redness was observed at 1 to 48 hours. All ocular reactions were resolved at 14 days.	91
New Zealand albino rabbits (n = 3; sex not provided)	Steartrimonium chloride and cetrimonium chloride (as Quartamin 60 W; 28% steartrimonium chloride: cetrimonium chloride, 80:20; 2% in distilled water; 0.1 mL)	No corneal opacity or irititis was observed. Conjunctival irritation was observed up to day 7. Swelling was observed up to 48 hours and continued in 2 rabbits until 72 hours. All reactions had resolved by day 14. The authors concluded that the mixture at 0.56% produced transient conjunctival irritation.	102
Male New Zealand albino rabbits (n = 3)	Behentrimonium chloride (10% in 0.5% methylcellulose in purified water; 0.1 mL). Rinsed after 30 seconds.	Grade 2 corneal opacity observed in 1 rabbit at 24 and 48 hours and grade 1 on day 22. 1 rabbit had grade 1 irititis from 1 hours to day 7. Grade 1-3 swelling between 1 and 72 hours; persisted until days 5, 9, and 22. Conclusion: behentrimonium chloride (10%) caused irreversible ocular damage.	63
Male New Zealand albino rabbits (n = 3)	Behentrimonium chloride (80%) in 0.5% methylcellulose in purified water (0.1 mL). Final concentration 6.25%. Rinsed after 30 seconds.	Corneal opacity at 24 hours. Irititis at 24 h, 1 rabbit until 72 hours. Conjuctival irritation (redness) from 1 to 72 hours; subsided after 7, 7, and 15 days. Swelling between 1 and 72 hours; subsided after 5, 6, and 18 days. Conclusion: behentrimonium chloride (6.25%) caused conjunctival irritation.	63
Male New Zealand albino rabbits (n = 3)	Behentrimonium chloride (3%) in 0.5% methylcellulose in purified water (0.1 mL). Rinsed after 30 seconds.	No corneal opacity. Irititis observed at 24 h in all rabbits and 1 at 72 hours. conjunctival irritation (redness) at 1 hours (3/3), 24 hours (2/3), and 48 hours (1/3). Conclusion: behentrimonium chloride (3%) caused transient conjunctival irritation.	63

(continued)

Table 9. (continued)

Species/Test (n)	Test Substance	Results	Reference
A male and a female rabbit	Choline chloride (70% aqueous)	Treated eyes were reddening and tearing after 10 minutes. Slight reddening persisted for 3 hours. No eye irritation or effects to the cornea were observed after day 1 and up to 8 days.	20
New Zealand White rabbits (n = 6)	Polyquaternium-28 (20%, 0.1 mL)	Average Draize scores were 13.3, 4.0, and 2.0 at 1 hour and 1 and 2 days; rinsing the eyes had little effect. Was rated as minimally irritating to the rabbit eye.	18
New Zealand White rabbits (n = 3)	Polyquaternium-47 (<30% in aqueous solution)	Conjuctival irritation was observed at 1 hour. All rabbits had moderate chemosis and 1 had moderate discharge. Initially, all the rabbits had conjuctival redness. At 24 hours, 1 rabbit still had conjuctival redness. No signs of corneal or iris irritation were observed. One animal had diarrhea on days 1 and 2 and alopecia on the face on day 3. Authors concluded that polyquaternium-47 was a slight ocular irritant.	77

24 hours, 4 rabbits had slight erythema. One rabbit had mucoid diarrhea during most of the observation time. All responses were resolved at 72 hours. The authors concluded that Polyquaterium-47 at <30% was a slight dermal irritant.

Polyquaternium-10 (4 g/kg) caused slight erythema to rabbits.[7] In another experiment, there was no irritation at 2% and there was moderate erythema at 5.0% and 10%. A shampoo containing polyquatermiun-10 (0.5%) was a severe skin irritant. A conditioner containing polyquaterium-10 (1%) was not a primary dermal irritant to rabbits on intact and abraded skin.

Dermal Sensitization

Straight and branched chain alkyl trimonium ingredients. A guinea pig maximization test was performed on cetrimonium chloride (as Quartamin 60W25; 25% cetrimonium chloride) using Dunkin-Hartley albino guinea pigs (n = 10/sex; control group n = 5/sex).[63] Induction on day 0 was by intradermal injections of 0.125% test substance in saline and/or Freud's complete adjuvant and on day 7 at 3% (0.75% active). Challenge was a topical application of the test substance at 0.5% (0.125% active ingredient; 0.5 mL) on day 21 and day 28. There were no deaths or clinical signs in the test group. At the first challenge, 3 males of the treatment group and 1 female in the control group had mild erythema. Three males and 1 female in the control group also had mild erythema on the vehicle-treated side. At 48 hours, no skin reactions were observed. Histopathology on all treated skin showed erythema. Reactivity to the vehicle was similar in both groups. SCCP concluded that the results were unclear.

A Buehler test on cetrimonium chloride (as Genamin CTAC; 30% cetrimonium chloride) using Pirbright white guinea pigs (n = 6) was conducted.[63] Dermal induction was at 4% (1.27% active) in distilled water; the challenge was at 1% (0.3% active ingredient). There was slight to well-defined erythema and very slight edema at the treatment sites during induction. There were no skin reactions in the treatment and controls groups at challenge. The authors concluded that the test substance was not a sensitizer under these conditions.

A guinea pig maximization test was performed using Dunkin Hartley albino guinea pigs (n = 10/sex) to test cetrimonium chloride (as Quartanim 60W25; 24%-26% cetrimonium chloride).[91] Induction was intradermal injections of Qartanim 60W25 (0.125%) and the challenge was topical administration of Quartamin 60W25 (0.5%) on 8 cm^2 under occlusion for 24 hours. There were no deaths, clinical signs, or changes in body weights. Skin reactions (erythema) were similar between the treatment and control groups.

A Buehler test was performed on steartrimonium chloride (as Genamin STAC; 79.8% steartrimonium chloride) using Pirbright white guinea pigs (n = 20; n = 10 in control group).[63] Induction was at 4% (3.192% active) in ethanol:water (80:20) under an occlusive patch on clipped skin for 6 hours. Induction was repeated on days 8 and 15. The challenge was on day 29 at 1% (0.798%) in isopropanol (instead of ethanol:water) on a naive site. The patch was left on for 6 hours. No clinical signs were observed. During induction, the treated group had slight, well-defined to severe erythema and very slight to well-defined edema at the treatment area. No skin reactions were observed after the challenge. The authors concluded that the test substance was not a sensitizer under these test conditions.

A guinea pig maximization test using Dunkin-Hartley guinea pigs (n = 10; n = 5 in control group) was performed on steartrimonium chloride: cetrimonium chloride (80:20; as

Quartamin 86W; 28% active [20.47%]) in distilled water or Freund complete adjuvant (FCA; 50%) and water.[63] Induction was 0.1% v/v (0.0204%) by intradermal injection then a topical patch at 5% (1.20%) on shaved skin for 48 hours. Challenge was at day 21 with a patch at 10% (2.4%) on the right shoulder and 5% (1.2%) on the left for 24 hours. There was well-defined or moderate to severe erythema at the induction sites in the test group at 24 and 48 hours. Slight erythema was noted at the induction sites of the control group at 24 hours and at 48 hours in 1 guinea pig. After topical induction, slight to well-defined erythema was noted at 24 hours in the test group; no reaction was noted in the control group. No skin reactions were observed at the challenge sites. The authors concluded that the test substance was not a sensitizer under these conditions.

Hartley guinea pigs (n = 10/sex in treated group; n = 5/sex in control group) were treated with a 10% solution of behentrimonium chloride (vehicle not stated) on a 8-cm^2 filter paper patch applied to clipped skin on the left flank for 6 hours, with occlusion on days 1, 8, and 15.[63] During the induction phase, the skin sites were examined for local effects 24 hours after each treatment. The challenge exposure consisted of a 0.5% solution of the test substance loaded onto a Finn chamber and applied to clipped skin on the right flank for 6 hours, with occlusion on day 29. A second challenge was performed on day 43 due to equivocal cutaneous reactions from the first challenge. One male animal from the treated group died on day 14, but this was considered by the investigators to be unrelated to treatment. A few treated animals (number not specified) showed slight to well-defined erythema during the induction phase. After the challenge, grade 1 erythema was observed in 3 of 19 animals at 24 hours and 5 of 19 animals at 48 hours. A grade 1 erythema was observed in 1 of 19 and a grade 2 erythema in 2 of 19 animals at 72 hours. No reactions were observed in control animals, after the challenge. The authors concluded that behentrimonium chloride was a sensitizer at 10%.

A Buehler test for sensitization was performed on behentrimonium chloride (as Genamin KDMP; 77% to 83% diluted to a 20% solution in ethanol:water [80:20]; 16%).[63] Female Pirbright white guinea pigs (n = 20, control n = 10) were treated with a 20% solution of the test substance on a 2 × 2 cm cellulose patch, applied to clipped skin on the left flank for 6 hours, with occlusion on days 1, 8, and 15. During the induction phase, the skin sites were examined for local effects 24 hours after each treatment. The challenge exposure consisted of a 0.8% solution of the test substance in isopropanol on a 2 × 2 cm occluded patch, applied to clipped skin on the right flank for 6 hours, on day 29. Skin reactions were scored 24 and 48 hours after patch removal. Animals were observed daily for signs of systemic toxicity and body weights were recorded on days 1 and 31. Treated animals showed slight to well-defined erythema and very slight edema at the site of treatment during the induction phase. No skin reactions were observed in the treated or control animals after challenge.

In a Buehler test using Dunkin Hartley guinea pigs (n = 10/sex), behentrimonium chloride (as Genamin KDMP; 77%-83%) was used at induction at 10% Genamin KDMP (7.7%-8.3%) and challenge at 0.5% (3.5%-4.25%) in Finn chambers.[91] No clinical signs were observed and weights were similar between groups. There was some well-defined erythema in some of the treatment groups. There were 3 reactions observed after challenge and no reactions at rechallenge.

