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Final Report of the Amended Safety Assessment of Quaternium-15 as Used in Cosmetics

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Abstract

Quaternium-15 is an antimicrobial agent used in cosmetics as a cosmetic preservative and antistatic agent. Little systemic toxicity was reported in most single-dose or repeated-dose animal studies. Quaternium-15 was an oral teratogen, but not a dermal teratogen, in rats at doses that exceeded the expected cumulative exposure from cosmetics. The frequency of sensitization increased in North America but not in Europe, where Quaternium-15 is used less often. In almost all animal and human studies, Quaternium-15 at 0.2% was not a sensitizer. The weight of evidence suggested that a 0.2% concentration is not a sensitizer and that cosmetic products containing Quaternium-15 up to that level are safe.

Keywords

safety; cosmetics; quaternium-15

The Cosmetic Ingredient Review (CIR) Expert Panel previously reported that Quaternium-15 was safe as a cosmetic ingredient at concentrations not exceeding those then in use.[1] The highest use concentration at that time was in the 0.1% to 1.0% range.

Recently, the CIR Expert Panel noted that the frequency of sensitization to Quaternium-15 in patch-tested patients has risen to almost 10% in clinical patch tests in North America. Although the incidence remained constant over several years, it was considered sufficiently high to reopen this safety assessment and consider all newly available data.

Chemistry

Definition and Structure

As given in the International Cosmetic Ingredient Dictionary and Handbook,[2] Quaternium-15 (CAS No. 4080-31-3; 51229-78-8) is a cosmetic preservative and antistatic agent. This quaternary ammonium salt with the empirical formula $C_9H_{16}ClN_4 \bullet Cl$ (MW = 251) has the structure shown in Figure 1.

According to Gottschalck and McEwen[2] and Windholz,[3] synonyms include the following:

N-(3-chloroallyl)hexaminium chloride
1-(3-chloroallyl)-3,5,7-triaza-1-azoniaadamantane chloride
1-(3-chloro-2-propenyl)-3,5,7-triaza-1-azoniatricyclo [3.3.1.1]decane chloride
Methenamine 3-chloroallyl chloride
3,5,7-triaza-1-azoiatricyclo[3.3.1.1]-decane, 1-(3-chloro-2-propenyl)-
Chloroallyl methenamine chloride

Physical and Chemical Properties

Quaternium-15 is a cream-colored powder with a pungent odor. It is readily soluble in water and practically insoluble in mineral oil.[4,5] Table 1 presents the available solubility data as a function of solvent.

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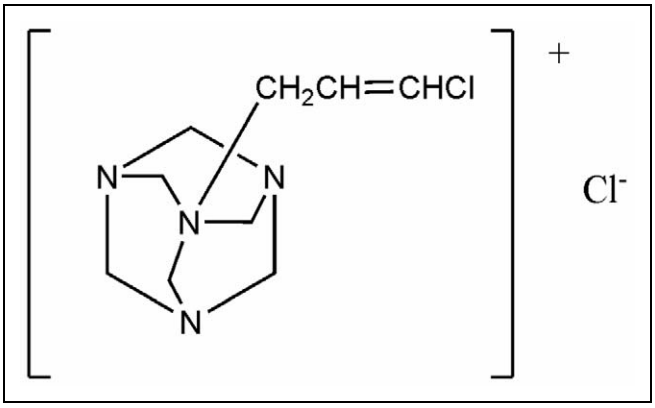

Figure 1. Quaternium-15.

Table 1. Solubility of Quaternium-15[4,5]

Solvent	Solubility[a]
Ethanol (absolute)	2.4
Isopropanol (anhydrous)	<0.1
Methanol (anhydrous)	20.8
Glycerine (99.5%)	12.6
Mineral oil	<.01
Propylene glycol, USP	18.7
Water	127.2

[a] Grams per 100 g of solvent at 25°C.

Reactivity

Croshaw[6] and Marouchoc[5] stated that Quaternium-15 is reasonably stable in the presence of nonionic, anionic, cationic, and proteinaceous ingredients over time and throughout a broad pH range of 4.0 to 10.5.

Sabourin[7] determined that reductions in detectable Quaternium-15 as a function of storage time occur when aqueous solutions of Quaternium-15 are stored at room temperature.

Rosen and Berke[8] and Croshaw[6] noted that Quaternium-15 decomposes when heated above 60°C. Decomposition products include pyrimidines and formamides.

Dow Chemical Co[9] stated that at high temperatures, such as those generated by a fire, the decomposition of Quaternium-15 may result in the release of toxic flammable vapors.

Release of Formaldehyde

Scott and Wolf[10] asserted that Quaternium-15 released formaldehyde in aqueous formulations. Jordan et al[11] stated that creams containing 0.1% or 2.0% Quaternium-15 also contained 0.01% or 0.2% (1000 or 2000 ppm) formaldehyde, respectively, according to polarographic analysis.

More recent testing was done by Dow Chemical Co[12] of an aqueous solution of 0.2% Quaternium-15 by C-13 nuclear magnetic resonance (NMR) spectroscopy. By analogy with the model carbonyls of acetone and acetaldehyde, formaldehyde was expected in the same region. The C-13 NMR spectra were scanned on the 11th, 28th, and 29th days of storage of the neat, 0.2% solution. As a reference for absorption, 1,4-dioxane was added for identification and quantitation. The solution of Quaternium-15 was stored at room temperature. There was no absorption detected in the target region in any spectra, indicating that there was no formaldehyde present in quantities greater than the estimated 70 μg/mL detection limit.

Dow Chemical Co[13] also analyzed a shampoo containing 0.1% Quaternium-15 for formaldehyde using the same method. A neat sample of the shampoo was scanned, and no significant absorption was detected in the range in which formaldehyde would be expected to absorb. The estimated detection limit of formaldehyde in this system was 60 μg/g.

Method of Manufacture

Quaternary compounds are prepared by reacting hexamine with the appropriate halocarbon in a nonaqueous solvent at room temperature.[10]

Analytical Methods

In addition to the analytical methods used to detect Quaternium-15 in the preceding sections, several other techniques have been used[14-19] to isolate and identify quaternary ammonium compounds, including the following:

- Calorimetric tests
- Gas chromatography
- Gas–liquid column chromatography and ion exchange resin columns
- Thin-layer chromatography (TLC)
- TLC with bioautographic visualization

Quaternium-15 has also been determined quantitatively by splitting off formaldehyde and subsequent reaction with dimedone.[20]

Impurities

Sabourin[7] stated that residual organics may be present in Quaternium-15 at concentrations less than 500 ppm but that 1,3-dichloropropene, specifically, was not detected at a detection limit of 1 ppm.

As described in the *Compendium of Cosmetic Ingredient Composition*,[21] Quaternium-15 typically assays at 94% minimum 1-(3-chloroallyl)-3,5,7-triaza-1-azoniaadamantane with 13.9% minimum ionic chloride.

Use

Cosmetic

According to information supplied to the US Food and Drug Administration (FDA) by industry as part of the Voluntary Cosmetic Ingredient Reporting Program (VCRP), Quaternium-15

was used in a total of 1015 cosmetic products in 1981; use concentrations were 1.0% or less.[1] Currently, VCRP data indicate that Quaternium-15 is used in 487 cosmetic products.[22] The results of a survey of current use concentrations conducted by the Personal Care Products Council[23] included uses at concentrations ranging from 0.000002% to 0.2%. Table 2 lists the available uses and use concentrations as a function of cosmetic product type, along with the total number of products in each category.

The European Union[24] regulations allow the use of Quaternium-15 up to 0.2% as a preservative in cosmetic products.

Inhalation Exposure of Cosmetics

Quaternium-15 is reported to be used in 12 aerosol/spray fixatives.

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.[25] In general, the smaller the particle, the farther into the respiratory tree the particle will deposit and the greater the impact on the respiratory system.[26]

Anhydrous hair spray particle diameters of 60 to 80 μm have been reported, and pump hair sprays have particle diameters of 80 μm or larger.[27] The mean particle diameter is about 38 μm in a typical aerosol spray.[28] In practice, aerosols should have at least 99% of particle diameters in the 10 to 110 μm range. This means that most aerosol particles are deposited in the nasopharyngeal region and are not respirable.

