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Final Report on the Safety Assessment of Quaternium-15

Quaternium-15 is used as an antimicrobial preservative in cosmetic products at concentration ranges from ≤0.1 to 1%. The cosmetic ingredient is not absorbed through human skin in significant amounts. Animal toxicity studies indicate that Quaternium-15 is mildly to moderately toxic depending on animal species, concentration, and route of exposure. Testicular atrophy and decreased spermatogenesis in immature rabbits were reported in one subchronic dermal study. Orally administered Quaternium-15 was teratogenic in rats when given by gavage to pregnant dams in doses ≥25 mg/kg per day. No teratogenic effects were observed at doses of 5 mg/kg per day. Dermally applied Quaternium-15 did not produce maternal toxicity, fetal toxicity, or fetal abnormalities at doses up to and including 500 mg/kg per day. No testicular effects were produced by Quaternium-15 in three subsequent studies on mature rabbits. Quaternium-15 was a moderate skin irritant in test animals at concentrations above 5%. Quaternium-15 was not a significant eye irritant in rabbits. Animal sensitization studies with Quaternium-15 produced conflicting results depending on the test methodologies used. Quaternium-15 does not appear to possess significant mutagenic activity. Primary irritation studies on Quaternium-15 in inert vehicles and formulations indicate that Quaternium-15 is not a primary skin irritant in humans. Quaternium-15 in inert vehicles may be a human sensitizer in some clinical patients. The sensitization potential of the preservative was much less pronounced when cosmetic products were tested on nonclinical subjects. Quaternium-15 may exhibit cross-sensitization with formaldehyde. Quaternium-15 is not a significant photosensitizing agent. Although Quaternium-15 is a potential skin sensitizer, it is concluded that Quaternium-15 is safe as a cosmetic ingredient at concentrations not exceeding those presently in use.

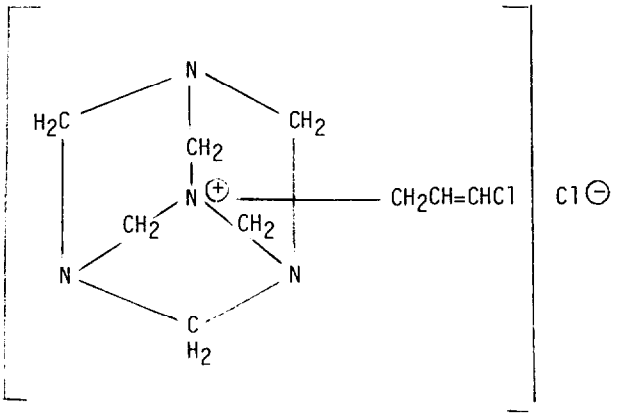
INTRODUCTION

Quaternium-15 is an antimicrobial agent that is effective at low concentrations. It is used in a wide variety of cosmetic products, including bath products, face and eye makeup, and skin care lotions.

CHEMICAL AND PHYSICAL PROPERTIES

Definition and Structure

Quaternium-15 (CAS No. 4080-31-3), a common cosmetic preservative, is a quaternary ammonium salt with the empirical formula $C_9H_{16}ClN_4$ Cl:



and a molecular weight of 251. Synonyms for Quaternium-15 include 1-(3-Chloroallyl)-3,5,7-Triaza-1-Azoniaadamantane Chloride and Chlorallyl Methenamine Chloride.[1,2]

Quaternium-15 used in cosmetics typically assays at 94% minimum 1-(3-chloroallyl)-3,5,7-triaza-1-azoniaadamantane with 13.9% minimum ionic chloride.[1,3] Residual organics may be present at concentrations less than 500 ppm, and 1,3-dichloropropene has not been detected to the detection limit of 1 ppm.[4]

Physical Properties

Quaternium-15 is a cream colored powder with a pungent odor. It is readily soluble in water and practically insoluble in mineral oil[3,5] (Table 1).

Reactivity

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 to 10.5. The average shelf-life of a Quaternium-15-containing product

TABLE 1. Solubility of Quaternium-15[5]

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

[a]g/100 g at 25°C.

is 2 years.[5,6] Aqueous solutions of Quaternium-15 were prepared and stored at room temperature, then aliquots were periodically removed and assayed for the presence of Quaternium-15 by differential pulse polarography. The results were as follows:

Concentration (%)	*0 h (ppm)*	*24 h (ppm)*	*1 week (ppm)*	*1 month (ppm)*	*3 months (ppm)*	*6 months (ppm)*	*1 year (ppm)*
2.0	22,900	21,175	19,455	18,000	15,010	9,380	8,900
20.0	200,000	207,170	196,900	135,800	98,700	87,900	56,400

A 23-month-old sample of 2% aqueous Quaternium-15 was also analyzed by differential pulse polarography. The sample was taken from a standard allergen test kit and contained 6300 ppm Quaternium-15.[7]

Quaternium-15 decomposes when heated above 60°C.[6,8] Decomposition products of Quaternium-15 include pyrimidines and formamides. At high temperatures, such as those generated by a fire, the decomposition of Quaternium-15 may result in the release of toxic flammable vapors.[9]

Quaternium-15 has been purported to release formaldehyde in aqueous formulations[10]; creams containing 0.1% or 2% Quaternium-15 also contained 0.01 or 0.2% (1000 or 2000 ppm) formaldehyde, respectively, according to polarographic analysis.[11]

An aqueous solution of 0.2% Quaternium-15 was analyzed for the presence of formaldehyde by C-13 nuclear magnetic resonance spectroscopy (NMR). A C-13 NMR reference spectrum was not available for formaldehyde; however, formaldehyde is expected to absorb in the vicinity of 190–200 ppm chemical shift, by analogy with the model carbonyls of acetone (204 ppm) and acetaldehyde (200 ppm). The C-13 NMR spectra were scanned on the 11th, 28th and 29th day of storage of the neat, 0.2% solution. 1,4-Dioxane was added as a reference absorption for identification and quantitation. The solution of Quaternium-15 was stored at room temperature. There was no absorption detected in the 150–250 ppm region in any spectra, indicating that there was no formaldehyde present in quantities greater than the estimated 70 μg/ml detection limit.[12]

A shampoo containing 0.1% Quaternium-15 was analyzed for formaldehyde

by C-13 NMR spectroscopy. A neat sample of the shampoo was scanned, and no significant absorption was detected in the 185–210 ppm shift range, where formaldehyde would be expected to absorb. The estimated detection limit of formaldehyde in this system was 60 μg/g shampoo.[13]

There is no rigorous chemical evidence available to support the claim that Quaternium-15 decomposes to ("releases") formaldehyde in aqueous solution.

Analytical Methods

Several techniques have been utilized for the isolation and identification of quaternary ammonium compounds, including colorimetric tests, gas chromatography, gas–liquid column chromatography and ion exchange resin columns.[14] Quaternium-15 has also been determined quantitatively by splitting off formaldehyde and subsequent reaction with dimedone.[15] Thin-layer chromatography (TLC) has been used to separate and identify Quaternium-15 and other preservatives in various formulations.[16–18] These TLC techniques have been further enhanced with bioautographic visualization. By growing bacteria on the TLC plates, the absence of bacterial growth at the preservative's location on the plate has enabled investigators to detect as little as 20 μg of Quaternium-15.[19]

Method of Manufacture

Quaternary compounds are prepared by reacting hexamine with the appropriate halocarbon in a nonaqueous solvent at room temperature.[10] The specific method for manufacturing Quaternium-15 is proprietary.

USE

Purpose in Cosmetics

Quaternium-15 is included in cosmetic formulations as an antimicrobial agent.[5,20]

Scope and Extent of Use in Cosmetics

The majority of the 13 cosmetic product categories listed by the Food and Drug Administration (FDA) contain products preserved by Quaternium-15. A total of 1015 cosmetic products reported to the FDA in 1981 by cosmetic companies participating in the voluntary cosmetic registration program contained Quaternium-15 in concentrations ranging from ≤0.1% to 1%.[21] This preservative is used in skin care lotions (284 products), eye makeup (290 products), facial makeup (212 products), and noncoloring hair products (142 products) and is found less frequently in bath products (22 formulations), fragrant powders (44 products), and other miscellaneous cosmetics.[21] The cosmetic product formu-

lation data that are made available by the FDA are compiled through voluntary filing of such data in accordance with Title 21 part 720.4 of the Code of Federal Regulations.[22] Ingredients are listed in prescribed concentration ranges under specific product type categories. Since certain cosmetic ingredients are supplied by the manufacturer at less than 100% concentration, the value reported by the cosmetic formulator may not necessarily reflect the actual concentration found in the finished product; the actual concentration in such a case would be a fraction of that reported to the FDA. The fact that data are only submitted within the framework of preset concentration ranges also provides the opportunity for overestimation of the actual concentration of an ingredient in a particular product. An entry at the lowest end of a concentration range is considered the same as one entered at the highest end of that range, thus introducing the possibility of a 2- to 10-fold error in the assumed ingredient concentration. See Table 2 for the list of cosmetic formulations containing Quaternium-15.

Quaternium-15 is also used by cosmetic manufacturers in Europe. The European Economic Community (EEC) has included Quaternium-15 (listed as methanamine 3-chloroallylochloride) on its list of regulated preservatives that cosmetic products may contain. The EEC's maximum authorized concentration of Quaternium-15 in cosmetic formulations is 0.2%.[23]

Surfaces and Frequency of Cosmetic Application

Products containing Quaternium-15 are applied to all areas of the skin, hair, nails, and mucous membranes and have the potential to come into contact with the eyes. These products are applied infrequently (cuticle softener) or up to several times a day (moisturizing lotions) and have the potential for remaining in contact with the skin for long periods of time over a span of several years.