Reproductive and Developmental Toxicity

Straight and Branched Chain Alkyl Trimonium Ingredients

In a range-finding study of laurtrimonium chloride (20, 50, 100, 200, 400 mg/kg/d), pregnant New Zealand White rabbits (n = 3) were orally administered the test substance from day 6 through 18 of pregnancy.[70] At 25 and 50 mg/kg, 1 in 3 rabbits died; at 100 mg/kg, 2 rabbits died; and at 400 mg/kg, all 3 rabbits died. There were embryonic effects (not defined) observed at 50 mg/kg.

In the main study, laurtrimonium chloride (2, 8, 24 mg/kg) was orally administered to pregnant New Zealand White rabbits (n = 13-14) from day 6 to 18 of pregnancy. The dams were killed on day 19 and necropsied. There were no adverse effects reported for the dams and no developmental or teratogenic effects observed.

The original safety assessment reported that cetrimonium bromide (25 mg/kg/d) administered on days 5 to 14 of gestation was not teratogenic in an oral study using rats.[1] Mild embryonic effects were observed at 50 mg/kg/d, but these were attributed to maternal toxicity rather than a teratogenic effect. There were no embryotoxic or teratogenic effects at lower doses. In an intraperitoneal study, cetrimonium bromide interfered with the embryonic development of mice at 10 mg/kg on day 8, 10, 12, or 14 of gestation 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 administered days 7 to 18 of gestation in a dermal study using rabbits. The only adverse effect observed was dermal irritation at the application sites. When tested in a dermal teratogenicity study, 2.5% steartrimonium chloride administered on days 6 to 15 of gestation was not maternally toxic, embryotoxic, or teratogenic.

Alkanol Trimonium Ingredients

Male rats were administered choline chloride (80 mg/kg/d) ip for 12 or 24 days.[93] Another group was administered choline chloride (10 to 12 mg/kg/d) in feed. The rats were necropsied at 2, 5, 8, and 12 days after the treatment period. There were no effects on body weight gain or weights of testes, epididymides, liver, kidney, and adrenals. After 12 days of treatment (one cycle of the seminiferous epithelium), epithelial vacuoles, spermatogonia with pyknotic nuclei and cellular debris were observed 2 days after the termination of treatment. Five days after termination of treatment, the seminiferous tubules were normal. After 24 days of treatment, a few tubules of stages I to

IV were observed on day 2 after treatment termination. Most spermatocytes were normal with some necrotic pachytene stages with an essential restoration to normal after 12 days.

Male rats (n = 25; strain not provided) were administered choline (25 mg/kg/d) ip for 12 or 24 days.[93] At 12 days, spermatogenesis was not changed. At 24 days, pachytene spermatocytes were decreased until day 5 posttreatment. Slight proliferation of spermatogonia was observed from day 5 posttreatment onward. By day 12 posttreatment, tubules showed almost normal cellular associations. The authors suggest that prolonged administration of excess choline may be toxic to male reproduction.

Pregnant mice (n = 7 – 16) were administered choline chloride (1250 to 20,000 mg/kg/d) in feed on gestations days 1 to 18.[20] There were no untreated controls. All groups except the lowest dose group had reduced maternal body weight gain. All fetuses were resorbed in the highest dose group but no resorptions were observed in the lowest dose group. At 4160 and 10 800 mg/kg/d, there was 35% and 69% embryonic/fetal lethality. Developmental toxicity was observed in all but the lowest dose group. There were no increases in malformations observed. An NOAEL was not determined for teratogenicity due to the lack of pups.

Genotoxicity

Straight and Branched Chain Alkyl Trimonium Ingredients

In a reverse mutation assay using *S typhimurium*, laurtrimonium chloride (0.004 to 0.4 µl/plate) was not cytotoxic or genotoxic, with or without metabolic activation.[70] In a forward mutation assay using L5178Y/TK+/− mouse lymphoma cells, laurtrimonium chloride (0.0038-0.050 µl/mL without metabolic activation and 0.012-0.16 µl/mL with metabolic activation) was not mutagenic. The results were negative in an unscheduled DNA synthesis assay of laurtrimonium chloride (0.004-0.1 µl/mL) using rat primary hepatocytes. In a bone marrow cytogentic assay, laurtrimonium chloride (16, 53.3, and 160 mg/kg) orally administered to male and female Sprague-Dawley rats for 5 days did not induce an increase in chromosomal aberrations.

In the original safety assessment, cetrimonium chloride was negative both with and without metabolic activation in an bacterial test up to 625 µg/plate, in forward-mutation and reverse-mutation tests at concentrations up to 50 µg/mL, and in a cell transformation assay up to 1.0 µg/mL.[1] Negative results were also obtained in a chromosome aberration test at concentrations up to 3.0 µg/mL, without metabolic activation and 10.0 µg/mL in tests with exogenous metabolic activation. Cetrimonium chloride (62.5 µg/plate) and cetrimonium bromide (no concentration provided) were both negative in bacterial tests. Cetrimonium chloride (1.0 µg/mL) was also negative in an in vitro cell transformation assay.

Cetrimonium chloride and steartrimonium chloride were not mutagenic in a reverse mutation assay using *S typhimurium* (TA98 and TA100).[70]

In an in vitro chromosome aberration test, V79 Chinese hamster cells were incubated with cetrimonium chloride (24%-26% cetrimonium chloride; 0.1-6.0 µg/mL without metabolic activation; 0.1-10.0 µg/mL with metabolic activation).[63] There were no increases in the number of cells with structural aberrations at any concentration with or without metabolic activation.

Salmonella typhimurium (TA98, TA100, TA1535, T1537) were incubated in steartrimonium chloride (as Genamin STAC; 79.8%; 4, 20, 100, 500, 2500, or 5000 µg/plate in ethanol) with and without metabolic activation.[63] This was repeated with lower concentrations of test material (0.8, 4, 20, 100, 500, and 2500 µg/plate). The test compound was toxic at 100 µg/plate, with and without metabolic activation. There was increase in the number of revertant colonies compared to controls with or without metabolic activation.

Salmonella typhimurium (TA98, TA100, TA1535, TA1537, TA1538) were incubated in steartrimonium chloride:cetrimonium chloride (80:20; as Quartamin 86 W; 28%; 0.15 to 50 µg/plate in acetone with metabolic activation; 1.5 to 500 µg/plate without metabolic activation).[63] The experiment was repeated with lower concentrations of test material (0.5 to 50 µg and 0.5 to 150 µg, respectively). There were incomplete bacterial lawns at 15 µg/plate, without activation, and 150 µg with activation. The test substance was toxic at 50 µg/plate, with and without activation. The test substance did not increase the number of revertant colonies compared to controls.

Behentrimonium chloride (as Genamin KDMP; 77% to 83%) was found not to be mutagenic, with or without metabolic activation, in a bacterial assay when tested at concentrations of 4 to 5000 µg active substance/plate in *S typhimurium* (TA98, TA100, TA1535, and TA1537).[63] Cytotoxicity occurred at 500 µg/plate without metabolic activation and at 2500 µg/plate with metabolic activation.

Tallowtrimonium chloride was not mutagenic in a reverse mutation assay using *S typhimurium* (TA98, TA100, TA1535, TA1537, and TA1538), with or without metabolic activation.[70] In another reverse mutation assay using *S typhimurium* (TA98, TA100, TA1535, TA1537, and TA1538), tallowtrimonium chloride was cytotoxic at 500 µg/plate and was genotoxic at 50 µg/plate with TA1538, with or without metabolic activation. The results were negative for the other strains. The study authors suggest that the positive result may be due to impurities.

Alkanol Trimonium Ingredients

Choline chloride did not produce any gene mutations, clastogenicity, or DNA damage when tested in vitro in studies reported by the United Nations Environment Programme (UNEP; Table 10).[20]

Polymeric Trimonium Ingredients

Polyquaternium-28 (20%) was negative in a reverse mutation assay using *S typhimurium* (TA1535, TA1538, TA98, and

Table 10. Genotoxicity Tests of Choline Chloride.[20]

Test		Concentration	Results
Ames test (2 separate tests)	*Salmonella typhimurium* TA98, TA100, TA1535, TA1537, TA1538 and *Escherichia coli* WP2uvrA	Up to 10,000 μg/plate and 5000 μg/plate with and without metabolic activation	No toxicity, no gene mutations
Chromosomal aberration test	Chinese hamster ovary cells	50 and 500 μg/mL with and without S9	Increase in simple aberrations in the absence of S9
Chromosomal aberration test (2 separate tests)	Chinese hamster ovary cells	Up to 5000 μg/mL.	No clastogenic effects; cytotoxicity began at 5000 μg/mL
Sister chromatid exchange	Chinese hamster ovary cells	500 μg/mL	No toxicity, no gene mutations
Sister chromatid exchange	Chinese hamster ovary cells	5000 μg/mL	Cytotoxicity began at 5000 μg/mL with S9, increased in SCEs with S9 was sporadic and not dose related
Sister chromatid exchange	Chinese hamster ovary cells	Up to 5000 μg/mL	No increase in SCEs
Gene conversion assay	*Saccharomyces cerevisiae* (D4)	12.5 – 50 mg/mL with and without metabolic activation	Negative

TA100), with or without metabolic activation.[18] In a micronucleus assay using the bone marrow cells of mice, polyquaternium-28 did not induce an increase in bone marrow polychromatic erythrocytes.

Polyquaternium-47 (< 30% in aqueous solution) was negative in a reverse mutation assay using *S typhimurium* (TA 1535, TA1537, TA100, TA98) and *E coli* (WP2 uvrA) with or without metabolic activation up to 5000 μg/plate.[77]

Polyquaternium-10 was not mutagentic in Ames test using *S typhimurium* (TA98, TA100, TA1535, Ta1537, TA1538) with or without metabolic activation.[7] It was also not mutagenic to Chinese hamster ovary cells up to 0.285% with or without metabolic activation. There was no increase in micronucleated polychromatophilic erythrocytes when Swiss mice were treated with polyquaternium-10 up to 0.4 g/kg.[7]

Carcinogenicity

Alkanol Trimonium Ingredients

Fischer 344 rats (n not provided) were administered choline chloride (500 mg/kg/d) in feed for 72 weeks followed by 30 weeks of observation.[84] Necropsy was performed at 103 weeks. There was no increase in the number of liver nodules, hepatocellular carcinomas, lung tumors, leukemia, or other tumors in the treated rats compared to controls.