Noncosmetic

Quaternium-15 is used as an antimicrobial agent in water-based metalworking fluids.[29]

Quaternium-15 is an approved indirect food additive.[30] It may be used in adhesives in contact with food, as a component of paper or paperboard in contact with aqueous and fatty foods, and in polyurethane resins used as food contact surfaces.

Absorption, Distribution, Metabolism, and Excretion

The absorption, distribution, metabolism, and excretion of Quaternium-15 have been studied in female Fischer 344 rats.[18] For these studies, either the hexamethylenetetramine ring or the chloropropene side chain was labeled. Three rats received either 5 or 75 mg/kg Quaternium-15 administered dermally or orally. In addition, 3 rats received 5 mg/kg by the intravenous (IV) route. Except for the IV studies, both the ring-labeled and side chain–labeled compounds were studied at each dose and route. Standard procedures for collecting urine, feces, blood, and expired air were used.

Almost complete absorption of Quaternium-15 (84%-88% of dose) was achieved in 48 hours after oral administration, whereas only 1% to 2% absorption occurred in that time after dermal application of dilute or concentrated solutions; absorption rates were not determined. Excretion of radioactivity from Quaternium-15 was bimodal, initially rapid (half-life = 1.3 ± 0.1 hours) and then slow (half-life = 22.7 ± 2.0 hours). Quaternium-15 that reached the systemic circulation was metabolized extensively. The extent of metabolism was influenced by the route of administration, because as little as 5% of ring-derived CO_2 was found after dermal application.

The only metabolite tentatively identified was formic acid (based solely on retention times). Although several other metabolites were found in the urine of rats, attempts to identify these compounds were unsuccessful.

Animal Toxicology

Acute Oral Toxicity

The oral toxicity of a cosmetic cleanser containing 0.2% Quaternium-15 was evaluated using rats (5 per group, sex and strain not given).[31] The undiluted product was administered by gavage (15 g/kg) and the rats were then observed for 7 days. One rat died 24 hours after intubation. The product was classified as nontoxic via ingestion with a median lethal dose (LD_{50}) greater than 15 g/kg.

Dow Chemical Co[32] administered Quaternium-15 (200, 400, 800, 1600, 3200, or 6300 mg/kg) by gavage to Fischer 344 CDF rats (6 males and 6 females per group). The LD_{50} for both male and female rats was reported to be 2664 mg/kg with a 95% confidence interval of 1836 to 3512 mg/kg.

Dow Chemical Co[33] administered a single oral dose of Quaternium-15 (126, 252, 500, 1000, or 2000 mg/kg; 10% aqueous solution) to Sprague-Dawley rats (5 males and 5 females per group). The group LD_{50} was calculated to be 1070 mg/kg with a 95% confidence interval of 768 to 1490 mg/kg Quaternium-15. The LD_{50} for male Sprague-Dawley rats was calculated to be 940 mg/kg with a 95% confidence interval of 612-1440 mg/kg.

Female Sprague-Dawley rats (5 per group) were administered a single oral dose of Quaternium-15 (252, 500, 1000, or 2000 mg/kg; 50% aqueous) by gavage. The LD_{50} for this experiment was 1552 mg/kg with a 95% confidence interval of 906 to 2684 mg/kg.

Four groups of 5 female New Zealand white rabbits were administered a single oral dose of Quaternium-15 (31.6, 63.0, 126.0, or 252.0 mg/kg; 50% aqueous solution). The LD_{50} was reported to be 78.5 mg/kg, and Quaternium-15 was classified as very toxic via oral ingestion.

The oral toxicity of Quaternium-15 was also evaluated in male chicks and male Hartley guinea pigs (5 per group). The animals were administered single oral doses of Quaternium-15 (ranging from 126 to 3980 mg/kg). Quaternium-15 was moderately toxic to guinea pigs and chicks, with LD_{50} values of 710 mg/kg and 2800 mg/kg, respectively.[32,33]

Table 3 summarizes these studies by the Dow Chemical Co.

Table 2. Historical and Current Cosmetic Product Uses and Concentrations for Quaternium-15

Product Category (no. used per category[30])		2007 Uses[22]	1981 Concentrations, %[1]	2007 Concentrations, %[23]
Baby products				
Shampoos (55)	2	30	≤1	0.05-0.1
Lotions, oils, powders, and creams (132)	1	1	>0.1-1	—
Other (138)	2	21	≤0.1	—
Bath products				
Oils, tablets, and salts (257)	4	3	≤0.1	—
Soaps and detergents (1329)	9	28	≤1	0.001-0.2
Bubble baths (262)	6	10	≤1	0.1
Capsules (4)	2	—	≤0.1	—
Other (239)	10	2	≤1	0.001-0.05
Eye makeup				
Eyebrow pencils (147)	1	—	≤0.1	—
Eyeliners (684)	31	4	≤1	0.1-0.2
Eye shadow (1196)	146	5	≤1	—
Eye lotions (177)	3	—	≤0.1	—
Eye makeup remover (131)	3	3	>0.1-1	0.05-0.06
Mascara (463)	88	37	≤1	0.004-0.2
Other (288)	17	4	≤1	—
Fragrance products				
Colognes and toilet waters (1288)	—	—	—	0.001
Powders (278)	32	4	≤1	0.1-0.2
Sachets (28)	5	—	≤0.1	—
Other (399)	6	—	≤0.1	—
Noncoloring hair care products				
Conditioners (1249)	26	24	≤1	0.2
Sprays/aerosol fixatives (371)	3	19	≤1	0.000002[a]
Permanent waves (141)	4	—	≤1	—
Rinses (47)	10	1	≤1	—
Shampoos (1403)	81	59	≤1	0.1-0.2
Tonics, dressings (1097)	4	27	≤1	0.1-0.2
Wave sets (50)	4	1	≤1	—
Other (716)	6	8	≤1	0.0002-0.05
Hair-coloring products				
Dyes and colors (2481)	3	—	≤1	—
Rinses (43)	—	1	—	—
Makeup				
Blushers (539)	18	7	≤1	—
Face powders (613)	10	15	≤1	—
Foundations (635)	61	12	≤1	0.002
Makeup bases (164)	102	2	≤1	—
Rouges (99)	8	—	≤1	—
Other (406)	13	3	≤1	—
Nail care products				
Cuticle softeners (18)	1	—	≤0.1	—
Creams and lotions (17)	2	—	≤0.1	—
Other (124)	5	3	≤1	—
Personal hygiene products				
Underarm deodorants (540)	2	—	≤1	0.1
Other (514)	—	7	—	0.05-0.2[b]
Shaving products				
Men's talcum (7)	1	—	≤0.1	—
Shaving cream (162)	—	1	—	—
Other (107)	1	1	>0.1-1	—
Skin care products				
Skin-cleansing creams, lotions, liquids, and pads (1368)	60	22	≤1	0.03-0.05
Face and neck creams, lotions, powder, and sprays (1195)	81[c]	14	≤1[c]	0.0004-0.1
Body and hand creams, lotions, powder, and sprays (1513)		35		0.1
Moisturizers (2039)	78	35	≤1	0.0002

(continued)

Table 2. (continued)

Product Category (no. used per category[30])		2007 Uses[22]	1981 Concentrations, %[1]	2007 Concentrations, %[23]
Night creams, lotions, powder, and sprays (343)	10	8	≤1	—
Paste masks/mud packs (418)	6	6	≤1	—
Skin fresheners (285)	6	2	≤1	—
Hormone preparations	2	NA[d]	>0.1-1	NA[d]
Wrinkle smoothers (removers)	1	NA[d]	>0.1-1	NA[d]
Other (1244)	18	13	≤1	—
Suntan products				
Suntan gels, creams, liquids, and sprays (156)	16	8	≤1	—
Indoor tanning preparations (200)	1	—	>0.1-1	—
Other (62)	3	1	>0.1-1	0.2
Total uses/ranges for Quaternium-15	1015	487	≤1	0.000002-0.2

Dash indicates not reported. NA, not applicable.
[a] 0.000002% in a nonaerosol hair spray.
[b] 0.2% in a body scrub, hand wash, and sunless tanner remover.
[c] This category was combined when the original safety assessment was performed and is now 2 separate categories.
[d] No longer included as a cosmetic product category.