Noncosmetic Use

Quaternium-15 is used as a preservative in adhesives and food packaging materials and is regulated by the FDA as an indirect food additive. The Code of Federal Regulations (CFR) authorizes Quaternium-15 (listed as 1-[3-chloroallyl]-3,5,7-triaza-1-azoniaadamantane chloride) for use as a "preservative only" in adhesives (21 CFR part 175.105), paper, or paperboard in contact with aqueous and fatty foods (21 CFR part 176.170), and polyurethane resins as food contact surfaces.[22] The over-the-counter (OTC) drug ingredient advisory review panel on topical analgesics, antirheumatics, otic, burn, sunburn treatment, and preventative products has made the following recommendation concerning Quaternium-15[24]:

> [It is] classified as an inactive ingredient or pharmaceutical necessity. When used in concentrations at the level of or above minimum effective dose, it is considered an active ingredient.

Quaternium-15 is also utilized as an antimicrobial agent in water-based metalworking fluids.[25]

TABLE 2. Product Formulation Data: Quaternium-15[21]

Product category	Total no. of formulations in category	Total no. containing ingredient	No. of product formulations within each concentration range (%)	
			>0.1–1	≤0.1
Baby shampoos	35	2	1	1
Baby lotions, oils, powders, and creams	56	1	1	–
Other baby products	15	2	–	2
Bath oils, tablets, and salts	237	4	–	4
Bubble baths	475	6	2	4
Bath capsules	3	2	–	2
Other bath preparations	132	10	2	8
Eyebrow pencil	145	1	–	1
Eyeliner	396	31	20	11
Eye shadow	2582	146	35	111
Eye lotion	13	3	–	3
Eye makeup remover	81	3	3	–
Mascara	397	88	55	33
Other eye makeup preparations	230	17	8	9
Fragrance powders (dusting and talcum, excluding aftershave talc	483	32	2	30
Sachets	119	5	–	5
Other fragrance preparations	191	6	–	6
Hair conditioners	478	26	13	13
Hair sprays (aerosol fixatives)	265	3	1	2
Permanent waves	474	4	3	1
Hair rinses (noncoloring)	158	10	1	9
Hair shampoos (noncoloring)	909	81	33	48
Tonics, dressings, and other hair grooming aids	290	4	3	1
Wave sets	180	4	2	2
Other hair preparations (non-coloring)	177	6	1	5
Hair dyes and colors (all types requiring caution statement and patch test)	811	3	1	2
Blushers (all types)	819	18	11	7
Face powders	555	10	4	6
Makeup foundations	740	61	14	47
Makeup bases	831	102	11	91
Rouges	211	8	1	7
Other makeup preparations (not eye)	530	13	4	9
Cuticle softeners	32	1	–	1
Nail creams and lotions	25	2	–	2
Other manicuring preparations	50	5	2	3
Bath soaps and detergents	148	9	5	4
Deodorants (underarm)	239	2	1	1
Men's talcum	13	1	–	1

TABLE 2. (Continued)

Product category	Total no. of formulations in category	Total no. containing ingredient	No. of product formulations within each concentration range (%)	
			>0.1–1	≤0.1
Other shaving preparation products	29	1	1	–
Skin cleansing preparations (cold creams, lotions, liquids, and pads)	680	60	18	42
Face, body, and hand skin care preparations (excluding shaving preparations)	832	81	27	54
Hormone skin care preparations	10	2	2	–
Moisturizing skin care preparations	747	78	35	43
Night skin care preparations	219	10	3	7
Paste masks (mud packs)	171	6	1	5
Skin fresheners	260	6	4	2
Wrinkle smoothers (removers)	38	1	1	–
Other skin care preparations	349	18	10	8
Suntan gels, creams, and liquids	164	16	8	8
Indoor tanning preparations	15	1	1	–
Other suntan preparations	28	3	3	–
1981 TOTALS		1015	354	661

BIOLOGY

Antimicrobial Activity

The antimicrobial activity of Quaternium-15 has been established by extensive testing. It is usually effective at concentrations of 0.1–0.2% by weight, being particularly effective against *Pseudomonas* and other bacteria and not as effective against yeasts and molds[5,6] (Table 3).

The minimum inhibitory concentration (MIC) of Quaternium-15 against *Enterobacter aerogenes* was determined using standard tube dilution techniques. Fresh and aged (3 months at room temperature) aqueous solutions of Quaternium-15 were tested. The MIC for fresh, 0.2% Quaternium-15 was 500 ppm. The MIC was 500 ppm for aged 0.2% Quaternium-15, 900 ppm for an aged 2.0% solution, and 3125 ppm for an aged 20.0% solution of Quaternium-15.[7]

Schanno et al.[26] found formulations of hair shampoos, conditioners, and hand creams containing 0.3 or 0.15% Quaternium-15 to be effective in 28-day trials against gram-negative *Escherichia coli* and *Pseudomonas aeruginosa*, gram-

TABLE 3. Minimum Concentration of Quaternium-15 for Inhibition of Microbial Growth[28]

pH	*Pseudomonas and other gram-negative bacteria*	*Yeasts*	*Molds*	*Cocci*	*Bacillus sp.*
4	100–900 ppm	350–2300 ppm	400–450 ppm	–	–
5.5	150–>4000 ppm	>4000 ppm	100–3300 ppm	150 ppm	200 ppm
7	150–2000 ppm	700–2000 ppm	750–4000 ppm	150–200 ppm	250 ppm

positive *Staphylococcus aureus*, the mold *Aspergillus niger*, and the yeast *Candida albicans*.

The effectiveness of Quaternium-15 as a preservative in mascara was tested in the laboratory and with human in-use tests for 9–11 weeks. Of seven preservatives tested, imidazolidinyl urea, Quaternium-15, and phenylmercuric acetate were most effective against *Staphylococcus epidermidis* and *P. aeruginosa*.[27]

Pharmacokinetics

The absorption, distribution, metabolism and excretion of Quaternium-15 have been studied in female Fischer 344 rats.[29] For these studies either the hexamethylenetetramine ring or the chloropropene side chain was labeled. The ring was uniformly labeled with ^{14}C; the side chain was labeled with ^{14}C at the C-2 position. Three rats received either 5 or 75 mg/kg of Quaternium-15 applied dermally or given orally. In addition, 3 rats received 5 mg/kg iv. Except for the iv studies, both the ring and side chain-labeled compounds were studied at each dose and route. Standard procedures for collecting urine, feces, blood, and so on were employed. Almost complete absorption of Quaternium-15 (84–88% of dose) was achieved in 48 h after oral administration, whereas only 1–2% 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 ($t_{1/2}$ = 1.3 ± 0.1 h), then slow ($t_{1/2}$ = 22.7 ± 2.0 h). Quaternium-15 that reached a systemic circulation was metabolized extensively. For example, after oral administration as much as 40–50% of the ^{14}C derived from the ring appeared in the expired air as "CO_2" (2-methoxyethanol:ethanolamine-soluble compounds). The extent of metabolism was influenced by the route of administration, since as little as 5% of ring-derived CO_2 was found after dermal application. Ion exclusion chromatography and high-performance liquid chromatography were used to identify the presence of metabolites in the urine of treated rats. 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 using GC-MS (chemical ionization) and fast bombardment mass spectrometry.

ANIMAL TOXICOLOGY

Acute Oral Toxicity

Quaternium-15 was moderately toxic via a single oral dose to male and female CDF (Fischer 344 derived) rats. Six male and six female rats per dose were given 200, 400, 800, 1600, 3200, or 6300 mg/kg Quaternium-15 by gavage and observed for signs of toxicity for 2 weeks. No rats died after receiving a dose of 1600 mg/kg or less. Five of the six male and five of the six female rats died after receiving 3200 mg/kg Quaternium-15, and all rats given 6300 mg/kg Quaternium-15 died within 24 h. No signs of toxicity were observed in rats given 200 or 400 mg/kg. Rats dosed with 800 or 1600 mg/kg Quaternium-15 were lethargic, had diarrhea, and had closure of the eyelids and/or lacrimation. In addition to these signs of toxicity, staining exudates of the nares and body tremors were observed in the 3200 mg/kg-dosed rats. No treatment-related changes were observed upon necropsy of surviving rats. The LD_{50} for both male and female rats was 2664 mg/kg with a 95% confidence interval of 1836–3512 mg/kg.[30]

Groups of 5 male and 5 female Sprague-Dawley rats were given a single oral dose of 126, 252, 1000, or 2000 mg/kg Quaternium-15 in a 10% aqueous solution. In the female rats, the number of deaths per group (5 animals total per group) in order of ascending dose was 2, 1, 0, 2, and 5, and the group LD_{50} was 1070 mg/kg with a 95% confidence interval of 768–1490 mg/kg Quaternium-15. In male rats, no deaths occurred in the 126 and 252 mg/kg-dosed group, 1 rat died of the 5 rats given 500 mg/kg, 2 rats died in the 1000 mg/kg group, and all 5 rats died following the single 2000 mg/kg dose of Quaternium-15. The LD_{50} for male Sprague-Dawley rats was 940 mg/kg with a 95% confidence interval of 612–1440 mg/kg.[31]

Four groups of 5 female Sprague-Dawley rats were given a single oral dose by gavage of a 50% aqueous solution of Quaternium-15 for total doses of 252, 500, 1000, or 2000 mg/kg Quaternium-15. The rats were evaluated for signs of in vivo toxicity and gross lesions. There were no signs of toxicity at doses of 252 or 500 mg/kg. Dosage of 1000 mg/kg Quaternium-15 resulted in slight lethargy, piloerection, and wetness in the perineal region after treatment. Rats given 2000 mg/kg were lethargic, had piloerection, had dark deposits around the eyes, and had diarrhea following treatment. No lesions were observed in rats submitted for pathological evaluation from the 2000 mg/kg group. No rats died after single doses of up to 1000 mg/kg Quaternium-15. Four of the five rats given 2000 mg/kg died, and the LD_{50} for this group was 1552 mg/kg with a 95% confidence interval of 906–2684 mg/kg.[31]

The oral toxicity of a cosmetic cleanser containing 0.2% Quaternium-15 was evaluated using 5 rats. Fifteen g/kg of the undiluted product was administered by gavage; the rats were then observed for 7 days. One rat died 24 h after intubation. The product was classified as nontoxic via ingestion with an LD_{50} greater than 15 g/kg.[32]

Of the species tested, New Zealand white rabbits were the most sensitive to Quaternium-15 via oral ingestion. Four groups of 5 female rabbits were given a single oral dose of 31.6, 63, 126, or 252 mg/kg in a 50% aqueous solution of Quaternium-15. No signs of toxicity were observed in rabbits given 31.6 mg/kg,

and there were no deaths in this group. Rabbits given 63–252 mg/kg Quaternium-15 were slightly lethargic following ingestion. One rabbit died after receiving 63 mg/kg, and all rabbits died after being given either 126 or 252 mg/kg Quaternium-15. The LD_{50} for this group was 78.5 mg/kg, which is classified as very toxic via oral ingestion.[31]

The oral toxicity of Quaternium-15 was also evaluated in male chicks and male Hartley guinea pigs. Groups of 5 animals were given 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}s of 710 mg/kg and 2800 mg/kg, respectively[31] (Table 4).