Clinical Assessment of Safety

Absorption, Distribution, Metabolism, and Excretion

Alkanol Trimonium Ingredients. Patients with dyskinesia and cerebellar ataxia were orally treated with choline chloride (150 and 220 mg/kg/d [10 and 16 g/d, respectively] for 2-6 weeks).[94] The patients developed fishy body odor, vomiting, salivation, sweating, and gastrointestinal effects.[44,95]

Fasting levels of choline in the plasma were reported to be around 10 μmol/L, ranging from 9 to 20 μmol/L.[96] A fishy odor from ingested choline has been reported, possibly due to excessive amounts of trimethylamine, a metabolite produced by bacteriological action and formation of methylamines.[44,95]

Total body carnitine was reported to be mostly contained in skeletal muscle carnitine (~20 g of carnitine in a 70-kg man, of which more than 19 g was reported to be in skeletal muscle).[97] The bioavailability of orally administered carnitine was reported to be ~16% to 18% at doses of 1 to 2 g and may be even lower at higher doses.[98,99] Once synthesized or absorbed, carnitine was reported to be eliminated from the body only via the urine as carnitine or acylcamitines. Renal tubules contain a saturable carnitine transport system that conserves most filtered carnitine but leads to large carnitine losses if the plasma concentration exceeds 60 to 90 μmol/L. Carnitine was reported to be absorbed at ~25% when consumed orally.[100]

Suspected and known coronary artery disease patients had blood analyzed after an overnight fast.[101] The average free carnitine was 41.9 ± 8.9 μmol/L; short-chain acylcarnitine, 10.5 ± 5.3 μmol/L; total acid-soluble carnitine, 52.4 ± 9.4 μmol/L, long-chain acylcarnitine, 2.8 ± .7 μmol/L, and total carnitine, 55.2 ± 9.9 μmol/L.

Participants (n = 8) were orally administered acetyl-L-carnitine (500 mg) in pill form after ovemight fasting.[102] Blood samples were collected at 0.33, 0.67, 1, 1.5, 2, 3, 4, 6, 8, and 12 hours. The area under the curve $(AUC)_{0-8}$ was 9879 ± 3.757 mL/h, AUC_{0-12} was 6.611 ± 3.757 mL/h, maximum concentration was 1.188 ± 1.316 hours, mean residence time was 4.5 ± 0.3 hours, elimination half life was 4.2 ± 1.6 hours, and the maximum time to reach maximum concentration was 3.1 ± 0.2 hours.

Clinical Testing

Alkanol trimonium ingredients. Participants with hyperthyroidism-related symptoms (n = 5) were orally

Table 11. Human Dermal Irritation Studies of Cetrimonium Chloride.

Test (Concentration and/or Amount)	N	Results	Reference
Webril (25%; 0.2 mL)	87	Nonirritating	126
Hill Top (25%; 0.2 mL)	87	Nonirritating	126
Finn (25%; 0.4 mL)	87	Nonirritating	126
Van der Bend (25%; 0.03 mL)	87	Nonirritating	126
4-hour Patch test (3.5%, 7.5%, 8.8%)	Not provided	Moderate irritant	127
24-hour Patch test (3.25%)	Not provided	Slightly to moderately irritating	63
1-hour Patch test (2.5%)	Not provided	Minimal skin irritant	63
24-hour Patch test (cosmetic formulation 3.52%; 50% solution)	Not provided	Nonirritating	63
48-hour Patch test (cosmetic formulation 3.2%; 50% solution)	Not provided	Nonirritating	63
24-hour Patch test (1%)	Not provided	Nonirritating	63
24-hour Patch test (formulation 2.0; 50% solution)	Not provided	Nonirritating	63
24-hour Patch test (cosmetic formulation 3.01%; 25% aqueous solution)	Not provided	Minimally irritating	63
24-hour Patch test (cosmetic formulation 4%; 10% aqueous solution)	Not provided	Not irritating	63
24-hour Patch test (styling gel formulation 0.5%; 25%, 50%, 75%, 100% solution)	Not provided	Nonirritating	63

administered L-carnitine (2 or 4 mg/kg/d) for 90 days.[103] Mild nausea and gastralgia were reported by 2 participants in the first week. No other adverse effects were reported.

Participants (n = 7) with dementia of the Alzheimer type were orally administered acetyl-L-carnitine (1 g/d) for 24 weeks.[104] Nausea and vomiting were the only reported adverse effects.

Patients with Alzheimer (n = 7) were orally administered choline chloride (10 g/d, 7.5 g of choline).[105] The patients developed nausea, diarrhea, and slight hypotension.

Participants, with and without cirrhosis, have been orally administered choline (6 g/d; form not stated) for 4 weeks with no liver toxicity.[106] Oral ingestion of choline (20 g/d) for 3 to 4 weeks have been associated with depression.[107] Mild, transient signs of Parkinson disease have been observed in patients with tardive dyskinesia after oral administration of choline (12.7 g/d).[108] Patients with tardive dyskinesia and Huntington disease who were orally administered choline (20 g/d) for 4 weeks had no adverse effects.[107]

The critical adverse effect from high intake of choline was reported to be hypotension, with corroborative evidence on cholinergic side effects (eg, sweating and diarrhea) and fishy body odor. The Tolerable Upper Intake Level (UL) for adults was reported to be 3.5 g/d.

Dermal Irritation

Straight and branched chain alkyl trimonium ingredients. Laurtrimonium bromide (7.5%) was applied to the volar surface of the arms of 11 white, healthy participants for 20 minutes for 8 consecutive days (excluding weekends, days 5 and 6).[109] An untreated site and a site treated with water served as controls. The amount of irritation increased with time. The transepidermal water loss (TEWL) measurement did not decrease after the weekend break and did not return to baseline for 20 days after treatment ended. Erythema was still present at 23 days.

In the original safety assessment, slight dermal irritation was observed during the induction phases of multiple human repeated insult patch tests (HRIPT) experiments of cetrimonium chloride (0.25%).[1]

Several studies of dermal irritation of products containing cetrimonium chloride, behentrimonium chloride, and cocotrimonium methosulfate are reported in Table 11. Cetrimonium chloride was not an irritant up to 1% and mixed results were reported above 1%. Behentrimonium chloride was not an irritant at 5.0% and cocotrimonium methosulfate at 0.42%.

The irritancy potential of a formulation containing behentrimonium chloride (5.0%, vehicle not provided) was tested in participants (n = 51; 5 male and 46 female participants) using a Finn chamber applied to the participants' backs, with occlusion, for 24 hours.[63] Deionized water was used as the negative control substance. No difference in irritant reactions between treatment and control sites was reported.

Alkanol trimonium ingredients. Bar soap and liquid body soap, with and without choline chloride (0.5%), were used by people (n = 25) with self-perceived sensitivity to choline chloride (0.5%) for 21 days.[20] Compared to controls, there was no difference in cumulative irritancy.

Polymeric trimonium ingredients. Polyquaternium-10 at 5% in water was not a dermal irritant to participants for 21 days of application.[7]

Dermal Sensitization

Straight and branched chain alkyl trimonium ingredients. In the original safety assessment, cetrimonium chloride was negative in 4 human repeated insult patch tests (HRIPT; n = 101-5202) at concentrations up to 0.25% for 100% active solutions and up to 0.4% for 25% active solutions.[1] HRIPTs of cetrimonium chloride and behentrimonium chloride are presented in Table 12. Cetrimonium chloride at 0.25% and behentrimonium chloride at 2.4% were not sensitizing in humans.

Alkanol trimonium ingredients. An HRIPT of choline chloride showed that it was not sensitizing at 0.5% (Table 12).

Polymeric trimonium ingredients. HIRPTs of polyquaternium-28 and polyquaternium-47 are presented in Table 12.

Table 12. HRIPTs of Cetrimonium Chloride, Behentrimonium Chloride, Choline Chloride, and Polyquaternium-28.

