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The skin irritation potential of quaternaries

M. M. RIEGER, *Morris Plains, NJ 07950.*

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Synopsis

It is frequently reported that the relative skin irritancy of surfactants depends on the ionic species formed by the surfactant. Thus the irritation potential of surfactants is widely assumed to follow the pattern below in which quaternaries are the most irritating: quaternaries > amphoterics > anionics > nonionics. The basis of this relative rating is examined below, with the conclusion that it requires modification for topically used quaternaries and surfactants in general.

INTRODUCTION

The evidence for the relative skin irritancy rating of surfactants—quaternaries > amphoterics > anionics > nonionics—dates back to the period following World War II when eye and skin irritancy were assessed via the well-known Draize animal procedures [as detailed in 1959 in ref. (1)]. In addition, the potential of any surfactant to cause skin irritation can be established by a variety of alternative toxicological test procedures, as reviewed by Drobeck (2). It is the objective of this review to examine the pertinence of these and related tests for the assessment of skin irritation that may result from accidental or deliberate contact with quaternary surfactants. In order to complete this effort, it will be necessary to include some details of the testing of irritancy of other groups of surfactants.

DEFINITION OF SKIN IRRITATION

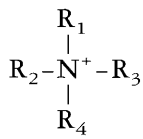
Skin irritation is defined simply as a transitory adverse response of skin to contact with the irritating species. Irritation may result from any corrosive or toxic substance, i.e., one that attacks the integument. Skin exposure to such a substance under normal cosmetic use may lead to changes in transepidermal water loss, dryness, tautness, scaling, and even erythema. The early investigators were limited to relying on erythema as the marker for skin irritation since they had no other means of assessing damage. Thus much of the data refers to the rate and level of reddening and the effect of concentration of the eliciting substance. It must be clearly recognized that erythema is a late effect of irritation, requiring that the applied substance itself reaches the dermal vasculature or that the

substance triggers the release or formation of a compound within the skin that upon further permeation (or via feedback) causes the hyperemia.

Erythema—even in the absence of urticaria—is also a sign of sensitization, but this is a reaction that is distinct from that of irritation. The elicitation of immune responses can be the result of the presence of epidermal immunocompetent cells or may take place only after systemic processing of the allergen. Immune responses to the application of quaternaries to the skin will not be discussed since they are evidently rare. Skin irritation in this review is, therefore, defined as a dermal reaction to topical administration. It is unrelated to dermal manifestations resulting from systemic administration of an irritant or to systemic toxicity.

DEFINITION OF QUATERNARY

In this review a quaternary is defined as a compound in which a nitrogen atom is covalently bound to four alkyl groups *regardless of pH* and, therefore, carries a positive charge *regardless of pH.* Thus amines and amphoterics, in which the charge on the nitrogen atom varies as a function of pH, are excluded. Despite this limitation, the number of cosmetically useful quaternaries is well over 500. For clarification, the defining quaternary structure is shown below:



Up to three of the covalently bonded R groups may be part of the same cyclic or aromatic system, as, e.g., in the well-known pyridinium salts. The hydrophobicity of quaternaries can be modified by the inclusion of a (poly)oxyethylene group in one or more of the substituent R groups. The counterion to the positively charged N– atom can be a halogen, a sulfate (ethosoulfate), a carboxylic acid, or, in fact, any species that can form an anion. In some cases, the anion-forming group may be part of one of the alkyl groups. A typical example of this is a betaine in which a zwitterionic species can be formed over a limited pH range.

Quaternary molecules are not unusual in nature. The N– atom in phosphatidylcholine is a true quaternary, and lecithin is a ubiquitous constituent of animal and plant tissue. Even though it is a quaternary, it is not regarded as toxic or irritant. The safety of lecithin leads to its use in parenteral nutrition. On the other hand, most of the cosmetically or pharmaceutically useful quaternaries are synthetic substances. Their ingestion or injection into mammals has elicited some toxic responses, especially at the dosages commonly employed by toxicologists.

The purity of synthetic quaternary substances is almost never described in the toxicological literature. In the early toxicological literature, identification was by (commercial) trade name (3). In retrospect, it is likely that most substances tested during this period included some amines and remnants of the alkylating agents. Unfortunately, details of this type are irretrievably lost, even though their significance to any skin irritation potential could be important.