Table 3. Acute Oral Median Lethal Doses (LD_{50}) Reported by Dow Chemical Co[32,33]

Animal	Preparation	LD_{50}, mg/kg	95% Confidence Interval, mg/kg
Sprague-Dawley rats, male	10% Aqueous solution	940	612-1440
Sprague-Dawley rats, female	10% Aqueous solution	1070	768-1490
Sprague-Dawley rats, female	50% Aqueous solution	1552	906-2684
CDF rats, male and female	Unspecified	2664	1836-3512
Chicks, male	Powder (in capsule)	2800	—
New Zealand white rabbits, female	50% Aqueous solution	78.5	45-136

Dash indicates not reported.

Acute Dermal Toxicity

Dow Chemical Co[32] evaluated the acute dermal toxicity of a 50% aqueous solution of Quaternium-15 using rabbits (2 males and 2 females per group). The entire trunk was shaved and then treated with Quaternium-15 (250, 500, 1000, or 2000 mg/kg). The treated site was covered by occlusive patches for 24 hours, rinsed, and made inaccessible by a collar for a further 72 hours. Animals were observed for 2 weeks.

Dermal responses at the treatment site were observed 24 hours after application and were as follows: moderate (4/13 rabbits) to marked (8/13) erythema, slight (5/13) or moderate (7/13) edema, and moderate (5/13) or marked (7/13) necrosis. Other observed signs of toxicity were lethargy, anorexia, and rapid shallow breathing. No treatment-related lesions were observed at necropsy. The mortality of the groups was as follows, given as dose (number dead/number treated): 250 mg/kg (1/4); 500 mg/kg (3/4); 1000 mg/kg (1/4); 2000 mg/kg (4/4). The acute percutaneous LD_{50} for Quaternium-15 was 605 mg/kg with a 95% confidence interval of 102 to 1559 mg/kg.[32]

The Dow Chemical Co[33] conducted an acute dermal toxicity study of Quaternium-15 using rabbits as described previously but with modifications. Five rabbits (males and females) were administered Quaternium-15 (252, 500, 1000, or 3980 mg/kg) on intact skin. Two groups of 3 rabbits (sex not stated) were administered Quaternium-15 (2000 mg/kg) to intact or abraded skin. An additional 2 groups of 3 rabbits (sex not stated) were administered powdered Quaternium-15 (3980 mg/kg) on intact or abraded skin. All animals administered Quaternium-15 on abraded skin died. The number of deaths following administration to intact skin was dose dependent. The combined acute percutaneous LD_{50} for the group was 565 mg/kg with a 95% confidence interval of 227 to 1400 mg/kg. Data comparing male and female rabbits were not provided.

The acute dermal toxicity of Quaternium-15 was evaluated using rats as described previously. Three groups of 2 rats (sex not given) were administered Quaternium-15 (500, 1000, or 2000 mg/kg: 50% aqueous solution). The test site was covered for 6.5 hours and then washed. All animals appeared normal during and after the exposure to Quaternium-15, and no animals died at any dose of the preservative.[33]

Short-Term Dermal Toxicity

The short-term dermal toxicity of Quaternium-15 was evaluated on the intact and abraded skin of sexually immature rabbits. Ten groups (5 males and 5 females each) were administered Quaternium-15 (0, 10, 25, 50, or 100 mg/kg/d; 20% wt/vol aqueous solution). One group of rabbits at each dose level received Quaternium-15 on abraded skin, and the

test sites of the other group were intact. The test material (tap water for the controls) remained in contact with the skin for 7 hours per day, 5 days per week, for 3 weeks. Animals administered 10 and 25 mg/kg/d did not differ from controls with respect to mortality, behavior, local skin reactions, body weights, hematological values, blood chemistry values, urine analyses, gross and microscopic lesions, or organ weight and ratio data.[34]

Subchronic Dermal Toxicity

A 13-week subchronic dermal toxicity test was conducted on a cleanser (wipe-off) containing Quaternium-15 (0.2%). Female rats (N = 15) were administered daily doses of the product 5 days per week at 3.0 mL/kg, which resulted in a dose of 3000 mg/kg/d Quaternium-15. The authors stated that this dose is approximately 60 times greater than that received from normal use of the product by a consumer. There was an untreated concurrent control group of 15 female rats. Body weight gains, blood and urine values, and organ weight values were all within normal limits for treated and control animals. Skin hyperkeratosis at the application site was confirmed by microscopic observation in 3 of the test animals. No cumulative systemic toxic effects were noted, and the product was considered safe for marketing.[35]

The dermal toxicity of prototype cosmetic formulations containing Quaternium-15 (0.1%, 1.0%, or 3.0%) was studied using rabbits. Five groups (5 males and 5 females each) were given daily applications of 1.0 mL/kg/d tap water (water control), 1.0 mg/kg/d base cosmetic formulation (base control), or 1 mL/kg/d prototype cosmetic formulations, which resulted in a dose of 1.04, 10.50, or 31.30 mg/kg/d of Quaternium-15. The test materials were administered 5 days a week for a total of 62 applications over 91 days. The test materials were administered in the morning, and residual materials were removed with a damp sponge at the end of each day. There were no treatment-related differences between test and control animals in mortality, signs of toxicity, local skin reactions, body weights, food consumption, clinical chemistry values, or pathological changes. Under these test conditions, dermal applications of 31.3 mg/kg/d or less did not present any hazard as a result of absorption of the test material through the skin.[36]

Chronic Toxicity

No published chronic toxicity studies were reported, and no unpublished chronic toxicity studies were provided.

Ocular Irritation

Rabbits (6, sex not given) were given a single instillation of pure Quaternium-15 (0.1 g) into the conjunctival sac of the right eye. The left eye served as an untreated control. The authors concluded that Quaternium-15 was practically nonirritating to the eye.[32]

The ocular irritancy of Quaternium-15 (1%, 3%, 5%, or 10% aqueous) was evaluated in New Zealand rabbits (4 in each group). The test material (1 mL) was instilled into the right eye 3 times per day for 5 days of the first week. The left eye served as a control. There were no signs of irritation or corneal injury in test or control animals throughout the 2-week study. However, test animals rubbed their eyes with their paws following instillation of Quaternium-15. Distilled water did not elicit this response in the control animal.[37]

A cleanser (wipe-off) containing Quaternium-15 (0.2%) was tested for eye irritation in rabbits (6, sex not given). A single application of the undiluted product was instilled in the eyes. Slight conjunctival irritation was observed in 3 rabbits, which cleared by day 4. The authors concluded that the product was a mild eye irritant.[38]

New Zealand rabbits (6, sex not given) were treated once in 1 eye with a mascara containing Quaternium-15 (0.2%). Slight conjunctivitis was observed 1 hour after treatment and cleared in 24 to 48 hours. There was no irritation of the cornea or iris. The authors concluded that mascara was mildly irritating.[39]

Two lots of a mascara containing Quaternium-15 (0.2%) were tested for ocular irritation on groups of rabbits (6, sex not given). Each rabbit had undiluted mascara (0.1 mL) instilled in 1 eye, with the other eye serving as a control. The eyelids were held together for several seconds after treatment, and the eyes were not rinsed. One lot of mascara caused slight conjunctivitis 1 hour after exposure, and this change cleared within 48 hours. Slight conjunctivitis was observed 1 hour after treatment with the other lot of mascara, but this change cleared in 72 hours. The authors concluded that the product was a mild eye irritant.[40]

Dermal Irritation

The skin irritancy of undiluted Quaternium-15 (0.5 g) was tested on the intact and abraded skin of 6 female New Zealand rabbits. Occlusive patches containing Quaternium-15 (0.5 g) were applied to an intact and to an abraded site on each animal and left in place for 24 hours. Test sites were scored immediately and 48 hours after patch removal. Observed irritation ranged from slight (2/6) to moderate erythema (2/6) and slight (5/6) to moderate edema (1/6). The Draize Primary Irritation Index (PII) was 1.2 (of a maximum of 8.0). The authors concluded that undiluted Quaternium-15 was a mild primary skin irritant.[32]