TABLE 4. Acute Oral LD_{50} Values[30–32]

Animal species, strain, and sex	*Preparation fed*	*LD_{50} (mg/kg)*	*95% confidence interval (mg/kg)*
Sprague-Dawley (S-D) rats, male	10% aqueous solution	940	612–1440
S-D rats, female	10% aqueous solution	1070	768–1490
S-D rats, female	50% aqueous solution	1552	906–2684
CDF rats, male and female	Unspecified	2664	1836–3512
Hartley guinea pigs, male	10% aqueous solution	710	–
Chicks, male	Powder (in capsule)	2800	–
New Zealand white rabbits, female	50% aqueous solution	78.5	45–136

Dermal Toxicity, Irritation, and Sensitization

Acute Dermal Toxicity

The acute percutaneous toxicity of a 50% aqueous solution of Quaternium-15 was evaluated using groups of 2 male and 2 female rabbits. The entire trunk of the rabbits was shaved, then treated with 250, 500, 1000, or 2000 mg/kg Quaternium-15. The treated site was covered by occlusive patches for 24 h, rinsed, and made inaccessible by a collar for a further 72 h. Animals were observed for 2 weeks for signs of toxicity. Topical responses of 13 rabbits at the treatment site were observed 24 h after application and were 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. Topical irritation data were reported without regard to dose concentration. 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: 250 mg/kg, 1/4 (number dead/number treated); 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 102–1559 mg/kg.[30]

Another acute percutaneous toxicity study of Quaternium-15 was conducted using rabbits as described above but with the following modifications. Groups of 5 rabbits of both sexes received 252, 500, 1000, or 3980 mg/kg on intact skin. Two groups of 3 rabbits were administered 2000 mg/kg Quaternium-15 to intact or abraded skin. An additional two groups of 3 rabbits each were given

3980 mg/kg powdered Quaternium-15 on intact or abraded skin. All animals given Quaternium-15 on abraded skin died. The number of deaths following administration to intact skin was dose dependent; 252 mg/kg, 2/5 (number dead/number treated); 500 mg/kg, 2/5; 1000 mg/kg, 3/5; 2000 mg/kg, 5/5 and 2/3; 3980 mg/kg, 4/5; 3980 mg/kg powdered Quaternium-15, 0/3. The combined acute percutaneous LD_{50} for the group was 565 mg/kg with a 95% confidence interval of 227–1400 mg/kg.[31]

The acute percutaneous toxicity of Quaternium-15 has also been evaluated using rats. Three groups of 2 rats were given 500, 1000, or 2000 mg/kg Quaternium-15 as a 50% aqueous solution; the test site was then covered for 6.5 h and finally washed. All animals appeared normal during and after the exposure to Quaternium-15, and no animals died at any concentration of the preservative.[31]

Subchronic Dermal Toxicity

The dermal toxicity of Quaternium-15 was evaluated on the intact and abraded skin of sexually immature rabbits. Ten groups of 10 rabbits (5 males and 5 females) were given 0, 10, 25, 50, or 100 mg/kg per day Quaternium-15 in a 20% (wt/vol) aqueous solution. One group of rabbits at each dose received Quaternium-15 on abraded skin, and the test sites of the other groups were intact. The test material (tap water for the controls) remained in contact with the skin 7 h per day, 5 days per week for 3 weeks. Animals given 10 and 25 mg/kg per day did not differ significantly 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. Animals in the 50 mg/kg group had slightly decreased spermatogenesis and slight skin irritation at the site of application. There were statistically significant decreases in absolute testes weight and in the testes to body and brain weight ratios in all animals given 100 mg/kg per day. 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.[33]

A follow-up study on groups of 7 male rabbits was conducted in an attempt to confirm the testicular atrophy and decreased spermatogenesis reported above. Sexually mature male rabbits were given 0 (water control), 25, 50, or 100 mg/kg per day Quaternium-15 5 days per week for 30 days. The Quaternium-15 was administered as a 20% (wt/vol) solution to abraded skin, and was washed off after 7 h of contact (plastic 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 dose related. At necrospy 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 depressed in animals receiving 50 and 100 mg/kg per day. 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.[34]

The subchronic dermal toxicity of prototype cosmetic formulations containing 3.0, 1.0, or 0.1% Quaternium-15 was studied using 50 rabbits. Five groups of 10 rabbits (5 males and 5 females) were given daily applications of 1.0 ml/kg per day tap water (water control), 1.0 mg/kg per day base cosmetic formulation (base control), or 1 ml/kg per day of the prototype cosmetic formulations, which resulted in a dose of 31.3, 10.5, or 1.04 mg/kg per day 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 per day or below did not present any hazard due to absorption of the test material through the skin.[35]

A 13-week subchronic dermal toxicity test was conducted on a cleanser (wipe-off) containing 0.2% Quaternium-15. Fifteen female rats were given daily doses 5 days per week of 3.0 ml/kg product, which resulted in a dose of 3000 mg/kg per day Quaternium-15. 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. Skin hyperkeratosis 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[36] (Table 5).

Primary Skin Irritation

The skin irritancy of undiluted Quaternium-15 was tested on the intact and abraded skin of 6 female New Zealand rabbits. Occlusive patches containing 0.5 g Quaternium-15 were applied to an intact and abraded site on each animal and left in place for 24 h. Test sites were scored immediately and 48 h 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 (max = 8.0). Undiluted Quaternium-15 was a mild primary skin irritant.[30]

Five rabbits were evaluated for primary skin irritation following exposure to powdered Quaternium-15 or a 10, 5, or 1% aqueous solution of Quaternium-15. 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 14-day period to the uncovered ear. The 1 and 5% aqueous solutions caused no irritation at any site. Ten percent aqueous Quaternium-15 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.[31]

The primary irritation of Quaternium-15 in aqueous solution and Eucerin* anhydrous ointment was evaluated using two groups of 10 albino guinea pigs of both sexes. The animals received ten 0.2-g applications (over 2 weeks) of 5 and 10% aqueous Quaternium-15 or 5 and 10% Quaternium-15–Eucerin to the shaved skin of the right flank. The test sites were not covered by occlusive patches, 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 four skin samples taken from animals treated with 10% aqueous Quaternium-15.[37]

Ten male guinea pigs received a single, open application of 5% Quaternium-15 in a Eucerin anhydrous preparation. No skin erythema or alteration was observed 6 and 24 h after application.[38]

A single insult, occlusive patch test was performed to evaluate the irritancy of a cleanser (wipe-off) containing 0.2% Quaternium-15. The undiluted product was administered to 9 rabbits, and 9/9 and 8/9 rabbits had slight erythema 2 and 24 h, respectively, after patch removal. The product was slightly irritating with a group PII of 0.78 of a maximum 4.0.[39]

Sensitization

A 10% solution (wt/vol) of Quaternium-15 in PPG-2 methyl ether/polysorbate 80 (9:1) was evaluated for sensitization potential in guinea pigs by a modification of the method of Maguire.[40] Ten guinea pigs were given four 48-h induction occlusive patches containing 0.1 ml of the test material. The third induction patch was accompanied by a 0.2 ml intradermal injection of Freund's adjuvant adjacent to the insult site. A second group of 10 guinea pigs served as a positive control and was given 10% epoxy resin in PPG-2 methyl ether/polysorbate 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 ten 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 h after the challenge application of Quaternium-15, and 2/4 of these reactions had cleared by 48 h. One of these reactions was possibly a weak sensitization response.[30]

Two guinea pig sensitization tests were conducted using 1% aqueous Quaternium-15 and one test was conducted with 10% Quaternium-15 in a 9:1 mixture of PPG-2 methyl ether and polysorbate 80. Ten animals per test preparation per method were used. The first method was identical to the previously described test,[40] with four occlusive induction patches, an injection of Freund's adjuvant, challenge after 2 weeks, and a positive control utilizing 15% epoxy resin in PPG-2 methyl ether and polysorbate 80. The second method consisted of a total of six 0.1-ml occlusive 24-h induction patches administered twice a

*Eucerin is 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.