EARLY SKIN TESTING PROCEDURES AND RESULTS

The study of the skin and eye irritation potential of quaternaries began about the time of WW II, and the ranking of surfactants was based primarily on the results of the Draize rabbit skin test. A few of the early data are shown in Table I, which is based on results of rabbit skin tests, as described by Draize (1). The data in Table I were obtained in 1967 using 0.5 ml of the undiluted quaternary (or 0.5 g of the dry) material on intact and abraded rabbit skin, establishing the high irritation potential of quaternaries (3). The concentrations tested are unrealistic and make it difficult to assess their pertinence to human skin irritancy at more modest (use) concentrations. It is also next to impossible to relate the results of these and similar tests to the irritancy potential of modern commercially available quaternaries; in addition, some of the trade-named test compounds are no longer available. These early investigators were forced to examine quaternaries that did not differ greatly from each other but were produced by different suppliers. Much of the early testing was probably tendentious and designed to confirm the innocuousness of the sponsor's compound. The excellent and comprehensive review by Cutler and Drobeck (3) shows the divergence of results due to differing test protocols that were inadequately controlled. Their review correctly notes that most studies concern irritation by accidental contact with antimicrobial quaternaries used in high concentrations. The efforts to categorize these results often resulted in some unexplainable irritancy ratings.

As noted, many of the studies reviewed by Cutler and Drobeck (3) were company-sponsored and not recorded in the published (journal) literature. Retrieval of these data today is difficult, and much of the material in the following paragraphs is based on their review.

In another study reviewed by Cutler and Drobeck (3), dihydrogenated tallow benzyldimonium chloride at 5% was rated as mild. It is also noteworthy that in a third study C_{12-16} alkyl dimethyl benzyl ammonium chloride (0.3%) was found only mildly irritating.

During the period up to about 1965–1970, it was common practice to rate dermal irritancy on the basis of (Draize) rabbit eye tests. These tests sometimes followed the protocol and included seven-day readings, while at other times scoring was terminated at the 48-hour reading.

Table I
Irritancy Ratings of Quaternaries (Draize Rabbit Skin Test)*

Test material	Irritancy rating
Di C_{8-10} alkyl benzyl methyl ammonium chloride (53.5%)	Severe
Di C_{8-10} alkyl dimethyl ammonium chloride (50.6%)	Severe
Didecyl dimethyl ammonium chloride (55.7%)	Severe
C_{12-16} alkyl dimethyl benzyl ammonium chloride (50%)	Severe
C_{12-16} alkyl dimethyl benzyl ammonium chloride (92.6% powder, i.e., Zephiran®)	Moderate
C_{12-18} alkyl dimethyl ethylbenzyl ammonium chloride plus C_{12-16} alkyl dimethyl benzyl ammonium chloride (50%)	Severe

* Reported by Cutler and Drobeck (3), based on data by Duprey and Hoppe (1970).

Finally, the Draize eye test procedure was modified by instillation of graded concentrations (in saline) from 0.063% up to 0.5% in order to assess the relative irritancy of various quaternaries. This test was intended to identify safe-use levels for surfactants used in finished formulations. The results [for details consult Table 2 in Cutler and Drobeck (3)] can be summarized as follows: quaternaries that include a benzyl group (benzalkonium types) appear to be milder than those with two fatty alkyl groups. Nevertheless, there is no compelling reason to conclude that human dermal irritancy shows the same pattern as eye damage in the Draize test. Table II below shows the maximum tolerated concentrations of active surfactants.

Draize's data and the ensuing discussion during the May 1952 Toilet Goods Association meeting (4) are important. Draize noted that different grades of sodium lauryl sulfate and different lots of the (supposedly) identical commercial grade elicited different levels of eye damage. Although Draize's readings suggest that 100% concentration of sorbitan esters were tolerated, one questioner reported that in his tests many non-ionics elicited corneal opacity. These important comments are included as an Appendix since few readers today have ready access to the TGA proceedings.

Despite these uncertainties, Hazelton (5) confirms the previously established order of cationics > anionics > nonionics as the best initial criterion for predicting eye irritation potential. How and where the same pattern was established for human skin irritation remains a mystery.

The intense research activity of the early fifties was followed by a hiatus. Researchers and formulators accepted the ratings of surfactants as skin irritants on the basis of the classic model (5).

The work of van der Valk *et al.* (6) initiated the period of research in which rabbit eye irritation and erythema were replaced by parametric measurements on human subjects. The work was based on the concept that skin exposed to surfactants would show enhanced TEWL long before visible eythema appeared. Their ranking of irritancy of surfactants (2%) based on evaporimetric scores is: sodium lauryl sulfate > cocobetaine > sodium laurate > polysorbate 60. These studies included a quaternary surfactant, as defined above, and their approach was followed by Berardesca *et al.* (7), who included another quaternary. Briefly, 0.03 ml/cm^2 of four different surfactants at different concentrations were applied to eight subjects once daily for three days to 16-cm^2 test sites

Table II
Maximum Tolerated Concentrations* of Active Surfactants

Surfactant	Concentration (%)
Lauralkonium chloride	0.5
Benzalkonium chloride	0.5
Benzethonium chloride	0.5
Cetethyl dimonium chloride	0.8
Stearalkonium chloride	3.0
Sodium lauryl sulfate	20.0
Octoxynol-9	5.0
Polysorbate 80	100.0

* Rabbit eye instillation; concentration at which no corneal or iris lesions were evident by the seven-day reading (4).

and allowed to dry. On the fourth day, the sites were occluded for 24 hours to produce hydration, and the skin surface water loss (g/m^2hr) was then recorded continuously for up to 25 minutes. The initially high skin surface water loss decayed in an exponential fashion. The data for a commercial skin wash are excluded here, since the component description is imprecise, and lactic acid was included at pH 5.0. On the other hand, the data for the four surfactants exposed to this so-called POST (post-occlusion stress test) are most revealing (Table III).