Substance	N	Results	Reference
Straight and branched chain alkyl trimonium Ingredients			
Hair-conditioning product containing cetrimonium chloride (0.5%)	107	Slight irritation during induction; no reaction at challenge	128
Hair gel containing cetrimonium chloride (0.5%)	105	No irritation or sensitization	129
Hair gel containing cetrimonium chloride (0.625%)	213	Transient, barely perceptible to mild nonspecific irritant responses accompanied by mild edema and mild to moderate dryness were observed in 32 participants at induction. At challenge, 2 participants displayed moderate hyperpigmentation without erythema. The authors stated that these responses were not considered evidence of clinically meaningful irritation nor were they considered allergic reactions.	130
Rinse-off conditioner (diluted to 10%) containing cetrimonium chloride (0.75%)	108	Nonirritation and not sensitizing	131
Hair-conditioning product containing cetrimonium chloride (0.5%)	107	Slight irritation at induction and no signs of sensitization	132
Hair treatment containing cetrimonium chloride (0.375%)	108	No irritation and no signs of sensitization	133
Hair styling product containing cetrimonium chloride (0.75%)	219	No signs of irritation or sensitization observed	134
Hair treatment product (25% and 50%) containing cetrimonium chloride (0.8%)	107	There was irritation activity in 50% and 80% of the participants, respectively, at induction.[135] There were no signs of sensitization at challenge.	135
Hair styling spray containing cetrimonium chloride (0.45%)	212	No signs of irritation or sensitization	136
Hair-conditioning product containing centrimonium [sic] methosulfate (diluted to 10%)	118	No signs of irritation or sensitization	137
Hair treatment containing cetrimonium chloride (0.125%), behentrimonium chloride (0.5%), and behentrimonium methosulfate (0.125%)	212	One grade 1 response was observed on one participant. There were no responses observed at challenge.	138
Test substance (50% in water) containing cetrimonium chloride (0.625%) and behentrimonium chloride (0.48%)	105	Grade 1 signs of erythema on 12 patients during induction. There were no signs of sensitization at challenge.	139
Test substance (50% in water) containing cetrimonium chloride (0.648%) and behentrimonium chloride (0.48%)	102	Mild erythema in 12% of the patients by the end of induction. There was no evidence of sensitization at challenge.	140
Rinse-off formulation containing behentrimonium chloride (3.4%; 0.2 g)	104	One participant showed erythema and edema after the first induction patch. No evidence of a sensitization reaction was observed.	63
Hair-conditioning product containing behentrimonium chloride (diluted to 10%)	101	No signs of irritation or sensitization	141
Hair styling product containing cocotrimonium methosulfate	103	No signs of irritation or sensitization	142
Hair styling product containing cocotrimonium methosulfate	100	No signs of irritation or sensitization	143
Hair styling product containing cocotrimonium methosulfate	113	No signs of irritation or sensitization	144
Hair styling product containing cocotrimonium methosulfate	113	No signs of irritation or sensitization	145
Alkanol trimonium Ingredients			
choline chloride (0.5% w/v aqueous)	200	No dermal sensitization observed	20
Polymers			
Polyquaternium 28 (5%, 0.2 mL)	104	No signs of irritation or sensitization	18
Polyquaternium-47 (20% induction, 5% challenge, 0.2 mL)	116	During induction, 16 participants showed faint erythema, 1 participant showed moderate erythema, and 13 participants showed severe erythema including papules. At challenge, 9 participants showed slight erythema. It was concluded to not be sensitizing at 5%.	77

Polyquaternium-28 and polyquaternium-47 were not sensitizing at 5%. Polyquaternium-10 was not sensitizing at up to 2%.[7]

Phototoxicity

Polymeric trimonium ingredients. Polyquaternium-28 (2%; 0.2 mL; vehicle not provided) was placed on patches and applied to the volar surfaces of the arms of participants (n = 10).[18] After 24 hours, 1 arm was exposed to UV-A for 15 minutes. The unirradiated arm served as the control. There were no reactions observed at 24 and 48 hours.

Photoallergy

Polymeric trimonium ingredients. Polyquaternium-28 (5%; 0.2 mL; vehicle not provided) was placed on patches and

applied to the volar surfaces of the arms of participants (n = 28) 6 times over 3 weeks.[18] One arm was exposed to UV-A (3.33 j/cm^2) and UV-B (90-126 mj) irradiation for 75 to 105seconds (depending on skin type) 24 hours after each application of the test substance. The unirradiated patches and untreated areas served as the controls. Two weeks after the completion of induction phase, a single application of the test substance (2%, 0.2 mL) followed by irradiation was administered to a naive site.

Minimal erythema or erythema and/or slight edema were observed in 26 of the irradiated sites during induction which was similar to untreated irradiated sites in 6 participants. In the challenge phase, minimal erythema was observed in 4 participants on treated irradiated sites and in 1 participant on a treated, nonirradiated site. No reactions were observed on untreated irradiated sites. Overall, slight irritation was observed on treated skin exposed to UV-A and UV-B irradiation, but polyquaternium-28 did not induce contact photoallergy in humans under these conditions.

Case Reports

One woman who had acute contact dermatitis and worked in a garden center patch tested positive for choline chloride (1% in water and petrolatum).[110] Ten control patients were negative.

Summary

The safety assessment for cetrimonium chloride, cetrimonium bromide and steartrimonium chloride was reopened to add structurally similar ingredients that include:

- straight and branched chain alkyl trimonium ingredients,
- alkanol trimonium ingredients, and
- polymeric trimonium ingredients.

Many of these ingredients vary only by the nontrimonium hydrocarbon chain length. Others also vary by branching and further functionalization by alcohols, esters, ethers, and polymers. It is expected that the toxicity of alkanol trimonium compounds would be similar to that of alkyl trimonium compounds and the available data may be extrapolated to the other ingredients in this safety assessment—a process usually termed read-across. To test the read-across reliability, similar toxicity end points were considered across the fourth substituent. Where data were available, the findings were similar.

Straight and branched chain alkyl trimonium ingredients in this safety assessment consist of cations each comprising a nitrogen atom bonded to 3 methyl groups and a simple alkyl chain, which can vary in length from 12 carbons to 28 carbons in length. These straight chain trimoniums are waxy solids at human physiological temperatures. Water solubility and volatility decrease and melting/boiling points increase as chain length increases. Cetrimonium bromide and myrtrimonium bromide are reported to be 99% pure.

The alkanol trimonium ingredients in this safety assessment differ from the alkyl trimonium ingredients by the addition of an ethoxy functional group attached to the trimonium core nitrogen. The simplest of these, and a major metabolite of all of the other members in the group, is choline.

The third group of trimoniums in this safety assessment differs from the other groups by their incorporation into a polymer backbone. Typical molecular weights of these polymers are usually above 400 000 g/mol. The residual monomer of polyquaternium-28 was <1%.

Trimoniums function mostly as hair-conditioning agents, antistatic agents, and surfactants. Several of these ingredients function as cosmetic biocides. Impurities for alkanol trimonium compounds include trimethylamine, ethylene glycol, organic impurities, color, heavy metals. The maximum content of the residual monomer for polyquatemium-28 was reported to be <1%.

The straight chain alkyl trimonium ingredients are reported to be used at 0.0005% to 10% in rinse-off products and 0.001% to 4% in leave-on products. The alkanol trimonium ingredients and related ethers/esters/acids are mostly used in skin care and makeup products. Dihydropropyltrimonium chloride was reported to be used in leave-on products at 0.05%. Choline chloride is considered GRAS as a nutrient by the FDA.

Straight and Branched Chain Alkyl Trimonium Ingredients

Orally administered cetrimonium bromide was poorly absorbed by the intestinal tract and was rapidly excreted in the urine and feces. Cetrimonium bromide and cetrimonium chloride did not penetrate the skin well. Cetrimonium bromide enhanced penetration (phenylazoaniline, benzocaine, and benzoic acid) at concentrations below 0.5% and inhibited penetration above 1.0% in artificial membranes. The oral LD_{50} of straight and branched chain alkyl trimoniums ranged from 490 to 2970 mg/kg for rats and 400 to 633 mg/kg for mice.

Tallowtrimonium chloride (4.0 mL/kg) applied to the intact and abraded skin of rabbits resulted in 100% mortality.

The short-term oral NOAEL for 5 weeks was 100 mg/kg for cetrimonium chloride for rats. Cetrimonium chloride at 0.5% produced mild, transient dermal irritation in rabbits. The short-term dermal NOEL for cetrimonium chloride was 10 mg/kg/d for rabbits. Chronic oral administration of cetrimonium bromide at 10 mg/kg/d for 72 weeks resulted in decreased body weights in rats.

Cetrimonium bromide and cetrimonium chloride were severe ocular irritants that caused irreversible damage at 25% and 10%, respectively, in in vitro studies. Steartrimonium chloride was rated an ocular irritant in several studies. Behentrimonium chloride caused irreversible ocular damage at 10%. In in vitro tests, laurtrimonium bromide, cetrimonium chloride, steartrimonium chloride, and behentrimonium chloride were rated mild to severe ocular irritants.

Cetrimonium chloride was reported to be a skin irritant at 25%, steartrimonium chloride at 20%, and behentrimonium was not an irritant at 25% in animals. Laurtrimonium bromide was dermally irritating in humans at 7.5%. Cetrimonium

bromide was not an irritant at 1%. Behentrimonium chloride was not irritating at 5.0%. Cetrimonium chloride at 3% and steartrimonium chloride at 3.192% were not sensitizers in guinea pigs. Behentrimonium chloride was a sensitizer at 16% in guinea pigs. Cetrimonium chloride at 0.25% and behentrimonium chloride at 2.4% were not sensitizing in humans. Laurtrimonium chloride (0.4 μg/plate), cetrimonium chloride (625 μg/plate), steartrimonium chloride (5000 μg/plate), and behentrimonium chloride (500 μg/plate) were not found to be genotoxic in bacterial tests. Tallowtrimonium chloride was genotoxic at 50 μg/plate in 1 *S typhimurium* strain. Laurtrimonium chloride and cetrimonium bromide produced embryonic effects at 50 mg/kg in rabbits when administered orally. There was no evidence of teratogenicity by 2.0% cetrimonium chloride in a dermal study using rabbits.

Alkanol Trimonium Ingredients

Orally administered choline was poorly absorbed by the intestinal tract and was recovered in the feces. Acetyl L-carnitine was reported to be maintained in the human body by dietary intake, some synthesis, and efficient renal reabsorption. Choline chloride did not penetrate the skin well.

After carnitine was injected into pregnant mice; of the amount that remained in the bodies, the highest concentrations of carnitine were in the liver, placenta, kidney, myocardium, and choroid plexus in the dam. Carnitine crossed the placental barrier and was present in the same proportions, but lower concentrations, in the fetuses.

Palmitoyl carnitine enhanced dermal penetration of Lucifer yellow and Ruthenium red across caco-2 monolayers; acetyl carnitine (with a shorter alkyl chain) did not.

Oral LD_{50} was reported to be between 3150 and ≥5000 mg/kg for choline chloride in rats and 3900 and 6000 mg/kg in mice.

No effects were observed in mice orally administered choline at 200 mg/kg/d for 28 days and rats administered 1.2 mmol/kg/k carnitine for 7 days.

Short-term ip administration of choline at 200 mg/kg for 28 days to mice had no effects on body weights, organ weights, hematological parameters, splenic cell counts, pathology of the organs, and clinical biochemistry. Short-term administration ip of choline at 50 mg/kg/d to guinea pigs for 8 weeks resulted in lung lesions.