TABLE 5. Animal Dermal Toxicity

Test type	*No. of animals*[a]	*Vehicle or product type*	*Quaternium-15 dose (mg/kg)*	*Condition of application site*	*Length of study (days)*	*Comments*	*Reference*
Acute: single 24-h occluded patch	4	50% aqueous solution	250	Intact	14	Topical responses included erythema, edema, and necrosis; lethargy, anorexia and shallow breathing observed; LD_{50}, 605 mg/kg	30
	4	50% aqueous solution	500	Intact	14		
	4	50% aqueous solution	1000	Intact	14		
	4	50% aqueous solution	2000	Intact	14		
Acute: single 24-h occluded patch	5	50% aqueous solution	252	Intact	14	Mortality from application to intact skin dose dependent; all animals given Quaternium-15 to abraded skin died; combined LD_{50}, 565 mg/kg	31
	5	50% aqueous solution	500	Intact	14		
	5	50% aqueous solution	1000	Intact	14		
	5	50% aqueous solution	2000	Intact	14		
	5	50% aqueous solution	3980	Intact	14		
	3	50% aqueous solution	2000	Abraded	14		
	3	50% aqueous solution	2000	Intact	14		
	3	None	3980	Intact	14		
	3	None	3980	Abraded	14		
Acute: single 6.5-h occluded patch	2 rats	50% aqueous solution	500	Intact	14	Exposure period limited to 6.5 h due to trauma caused by restraint; no animals died	31
	2 rats	50% aqueous solution	1000	Intact	14		
	2 rats	50% aqueous solution	2000	Intact	14		
Subchronic: 7½ h contact, 5 days/week	5	Tap water	Control	Intact	21	No toxicity in 10 and 25 mg/kg per day groups; slightly decreased spermatogenesis in 50 and 100 mg/kg	33
	5	Tap water	Control	Abraded	21		
	5	20% in water	10	Intact	21		

	5	20% in water	10	Abraded	21	per day intact and abraded groups; decreased testicular weights and application site irritation in 100 mg/kg per day intact and abraded groups	
	5	20% in water	25	Intact	21		
	5	20% in water	25	Abraded	21		
	5	20% in water	50	Intact	21		
	5	20% in water	50	Abraded	21		
	5	20% in water	100	Intact	21		
	5	20% in water	100	Abraded	21		
Subchronic: 7 h contact, 5 days/week	7 male	Tap water	Control	Abraded	30	Dose-related inflammation at application site; liver weights depressed in 50 and 100 mg/kg per day groups; no other signs of toxicity or testicular effects were treatment related	34
	7 male	20% in water	25	Abraded	30		
	7 male	20% in water	50	Abraded	30		
	7 male	20% in water	100	Abraded	30		
Subchronic: ~8 h contact, 5 days/week	10	0.1% in prototype cosmetic	1.04	Intact	91	No treatment-related signs of toxicity	35
	10	1% in prototype cosmetic	10.5	Intact	91		
	10	3% in prototype cosmetic	31.3	Intact	91		
Subchronic: 5 days/week	15	0.2% in cleanser (wipe-off)	3000	Intact	91	Minimal skin irritation in 3 animals; no other signs of toxicity as compared to an untreated, concurrent control group	36

[a]Rabbits, unless otherwise specified.

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. Tests sites were observed for sensitization response 24 and 48 h after challenge application. No animals reacted to the 1% aqueous Quaternium-15, 7/10 animals were sensitized to 10% Quaternium-15, and 18/20 animals were sensitized to the positive control. Quaternium-15 appeared to be a skin sensitizer in situations where gross contact is likely or in the presence of a penetrating solvent. Prolonged and/or repeated skin contact with "gross" amounts of Quaternium-15 was not advised.[41]

An extensive study comparing several sensitization testing methods was conducted by Marzulli and Maguire.[42] Quaternium-15 was one of 11 compounds tested in 5 guinea pig sensitization assays. Each test was conducted three 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 in the challenge phase of the test. The assay methods and results were:

The Draize[43] guinea pig technique involved a series of 10 intradermal injections administered three times a week and a challenge injection administered to an untreated site after a 2-week nontreatment period. No sensitization was produced by 0.1% Quaternium-15 in saline.

In the Buehler[44] assay, 0.5 ml of the test material was administered in occlusive, 6-h patches on days 1, 7, and 14. On day 24, a 24-h occlusive challenge patch was administered to an untreated site, and the sites were scored for reactions to Quaternium-15, 24 and 48 h after patch removal. No reactions were observed in the Buehler assay.

A Magnusson and Kligman[45] maximization test resulted in a large proportion (55%) of guinea pig sensitization to Quaternium-15. The animals were given three sets of two intradermal injections to an induction site on the back: (1) complete Freund's adjuvant in distilled water, (2) 5% Quaternium-15 in saline, and (3) 10% Quaternium-15 in saline and Freund's adjuvant. The injections were given over 6 days, and if the induction site was not significantly irritated, 5% sodium lauryl sulfate was administered to produce irritation. On day 7, a 0.5 ml occlusive patch (5% Quaternium-15 in petrolatum) was administered to the induction site. After a 2-week nontreatment period, a 24-h occlusive challenge patch was administered to a clipped area of the flank. The number of sensitization reactions recorded for the three rounds were 9/10, 2/10, and 5/9. Quaternium-15 was a guinea pig sensitizer in the maximization test.

In the split-adjuvant assay,[46] a dressing containing a window was placed over a clipped, shaved, and frozen (by dry ice) induction site on the guinea pig's flank. Two tenths of a milliliter of test material was applied through the window, under an occlusive patch for 48 h. 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 complete Freund's adjuvant directly adjacent to the induction site. A challenge was made on day 22 using the maximization test technique. Quaternium-15 was a sensitizer in this assay, causing 11/30 reactions.

The final test was a cyclophosphamide/complete Freund's adjuvant bioassay.[46] Three days prior to induction, test animals were given an intraperitoneal injection of 150 mg cyclophosphamide/kg body weight. A dressing containing a window was applied to a clipped, shaved, and frozen induction site on the flank, under occlusive patches; patches containing 0.2 ml test material were administered through the window on days 0, 1, 2, 3, and 4. The patch on day 4 was accompanied by injections of complete Freund's adjuvant as described in the split-adjuvant assay. A final 6-h 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. Quaternium-15 was a sensitizer in this assay, with 9/30 animals reacting to the challenge patch.

A group of 30 albino guinea pigs of both sexes were given four intracutaneous injections on four consecutive days of 0.5 ml isotonic 0.5% Quaternium-15. These injections resulted in a dose of 1 mg Quaternium-15 per animal per day. A 0.03 ml injection of complete Freund's additive was administered with the first and third induction injections. An additional group of 10 guinea pigs was given 0.5 ml intracutaneous injections of 0.5% Quaternium-15 for 10 consecutive days, excluding Sunday. After an 18-day nontreatment period, a challenge test was conducted with 1 and 5% Quaternium-15 in Eucerin anhydrous ointments. 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. In the group of 30 animals, 2 guinea pigs had weakly visible erythema 24 h 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 6/10 animals in the second group, and no microscopic indication of contact sensitization was observed.[38]

Groups of 10 male Hartley albino guinea pigs were used to evaluate the skin sensitization potential of four samples of Quaternium-15. The samples were tested as 2% aqueous solutions (40 animals) and 2/4 samples were additionally tested as 2% suspensions in petrolatum (20 animals). 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 four induction applications per test or control preparation, then 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.[47]

Twenty guinea pigs that had previously been used in a 2-week primary irritation study with 5 and 10% Quaternium-15 were evaluated for sensitization to Quaternium-15 following a 16-day nontreatment period. Challenge tests consisted of single, simultaneous applications of 0.1, 0.5, 1, and 5% Quaternium-15 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% solu-

tions of Quaternium-15 were applied to previously untreated sites. No inflammatory reactions were observed 24 and 48 h after challenge.[37]

Ten guinea pigs were given 15 cutaneous applications of 5% aqueous Quaternium-15 over a 3-week period. After a 1-week nontreatment period, a challenge test with 0.1, 0.5, 1, and 5% aqueous Quaternium-15 was performed on a previously untreated site. Challenge applications were repeated once a week for 6 weeks. No sensitization reactions were observed.[37]

Two groups of 10 guinea pigs were used in an intracutaneous sensitization test of Quaternium-15. The first group was given four consecutive daily injections of 0.5% Quaternium-15 in 0.9% NaCl, for a 4-day total of 4 mg Quaternium-15 per animal. Animals also received an injection of complete Freund's adjuvant on days 1 and 3. Animals were challenged 14 days after induction with 0.1, 0.5, 1, and 5% Quaternium-15 in aqueous solution and Eucerin ointment. An additional five challenges were administered at weekly intervals. There were no sensitization reactions in this group. The second group of 10 guinea pigs had sensitization reactions to 2,4-dinitro-1-chlorobenzene in a previous study. The animals were given four intracutaneous injections of 0.03 ml Freund's additive. Following 3 nontreatment days, the animals received ten 0.5 ml injections of 5% Quaternium-15 in saline. The injections were given daily, 5 days a week for 2 weeks. The challenge test with 1 and 5% Quaternium-15 in aqueous solution and Eucerin ointment followed an 18-day nontreatment period. Subsequent challenge tests with 0.1, 0.5, 1, and 5% solutions and ointments were repeated weekly for 6 weeks. No sensitization reactions to Quaternium-15 were observed throughout the study.[37]

Ocular Toxicity

Six rabbits were given a single 0.1 g instillation of pure Quaternium-15 into the conjunctival sac of the right eye. The left eye served as an untreated control. Three rabbits had slight irritation and one animal had moderate irritation and a slight discharge after 24 h. All signs of irritation had resolved by 72 h, and the group ocular irritation score was 1.7 (max = 110). An additional three rabbits had 0.1 g instilled into the right eye, then the eye was rinsed after 30 seconds. Two of the three rabbits had slight irritation at 24 h, which disappeared by 48 h postexposure. The group eye irritation score was 1.3. Under these test conditions, Quaternium-15 was practically nonirritating to the eye.,[30]

The ocular irritancy of 1, 3, 5, and 10% aqueous solutions of Quaternium-15 was evaluated in 4 New Zealand rabbits. One milliliter of the test material was instilled into the right eye three times/day for 5 days of the first week. The left eye served as a control. One-tenth milliliter of the respective solutions was instilled 3 times/day, 5 days a week for the remaining 2 weeks of the study. An additional control rabbit was used in the second and third weeks of the study, and it was given 0.1 ml distilled water in both eyes on the same treatment schedule. There were no signs of irritation or corneal injury in test or control animals throughout the 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.[48]

A cleanser (wipe-off) containing 0.2% Quaternium-15 was tested for eye irri-

tation in rabbits. A single application of the undiluted product was instilled in the eyes of 6 rabbits. Slight conjunctival irritation was observed in 3 rabbits, which cleared by day 4. The product was a mild eye irritant.[49]

Six New Zealand rabbits were treated once in one eye with a mascara containing 0.2% Quaternium-15. Slight conjunctivitis was observed 1 h after treatment and cleared in 24–48 h. There was no irritation of the cornea or iris. The mascara was mildly irritating.[50]

Two lots of a mascara containing 0.2% Quaternium-15 were tested for ocular irritation on groups of 6 rabbits. Each rabbit had 0.1 ml of undiluted mascara instilled in one 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 h after exposure, and this change cleared within 48 h. Slight conjunctivitis was observed 1 h after treatment with the other lot of mascara, but this change cleared in 72 h. The product was a mild eye irritant[51] (Table 6).