Rabbits (5, sex not given) were evaluated for primary skin irritation following exposure to powdered Quaternium-15 or an aqueous solution of Quaternium-15 (1%, 5%, or 10%). Two rabbits received either a wet or dry occlusive patch of the powdered ingredient to an intact (ten 0.5-mL applications over 14 days) and an abraded (three 0.5-mL applications on 3 consecutive days) site on the abdomen. The rabbits receiving solutions followed the same dosing regimen (occlusive patch) on intact and abraded sites on the abdomen and also received ten 0.1-mL applications over a 10-day period to the uncovered ear. The 1% and 5% aqueous solutions caused no irritation at any

site. Aqueous Quaternium-15 at 10% caused no irritation to the ear, slight erythema to intact and abraded skin, and slight exfoliation, crusting, and scarring to abraded sites. The dry patch of undiluted Quaternium-15 did not irritate intact skin and was slightly irritating to abraded skin as manifested by erythema and edema. The occlusive moistened patch of pure Quaternium-15 was moderately irritating to intact and abraded skin and caused erythema, edema, necrosis, crusting, and scarring of the test site.[33]

The primary irritation of Quaternium-15 in aqueous solution and an anhydrous ointment (a cream formulation of a 50% water-in-oil emulsion containing petrolatum, mineral oil, mineral wax, wool wax alcohols, and 0.05% of the preservative Bronopol) was evaluated using 2 groups of 10 male and female albino guinea pigs. The animals were administered 10 applications (over 2 weeks) of 5% or 10% aqueous Quaternium-15 (0.2 g) or 5% or 10% Quaternium-15 ointment (0.2 g) to the shaved skin of the right flank. The test sites were not covered and the test material was not washed off between applications. There were no signs of irritation in any test animal and no indications of toxic reaction or irritation in 4 skin samples taken from animals treated with 10% aqueous Quaternium-15.[41]

The Dow Chemical Co[42] applied a single, open application of Quaternium-15 (5%) to 10 male guinea pigs in an anhydrous preparation. No skin erythema or alteration was observed 6 and 24 hours after application.

A single insult occlusive patch test was performed to evaluate the irritancy of a cleanser (wipe-off) containing Quaternium-15 (0.2%). The undiluted product was administered to 9 rabbits; 9 of 9 and 8 of 9 rabbits had slight erythema 2 and 24 hours, respectively, after patch removal. The authors concluded that the product was slightly irritating with a group PII of 0.78 of a maximum 4.0.[43]

Dermal Sensitization

Numerous guinea pig sensitization tests were conducted with various concentrations resulting in mixed results. These studies are summarized below.

Quaternium-15 (10% wt/vol in PPG-2 methyl ether/polysorbate 80; 9:1; 0.1 mL) was evaluated for sensitization potential in guinea pigs. Guinea pigs (10, sex not given) were given four 48-hour induction occlusive patches containing the test material. The third induction patch was accompanied by an intradermal injection of Freund's adjuvant (0.2 mL) adjacent to the insult site. A second group of 10 guinea pigs served as positive controls and were treated with epoxy resin in PPG-2 methyl ether/polysorbate (10%) on the same schedule. After a 2-week nontreatment period, the animals were challenged with the test material (uncovered) and also challenged with the vehicle as a further control. Seven of the 10 positive control animals had a sensitization response to the epoxy resin. No test animals had reactions at challenge to the vehicle. Four guinea pigs had very slight (2 on a scale of 1-5) erythema and edema 24 hours after the challenge application of Quaternium-15, and 2 out of 4 of these reactions had cleared by 48 hours. One of these reactions was possibly a weak sensitization response.[32]

Two guinea pig sensitization tests were conducted using aqueous Quaternium-15 (1%) and 1 test was conducted with Quaternium-15 (10% in a 9:1 mixture of PPG-2 methyl ether and polysorbate 80). In the first test, the guinea pigs (10, sex not given) were treated with 4 occlusive induction patches, an injection of Freund's adjuvant, challenge after 2 weeks, and a positive control using epoxy resin (15% in PPG-2 methyl ether and polysorbate 80). The second test consisted of a total of six 0.1-mL occlusive 24-hour induction patches administered twice a week for 3 weeks. After a 2-week nontreatment period, the animals were given a single, open challenge application along with a solvent control application. Test sites were observed for sensitization response 24 and 48 hours after challenge application. No animals reacted to the 1% aqueous Quaternium-15, 7 of 10 animals were sensitized to 10% Quaternium-15, and 18 of 20 animals were sensitized to the positive control. Quaternium-15 appeared to be a skin sensitizer in situations where contact with high concentrations is likely or in the presence of a penetrating solvent. Prolonged or repeated skin contact with high concentrations of Quaternium-15 was not advised by the authors.[44]

An extensive study comparing several sensitization testing methods was conducted by Marzulli and Maguire.[45] Quaternium-15 was 1 of 11 compounds tested in 5 guinea pig sensitization assays. Each test was conducted 3 times with 10 animals, for a total of 30 animals per assay, unless otherwise noted. The Draize test was conducted with 0.1% Quaternium-15 in saline, and all other tests used 5% Quaternium-15 in petrolatum. No sensitization was produced by 0.1% Quaternium-15 in saline.

In the Buehler assay, 0.5 mL of the test material was administered in occlusive, 6-hour patches on days 1, 7, and 14. On day 24, a 24-hour occlusive challenge patch was administered to an untreated site, and the sites were scored for reactions to Quaternium-15 at 24 and 48 hours after patch removal. No reactions were observed in the Buehler assay.

A Magnusson and Kligman maximization test resulted in a large proportion (55%) of guinea pig sensitization to Quaternium-15. The authors concluded that Quaternium-15 was a guinea pig sensitizer in the maximization test.

In the split-adjuvant assay, a dressing containing a window was placed over a clipped, shaved, and frozen (by dry ice) induction site on the guinea pig's flank.[45] Test material (0.2 mL) was applied through the window, under an occlusive patch for 48 hours. Similar patches were applied on days 2, 4, and 7, and the patch on day 4 was accompanied by two 0.075-mL injections of Freund's complete adjuvant directly adjacent to the induction site. A challenge was made on day 22 using the maximization test technique. Eleven reactions were reported in 30 tests. The authors concluded that Quaternium-15 was a sensitizer in this assay.

A cyclophosphamide/Freund's complete adjuvant bioassay of Quaternium-15 was conducted. Three days before induction, test animals were given an intraperitoneal injection of

cyclophosphamide (150 mg/kg). A dressing containing a window was applied to a clipped, shaved, and frozen induction site on the flank, under occlusive patches; patches containing the test material (0.2 mL) were administered through the window on days 0, 1, 2, 3, and 4. The patch on day 4 was accompanied by injections of Freund's complete adjuvant as described in the split-adjuvant assay. A final 6-hour occlusive application of Quaternium-15 was made to the induction site on day 9. The guinea pigs were challenged on day 22 using the maximization test technique. Nine of 30 animals reacted to the challenge. The authors concluded that Quaternium-15 was a sensitizer in this assay.[45]

A group of albino guinea pigs (30; both sexes) were given 4 intracutaneous injections on 4 consecutive days of Quaternium-15 (0.5%; 0.5 mL of isotonic solution; 1 mg/d). An injection of Freund's complete adjuvant (0.03 mL) was administered with the first and third induction injections. An additional group of guinea pigs (n = 10) were given intracutaneous injections of Quaternium-15 (0.5%; 0.5 mL) for 10 consecutive days, excluding Sunday. After an 18-day nontreatment period, a challenge test was conducted with Quaternium-15 (1% and 5%) in an anhydrous ointment. The ointment was administered to intact, not pretreated, skin on the guinea pig flank. The challenge application was repeated once a week for 6 weeks. Two guinea pigs had weakly visible erythema 24 hours after challenge. The reaction site had been treated with 1% Quaternium-15 in 1 animal and 5% Quaternium-15 in the other animal. No other contact reactions were observed in either the 30- or 10-animal group. Skin samples were evaluated microscopically from 6 of 10 animals in the second group; no microscopic indication of contact sensitization was observed.[42]

Groups of 10 male Hartley albino guinea pigs were used to evaluate the skin sensitization potential of 4 samples of Quaternium-15. The samples were tested as 2% aqueous solutions (n = 40) and 2 of 4 samples were additionally tested as 2% suspensions in petrolatum (n = 20). Two positive control groups received either 10% epoxy resin in PPG-2 methyl ether and polysorbate 80 (9:1) or 37% formaldehyde. Animals were given 4 induction applications per test or control preparation and then were challenged with the respective material after a 2-week nontreatment period. All animals receiving Quaternium-15 during the induction phase were also challenged with 37% formaldehyde to test for cross-sensitization. Positive sensitization responses (slight to moderate erythema) were observed in all 20 positive control animals. No sensitization was observed in any test animal challenged with Quaternium-15 or formaldehyde. Two percent Quaternium-15 was not considered a skin sensitizer.[46]

The Dow Chemical Co[47] used guinea pigs (20, sex not given) that had previously been used in a 2-week primary irritation study with Quaternium-15 (5% and 10%) to evaluate for sensitization to Quaternium-15 following a 16-day nontreatment period. Challenge tests consisted of single, simultaneous applications of Quaternium-15 (0.1%, 0.5%, 1%, and 5%) in aqueous solutions and Eucerin ointment suspensions. The lower concentrations (0.1% and 0.5%) were applied to the induction sites, and the 1% and 5% solutions of Quaternium-15 were applied to previously untreated sites. No inflammatory reactions were observed 24 and 48 hours after challenge.