Short-term nasally administered choline at 200 mg/kg to Balb/c mice for 10 days had no effects on body weights, organ weights, hematological parameters, splenic cell counts, pathology of the organs, and clinical biochemistry. Subchronic ip administration of choline chloride to rats resulted in changes in the relative weights and/or cells of the liver, bronchiolar epithelium, thymus, and peripheral lymph nodes.

Chronic oral administration of choline had no adverse effects on rats (72 weeks), mice (20-24 months), and baboons (3-4 years). Chronic ip administration of choline to rats at 25 mg/d for 5 days/week for 10 weeks resulted in adverse effects to the lungs.

Choline chloride was not a dermal irritant to rabbits at 70%. Choline chloride was not sensitizing at 0.5% in humans. Choline chloride, carnitine, and carnitine HCl may be ocular irritants. Choline chloride was not found to be genotoxic. There was no increase in the number of liver nodules, hepatocellular carcinomas, lung tumors, leukemia, or other tumors in rats treated with 500 mg/kg/d of choline chloride.

Choline at 25 mg/kg/d ip was toxic to male rat reproduction after 24 days. In mice, there was no developmental toxicity at 1250 mg/kg. There were maternal and developmentat toxicity above this dose.

In humans, choline levels in plasma range from 9 to 20 μmol/L. Carnitine is only eliminated from the body via the urine as carnitine or acylcarnitines.

Carnitine at 2 mg/kg/d and acetyl-L-carnitine at 1 g/d orally caused nausea and vomiting in humans. Choline chloride at 10 g/d caused slight hypertension in 1 study and fishy odor, vomiting, salivation, sweating, and gastrointestinal effects. Choline did not induce liver toxicity at 6 g/d. Mild, transient signs of Parkinson disease developed in patients with tardive dyskinesia, at 12.7 g/d but no other adverse effects at 20 g/d.

Polymeric Trimonium Ingredients

The oral LD_{50} of quaternium-28 and quaternium-47 for rats was reported to be >5 g/kg.

In rabbits, polyquaternium-28 was rated a minimal ocular irritant at 20% and polyquaternium-47 was a slight ocular irritant. Polyquaternium-33 was listed as an ocular irritant.

Polyquaternium-47 was a slight dermal irritant at <30% in rabbits. Polyquaternium-28 and polyquaternium-47 were not sensitizing at 5% in humans.

Polyquaternium-28 and polyquaternium-47 were not genotoxic in reverse mutation assays. Polyquaternium-28 was not found to be genotoxic.

Polyquaternium-28 was not phototoxic or photoallergic in humans.

Discussion

The Expert Panel noted gaps in the available safety data for some of the trimoniums in this safety assessment. The available data on many of the trimoniums are sufficient, however, and similarity between structural activity relationships, biologic functions, and cosmetic product usage suggests that the available data may be extrapolated to support the safety of the entire group.

For example, these trimoniums vary by chain length; presence of branching; further functionalization by an alcohol, ester, or ether; or polymerization. Where available, the information for multiple ingredients is consistent; for example the smallest of the alkyl substituted ingredients, laurtrimonium bromide, has been shown to not penetrate skin after 2 days of application. The larger trimonium ingredients have similar results, suggesting that the various length ingredients in this assessment will also not penetrate the skin. The Panel noted

that despite the structural diversity in the ingredients group, the dominant feature of the group is its trimonium moiety. The similarity in their physical properties and uses justify their consideration together in this assessment.

The Expert Panel noted that most of genotoxicity data were on choline chloride and data on the other ingredients was lacking. The lack of dermal penetration, combined with the negative results of the bacterial genotoxicity assays, supports the absence of any genotoxic risk from the use of these ingredients in cosmetics.

The Expert Panel noted the new uses of trimoniums in baby products. These new uses are reported to be at very low concentrations (<1%) and are at a level lower than that which caused irritation. Coupled with the lack of dermal penetration, the low concentration of use levels provides assurance that the use of these ingredients in baby products would not present any risk of systemic or dermal adverse effects. It was also noted that the literature lacks clinical reports of irritation and/or sensitization by the trimonium· compounds, including the ingredients that are used in hundreds of products (ie, cetrimonium chloride and behentrimonium chloride).

Ocular irritation was moderate at 2.0% for cetrimonium chloride and mild for behentrimonium chloride at 3.0%. All the uses of eye-related products that contain trimonium ingredients were reported to be at 0.3% or less, well below these concentrations. The other straight and branched chain alkyl trimonium ingredients for which there is data (laurtrimonium chloride, steartrimonium chloride, behentrimonium chloride) were irritating at higher concentrations; choline chloride was minimally irritating at 70%, and polyquaternium-28 was minimally irritating at 20%. While there are uses in lipstick and eye care that would involve exposure of mucous membranes, the concentrations of trimonium in such products is very low (< 1%). Adequate data available to support that such levels in these product types would not result in toxic systematic exposure.

The Expert Panel recognized that these ingredients (ie, cetrimonium bromide and palmitoyl carnitine) can enhance the penetration of other ingredients through the skin (eg, phenylazoaniline, benzocaine, and benzoic acid). The Panel cautioned that care should be taken in formulating cosmetic products that may contain these ingredients in combination with any ingredients whose safety was based on their low dermal absorption, or where dermal absorption was a concern.

The potential adverse effects of inhaled aerosols depend on the specific chemical species, the concentration, the duration of the exposure, and the site of deposition within the respiratory system. In practice, aerosols should have at least 99% of their particle diameters in the 10 to 110 μm range and the mean particle diameter in a typical aerosol spray has been reported as ~38 μm. Particles with an aerodynamic diameter of ≤10 μm are respirable. In the absence of inhalation toxicity data, the Panel determined that trimoniums can be used safely in hair sprays, because aerosol particle size is not respirable.

The Expert Panel was not concerned about the plant and animal derived components of ingredients in this group. The extensive amount of processing to extract the alcohol moieties removed any concern about residual pesticides, heavy metals, or prions.

Conclusion

The CIR Expert Panel concluded that the following ingredients are safe in the present practices of use and concentration described in this safety assessment (ingredients not in current use identified with an *), when formulated to be nonirritating:

- laurtrimonium bromide*
- laurtrimonium chloride
- myrtrimonium bromide
- cetrimonium chloride
- cetrimonium bromide
- cetrimonium methosulfate
- steartrimonium bromide*
- steartrimonium chloride
- steartrimonium methosulfate*
- behentrimonium chloride
- behentrimonium methosulfate
- octacosatrimonium chloride*
- ceteartrimonium chloride*
- hydrogenated tallowtrimonium chloride*
- hydrogenated palmtrimonium chloride*
- soytrimonium chloride
- tallowtrimonium chloride
- cocotrimonium chloride
- cocotrimonium methosulfate
- octyldodecyltrimonium chloride*
- dodecylhexadecyl-trimonium chloride*
- choline chloride*
- stearoxypropyltrimonium chloride*
- lauroyl ethyltrimonium methosulfate*
- myristoyl ethyltrimonium methosulfate*
- palmitoyl ethyltrimonium methosulfate*
- stearoyl ethyltrimonium methosulfate*
- cocoylcholine methosulfate*
- carnitine
- carnitine HCl
- carnitine hydroxycitrate
- carnitine PCA*
- acetyl carnitine*
- acetyl carnitine HCl
- palmitoyl carnitine
- polyquaternium-37
- polyquaternium-14*
- polyquaternium-28
- polyquaternium-32
- polyquaternium-33*
- polyquaternium-35
- polyquaternium-36*
- polyquaternium-45*
- polyquaternium-47
- polyquaternium-48*

- polyquaternium-53*
- polyquaternium-63*
- polyquaternium-73*
- polyquaternium-91*
- acrylamide/ethyltrimonium chloride acrylate/ethalkonium chloride acrylate copolymer*
- acrylamidopropyl trimonium chloride/acrylamide copolymer and
- acrylamidopropyltrimonium chloride/acrylates copolymer.

Author's Note

Unpublished sources cited in this report are available from the Director, Cosmetic Ingredient Review, 1101 17th St, Suite 412, Washington, DC 20036, USA.