Mutagenicity

Quaternium-15 was evaluated for mutagenic activity in an Ames Standard Plate Incorporation Test using five strains of *Salmonella typhimurium*, TA98, TA100, TA1535, TA1537, and TA1538. Quaternium-15 was not mutagenic in concentrations of 25–500 μg/plate with or without metabolic activation.[52]

The genotoxic activity of Quaternium-15 was evaluated in a rat hepatocyte unscheduled DNA synthesis assay. Concentrations of 4×10^{-8} to 2×10^{-1} M Quaternium-15 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 significant DNA synthesis was observed in any other test concentration of Quaternium-15, which indicated a lack of genotoxic activity under the test conditions.[53]

Teratogenicity

An extensive study on the teratogenicity of orally administered Quaternium-15 was performed 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 among rats administered 100, 200, or 400 mg/kg per day Quaternium-15. Based on these results, groups of 33 or 34 bred rats were given 0, 5, 25, or 75 mg/kg per day Quaternium-15 via gavage on days 6–15 (period of most organogenesis) of gestation. The Quaternium-15 was administered in aqueous solution, and control animals were given an equivalent volume of distilled water. Food and water consumption during gestation was monitored, and during the first 14 days of pregnancy dams of the 25 and 75 mg/kg groups ate significantly less food than controls. The 75 mg/kg group also consumed significantly less water on days 9–11 and significantly

TABLE 6. Animal Primary Skin Irritation, Sensitization and Ocular Toxicity

Test type[a]	*No. of animals and species*	*Vehicle or product type*	*Quaternium-15 dose*	*Length of study*	*Comments*	*Reference*
			PRIMARY SKIN IRRITATION			
Single insult: 24-h occluded patch	6 rabbits	None	0.5 g	48 h	Redness (4/6) and swelling (6/6) observed; PII = 1.2 out of 8.0 max; mild irritant	30
Repeat insult: occluded patch	1 rabbit	Water	1%	14 days	No irritation to intact or abraded skin or skin of the ear; not an irritant	31
	1 rabbit	Water	5%	14 days	No irritation at ear, intact, or abraded sites; not an irritant	
	1 rabbit	Water	10%	14 days	No irritation to ear site; redness on intact and abraded sites; scabbing and scarring at abraded sites; mild irritant	
	1 rabbit	–	100%	14 days	Dry patch slightly irritating to abraded skin; mild irritant	
	1 rabbit	–	100%	14 days	Wet patch moderately irritating to intact and abraded skin causing redness, swelling, necrosis, scabbing and scarring; moderate irritant	
Repeat insult: non-occluded	10 guinea pigs	Water and Eucerin	5%	14 days	No irritation observed; skin biopsies from 4 animals in 10% group were normal; not an irritant	37
	10 guinea pigs	Water and Eucerin	10%	14 days		
Single insult: non-occluded	10 guinea pigs	Eucerin	5%	24 h	No irritation 6 and 24 h after application; not an irritant	38
Single insult: occluded patch	9 rabbits	Cleanser (wipe-off)	0.2%	48 h	Slight erythema 2 and 24 h after patch removal; PII = 0.78 out of 4.0 max; product slightly irritating	39
			SENSITIZATION[b]			
Maguire: occluded induction patches, injections of	10 guinea pigs	PPG-2 methyl ether/polysorbate 80	10%	21 days	7/10 positive reactions in positive controls; erythema and edema in 4/10 test animals, one reaction possibly sensitization; no test animals reacted to vehicle control; probably not significant sensitizer	30
Freund's adjuvant, open challenge	10 guinea pigs	PPG-2 methyl ether/polysorbate 80%	Positive control (10% epoxy resin)	21 days		

Maguire or induction patches with no injections	10 guinea pigs	Water	1%	21 or 37 days	18/20 positive reactions to positive control; no reactions to 1% Quaternium-15; 7/10 sensitization reactions to 10% Quaternium-15; sensitizer at 10% in penetrating solvent	41
	10 guinea pigs	Water	1%	21 or 37 days		
	10 guinea pigs	PPG-2 methyl ether/polysorbate 80	10%	21 or 37 days		
	20 guinea pigs	PPG-2 methyl ether/polysorbate 80	Positive control (15% epoxy resin)	21 or 37 days		
Draize: induction and challenge intradermal injections	30 guinea pigs	Saline	0.1%	6 weeks	No reactions; not a sensitizer	42
Buehler: occluded induction and challenge patches	30 guinea pigs	Petrolatum	5%	4 weeks	No reactions; not a sensitizer	
Magnusson and Kligman maximization test	29 guinea pigs	Petrolatum	5%	3 weeks	16 positive reactions; sensitizer	
Spli-adjuvant: occlusive patches and injections of Freund's adjuvant	30 guinea pigs	Petrolatum	5%	3 weeks	11 positive reactions; sensitizer	
Cyclophosphamide/complete Freund's adjuvant: occluded patches and injections of cyclophosphamide and Freund's adjuvant	30 guinea pigs	Petrolatum	5%	3 weeks	9 positive reactions; sensitizer	
Intracutaneous induction injections, 7 challenge patches at weekly intervals	30 guinea pigs 10 guinea pigs	Eucerin	1 and 5%	10 weeks	30 and 10 animal groups received 4 and 10 induction injections, respectively, of 0.5% isotonic Quaternium-15; 2/30 animals had erythema at challenge; no reactions in other group; probably not a sensitizer	38

TABLE 6. (Continued)

Test type[a]	*No. of animals and species*	*Vehicle or product type*	*Quaternium-15 dose*	*Length of study*	*Comments*	*Reference*
Four induction applications, challenged with Quaternium-15 and formaldehyde	10 guinea pigs	Water	2%	18 days	All positive control animals were sensitized; test animals were challenged with Quaternium-15 and 37% formaldehyde; no sensitization responses to either material; cross-sensitization with formaldehyde unlikely; 2% Quaternium-15 not a sensitizer	47
	10 guinea pigs	Water	2%	18 days		
	10 guinea pigs	Water	2%	18 days		
	10 guinea pigs	Water	2%	18 days		
	10 guinea pigs	Petrolatum	2%	18 days		
	10 guinea pigs	Petrolatum	2%	18 days		
	10 guinea pigs	PPG-2 methyl ether/polysorbate 80	Positive control (10% epoxy resin)	18 days		
	10 guinea pigs	Water	Positive control (37% formaldehyde)	18 days		
Ten induction applications, simultaneous challenge with 4 concentrations of Quaternium-15	20 guinea pigs	Water and Eucerin	0.1, 0.5, 1, and 5%	30 days	No reactions; not a sensitizer	37
Fifteen induction applications, 7 challenge applications at weekly intervals	10 guinea pigs	Water	0.1, 0.5, 1, and 5%	10 weeks	No reactions; not a sensitizer	37

Induction injection, 6 challenge applications at weekly intervals	20 guinea pigs	Water and Eucerin	0.1, 0.5, 1, and 5%	10 weeks	10 animals had been sensitized in an earlier study to 2,4-dinitro-1-chlorobenzene; no reactions in any animals; not a sensitizer	37
			OCULAR TOXICITY			
Modified Draize	6 rabbits	–	0.1 g	7 days	Eyes were not washed; redness (4/6) and discharge (1/6) observed which cleared in 72 h; ocular irritation score = 1.7 of 110; practically nonirritating	30
	3 rabbits	–	0.1 g	7 days	Eyes were washed 30 sec after instillation of test material; irritation score = 1.3 out of 110; practically nonirritating	
Repeat applications	4 rabbits	Water	1, 3, 5, or 10%	21 days	No irritation observed; test animals "pawed" at their eyes after instillation; not an eye irritant	48
Single application	6 rabbits	Cleanser (wipe-off)	%	4 days	Slight conjunctival irritation in 3/6 rabbits which cleared by day 4; mild eye irritant	49
Single application	6 rabbits	Mascara	0.2%	7 days	Slight conjunctivitis which cleared by 48 h; mildly irritating	50
Single application, no wash	6 rabbits 6 rabbits	Mascara Mascara	0.2% 0.2%	7 days 7 days	1 animal in each group had slight conjunctivitis which cleared by 72 h; mildly irritating	51

[a]See text for complete description of procedure.
[b]"Dose" and "vehicle" refer to dose and vehicle of challenge application

more water on days 18–20 of gestation. Dams were killed on day 21 of gestation and the fetuses examined.

Seventy-five mg/kg per day of Quaternium-15 caused significant maternal and fetal toxicity and embryonic malformations as compared to 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 weight (g liver/100 g body weight) was significantly increased over controls. Fetal resorption was increased over concurrent control resorptions, and the fetuses weighed significantly less than controls. Eleven fetuses in seven litters had major malformations, primarily of the eye (microphthalmia). A significant increase in minor malformations was indicative of delayed development.

Twenty-five mg/kg per day Quaternium-15 did not cause maternal toxicity. However, pregnant rats had a transient decrease in food consumption. Twenty-five mg/kg per day Quaternium-15 did result in significant fetal malformations; major malformations were found in 10 fetuses in nine litters. The predominant malformation was eye anomalies. The administration of 5 mg/kg per day Quaternium-15 did not produce any evidence of maternal or fetal toxicity or malformations.