Guinea pigs (10, sex not given) were administered 15 cutaneous applications of Quaternium-15 (5% aqueous) over a 3-week period. After a 1-week nontreatment period, a challenge test with Quaternium-15 (0.1%, 0.5%, 1%, and 5% aqueous) was performed on a previously untreated site. Challenge applications were repeated once a week for 6 weeks. No sensitization reactions were observed.

Two groups of 10 guinea pigs (sex not given) were used in an intracutaneous sensitization test of Quaternium-15. The first group was given 4 consecutive daily injections of Quaternium-15 (0.5% in 0.9% saline), for a 4-day total of 4 mg Quaternium-15 per animal. Animals also received an injection of Freund's complete adjuvant on days 1 and 3. Animals were challenged 14 days after induction with Quaternium-15 (0.1%, 0.5%, 1%, and 5% aqueous) and an ointment. An additional 5 challenges were administered at weekly intervals. There were no sensitization reactions in this group. The second group of guinea pigs had sensitization reactions to 2,4-dinitro-1-chlorobenzene in a previous study. The animals were given 4 intracutaneous injections of Freund's adjuvant (0.03 mL). Following 3 nontreatment days, the animals received 10 injections of Quaternium-15 (5% in saline; 0.5 mL). The injections were given daily, 5 days a week for 2 weeks. The challenge test with Quaternium-15 (1% and 5% aqueous) and Eucerin ointment followed an 18-day nontreatment period. Subsequent challenge tests with 0.1%, 0.5%, 1.0%, and 5.0% solutions and ointments were repeated weekly for 6 weeks. No sensitization reactions to Quaternium-15 were observed throughout the study.[41]

Reproductive and Developmental Toxicity

Oral

The Dow Chemical Co[48] conducted a study on the teratogenicity of orally administered Quaternium-15 using Fischer 344 rats. Initially, a probe study was conducted to determine the maximum tolerated dose of Quaternium-15 in pregnant rats. Maternal toxicity (decreases in body weight, body weight gain, and water consumption; increased liver weight relative to body weight) and embryo lethality (increased resorption incidence) were observed in rats administered Quaternium-15 (100, 200, or 400 mg/kg/d). Based on these results, groups of 33 or 34 pregnant rats were administered Quaternium-15 (0, 5, 25, or 75 mg/kg/d aqueous) via gavage on days 6 to 15 (period of major organogenesis) of gestation. Control animals were administered an equivalent volume of distilled water. Dams were killed on day 21 of gestation and the fetuses were examined.

A dose of 75 mg/kg/d of Quaternium-15 not only caused maternal and fetal toxicity but also caused embryonic malformations compared with control animals. Dams consumed less food and water than controls, with the subsequent decreases in body weight and body weight gain. The relative maternal liver weights (grams of liver per 100 g of body weight) were

increased over controls. Fetal resorption was increased over concurrent control resorptions, and the fetuses weighed less than controls. Eleven fetuses in 7 litters had major malformations, primarily of the eye (microphthalmia). An increase in minor malformations was indicative of delayed development.

A dose of 25 mg/kg/d Quaternium-15 did not cause maternal toxicity; however, pregnant rats had a transient decrease in feed consumption. This dose did result in fetal malformations; major malformations were found in 10 fetuses in 9 litters. The predominant malformations were eye anomalies. The administration of 5 mg/kg/d Quaternium-15 did not produce any evidence of maternal or fetal toxicity or malformations. The authors concluded that Quaternium-15 orally administered at 25 and 75 mg/kg/d on days 6 to 15 of gestation was teratogenic in Fischer 344 rats. However, 5 mg/kg/d Quaternium-15 was below this teratogenic threshold.[48]

Dermal

In the dermal toxicity study (see Short-Term Dermal Toxicity), rabbits in the 50-mg/kg group had slightly decreased spermatogenesis and slight skin irritation at the site of application. There were decreases in absolute testes weights, testes to body weight ratios, and testes to brain weight ratios in all animals given 100 mg/kg/d. Decreased spermatogenesis was observed in 4 of 5 males receiving 100 mg/kg on intact skin and in 3 of 5 males receiving the same dose on abraded skin; irritation at the test site was also observed. All other parameters in the 50- and 100-mg/kg groups were comparable to controls.[34]

A follow-up study on 7 male rabbits was conducted in an attempt to confirm the testicular atrophy and decreased spermatogenesis reported previously. Sexually mature male rabbits were administered Quaternium-15 (0, 25, 50, or 100 mg/kg/d; 20% wt/vol solution) 5 days per week for 30 days. The Quaternium-15 was administered to abraded skin and then washed off after 7 hours of contact (collars prevented ingestion of the test material from the test site). Chronic inflammation, degeneration, and necrosis of the epidermis and dermis with thickening and keratinization at the application site were reported to be dose related. At necropsy, 30 days after the start of treatment, incidental lesions, including disseminated lymphosarcoma and focal hypoplasia of the seminiferous tubules of the testes, were observed in control and test animals and were not considered clinically significant. Liver weights were significantly decreased in animals receiving 50 and 100 mg/kg/d. Hematological and blood chemistry values were within normal limits. No signs of systemic toxicity were apparent in any animals throughout the test, and no testicular effects were attributed to treatment with Quaternium-15.[47]

Dow Chemical Co[49] conducted a study on the dermal teratogenicity of Quaternium-15 using 25 female Fischer 344 rats. Quaternium-15 (250 or 500 mg/kg/d; 50% aqueous) was dermally administered on days 6 to 15 of gestation. The application sites were placed under occlusive patches; the patches were removed only long enough to apply the daily dose of Quaternium-15. There were no statistical differences between test and control groups with respect to maternal toxicity, fetotoxicity, or fetal alterations. The incidence of resorptions in the 250 mg/kg group was increased compared with controls. There was no difference in incidences of resorptions between controls and the high-dose group. The authors concluded that Quaternium-15 in doses up to and including 500 mg/kg/d were not teratogenic in rats, consistent with the low rate of dermal absorption for this compound.

Genotoxicity

Quaternium-15 was evaluated for mutagenic activity in an Ames test using 5 strains of *Salmonella typhimurium* (TA98, TA100, TA1535, TA1537, and TA1538). Quaternium-15 was not mutagenic in concentrations of 25 to 500 μg per plate with or without metabolic activation.[50]

The genotoxic activity of Quaternium-15 was evaluated in a rat hepatocyte unscheduled DNA synthesis assay. Quaternium-15 (4×10^{-8} to 2×10^{-1} M) and tritiated thymidine were added to primary cultures of hepatocytes isolated from male CDF Fischer 344 rats. Incorporation of tritiated thymidine into nuclei as visualized by autoradiography would indicate unscheduled DNA synthesis. Quaternium-15 was toxic to the hepatocyte cultures at concentrations of 4×10^{-4} M and greater. No DNA synthesis was observed in any other test concentration of Quaternium-15, which indicated a lack of genotoxic activity under the test conditions.[51]

Clinical Assessment of Safety

Irritation and Sensitization

Healthy Volunteers

Cumulative irritation test. A moisturizer containing Quaternium-15 (0.3%) was tested for irritancy in a 21-day cumulative irritation study. The moisturizer was applied to the same test site (on the back) of 10 individuals for 21 consecutive days. The test sites were covered with occlusive patches for 23 hours, rinsed, and then scored for irritation 1 hour later. A fresh patch was reapplied immediately after scoring. One panelist had barely perceptible erythema on day 3, and no other reactions were observed. The authors concluded that the product was essentially nonirritating with a calculated irritation score of 0.83 (maximum = 630).[52]

Single-insult patch test. The irritancy of a cleanser (wipe-off) containing Quaternium-15 (0.2%) was evaluated in a single-insult patch test. An occlusive patch of the undiluted product was administered to panelists (20; age, sex, and skin type not given). A reference control was administered in the same fashion as the test material. One panelist reacted with slight irritation to the test material, and 1 panelist had a similar reaction to the control material. The product was not considered an irritant with reference to the control material by the authors.[43]

Single, 24-hour occlusive patches containing Quaternium-15 (0.5%, 1.0%, and 2.5%) in ointment were applied to panelists (n = 10) by the Dow Chemical Co.[42] This procedure

resulted in 0.50, 1.25, and 2.50 mg of Quaternium-15 coming into contact with test sites on the back. Test sites were scored for irritation upon patch removal and 24 hours later. No visible reactions were observed, and 2.5% Quaternium-15 was not considered a skin irritant by the authors.