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References

1. Andersen FA. Final report on the safety assessment of cetrinonium chloride, cetrimonium bromide, and steartrimonium chloride. *Int J Toxicol.* 1997;16:195-220.
2. Gottschalck TE, Bailey JE. *International Cosmetic Ingredient Dictionary and Handbook.* 12th ed. Washington, DC: Personal Care Products Council (formerly the Cosmetic, Toiletry, and Fragrance Association.); 2008.
3. Schwan-Jonczyk A, Lang G, Clausen T, Kohler J, Schuh W, Liebscher KD. *Hair Preparations.* 6th ed. New York, NY: Wiley-VCH; 2002:99-140.
4. Bartnik FG, Wingen F. Percutaneous absorption of dodecyltrimethylammonium bromide, a cationic surfactant, in the rat. *Food Cosmet Toxicol.* 1979;17(6):633-637.
5. Frantz SW, Tallant MJ, Grosse CM, Ballantyne B. Skin penetration potential of cationic cellulose ethers following single cutaneous application to Fischer 344 rats. *J Toxicol Cutaneous Ocul Toxicol.* 1989;8(3):279-290.
6. Barel AO, Paye M, Maibach HI. *Handbook of Cosmetic Science and Technology.* 3rd ed.; 2009.
7. Andersen FA. Final report on the safety assessment of polyquaternium-10. *J Am Coll Toxicol.* 1988;7(3):335-351.
8. Andersen FA. Annual review of cosmetic ingredient safety assessments: 2005/2006. *Int J Toxicol.* 2008;27(suppl 1):77-142.
9. O'Lenick J, Price SNC. Surfactants. In: Schlossman ML, ed. *The Chemistry and Manufacture of Cosmetics.* Vol. III. 3rd ed. Carol Stream, IL: Allured Publishing Corportation; 2002:963-998.
10. Dekker M. *Reactions and Synthesis in Surfactant Systems.* Vol. 100. John Texter; 2001.
11. Akisada H, Kuwahara J, Koga A, Motoyama H, Kaneda H. Unusual behavior of CMC for binary mixtures of alkyltrimethylammonium bromides: dependence on chain length difference. *J Colloid Interface Sci.* 2007;315(2):678-684.
12. Croda Inc. Material safety data sheet; Behentrimonium chloride (in isoporopanol). 2004:1-4.
13. Croda Inc. Material safety data sheet; Behentrimonium chloride (in cetearyl alcohol). 2009:1-4.
14. Harmonized information on raw material identification according to TEGEWA (Version 2007). 2008:1-6.
15. Mallinckrodt Baker, Inc. MSDS: Choline chloride. http://www.jtbaker.com/msds/englishhtml/c4092.htm. Accessed 2009.
16. Evangeliou A, Vlassopoulos D. Carnitine metabolism and deficit—When supplementation is necessary? *Curr Pharm Biotechnol.* 2003;4(3):211-219.
17. Arcos Organics NV. Material Safety Data Sheet: L-Carnitine, 99+%. http://www.coleparmer.com/catalog/Msds/14442.htm. Accessed 2009.
18. National Industrial Chemicals Notification and Assessment Scheme. [3-(Methacryloyl amino) propyl] trimethylammonium chloride, polymer with N-vinyl-2-pyrrolidone. Australia, Report No. NA/89. 1999:1-15.
19. Vian L, Vincent J, Maurin J, Fabre I, Giroux J, Cano JP. Comparison of three *in vitro* cytotoxicity assays for estimating surfactant ocular irritation. *Toxicol In Vitro.* 1995;9(2):185-190.
20. Organization for Economic Co-Operation and Development. Choline Chloride Cas No: 67-48-1. United Nations Environment Programme Chemicals Branch. 2006. http://www.chem.unep.ch/irptc/sids/oecdsids/67481.pdf. Accessed April 22, 2010.
21. Parchem. Acetyl-L-carnitine HCl. http://www.parchem.com/chemical-supplier-distributor/Acetyl-L-Carnitine-HCL-001767.aspx. 2010. Accessed January 15, 2010.
22. Food and Drug Administration (FDA). Frequency of use of cosmetic ingredients. *FDA Database.* Washington, DC: FDA; 2009.
23. Personal Care Products Council. Concentration of Use—Acetamidoethoxybutyl Trimonium Chloride, Acetamidoethyl PG-Trimonium Chloride, Acetamidopropyl Trimonium Chloride, Acrylamide/Ethyltrimonium Chloride Acrylate/Ethalkonium Chloride Acrylate Copolymer, Acrylamidopropyltrimonium Chloride/Acrylamide Copolymer, Acrylamidopropyltrimonium Chioride/Acrylates Copolymer, Babassuamidopropyltrimonium Chloride, Babassuamidopropyltrimonium Methosulfate, Behenamidopropyltrimonium Methosulfate, Behenoyl PG-Trimonium Chloride, Behentrimonium Chloride, Behentrimonium Methosulfate, Behenyl PG-Trimonium Chloride, Cetrimonium Bromide, Cetrimonium Chloride, Cetrimonium Methosulfate, Cetrimonium Saccharinate, Cetrimonium Tosylate, Ceteartrimonium Chloride, Cinnamidopropyltrimonium Chloride, Cocamidopropyltrimonium Chloride, Cocotrimonium Chloride, Cocotrimonium Methosulfate, Dihydroxypropyltrimonium Chloride, Distearoylpropyl Trimonium Chloride, Dodecyihexadecyltrimonium Chloride, Galactoarabinan Hydroxypropyltrimonium Chloride, Hydrogenated Palmtrimonium Chloride, Hydrogenated Tallowtrimonium Chloride, Hydroxystearmidopropyl Trimonium Chloride, Hydroxystearamidopropyl Trimonium Methosulfate, Isostearoyl PG-Trimonium Chloride, Lactamidopropyl Trimonium Chloride,

Lauroyl Ethyltrimonium Methosulfate, Lauroyl PG-Trimonium Chloride, Laurtrimonium Bromide, Laurtrimonium Chloride, Laurtrimonium Trichiorophenoxide, Locust Bean Hydroxypropyltrimonium Chloride, Myristoyl Ethyltrimonium Methosulfate, Myrtrimonium Bromide, Octacosatrimonium Chloride, Octyldodecyltrimonium Chloride, Oleoyl PG-Trimonium Chloride, Olivamidopropyltrimonium Chloride, Palmamidopropyl Trimonium Methosulfate, Palmitamidopropyltrimonium Chloride, Palmitoyl Ethyltrimomum Methosulfate, Palmitoyl PG-Trimonium Chloride, Ricinoleamidopropyltrimonium Chloride, Ricinoleamidopropyltrimonium Methosulfate, Shea Butteramidopropyltrimonium Chloride, Soytrimonium Chloride, Stearamidopropyl Trimonium Methosulfate, Stearoxypropyltrimonium Chloride, Stearoyl Ethyltrimonium Methosulfate, Stearoyl PG-Trimonium Chloride, Steartrimonium Bromide, Steartrimonium Chloride, Steartrimonium Methosulfate, Steartrimonium Saccharinate, Stearyl PG-Trimonium Chloride, Tallowtrimomum Chloride and Undecylenamidopropyltrimonium Methosulfate. 2010; 1-6.

24. Personal Care Products Council. 2010. Concentration of use by FDA product category choline chloride, cocoylcholine methosulfate, carnitine, carnitine HCl, carnitine hydroxycitrate, carnitine PCA, palmitolyl carnitine, acetyl carnitine, acetyl carnitine HCl, polyquaternium-37, polyquaternium-14, polyquaternium-28, polyquaternium-32, polyquaternium-33, polyquaternium-35, polyquaternium-36, polyquaternium-45, polyquaternium-47, polyquaternium-48, polyquaternium-53, polyquaternium-63, polyquaternium-73, polyquaternium-91.
25. Jensen PA, Obrien D. Industrial hygiene. In: Willeke K, Baron PA, eds. *Aerosol Measurment. Principles Techniques and Applications*. New York, NY: John Wiley and Sons, Inc.; 1993: 538-540.
26. James AC, Stahlhofen W, Rudolf G. Deposition of inhaled particles. *Ann ICRP*. 1994;24(1-3):231-232.
27. Oberdorster G, Oberdorster E, Oberdorster J. Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles. *Environ Health Perspect*. 2005;113(7):823-839.
28. Lehman-McKeeman LD. Absorption, distribution, and excretionof toxicants. In: Klassen CD, eds. *Casarett and Doull's Toxicology: The Basic Science of Poisons*. 7th ed. New York, NY: McGraw-Hill Companies, Inc.; 2008:131-159.
29. Bower D. 1999. Unpublished information on hair spray particle sizes provided at the September 9, 1999 CIR Expert Panel meeting.
30. Johnson MA. The influence of particle size. *Spray Technology and Marketing*.; 2004:24-27.
31. European Commision. European Commission Health and Consumers Cosmetics - Cosing. http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=31354. Accessed July 15, 2010.
32. European Commision. European Commision Health and Consumers Cosmetics - Cosing Annex III. http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.results&annex=III&search. Accessed July 15, 2010.
33. Scientific Committee on Consumer Safety. Opinion on clarification of Annex II, entry 168 of the cosmetic directive: Choline salts and their esters e.g. choline chloride (INN). Brussels, European Commision. 2009. http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=31354. Accessed April 15, 2010.
34. Scientific Committee on Consumer Products. Opinion on choline chloride. United Nations Environment Programme Chemicals Branch. 4-15-2008. Accessed April 22, 2010.
35. McLoughlin DM, O'Brien J, McManus JJ, Gorelov AV, Dawson KA. A simple and effective separation and purification procedure for DNA fragments using dodecyltrimethylammonium bromide. *Bioseparation*. 2000;9(5):307-313.
36. Food and Drug Administration (FDA). Food and Drugs - Substances generally recognized as safe - Nutrients. 2010. 21CFR: 182. 8252 Choline Chloride
37. PDR Network. Herbals & Supplements: Choline. 2009. http://www.pdrhealth.com/drugs/altmed/altmed-mono.aspx?contentFileName=ame0336.xml&contentName=Choline&contentId=492. Accessed May 15, 2010.
38. PDR Network. Herbals & Supplements: Propionyl-L-camitine. 2009. http://www.pdrhealth.com/drugs/altmed/altmed-mono.aspx?contentFileName=ame0462.xml&contentName=Propionyl-L-carnitine&contentId=617. Accessed May 15, 2010.
39. Herzberg GR, Sheikholislam B, Lerner J. Cationic amino acid transport in chicken small intestine. *Comp Biochem Physiol A Comp Physiol*. 1971;41(1):229-247.
40. Herzberg GR, Lerner J. Intestinal absorption of choline in the chick. *Biochim Biophys Acta*. 1973;307(1):234-242.
41. Kuczler FJ, Nahrwold DL, Rose RC. Choline influx across the brush border of guinea pig jejunum. *Biochim Biophys Acta*. 1977; 465(1):131-137.
42. Sheard NF, Zeisel SH. An in vitro study of choline uptake by intestine from neonatal and adult rats. *Pediatr Res*. 1986;20(8): 768-722.
43. Politzer Shronts E. Essential nature of choline with implications for total parenteral nutrition. *J Am Diet Assoc*. 1997;97(6):639-646.
44. Zeisel SH, Wishnod JS, Blusztajn JK. Formation of methylamines from ingested choline and lecithin. *J Pharmacol Exp Ther*. 1983; 225(2):320-324.
45. Le Kim D, Betzing H. Intestinal absorption of polyunsaturated phosphatidycholine in the rat. *Hoppe Seylers Z Physiol Chem*. 1976;357(9):1321-1331.
46. Institute of Medicine, National Academy of Sciences USA. *Dietary Reference Intakes for Folate, Thiamin, Niacin, Vitamin B12, Panthothenic Acid, Biotin, and Choline*. Washington, DC: National Academy Press; 1998:390-422.
47. Mehta AK, Arora N, Gaur SN, Singh BP. Acute toxicity assessment of choline by inhalation, intraperitoneal and oral routes in Balb/c mice. *Regul Toxicol Pharmacol*. 2009;54(3):282-286.
48. Zeisel SH, Wurtman RJ. Developmental changes in rat blood choline concentration. *Biochem J*. 1981;198(3):565-570.
49. Comford EM, Comford ME. Nutrient transport and the blood-brain barrier in developing animals. *Fed Proc*. 1986;45(7):2065-2072.
50. Zeisel SH, Story DL, Wurtman RJ, Brunengraber H. Uptake of free choline by isolated perfused rat liver. *Proc Natl Acad Sci U S A*. 1980;77(8):4417-4419.