The investigators concluded that 25 and 75 mg/kg per day Quaternium-15 orally administered on days 6–15 of gestation was teratogenic in Fischer 344 rats. However, 5 mg/kg per day Quaternium-15 was below this teratogenic threshold.[54]

The dermal teratogenicity of Quaternium-15 was evaluated in groups of 25 bred, female Fischer 344 rats. Two hundred fifty or 500 mg/kg per day Quaternium-15 was dermally administered to the rats in a 50% aqueous solution on days 6–15 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 as compared to controls. There was no difference in incidences of resorptions between controls and the high-dose group. Quaternium-15 in doses up to and including 500 mg/kg per day was not teratogenic in rats. The results are consistent with the low rate of dermal absorption for this compound.[55]

CLINICAL ASSESSMENT OF SAFETY

Primary Skin Irritation

Contact Irritation in Healthy Individuals

Ten male and ten female panelists participated in a single-insult patch test to determine the irritancy of a 2% aqueous Quaternium-15 solution. A closed patch was applied to the shoulder area and kept in place for 24 h. The test sites were scored for irritation 20 minutes and 24 h after patch removal. Slight erythema was observed in three panelists at 20 minutes but cleared at 1 h after patch removal. No other signs of irritation were observed, and 2% aqueous Quaternium-15 was not considered a primary irritant to healthy skin.[56]

Single, 24-h occlusive patches containing 0.5, 1.0, and 2.5% Quaternium-15 in Eucerin were applied to 10 panelists. This procedure resulted in 0.5, 1.25, and 2.5 mg Quaternium-15 coming into contact with test sites on the back. Test sites were scored for irritation upon patch removal and 24 h later. No visible reactions were observed, and 2.5% Quaternium-15 was not considered a skin irritant.[38]

A cream shampoo was formulated with 0.2, 0.5, or 0% (control) Quaternium-15 and evaluated for irritation using 16 panelists. The test materials remained in contact with the test areas for 3 h. No irritation was observed.[38]

The irritancy of a cleanser (wipe-off) containing 0.2% Quaternium-15 was evaluated in a single-insult patch test. An occlusive patch of the undiluted product was administered to 20 panelists. A reference control was also 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.[39]

A moisturizer containing 0.3% Quaternium-15 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 23 h, rinsed, then scored for irritation 1 h 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 product was essentially nonirritating with a calculated irritation score of 0.83 (max = 630).[57]

Contact Irritation in Eczema Patients

Twenty eczema patients participated in a study to evaluate the irritancy of a 2% aqueous solution of Quaternium-15. Three patients were sensitive to formaldehyde, five were sensitive to nickel, four to phenylmercuryborate, and the remaining eight patients had eczema attributable to a variety of other agents. Twenty-four hour occlusive patches were administered to the shoulder area, and sites were observed for irritation 20 minutes and 24 h after patch removal. There were no positive reactions to 2% Quaternium-15. Quaternium-15 was not a primary irritant in eczema patients.[56]

Quaternium-15 in Eucerin was tested for primary skin irritation in 10 eczema patients. Single 24-h occlusive patches containing 0.5, 1.0, and 2.5% Quaternium-15 were applied to noneczematous sites. Test sites were scored for irritation at patch removal and 24 h later. No irritation was observed, and the test material was not a primary skin irritant.[38]

Three preparations of a hand cream were evaluated for skin irritancy in 32 eczema patients. The three formulations contained 0 (control), 0.2, or 0.5% Quaternium-15. The test materials were applied as 24-h occlusive patches to an eczema-free site on the shoulder, and sites were evaluated for irritation at patch removal and again 24 h later. The cream containing 0.2% Quaternium-15 did not produce irritation. Erythema was observed in 1 patient receiving 0.5% Quaternium-15, and 3 patients receiving the control cream. There was no significant difference in the number of reactions in test and control groups.[38]

Formulations of a cream shampoo varying in Quaternium-15 content were

evaluated for irritation in eczema patients using 24-h occlusive patches as outlined above. The test formulations of the shampoo contained 0 (control), 0.2, or 0.5% Quaternium-15. Fifty-six (group 1) patients were given the undiluted shampoos. Forty-two (group 2) patients received the shampoos as a 50% solution in Eucerin, and 38 patients (group 3) were given the three formulations as a 25% solution in Eucerin. For the most part, the same patients were used in groups 2 and 3. Forty-four of the 56 patients given undiluted formulations had severe erythema 24 and 48 h after application of the three formulations. Microscopic changes indicated an irritant reaction resembling "hyperemia" rather than a dermatitic reaction. In group 2 (50% solution), 6/42 patients had severe erythema at test sites, and 14/42 had slight erythema. Fifteen patients in group 3 (25%) reacted to the three formulations with erythema. Dilution of the shampoos with Eucerin produced, overall, a lessening of the severity of the erythematous reactions.[38]

Irritation and Sensitization

Irritation and Sensitization in Healthy Subjects

Repeated insult patch test

A modified Draize procedure was used to evaluate Quaternium-15 for sensitization in 183 subjects. The Draize[58] sensitization test consists of a series of ten 24-h induction patches and a challenge patch following a 10- to 14-day nontreatment period. The induction patches are administered every other day, with a 24-h rest between each application. Five percent Quaternium-15 was used for induction and challenge patches. One subject had a sensitization reaction at challenge.[42]

A modified Draize repeated insult patch test was performed using 1% aqueous Quaternium-15. One hundred sixty male and female Caucasian panelists completed the study. Nine 24-h 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 1, 0.3, and 0.1% Quaternium-15 in distilled water. Challenge sites were evaluated for sensitization reactions 24 h after application. During the induction phase, repetitive application of 1% Quaternium-15 demonstrated a potential for cumulative irritation. Eleven 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 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 and was administered to 10 of the 11 previously sensitized subjects and 8 control subjects. Seven of the ten reactive subjects were reconfirmed as sensi-

tive to Quaternium-15, 2/10 had inconclusive reactions, and 1/10 subjects had no reaction and was reclassified as not sensitized, giving a total of 7/11 original reactors classified as sensitized. There were no reactions in control subjects. These studies indicated that 1% aqueous Quaternium-15 caused a significant incidence of contact sensitization in the original 160-member test panel.[59]

Seventy-two subjects completed a repeated insult patch test (RIPT) to evaluate the irritancy and sensitization of a prototype underarm deodorant. The deodorant formulation contained 2% Quaternium-15 and a control formulation had 2% ethanol replacing the Quaternium-15. Induction and challenge patches consisted of 0.5 ml undiluted material administered under occlusive patches and remained in contact with the test site for 24 h. 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.[60]

A mascara containing 0.2% Quaternium-15 was tested in a 6-week modified Draize-Shelanski RIPT on 206 healthy human subjects. Ten 24-h occlusive patches containing 0.1 g of undiluted product were administered three 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 panelists, and erythema and edema or induration were observed in 3 panelists 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.[61]

A cleanser (wipe-off) containing 0.2% Quaternium-15 did not produce irritation or sensitization in 97 subjects participating in an RIPT. One subject had slight erythema after the last of nine 24-h induction patches, and no reactions were observed at challenge.[62]

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 two moisturizing products. Both moisturizers contained 0.3% Quaternium-15. One hundred eight subjects completed the study for one product, and 101 subjects participated in the second study. There were no reactions to the products, and neither moisturizer was an irritant or a sensitizer.[63,64]

A moisturizer containing 0.3% Quaternium-15 was evaluated for irritation and sensitization using 205 individuals. A modified Draize-Shelanski RIPT was conducted as described above. 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.[64]

Maximization tests

Twenty-five healthy adults participated in a maximization test[65,66] to determine the contact sensitivity of a cuticle cream containing 0.2% Quaternium-15.

The material was pretested in order to determine if sodium lauryl sulfate (SLS) would be needed to produce irritation at the test sites. The cuticle cream was nonirritating in a single 48-h occlusive patch test, so induction sites were pretreated with 1.5% SLS. Five induction patches were administered to the same site over a period of 14 days. Each occlusive patch contained 0.3 g of undiluted product and remained in contact with the test site for 48 h. After 10 days of nontreatment, a fresh site was pretreated with 5% SLS, and a 48-h occlusive challenge patch was applied. Challenge sites were observed for sensitization reactions immediately and 24 h after patch removal. No reactions were observed in the 25 subjects. The product was not a contact sensitizer under the test conditions.[67]

A mascara formulation containing 0.3% Quaternium-15 was assessed for sensitization in a maximization test. Twenty-five panelists completed the study. The procedure was identical to that described in the preceding paragraph, except induction sites were pretreated with 2.5% SLS and the challenge site was pretreated with 5–10% SLS. There were no instances of contact sensitization, and the product was not considered a sensitizer.[68]

Prophetic patch and use test

The irritancy and sensitization of a mascara containing 0.2% Quaternium-15 was evaluated in a prophetic patch and use test. One hundred two female subjects completed the study. A preinduction patch was applied to the right arm of each panelist. A postinduction patch was applied to the same area of the left arm. Each occlusive patch was administered for 48 h; sites were scored 1 h and 24 h 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.[69]

Three shades of a mascara containing 0.2% Quaternium-15 were tested for irritation and sensitization in a modified Schwartz-Peck procedure. None of 221 panelists completing the study had a reaction to the preinduction 48-h occlusive patch. Each panelist was tested with all three shades at the pre- and post-induction 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-h occlusive patch and 24 h later. One panelist had erythema and edema or induration at the second scoring of 2/3 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 three strongly positive reactions to other products applied simultaneously. These products were described as being neither irritants nor sensitizers.[70]

Two other mascaras containing 0.2% Quaternium-15 were tested in modified Schwartz-Peck Procedures as described above. One product was tested

using 114 subjects, and no irritation or sensitization was reported or observed in any phase of the study.[71] The second mascara was tested using 213 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 negative patch tests indicated that the product was probably not a potent sensitizer.[72]

Irritation and Sensitization in Sensitized Patients

Formaldehyde-sensitive individuals

Quaternium-15 is thought to elicit sensitization reactions in humans due to its formaldehyde-releasing properties.[73] Two commercial creams containing 0.1% (100 ppm formaldehyde released) Quaternium-15 were tested on 9 formaldehyde-sensitive individuals. Four patches per subject were applied at time zero, the sites were scored and patches reapplied at 72 and 120 h, with a final observation at 168 h. Six of the nine subjects had an allergic response (infiltrated, confluent, papulovesicular response) to the first cream, with three reactions observed at 72 h, one at 120 h, and two reactions observed at 168 h. The second cream produced five allergic responses, two at 72 and 120 h, and one reaction observed at 168 h. These responses were very similar to those induced by aqueous formaldehyde solutions containing 60–100 ppm formaldehyde. Quaternium-15 can produce dermatitis in formaldehyde-sensitive individuals.[11]