A cream shampoo was formulated with Quaternium-15 (0%, 0.2%, or 0.5%) and evaluated for irritation on 16 panelists. The test materials remained in contact with the test areas for 3 hours. No irritation was observed.[42]

Panelists (10 males and 10 females) participated in a single-insult patch test to determine the irritancy of Quaternium-15 (2% aqueous). A closed patch was applied to the shoulder area and kept in place for 24 hours. The test sites were scored for irritation 20 minutes and 24 hours after patch removal. Slight erythema was observed in 3 panelists at 20 minutes but cleared at 1 hour after patch removal. No other signs of irritation were observed. The authors concluded that 2% aqueous Quaternium-15 was not considered a primary irritant to healthy skin.[53]

Repeated insult patch tests. A cleanser (wipe-off) containing Quaternium-15 (0.2%) did not produce irritation or sensitization in 97 subjects (16-70 years of age; 96 females and 1 male) participating in a repeated-insult patch test (RIPT; n = 97). One subject had slight erythema after the last of nine 24-hour induction patches, and no reactions were observed at challenge.[54]

Two modified Draize-Shelanski RIPTs involving 10 induction patches and a challenge patch at the induction site and/or a naive site were conducted using 2 moisturizing products on 108 and 101 individuals, respectively. Both moisturizers contained Quaternium-15 (0.3%). There were no reactions to the products, and the authors concluded that neither moisturizer was an irritant or a sensitizer.[55,56]

A mascara containing Quaternium-15 (0.2%) was tested in a 6-week modified Draize-Shelanski RIPT on 206 healthy human subjects. Ten 24-hour occlusive patches containing 0.1 g of undiluted product were administered 3 times a week during the induction phase. Test sites were scored for irritation to the product after removal of the induction patches. A final challenge patch was administered after a 12-day nontreatment period. Erythema was observed in 8 subjects, and erythema and edema or induration was observed in 3 subjects during the induction phase. Four panelists reacted at challenge with erythema (1 subject); erythema and edema or induration (2 subjects); or erythema, edema/induration, and vesiculation (1 subject). One of the panelists was thought to have been previously sensitized to Quaternium-15, 1 reactor was considered an irritant reaction, and the other 2 reactors may represent sensitization to the product.[57]

Three shades of a mascara containing Quaternium-15 (0.2%) were tested for irritation and sensitization in a modified Schwartz-Peck procedure. None of the 221 panelists completing the study had a reaction to the preinduction 48-hour occlusive patch. Each panelist was tested with all 3 shades at the preinduction and postinduction applications. The panelists were given a choice of shade for use during the 4-week induction period and instructed to use the product at least once a day. The challenge patch was scored for irritation and sensitization at removal of the 48-hour occlusive patch and 24 hours later. One panelist had erythema and edema or induration at the second scoring of 2 of the 3 shades of the mascara. Subsequent retesting elicited no reactions to either formulation, and the reaction in this 1 panelist was attributed to the application of the patches being in close proximity to 3 strongly positive reactions to other products applied simultaneously. These products were described by the authors as being neither irritants nor sensitizers.[58]

Two other mascaras containing Quaternium-15 (0.2%) were tested in modified Schwartz-Peck procedures. One product was tested using 114 healthy subjects, and no irritation or sensitization was reported or observed in any phase of the study.[59]

The second mascara was tested using 213 healthy subjects. There were no reactions to the preinduction or challenge patches. Two subjects experienced reactions to the product after 1 or 4 weeks of use. The reactions consisted of periorbital edema and mild conjunctivitis. These reactions may indicate a low degree of sensitization; however, the authors concluded that the negative patch tests indicated that the product was probably not a potent sensitizer.[60]

A moisturizer containing Quaternium-15 (0.3%) was evaluated for irritation and sensitization using 205 healthy subjects. A modified Draize-Shelanski RIPT was conducted as described previously. Challenge patches were applied to induction and untreated sites. Erythema or erythema with edema or induration was observed in 2 subjects during the induction. Ten subjects reacted with erythema at challenge. None of these reactions was considered significant or indicative of irritation or sensitization.[56]

The irritancy and sensitization of a mascara containing Quaternium-15 (0.2%) was evaluated in a prophetic patch and use test by Hill Top Research.[61] A preinduction patch was applied to the right arm of each of 102 female panelists. A postinduction patch was applied to the same area of the left arm. Each occlusive patch was administered for 48 hours; sites were scored 1 hour and 24 hours after patch removal. The induction period consisted of unsupervised daily use of the product for 4 weeks. No irritation was reported during the induction period. One panelist had a marked reaction to the challenge patch. This panelist was rechallenged and again had a severe reaction indicative of sensitization. The panelist was then exposed to the components of the products: parabens in alcohol/water mixture, disodium EDTA in water, chloroxylenol in yellow petrolatum, dihydroabietyl alcohol in yellow petrolatum, the water phase, and the oil phase of the product. The subject was definitely sensitized to parabens and chloroxylenol and was possibly sensitized to the water and oil phases.

Marzulli and Maguire[45] performed a modified Draize procedure to evaluate Quaternium-15 (5%) for sensitization in 183 healthy subjects. The test consisted of a series of ten 24-hour induction patches and a challenge patch following a 10- to 14-day nontreatment period. The induction patches were administered every other day, with a 24-hour rest between each

application. One subject had a sensitization reaction at challenge.

Dow Chemical Co[62] conducted a modified Draize repeated insult patch test using Quaternium-15 (1% aqueous) on 160 Caucasian panelists (males and females). Nine 24-hour occlusive patches were administered over 3 weeks during the induction phase of the study. After a 2-week nontreatment period, challenge patches were administered to induction and nontreated sites with Quaternium-15 (1.0%, 0.3%, and 0.1% in distilled water). Challenge sites were evaluated for sensitization reactions 24 hours after application. During the induction phase, repetitive application of Quaternium-15 demonstrated a potential for cumulative irritation; 11 subjects had positive responses to 1% Quaternium-15 at challenge. The investigators initially considered 8 of these responses true sensitization and the remaining 3 questionable sensitization responses. The results of the 0.3% and 0.1% challenge patches were not reported.