51. Acara M Rest J. Regulation of plama choline by the renal tubule: bidirectional transport of choline. *Am J Physiol.* 1973;225(5): 1123-1128.
52. Rennick B, Acara M, Glor M. Relations of renal transport rate, transport maximum, and competitor potency for tetraethylammonium and choline. *Am J Physiol.* 1977;232(5):F443-F447.
53. Bianchi G, Azzone GF. Oxidation of choline in rat liver mitochondria. *J Biol Chem.* 1964;239:3947-3955.
54. Weinhold PA, Sanders R. The oxidation of choline by liver slices and mitochondrea during liver development in the rat. *Life Sci.* 1973;13:621-629.
55. Kennedy EP and Weiss SB. The function of cytidine coenzymes in the biosynthesis of phospholipids. *J Biol Chem.* 1956;222(1): 193-214.
56. Vance DE. Boehringer Mannheim Award lecture. Phosphatidylcholine metabolism: masochistic enzymology, metabolic regulation, and lipoprotein assembly. *Biochem Cell Biol.* 1990;68(10): 1151-1165.
57. Bremer J, Greenberg D. Methyl transfering enzyme system of microsomes in the biosynthesis of lecithin (phospatidylcholine). *Biochim Biophys Acta.* 1961;46:205-216.
58. Vance DE, Ridgway ND. The methylation of phosphatidylethanolamine. *Prog Lipid Res.* 1988;27(1):61-79.
59. Blusztajn JK, Zeisel SH, Wurtman RJ. Synthesis of lecithin (phosphatidylcholine) from phosphatidylethanolamine in bovine brain. *Brain Res.* 1979;179(2):319-327.
60. Crews FT, Calderini G, Battistella A, Toffano G. Age-dependent changes the methylation of rat brain phospholipids. *Brain Res.* 1981;229(1):256-259.
61. Yang EK, Blusztajn JK, Pomfret EA, Zeisel SH. Rat and human mammary tissue can synthesize choline moiety via the methylation of phosphatidylethanolamine. *Biochem J.* 1988;256(3):821-828.
62. Rebouche CJ. Overview of physiological and pharmacological actions. 2004. http://ods.od.nig.gov/News/Carnitine_Conference_Summary.aspx.
63. Scientific Committee on Cosmetic Products (SCCP). Opinion on alkyl (C16, C18, C22) trimethylammonium chloride for uses other than as a preservative. 3-17-2006:1-45.
64. Scientific Committee on Cosmetic Products and Non-Food Products Intended for Consumers. Choline chloride. European Commission Enterprise and Industry. 12-9-2003. Accessed April 22, 2010.
65. Kim CS, Roe CR. Maternal and fetal tissue distribution of L-carnitine in pregnant mice: low accumulation in the brain. *Fundam Appl Toxicol.* 1992;19(2):222-227.
66. Hagen LE, Berge RK, Farstad M. Different subcellular localization of palmitoyl-L-carnitine hydrolysis in human and rat liver. *Febs Lett.* 1979;104(2):297-299.
67. Yoon KA, Burgess DJ. Effect of cationic surfactant on transport of model drugs in emulsion systems. *J Pharm Pharmacol.* 1997; 49(5):478-484.
68. Hochman JH, Fix JA, LeCluyse EL. In vitro and in vivo analysis of the mechanism of absorption enhancement by palmitoylcarnitine. *J Pharmacol Exp Ther.* 1994;269(2):813-822.
69. O'Hagan DT, Critchley H, Farraj NF, et al. Nasal absorption enhancers for biosynthetic human growth hormone in rats. *Pharm Res.* 1990;7(7):772-776.
70. Environmental Protection Agency (EPA). Index of robust summaries: FND Cationics HPV chemicals challenge. Appendix A. 12-13-2001. http://www.epa.gov/chemrtk/pubs/summaries/fatnitro/c13407rs.pdf.
71. Cortesi R, Esposito E, Menegatti E, Gambari R, Nastruzzi C. Effect of cationic liposome composition on in vitro cytotoxicity and protective effect on carried DNA. *Int J Pharm.* 1996;139: 69-78.
72. Bigliardi PL, Herron MJ, Nelson RD, Dahl MV. Effects of detergents on proliferation and metabolism of human keratinocytes. *Exp Dermatol.* 1994;3(2):89-94.
73. Al-Adham I. Si, Dinning AJ, Eastwood IM, Austin P, Collier PJ. Cell membrane effects of some common biocides. *J Ind Microbiol Biotechnol.* 1998;21(1-2):6-10.
74. Breen PJ, Compadre CM, Fifer EK, Salari H, Serbus DC, Lattin DL. Quaternary ammonium compounds inhibit and reduce the attachment of viable *Salmonella typhimurium* to poultry tissue. *J Food Sci.* 1995;60(6):1191-1196.
75. Ho IK, Loh HH, Way EL. Toxic interaction between choline and morphine. *Toxicol Appl Pharmacol.* 1979;51(2):203-208.
76. Agut J, Font E, Sacristan A, Ortiz JA. Dissimilar effects in acute toxicity studies of CDP-choline and choline. *Arzneimittelforschung.* 1983;33(7A):1016-1018.
77. National industrial chemicals notification and assessment. Full public report: Merquat 2001. Sydney, Australia: 2002:1-19.
78. Isomaa B, Bjondahl K. Toxicity and pharmacological properties of surface-active alkyltrimthylammonium bromides in the rat. *Acta Pharmacol Toxicol (Copenh).* 1980;47(1):17-23.
79. Comatzer WE. Toxicity of choline and dimethylethanolamine in the guinea pig and the rat. *Proc Soc Exp Biol Med.* 1954;85(4): 642-643.
80. Gazola VA, Lopes G, Dias RM, Curi R, Bazotte RB. Comparative effects of diet supplementation with l-carnitine and dl-carnitine on ammonia toxicity and hepatic metabolism in rats. *Acta Pharmacol Sin.* 2001;24(4):305-310.
81. International Research and Development Corporation. Subchronic percutaneous toxicity study (twenty-eight days) in rabbits. Adogen 444 (54.5% cetrimonium chloride). 1979:1-44.
82. Sahu AP. Effect of choline and mineral fibres (chrysotile asbestos) on guinea-pigs. *IARC Sci Publ.* 1989;(90):185-189.
83. Sahu AP, Saxena AK, Singh KP, Shanker R. Effect of chronic choline administration in rats. *Indian J Exp Biol.* 1986;24(2): 91-96.
84. Shivapurkar N, Hoover KL, Poirier LA. Effect of methionine and choline on liver tumor promotion by phenobarbital and DDT in diethylnitrosamine-initiated rats. *Carcinogenesis.* 1986;7(4): 547-550.
85. Muma NA, Rowell PP. Effects of chronic choline and lecithin on mouse hippocampal dendritic spine density. *Exp Aging Res.* 1988; 14(2-3):137-141.
86. Muma NA, Rowell PP, Schultz GS. Effects of long-term dietary choline and phosphatidylcholine administration on muscarinic receptors in aged mouse brain. *Neurol Res.* 1988;10(3):130-135.
87. Lieber CS, Leo MA, Mak KM, DeCarli LM, Sato S. Choline fails to prevent liver fibrosis in ethanol-fed baboons but causes toxicity. *Hepatology.* 1985;5(4):561-572.