Several preparations containing various amounts of Quaternium-15 were tested for sensitization in 6 formaldehyde-sensitive subjects. Cutaneous tests were conducted with:

1. 2% formaldehyde solution as a control
2. Hand cream containing:
   a. 0.2% Quaternium-15
   b. 0.5% Quaternium 15
   c. 0% Quaternium-15
3. Cream shampoo containing:
   a. 0.2% Quaternium-15
   b. 0.5% Quaternium-15
   c. 0% Quaternium-15
4. 5% Quaternium-15 in Eucerin
5. 5% Quaternium-15
6. 20% Quaternium-15

The test sites were evaluated for sensitization 24 and 48 h after application of the test material. All 6 panelists had positive reactions to the formaldehyde control. Slight erythema was observed in 1 panelist with all three hand cream formulations, indicating that Quaternium-15 was not the irritating component. Four panelists had erythema with or without edema and induration after contact with the cream shampoo. These reactions were observed with all three preparations, again indicating that an ingredient other than Quaternium-15 was responsible

for the reactions. There were no reactions to the 5% aqueous solution of Quaternium-15, one erythematous reaction to 5% Quaternium-15 in Eucerin, and 4/6 reactions (slight erythema) to the 20% aqueous solution. The sensitivity results obtained from tests with Quaternium-15 in Eucerin are invalid because the vehicle, Eucerin, contains 2-Bromo-2-Nitropropane-1,3-Diol, a known formaldehyde releaser.[74,75] Quaternium-15 did not produce cross-sensitization in formaldehyde-sensitive individuals.[38]

Quaternium-15 was tested using 12 formaldehyde-sensitive individuals in several concentrations and vehicles. The test materials were 0.1, 0.5, 1, and 5% aqueous solutions and 0.1 and 1% Quaternium-15 in aqueous and anhydrous Eucerin. The 12 subjects had had no previous contact with Quaternium-15. Three panelists reacted to 0.5, 1, and 5% 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 three reactors also had slight reactions to 1% Quaternium-15 in aqueous Eucerin. The sensitivity results obtained from tests with Quaternium-15 in Eucerin are invalid because the vehicle, Eucerin, contains 2-Bromo-2-Nitropropane-1,3-Diol, a known formaldehyde releaser.[74,75] These reactions were considered to be sensitization.[37]

Contact dermatitis group studies

The North American Contact Dermatitis Group (NACDG) reported the incidence of skin sensitization for a 2% aqueous Quaternium-15 solution. The patients screened by NACDG were those of individual or group private practices or of university dermatology clinics. Some patients were referred specifically for NACDG patch tests. In 1978–1979, 77 of 1985 subjects (4%) had a positive reaction to Quaternium-15. One thousand seven hundred fifty-four subjects were tested in 1979–1980, and 54 (3%) subjects reacted positively to Quaternium-15.[76] Of the 3739 patients in these 4 years of testing, 487 cases of cosmetics-related dermatitis were identified. One hundred forty-nine of these 487 patients 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.[77] Also in conjunction with these NACDG studies, 20/27 individuals who had reacted positively to Quaternium-15 were patch tested with Quaternium-15, formaldehyde, and compounds thought to release formaldehyde; 14/20 demonstrated sensitivity to Quaternium-15; 15/20 were sensitive to formaldehyde, 1/20 was sensitive to Bronopol, 7/18 were sensitive to hexamethylene tetramine, 0/20 reacted to imidazolidinyl urea, and 1/20 was sensitive to dimethylol dihydroxy ethylene urea.[78]

The 1980–1981 and 1981–1982 results of the NACDG standard screening assay for Quaternium-15 have also been released. In 1980–1981, 1818 patients were tested with 2% aqueous Quaternium-15, and 69 (4%) patients had positive reactions. In 1981–1982, 1348 patients were tested with 2% aqueous Quaternium-15 and 1006 patients were tested with 2% Quaternium-15 in petrolatum. Fifty-nine (4%) patients reacted to the aqueous Quaternium-15 and 29 (3%) patients reacted to Quaternium-15 in petrolatum. Therefore, between 1978 and

1982, NACDG patient sensitization to Quaternium-15 remained consistent at 3–4%.[79]

In a study similar to those conducted by the NACDG, seven French clinics of dermatology were involved in testing eczema patients for sensitivity to cosmetic and drug preservatives. Quaternium-15 was among the 29 preservatives tested, and it was administered according to the International Contact Dermatitis Research Group recommendations and procedures. Quaternium-15 was tested at 2% in petroleum jelly on a population of 465 patients. Four patients had positive patch tests for Quaternium-15. Quaternium-15 was a weak sensitizer.[80]

Photosensitization

A series of 13 test and control materials were evaluated for photosensitization using 50 panelists. The test material (0.1 ml) was applied to the test area for 60 seconds. The sites were then exposed to ultraviolet (UV) radiation (Fischer quartz sunlamp model 88) for 30 seconds at a distance of 12 inches from the UV source. Individuals were subjected to this exposure 5 days a week for 5 weeks. Challenge exposures were performed 3 weeks after the final induction exposure. Each panelist did not necessarily receive all 13 test materials. There were no reactions during induction or challenge to 1% Quaternium-15 in water, 0.25% Quaternium-15 in a facial gel cleanser, 0.75% Quaternium-15 in newly prepared hand lotion, 0.1% Quaternium-15 in aged hand lotion, facial gel cleanser control (no Quaternium-15; administered as a challenge only), fresh hand lotion control (no Quaternium-15; challenge only), aged hand lotion control (no Quaternium-15; challenge only), a petrolatum control, or a methanol control. Two percent 3,3',4'5-tetrachlorosalicylanilide (TCSA) in methanol or petrolatum was administered to 20 panelists as a positive control. Nine of ten and 8/10 individuals were photosensitized to TCSA in methanol and petrolatum, respectively. Two percent Quaternium-15 in methanol produced a reaction in 3/20 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.[81]

A cleansing product containing 0.2% Quaternium-15 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 three times the individual's minimal erythema dose (MED), which had been determined prior to product testing. The UV source was a Xenon Arc Solar Simulator (150W), which was filtered to produce a continuous spectrum in the UVA and UVB region (290–400 nm). This process was repeated twice a week for a total of six exposures. Following a 10-day nontreatment period, a challenge patch was applied to a previously untreated site. After 24 h, the patch was removed, and the site was evaluated, then irradiated for 3 minutes using a Schott WG345 filter over the light source. Challenge sites were scored for reactions 15 minutes, 24, 48, and 72 h following UV exposure. No reactions were observed, and the cleanser was not a photosensitizer[82] (Table 7).

TABLE 7. Clinical Studies—Skin Irritation, Sensitization and Photosensitization

Test type[a]	*No. of subjects*	*Patient clinical history*	*Vehicle or product type*	*Concentration of Quaternium-15 (%)*	*Comments*	*Reference*
			PRIMARY SKIN IRRITATION			
Single, 24-h occluded patch	20	None	Water	2	Slight erythema at 20 min in 3 subjects; not a primary irritant	56
Single, 24-h occluded patch	10	None	Eucerin	0.5, 1.25, and 2.5	No reactions; not a primary irritant	38
Single, 24-h occluded patch	20	Eczema	Water	2	No reactions; not a primary irritant	56
Single, 24-h occluded patch	10	Eczema	Eucerin	0.5, 1.25, and 2.5	No reactions; not a primary irritant	38
Single, 3-h patch	16	None	Cream shampoo	0, 0.2, or 0.5	No reactions; not a primary irritant	38
Single patch	20	None	Cleanser (wipe-off)	0 and 0.2	Slight irritation in 1 subject to test and control; Quaternium-15 not considered to be the irritant	83
21-day cumulative irritation	10	None	Moisturizer	0.3	Slight erythema in 1 subject on day 3; irritation score of 0.83 (max = 630); practically nonirritating	57
Single, 24-h occluded patch	32	Eczema	Hand cream	0, 0.2, or 0.5	Erythema in 1 subject given 0.5% and 3 subjects given control; Quaternium-15 not considered to be the irritant	38
Single, 24-h occluded patch	56	Eczema	Cream shampoo	0, 0.2, or 0.5	44/56 had severe erythema; 6/42 and 14/42 had severe or slight erythema, respectively; 15/38 had slight erythema; reactions were to control and test materials; Quaternium-15 was not considered to be the irritant	38
	42	Eczema	50% cream shampoo in Eucerin	0, 0.1, or 0.25		
	38	Eczema	25% cream shampoo in Eucerin	0, 0.05, or 0.125		

SENSITIZATION

Patch test	12	Formaldehyde sensitive	Water and/or Eucerin	0.1, 0.5, 1, and 5	3 patients had sensitization reactions to 0.5, 1, and 5%	37
Scratch test	1985	None (?)	Water	2	77/1985 and 54/1754 positive reactions; a sensitizer	76
	1754	None (?)	Water	2		
Patch test	149	Cosmetic-related contact dermatitis	Not specified	2	32 positive reactions; a sensitizer; *note*, these 149 patients are a subgroup of the 3739 reported above by NACDG (1980)	77
Patch test	20	Quaternium-15 sensitive	Not specified	Tested with Quaternium-15 and formaldehyde	14/20 positive reactions to Quaternium-15; 15/20 positive reactions to formaldehyde; *note*, these 20 patients are a subgroup of the 3739 reported above by NACDG (1980)	78
Scratch test	1818	None (?)	Water	2	69/1818, 59/1348, and 29/1006 positive reactions; a sensitizer	79
	1348	None (?)	Water	2		
	1006	None (?)	Petrolatum	2		
RIPT	183	None	Not specified	5	1 subject sensitized	42
RIPT	160	None	Water	1	11 subjects reacted at 1st challenge; 7/10 considered truly sensitized at subsequent challenges; a patch test using 9/11 initial reactors elicited no sensitization response to 0.01% formaldehyde; 1% Quaternium-15 was a significant sensitizer	59
Patch test	6	Formaldehyde sensitive	Hand cream	0, 0.2, and 0.05	All panelists were tested with all solutions; 1/6 reacted to all hand cream formulations; 4/6 reacted to all shampoo formulations; 0/6 reactions to 5% aqueous solution; 1/6 reacted to 5% Eucerin; 4/6 reacted to 20% aqueous solution; interpreted as not exhibiting cross-sensitization with formaldehyde	38
			Cream shampoo	0, 0.2, and 0.5		
			Eucerin	5		
			Water	5 and 20		