Two supplemental challenge patches were administered to the 11 reactors and to a control group selected from the 149 subjects considered not to have been sensitized to Quaternium-15. The first supplementary challenge patch was performed with 0.01% (100 ppm) formaldehyde. Nine of the 11 sensitized subjects participated, along with 11 control subjects. Three of the sensitized population had minimal to definite erythema, but the investigators did not consider these reactions evidence of contact sensitization. The final challenge was with 1% aqueous Quaternium-15 administered to 10 of the 11 previously sensitized subjects and 8 control subjects. Seven of the 10 reactive subjects were reconfirmed as sensitive to Quaternium-15, 2 of 10 subjects had inconclusive reactions, and 1 of 10 subjects had no reaction and was reclassified as not sensitized, giving a total of 7 of the 11 original reactors classified as sensitized. There were no reactions in control subjects. The authors concluded that these studies indicated that 1% aqueous Quaternium-15 caused a significant incidence of contact sensitization in the original 160-member test panel.[62]

Subjects (72; age, sex, and skin type not given) completed an RIPT to evaluate the irritancy and sensitization of a prototype underarm deodorant. The deodorant formulation contained Quaternium-15 (2%), and a control formulation contained ethanol (2%) to replace the Quaternium-15. Induction and challenge patches consisted of 0.5 mL of undiluted material that was administered under occlusive patches and remained in contact with the test site for 24 hours. Nine induction patches were applied over 3 weeks, and the challenge patch was administered after a 3-week nontreatment period. Both formulations caused slight irritation in approximately one third of the subjects. No panelists were sensitized by either formulation.[63]

Maximization tests. A mascara formulation containing Quaternium-15 (0.3%) was assessed for sensitization in a maximization test by Ivy Research Laboratories.[64] Twenty-five panelists completed the study. The mascara was nonirritating in a single 48-hour occlusive patch test, so induction sites were pretreated with sodium lauryl sulfate (SLS) (2.5%) and the challenge site was pretreated with 5% to 10% SLS. There were no instances of contact sensitization, and the authors concluded that the product was not considered a sensitizer.

Healthy adults (25; age, sex, and skin type not given) participated in a maximization test to determine the contact sensitivity of a cuticle cream containing Quaternium-15 (0.2%) conducted by Ivy Research Laboratories.[65] The cuticle cream was nonirritating in a single 48-hour occlusive patch test, so induction sites were pretreated with 1.5% SLS. Five induction patches were administered to the same site over 14 days. Each occlusive patch contained 0.3 g of undiluted product and remained in contact with the test site for 48 hours. After 10 days of nontreatment, a fresh site was pretreated with 5% SLS, and a 48-hour occlusive challenge patch was applied. Challenge sites were observed for sensitization reactions immediately and 24 hours after patch removal. No reactions were observed. The authors concluded that the product was not a contact sensitizer under the test conditions.

Cross-sensitization with formaldehyde. Jordan et al[11] tested 2 commercial creams containing Quaternium-15 (0.1%; 100 ppm formaldehyde released) on 9 formaldehyde-sensitive individuals. Four patches per subject were applied at time zero, the sites were scored, and patches were reapplied at 72 and 120 hours, with a final observation at 168 hours. Allergic responses to the first cream were observed in 6 of 9 subjects (infiltrated, confluent, papulovesicular response), with 3 reactions observed at 72 hours, 1 at 120 hours, and 2 at 168 hours. The second cream produced 5 allergic responses: 2 at 72 hours, 2 at 120 hours, and 1 at 168 hours. The authors stated that these responses were very similar to those induced by aqueous formaldehyde solutions containing 60 to 100 ppm formaldehyde. The authors concluded that Quaternium-15 can produce dermatitis in formaldehyde-sensitive individuals.

Quaternium-15 in several concentrations and vehicles was tested using formaldehyde-sensitive individuals ($n = 12$). The test materials were Quaternium-15 (0.1%, 0.5%, 1.0%, and 5.0% aqueous) and Quaternium-15 (0.1% and 1.0% aqueous and anhydrous ointment). The subjects had had no previous contact with Quaternium-15. Three panelists reacted to 0.5%, 1.0%, and 5.0% aqueous Quaternium-15. The severity of the reaction increased with the concentration of Quaternium-15, from slight erythema to pronounced erythema with papulovesicles. Two of these 3 reactors also had slight reactions to 1% Quaternium-15 in aqueous ointment. These reactions were considered to be sensitization.[41] The sensitivity results obtained from tests with Quaternium-15 in ointment were considered invalid because the vehicle contains 2-bromo-2-nitropropane-1,3-diol, a known formaldehyde releaser.[66,67]

Several preparations containing various amounts of Quaternium-15 were tested for sensitization in 6 formaldehyde-sensitive subjects. Cutaneous tests were conducted with formaldehyde solution (2%; control), hand cream containing Quaternium-15 (0%, 0.2%, and 0.5%), cream shampoo containing Quaternium-15 (0%, 0.2%, and 0.5%), Quaternium-15 (5%)

in Eucerin, and Quaternium-15 (5% and 20%). The test sites were evaluated for sensitization 24 and 48 hours after application of the test material. All 6 panelists had positive reactions to the formaldehyde control. These reactions were observed with all 3 preparations, again indicating that an ingredient other than Quaternium-15 was responsible for the reactions. In the 6 subjects, there were no reactions to the 5% aqueous solution of Quaternium-15, 1 erythematous reaction to 5% Quaternium-15 in Eucerin, and 4 reactions (slight erythema) to the 20% aqueous solution. The authors concluded that Quaternium-15 did not produce cross-sensitization in formaldehyde-sensitive individuals.[42] The sensitivity results obtained from tests with Quaternium-15 in Eucerin were considered invalid because the vehicle, Eucerin, contains 2-bromo-2-nitropropane-1,3-diol, a known formaldehyde releaser.[66,67]

Hectorne and Fransway,[68] in a cross-reactivity study, patch tested 708 dermatological patients for diazolidinyl urea. The 58 patients who tested positive were patch tested for combinations of diazolindinyl urea, other formaldehyde releasers (including Quaternium-15), and formaldehyde. None of the patients tested positive for diazolindinyl urea and Quaternium-15; 14 (24%) tested positive for diazolindinyl urea, formaldehyde, and Quaternium-15; and 1 (2%) tested positive for diazolindinyl urea, formaldehyde, 2-bromo-2-nitropropane-1,3-diol, and Quaternium-15. The authors concluded that sensitization to formaldehyde may not explain all cases of sensitization to formaldehyde releasers.

Dermatological Patients. The North American Contact Dermatitis Group (NACDG) reported the incidence of skin sensitization for Quaternium-15 (2% aqueous). The patients screened by NACDG were patients from individual or group private practices or from university dermatology clinics. Some patients were referred specifically for NACDG patch tests. From 1978 to 1979, 77 of 1985 subjects (4%) had a positive reaction to Quaternium-15. From 1979 to 1980, 1754 subjects were tested and 54 (3%) subjects reacted positively to Quaternium-15.[69]

Of the 3739 patients in these 4 years of testing, 487 cases of cosmetics-related dermatitis were identified. Of 487 patients, 149 were patch tested with some or all of the ingredients of products associated with contact dermatitis, and 32 of the 149 patients had cutaneous reactions to Quaternium-15.[70]

In conjunction with these NACDG studies, 20 of 27 individuals who had reacted positively to Quaternium-15 were patch tested with Quaternium-15, formaldehyde, and compounds thought to release formaldehyde. Sensitivity to Quaternium-15 was demonstrated in 14 of the 20 individuals; 15 of 20 were sensitive to formaldehyde, 1 of 20 was sensitive to Bronopol, 7 of 18 were sensitive to hexamethylene tetramine, none of the 20 reacted to imidazolidinyl urea, and 1 of 20 was sensitive to dimethylol dihydroxy ethylene urea.[71]

The results of the NACDG[72] standard screening assay for Quaternium-15 reported that from 1980 to 1981, 1818 patients were tested with Quaternium-15 (2% aqueous). Positive reactions were observed in 69 (4%) patients. From 1981 to 1982, 1348 patients were tested with Quaternium-15 (2% aqueous) and 1006 patients were tested with Quaternium-15 (2% in petrolatum). Reactions to the aqueous Quaternium-15 were observed in 59 (4%) patients, and 29 (3%) patients reacted to Quaternium-15 in petrolatum. Therefore, between 1978 and 1982, NACDG patient sensitization to Quaternium-15 remained consistent at 3% to 4%.

Numerous studies reported testing eczema patients for irritation or sensitivity to Quaterium-15. Mixed results were reported ranging from no irritation[41,53] to weak sensitizer.[73]

Numerous studies reported on the incidence of reactions to Quaternium-15 in dermatological patients. Mixed results were reported ranging from no reactions[74] to observed reactions.[74-85] Women were affected twice as often as were men.[86]

A large body of literature has reported positive reactions to Quaternium-15.[87-100] Positive reactions to Quaternium-15 ranged from 0.2% to 23.3% in the general population. In healthcare workers, 1 study reported reactions in 34% of health care workers tested.[101]

Hogeling and Pratt[102] performed a retrospective chart review of 100 children (aged 4-18) who were patch tested between 1996 and 2006. Positive reactions to Quaternium-15 were recorded for 4% of the children.