88. Sahu AP, Singh KP, Shukla LJ, Shanker R. Choline and mica dust induced pulmonary lesions in rats. *Ind Health*. 1985;23(2): 135-144.
89. Sanchez L, Mitjans M, Infante MR, Vinardell MP. Assessment of the potential skin irritation of lysine-derivative anionic surfactants using mouse fibroblasts and human keratinocytes as an alternative to animal testing. *Pharm Res*. 2004;21(9):1637-1641.
90. Butler NJ, Langley GR, Winwood J. A cell suspension agar diffusion test using neutral red release to assess the relative irritancy potential of cosmetic ingredients. *Int J Cosmet Sci*. 1993;15(1): 33-42.
91. Scientific Committee on Consumer Safety. Opinion on clarification of Annex II, entry 168 of the cosmetic directive: Choline salts and their esters e.g. choline chloride (INN). Brussels, European Commision. 2009. http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=31354. Accessed April 15, 2010.
92. Hill Brothers Chemical Co. *Material Safety Data Sheet*. 2003:1-3.
93. Vachhrajani KD, Sahu AP, Dutta KK. Excess choline availability: a transient effect on spermatogenesis in the rat. *Reprod Toxicol*. 1993;7(5):477-481.
94. Davis KL, Bergfeld WF, Hollister LE. Choline for tardive dyskinesia. *N Engl J Med*. 1975;293(3):152.
95. Zeisel SH, da Costa KA, Franklin PD, et al. Choline, an essential nutrient for humans. *FASEB J*. 1991;5(7):2093-2098.
96. Savendahl L, Mar MH, Underwood LE, Zeisel SH. Prolonged fasting in humans results in diminished plasma choline concentrations but does not cause liver dysfunction. *Am J Clin Nutr*. 1997;66(3):622-625.
97. Brass EP. Parmacokinetic considerations for the therapeutic use of carnitine in hemodialysis patients. *Clin Ther*. 1995;17(2): 176-185.
98. Harper P, Elwin CE, Cederblad G. Pharmacokinetics of bolus intravenous and oral doses of l-carnitine. *Eur J Clin Pharmacol*. 1988;35(1):69-75.
99. Sahajawalla CG, Helton ED, Purich ED, Hoppel CL, Cabana BE. Multiple-dose pharmacokinetics and bioequivalence of l-carnitine 330-mg tablet versus 1-g chewable tablet versus enteral solution in healthy adult male volunteers. *J Pharm Sci*. 1995;84(5): 627-633.
100. Winter S, Birek L, Walker T, et al. Therapy of metabolic disorders with intravenous (IV) access ports and long term intravenous L-carnitine therapy. *Southeast Asian J Trop Med Public Health*. 1999;30(suppl 2):152-153.
101. Krahenbuhl S, Reichen J. Carnitine metabolism in patients with chronic liver disease. *Hepatology*. 1997;25(1):148-153.
102. Kwon OS, Chung YB. HPLC determination and pharmacokinetics of endogenous acetyl-L-carnitine (ALC) in human volunteers orally administered a single dose of ALC. *Arch Pharm Res*. 2004;27(6):676-681.
103. Benvenga S, Ruggeri RM, Russo A, Lapa D, Campenni A, Trimarchi F. Usefulness of L-carnitine, a naturally occurring peripheral antagonist of thyroid hormone action, in iatrogenic hyperthyroidism: a randomized, double-blind, placebo-controlled clinical trial. *J Clin Endocrinol Metab*. 2001;86(8): 3579-3594.
104. Rai G, Wright G, Scott L, Beston B, Rest J, Exton-Smith AN. Double-blind, placebo controlled study of acetyl-l-carnitine in patients with Alzheimer's dementia. *Curr Med Res Opin*. 1990; 11(10):638-647.
105. Boyd WD, Graham-White J, Blackwood G, Glen I, McQueen J. Clinical effects of choline in Alzheimer senile dementia. *Lancet*. 1977;2(8040):711.
106. Chawla RK, Wolf DC, Kutner MH, Bonkovsky HL. Choline may be an essential nutrient in malnourished patients with cirrhosis. *Gastroenterology*. 1989;97(6):1514-1520.
107. Davis KL, Hollister LE'Berger PA, Vento AL. Studies on choline chloride in neuropsychiatric disease: Human and animal data. *Psychopharmacol Bull*. 1978;14(4):56-58.
108. Gelenberg AJ, Doller-Wojcik J, Growdon JH. Choline and lecithine in the treatment of tardive dyskinesia: Preliminary results from a pilot study. *Am J Psychiatry*. 1979;136(6): 772-776.
109. Wilhelm KP, Freitag G, Wolff HH. Surfactant-induced skin irritation and skin repair: evaluation of a cumulative human irritation model by noninvasive techniques. *J Am Acad Dermatol*. 1994;31(6):981-987.
110. Fischer T. Contact allergy to choline chloride. *Contact Dermatitis*. 1984;10(5):316-317.
111. Environmental Protection Agency. *Estimation Programs Interface SuiteT for Microsoft® Windows*. 2010. (v 4.00): Washington, DC: United States Environmental Protection Agency. http://www.epa.gov/oppt/exposure/pubs/episuite.htm
112. Gautheron P, Giroux J, Cottin M, et al. Interlaboratory assessment of the bovine corneal opacity and permeability (BCOP) assay. *Toxicol In Vitro*. 1994;8(3):381-392.
113. Vinardell MP, Gonzalez S, Infante MR. Adaptation of hemoglobin denaturation for assessment of ocular irritation of surfactants and manufactured products. *J Toxicol*. 1999;18(4):375-384.
114. Catroux P, Rougier A, Dossou KG, Cottin M. The silicon microphysiometer for testing ocular toxicity in vitro. *Toxicol In Vitro*. 1993;7(4):465-469.
115. Anonymous. CAMVA and BCOP evaluation of hair conditioning product containing cetrimonium methosulfate. 2008:1-3.
116. Tani N, Kinoshita S, Okamoto Y, et al. Interlaboratory validation of the in vitro eye irritation tests for cosmetic ingredients. (8) Evaluation of cytotoxicity tests on SIRC cells. *Toxicol In Vitro*. 1999;13(1):175-187.
117. Ohno Y, Kaneko T, Kobayashi T, et al. First phase validation of the *in vitro* eye irritation tests for cosmetic ingredients. *In Vitro Toxicol*. 1994;7:89-94.
118. Okumura Arashima M, Ohuchi J, et al. Interlaboratory validation of the *in vitro* eye irritation tests for cosmetic ingredients. (10) Evaluation of cytotoxicity test on CHL cells. *Toxicol In Vitro*. 1999;13(1):199-208.
119. Matsukawa K, masuda K, Kakishima H, et al. Interlaboratory validation of the *in vitro* eye irritation tests for cosmetic ingredeints. (11) EYTEX™. *Toxicol In Vitro*. 1999;13(1):209-217.
120. Hagino S, Kinoshita S, Tani N, et al. Interlaboratory validation of in vitro eye irritation tests for cosmetic ingredients. (2) Chorioallantoic membrane (CAM) test. *Toxicol In Vitro*. 1999;13(1): 99-113.

121. Anonymous. Executive summary for the assessment of eye irritation potential using the in vitro tests 10-day CAMVA and BCOP of a hair conditioning product containing steartrimonium chloride (0.75%). 2007:1-3.
122. Anonymous. Executive summary for the assessment of eye irritation potential using the in vivtro tests 10-day CAMVA and BCOP for hair conditioning product containing behentrimonium chloride (5.0%). 2007:1-3.
123. Muir CK. Surfactant-induced opacity of bovine isolated cornea: an epithelial phenomenon? *Toxicol Lett.* 1987;38(1-2):51-54.
124. Okamoto Y, Ohkoshi K, Itagaki H, et al. Interlaboratory validation of the *in vitro* eye irritation tests for cosmetic ingredients. (3) Evaluation of the Haemolysis test. *Toxicol In Vitro.* 1999; 13(1):115-124.
125. Jester JJ, Li HF, Petroll M, et al. Area and depth of surfactant-induced corneal injury correlates with cell death. *Invest Ophthalmol Vis Sci.* 1998;39(6):922-936.
126. York M, Basketter DA, Cuthbert JA, Neilson L. Skin irritation testing in man for hazard assessment - evaluation of four patch systems. *Hum Exp Toxicol.* 1995;14:729-734.
127. Basketter DA, Chamberlain M, Griffiths HA, York M. The classification of skin irritants by human patch test. *Food Chem Toxiciol.* 1997;35:845-852.
128. Hill-Top Research Inc. A repeat insult patch test in healthy volunteers to investigate the irritation potential of ten products and to confirm that the application of the products under maximised conditions does not induce delayed contact allergic responses, following repeated cutaneous patch applications. This report is for product "hair conditioning product no 471289". 2002:1-26.
129. Institut D'Expertise Clinique. Sensitisation and cutaneous compatibility study fo a hair styling product containting 0.5% Cetrimonium Chloride. Report No. B080274RD7. 2008.
130. Reliance Clinical Testing Services, Inc. Repeated insult patch test of a hair treatment (gel) containing 0.625% cetrimonium chloride (product DT015160). 2005:1-19.
131. Institut D'Expertise Clinique. Sensitisation and cutaneous compatibility study of a rinse-off conditioner containing 0.75% cetrimonium chloride. 2008:1-59.
132. Hill-Top Research Inc. A repeat insult patch test in healthy volunteers to investigate the irritaion potential of ten products and to confirm that the application of the products under maximised conditions does not induce delayed contact allergic responses, following repeated cutaneous patch applications - report is for "hair conditioning product no. 487170". 2002:1-26.
133. TKL Research, Inc. Human repeat insult patch test with challenge of a hair treatment containing 0.375% cetrimonium chloride. 2006:1-39.
134. Product Investigations, Inc. Determination of the irritating and sensitizing propensities of Red shine milk #U00241.05 on human skin (hair styling product containing 0.75% cetrimonium chloride). 2000:1-8.
135. Product Investigations, Inc. Human repeated insult patch test of FLA No. 487309-batch DG DU 10/07/2002 (hair treatment gel containing 0.8% cetrimonium chloride). 2003:1-15.
136. Product Investigations, Inc. Determination of the irritating and sensitizing propensities of 85761 PUR2 on human skin (hair styling spray containing 0.45% cetrimonium chloride). 2009: 1-20.
137. Anonymous. Results of the sensitization test with hair conditioning product (diluted to 10%) containing cetrimonium methosulfate. 2008:1-3.
138. Product Investigations, Inc. Determination of the irritating and sensitizing propensities of 86092 PUR on human skin (hair treatment containing 0.5% behentrimonium chloride, 0.125% cetrimonium chloride and 0.125% behentrimonium methosulfate). 2009:1-20.
139. IS Consultancy Limited. Human repeat insult patch test of material no, 487343 (hair treatment containing 0.648% cetrimonium chloride and 0.48% behentrimonium chloride). 2002:1-23.
140. IS Consultancy Limited. Human repeat insult patch test of a hair treatment containing 0.625% cetrimonium chloride and 0.48% behentrimonium chloride. 2002:1-29.
141. Anonymous. Executive summary of human repeat insult patch test of a hair condiditoning product (diluted to 10%) containing behentrimonium chloride (5.0%). 2007:1-3.
142. Anonymous. Executive summary of human repeat insult patch test of a hair styling product containing cocotrimonium methosulfate (0.42%). 2007:1-3.
143. Anonymous. Executive summary of human repeat insult patch test of a hair styling product containing cocotrimonium methosulfate (0.42%). 2006:1-3.
144. Anonymous. Results of the sensitization test with hair styling product containing cocotrimonium methosulfate (0.42%). 2005:1-3.
145. Anonymous. Results of the sensitization test with hair styling product containing cocotrimonium methosulfate (0.42%). 2004:1-5.