TABLE 7. (Continued)

Test type[a]	*No. of subjects*	*Patient clinical history*	*Vehicle or product type*	*Concentration of Quaternium-15 (%)*	*Comments*	*Reference*
Patch test	9	Formaldehyde sensitive	2 cream products	0.1	6/9 and 5/9 sensitization reactions to creams; concluded that Quaternium-15 produced formaldehyde dermatitis	11
RIPT	72	None	Underarm deodorant	2 and 0 (control)	Slightly irritating in 9/72 subjects; no reactions at challenge; not a sensitizer	60
RIPT	206	None	Mascara	0.2	4 panelists reacted at challenge; 3 considered true sensitization reactions, 1/3 may have been previously sensitized; a sensitizer	
RIPT	97	None	Cleanser (wipe-off)	0.2	No reactions at challenge; not a sensitizer	61
RIPT	108	None	Moisturizer	0.3	No reactions; not an irritant or sensitizer	63
RIPT	101	None	Moisturizer	0.3	No reactions; not an irritant or sensitizer	
RIPT	205	None	Moisturizer	0.3	10 subjects had erythema at challenge; not considered significant; not an irritant or sensitizer	64
Maximization test	25	None	Cuticle cream	0.2	No reactions; not a sensitizer	67
Maximization test	25	None	Mascara	0.3	No reactions; not a sensitizer	68
Prophetic patch and use test	102	None	Mascara	0.2	1 subject had marked reaction at challenge; at rechallenge with individual ingredients, subject reacted positively to parabens, chloroxylenol, and aqueous phase	69

Prophetic patch and use test	221	None	Mascara, 3 shades	0.2	1 panelist reacted to 2/3 shades at challenge; unable to duplicate reaction at rechallenge; not an irritant or sensitizer	70
Prophetic patch and use test	114	None	Mascara	0.2	No reactions; not an irritant or sensitizer	71
Prophetic patch and use test	213	None	Mascara	0.2	2 subjects experienced periorbital edema and mild conjunctivitis during use/induction phase; no reactions at challenge; possibly a sensitizer	72
			PHOTOSENSITIZATION			
Photosensitization	50	None	Water	1	UV source: Fischer quartz sunlamp mode 188; no positive reactions except: 17/20 given TCSA (positive control), 3/20 given 2% Quaternium-15 in methanol—thought to be an irritant; ≤1% Quaternium-15 in formulation not a photosensitizer	81
			Facial gel cleanser	0 and 0.25		
			"Fresh" hand lotion	0 and 0.75		
			"Aged" hand lotion	0 and 0.1		
			Petrolatum	Controls: 0 and 2% TCSA		
			Methanol	Controls: 0 and 2% TCSA		
			Methanol	2		
Photosensitization	25	None	Cleanser	0.2	UV source: Xenon arc solar simulator, 290–400 nm; no reactions; not a photosensitizer	82

[a]See text for amplification of test procedures.

SUMMARY

Quaternium-15 is a quaternary ammonium salt that is readily soluble in water. It is reasonably stable in a pH range of 4 to 10.5. Quaternium-15 decomposes at temperatures greater than 60°C.

Quaternium-15 is used as an antimicrobial preservative in a variety of cosmetic products including skin care lotions, eye and facial makeup, shampoos, and baby products. The concentration of Quaternium-15 in these products ranges from ≤0.1 to 1%.

Results of several studies of the antimicrobial activity of Quaternium-15 have indicated the preservative is effective against yeast, molds, and bacteria.

Animal toxicity studies indicate that Quaternium-15 is mildly to moderately toxic depending on animal species, concentration, and route of administration of the preservative. It is moderately toxic to rats, guinea pigs, and chicks when administered as a single oral dose; the rabbit is more sensitive. Dermal administration of the preservative was also moderately toxic to rabbits. Acute dermal administration resulted in dose-dependent local irritation, lethargy, and anorexia as well as mortality at doses greater than 250 mg/kg per day. Testicular atrophy and decreased spermatogenesis in immature rabbits were reported in one subchronic dermal study. However, no testicular effects were noted in three subsequent studies on mature rabbits involving products containing, or aqueous solutions of, Quaternium-15.

Quaternium-15 is a moderate skin irritant in test animals. The irritancy of the preservative is directly related to the concentration coming into contact with the skin. The maximum concentration of Quaternium-15 tolerated without irritation was 5%.

Animal sensitization studies with Quaternium-15 produced conflicting results. In 12 guinea pig sensitization tests employing several different methodologies, Quaternium-15 was a sensitizer in 4 studies, a nonsensitizer in 6 studies, and its sensitization was questionable in the remaining 2 studies.

Quaternium-15 was not a significant eye irritant in rabbits.

Quaternium-15 does not appear to possess significant mutagenic activity.

Orally administered Quaternium-15 is teratogenic in rats when given by gavage to pregnant dams in doses ≥25 mg/kg per day. Fetal resorption was increased, and the predominant malformation in the pups was eye anomalies. No teratogenic effects were observed at doses of 5 mg/kg per day. Dermally applied Quaternium-15 did not produce maternal toxicity, fetal toxicity, or fetal abnormalities at doses up to and including 500 mg/kg per day.

Clinical studies on the toxicity of Quaternium-15 generally utilized much lower concentrations than the animal studies; concentrations approximated those actually found in cosmetics. Primary irritation studies on Quaternium-15 in inert vehicles and formulations indicate that Quaternium-15 is not a primary skin irritant in humans.

Quaternium-15 in inert vehicles is a human sensitizer. In RIPTs, 8/343 individuals were sensitized, and in North American Contact Dermatitis testing, 288/7911 subjects had definite sensitization reactions to Quaternium-15. Sensitization by the preservative was much less pronounced when products were tested. Only 3/789 panelists were sensitized in RIPTS using cosmetics containing up to

2% Quaternium-15. There was 1 questionable sensitization reaction in maximization and prophetic patch/in-use testing with 50 and 650 subjects, respectively. The question of whether Quaternium-15 produced cross-sensitization with formaldehyde is still under debate. In 3 studies involving formaldehyde-sensitive patients, 3/12 and 0/6 people reacted to patch applications of Quaternium-15 in an inert vehicle; however, these results are questionable since some of the tests were conducted with Eucerin, a product that contains a known formaldehyde releaser. Nine formaldehyde sensitive patients tested with two Quaternium-15-containing products had 5/9 reactions to one product and 6/9 reactions to the other product.

Quaternium-15 is not a significant photosensitizing agent. There were no reactions reported for 2 studies involving 75 subjects.

DISCUSSION

Quaternium-15 is a teratogen in rats when administered orally in doses ≥ 25 mg/kg body weight; however, Quaternium-15 is not a teratogen in rats when administered dermally in doses up to 500 mg/kg per day. Quaternium-15 is a charged compound, highly soluble in water, and may not have induced terata in dermal studies because it was not absorbed through the skin in significant amounts. Conditions that favor rapid absorption from the skin, therefore, might be expected to increase the risk of teratogenicity. It should be noted that the teratogenicity studies, as well as the metabolism study, were performed in the species that is the least sensitive to Quaternium-15 in terms of acute toxicity. The results of the teratogenicity and metabolism studies cannot be extrapolated to man.

Contradictory analytical data surround the issue of formaldehyde release by Quaternium-15. Formaldehyde was detected in aqueous solutions of Quaternium-15 by polarographic and colorimetric analyses.[11] Carbon-13 NMR analysis detected no formaldehyde in an aqueous solution of Quaternium-15 or in a product containing Quaternium-15.[12,13] However, clinical experience indicates that formaldehyde-sensitive individuals may also be sensitive to Quaternium-15.

Quaternium-15 is a human sensitizer, but testing results are dependent on the population studied. On normal skin, Quaternium-15 in inert vehicles sensitized 2.3% and 3.6% of the test population in RIPTs and patch tests, respectively. Products containing Quaternium-15 tested on normal skin sensitized 0.4% of the test populations in RIPTs and $\leq 0.1\%$ (1 questionable reactor in an in-use test) in maximization tests, prophetic patch/in-use tests, and photosensitizatiion studies. Three of 12 (25%) and 0/6 (0%) formaldehyde-sensitive subjects reacted to Quaternium-15 in inert vehicles. In a panel of 9 formaldehyde-sensitive individuals tested with two Quaternium-15 containing products, 5 of 9 (55.6%) subjects reacted to one product and 6 of 9 (66.7%) subjects reacted to the second product. In further testing of sensitive individuals isolated by the North American Contact Dermatitis Group (NACDG) patch test screening (i.e., isolated from the 1978–1980 population of 3739 subjects), 21.5% of a group of 149 individuals with cosmetically related contact dermatitis reacted to aqueous Quaternium-15.

Large-scale screening tests conducted by the NACDG were performed with

test solutions of aqueous Quaternium-15 which had been stored at room temperature and used for up to 2 years. Differential pulse polarographic analysis demonstrated that Quaternium-15 in aqueous solution breaks down into unknown products over time; after 23 months, a 2% (estimated) solution contained only 6300 ppm Quaternium-15, a loss of 68.5%. Consequently, these assays were possibly testing the unknown breakdown products of Quaternium-15 as well as the ingredient itself.[4]

CONCLUSION

Although Quaternium-15 is a potential skin sensitizer, the CIR Expert Panel concludes that Quaternium-15 is safe as a cosmetic ingredient at concentrations not exceeding those presently in use.

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