Many studies assessed sensitivity to Quaternium-15 over time.[86,103,104]

Photosensitization

A cleansing product containing Quaternium-15 (0.2%) was not a photosensitizer in a test involving 25 panelists. An occlusive patch containing undiluted product was administered to a test site on the back. Twenty-four hours later, the patch was removed, and the site was evaluated. The site was then irradiated with 3 times the individual's minimal erythema dose, which had been determined before product testing. The ultraviolet (UV) source was filtered to produce a continuous spectrum in the UVA and UVB regions (290-400 nm). This process was repeated twice a week for a total of 6 exposures. Following a 10-day nontreatment period, a challenge patch was applied to a previously untreated site. After 24 hours, the patch was removed, and the site was evaluated and then irradiated for 3 minutes. Challenge sites were scored for reactions 15 minutes and 24, 48, and 72 hours following UV exposure. No reactions were observed. The authors concluded that the cleanser was not a photosensitizer.[105]

The Dow Chemical Co[106] evaluated a series of 13 test materials containing 0.1% to 1.0% Quaternium-15 for photosensitization using 50 healthy panelists. The test material (0.1 mL) was applied to the test area for 60 seconds. The sites were then exposed to UV radiation for 30 seconds at a distance of 12 inches (30 cm) from the UV source (spectrum not given). Individuals were subjected to this exposure 5 days a week for 5 weeks. Challenge exposures were performed 3 weeks after the final induction exposure. There were no reactions during

induction or challenge to Quaternium-15 products tested or to the petrolatum or methanol control.

3,3′,4′5-Tetrachlorosalicylanilide (TCSA; 2%) in methanol or petrolatum was administered to 20 panelists as a positive control. Nine of 10 and 8 of 10 individuals were photosensitized to TCSA in methanol and petrolatum, respectively. Quaternium-15 (2%) in methanol produced a reaction in 3 of 20 subjects concurrently in a state of hypersensitivity to TCSA. These reactions were irritant in nature and were not produced again at subsequent challenge applications. Quaternium-15 at concentrations of 1% or lower in aqueous-based formulations was not a photosensitizer under these test exposure conditions.[106]

Case Reports

Numerous case reports involving reactions to Quaternium-15 have been reported in the literature, many involving patients exposed to Quaternium-15–containing products at work.[107-112]

Summary

Quaternium-15 is an antimicrobial agent that is effective at low concentrations. Quaternium-15 is used in cosmetics assays at more than 94% with more than 13.9% minimum ionic chloride. It is readily soluble in water and practically insoluble in mineral oil. Quaternium-15 is reasonably stable in the presence of nonionic, anionic, cationic, and proteinaceous ingredients over time and throughout a broad pH range. Decomposition products of Quaternium-15 include pyrimidines and formamides. Quaternium-15 has been purported to release formaldehyde in aqueous formulations, but other information suggests that it is not a formaldehyde releaser. Various forms of TLC have been used to separate and identify Quaternium-15. Quaternary compounds are prepared by reacting hexamine with the appropriate halocarbon in a nonaqueous solvent at room temperature.

Cosmetic use of Quaternium-15 has decreased from 1015 products reported in 1981 to 487 products in 2007. Use concentrations were less than or equal to 1.0% in 1981 and ranged from 0.000002% to 0.2% in 2008 with more detailed reporting. Quaternium-15 may be used at concentrations up to 0.2% in Europe.

Almost complete absorption of Quaternium-15 was achieved in 48 hours after oral administration to rats, whereas only 1% to 2% absorption occurred in that time after dermal application of dilute or concentrated solutions. As little as 5% of ring-derived CO_2 was found after dermal application. Excretion of radioactivity from Quaternium-15 was bimodal. Quaternium-15 that reached the systemic circulation was metabolized extensively. The only metabolite tentatively identified was formic acid.

The oral LD_{50} ranged from 940 to 2664 mg/kg for rats; the oral LD_{50} was 2800 mg/kg for chicks and 78.5 mg/kg for rabbits. Dermal application up to 3980 mg/kg of Quaternium-15 in a powder to rabbits had no observable effect.

Short-term application of Quaternium-15 to the abraded or intact skin of rabbits produced no observable effects up to 100 mg/kg. Subchronic application of Quaternium-15 at 0.2% to rats resulted in no cumulative systemic toxic effects. Chronic toxicity data were not available.

Quaternium-15 is a mild eye irritant in rabbits. Quaternium-15 was found to be a mild to moderate dermal irritant. Multiple sensitization assays using guinea pigs produced both negative and positive sensitization results.

Quaternium-15 at 75 mg/kg/d administered orally to rats caused maternal and fetal toxicity and embryonic malformations; 25 mg/kg/d did not cause maternal toxicity but did result in fetal malformations. In a dermal application study, doses up to 500 mg/kg/d were not teratogenic in rats. The oral no-observable-effects level for developmental effects was 5 mg/kg/d.

Quaternium-15 was not genotoxic in either the Ames test or the hepatocyte unscheduled DNA synthesis assay.

In extensive testing using healthy volunteers, various formulations containing Quaternium-15 at 0.2% to 0.3% were nonirritating to mildly irritating. Quaternium-15 at 1% was sensitizing, but Quaternium-15 at 2% was not sensitizing in another test. Quaternium-15 was found to produce dermatitis in formaldehyde-sensitive individuals.

In retrospective studies by the NACDG, there was an increase in the incidence of allergic reactions to Quaternium-15 over time, but the percentage has stabilized at around 9% to 10% over the past 10 years.

In various patch tests of Quaternium-15 in dermatological patients, positive reactions were observed in 0% to 22.3% of the patients. Quaternium-15 was not an irritant to eczema patients and was not a photosensitizer. Results of testing for cross-sensitization between formaldehyde and Quaternium-15 in dermatitis patients were mixed. Only 1 of many RIPTs of products containing 0.2% Quaternium-15 resulted in sensitization reactions.

There are numerous case reports of persons developing contact dermatitis from products containing Quaternium-15.

Discussion

The CIR Expert Panel recognizes that there are gaps in the data regarding use and concentration of this ingredient. However, the overall information available on the types of products in which this ingredient is used and at what concentration indicates a pattern of use, which was considered by the Expert Panel in assessing safety.

In evaluating the sensitization data, the Expert Panel noted that the maximum reported concentration of use of Quaternium-15 in cosmetics was 0.2%. In numerous animal and human studies, Quaternium-15 at 0.2% was not a sensitizer except for 1 study[57] in which 2 of 206 subjects were thought to have been sensitized to a mascara containing 0.2% Quaternium-15. However, it is not clear from the study that Quaternium-15 was the actual sensitizer. The panel concluded

that the weight of evidence suggests that a 0.2% concentration is not a sensitizer.

The frequency of sensitization reports has increased since the original safety assessment in 1986. It was noted that the increase in sensitization occurred in North America and not in Europe, where Quaternium-15 is used less often. This increase in sensitization occurred despite the decrease in use concentration in cosmetic products. The Expert Panel also noted that the increase in sensitization was in a selected population of dermatology patients tested by NACDG.

The Expert Panel concluded that the weight of the evidence required a limit of 0.2%.

The previous report indicated that Quaternium-15 was a potential formaldehyde releaser. Current evidence documents that Quaternium-15 does not release formaldehyde in formulation.

The Expert Panel determined that in the absence of inhalation toxicity data, Quaternium-15 can be used safely in hair sprays, because the ingredient particle size is not respirable. The panel reasoned that the particle size of aerosol hair sprays ($\sim$38 μm) and pump hair sprays (>80 μm) is large compared with respirable particulate sizes ($\leq$10 μm).

Quaternium-15 is a teratogen in rats when administered orally in doses of 125 mg per kilogram of body weight; however, Quaternium-15 is not a teratogen in rats when administered dermally in doses up to 500 mg/kg/d, which exceeds the expected cumulative exposure.

Amended Conclusion

Quaternium-15 is safe as a cosmetic ingredient in the practices of use in this safety assessment at concentrations not to exceed 0.2%.

Declaration of Conflicting Interests

No potential conflict of interest relevant to this article was reported. F. Alan Andersen and Lillian C. Becker are employed by the Cosmetic Ingredient Review.

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