Clinical signs of irritation (erythema and microvesiculation) occurred at the alkyl sulfate site. Benzalkonium chloride appears to be the most irritating overall, in light of the low concentration tested. Unfortunately, no data were obtained at comparably low concentrations of the alkyl sulfate. The data are crucial for establishing some relative rankings. The innocuousness of the betaine in this test is surprising since it is a quaternary. These data bear only little resemblance to the Draize eye test results of Hazelton (4), some of which are included in Table IV.

It is difficult to assess the validity of early testing via rabbit eye or skin tests in light of more modern test approaches. The differences between the POST on humans of Berardesca *et al.* (7) (Table III) and the rabbit eye test scores of Hazelton (5) (Table IV) are significant and strongly suggest that skin irritancy, presumably measured via TEWL, is not the same phenomenon as eye opacity in the Draize rabbit eye test.

A detailed interpretation of Hazelton's data is difficult. The concentrations of the surfactants—as shown in Table IV—might not have been the concentrations introduced into rabbit eyes. Even if all substances were used at the level of 1% as mentioned elsewhere in the text, the quaternaries are clearly the most damaging. On the other hand, Hazelton observed differences in the eye irritancy potential of nonionic ethers (laureth-4 vs polysorbate 80).

It is still a questionable approach to equate skin irritancy potential with irritation observed in rabbit eye tests. The approach taken by van der Valk *et al.* (6) and Berardesca *et al.* (7) differs radically from earlier attempts to assess skin irritancy: the skin of normal human subjects is used, and irritancy is quantified by water loss.

Water imbibition by the stratum corneum (8) is evidently maximal for the tested anionic surfactant. The water loss data after 25 minutes of drying suggest that the betaine and the quaternary affect the skin by a mechanism different from that of sodium lauryl sulfate. It would seem wise, therefore, to abandon efforts to search for a single mechanism for explaining the skin irritancy of surfactants.

Table III
Skin Surface Water Loss in POST

Surfactant	Concentration (%)	Skin surface water loss ($g/m^2/h$)	
		1st Min	25th Min
Sodium lauryl sulfate	7	64.0	27.1
Cocamido betaine	7	44.7	12.1
Benzalkonium chloride	1	33.8	10.3
Sorbitan monolaurate	10	36.1	7.8
Water (control)		40.5	8.7

Table IV
Irritation Potential in Draize Test

Surfactant	Concentration (%)	Type	Irritation potential*
Roccal (benzalkonium chloride)	50	Cationic	1
Hyamine 2389 (CTAC)	50–52	Cationic	1
Duponol WAT (TEA lauryl sulfate)	50	Anionic	20
Ivory soap	100	Anionic	>10
Tween 80 (polysorbate 80)	100	Nonionic	100
Brij 30 (laureth-4)	100	Nonionic	20

* As graded by Hazelton (5).

The parametric approach based on the work of van der Valk *et al.* (6) and Berardesca *et al.* (7) fails to meet the needs of the formulator to identify the transitory or minimal irritancy caused by surfactant-containing products used on the skin. Avoidance of the phenomena of scaling and tightness are likely to benefit consumers more than additional patch testing with conclusions based on erythema. Nevertheless, Willis *et al.* (9) performed closed patch tests of benzalkonium chloride and sodium lauryl sulfate at different concentrations. Their tests on 42 healthy males showed the following pattern of total positive patch tests after 48 hours (15 µl/Finn Chamber):

Surfactant concentration	0.5%	1.0%	2%	5%
Benzalkonium chloride	10	7	—	—
Sodium lauryl sulfate	—	6	12	20

There can be little doubt about the fact that irritancy based on acute patch testing is higher for benzalkonium chloride than for sodium lauryl sulfate. Most of these and related studies are flawed by uncertainties about the test substances: Cocamidopropyl betaine may contain unalkylated derivatives of N,N-dimethylpropylamine. Sodium lauryl sulfate is a generic mix that may be based on natural or synthetic alkanols. Benzalkonium chloride is a notoriously variable mix of alkyl-derived quaternaries. Thus the investigators and future students cannot be certain of exactly what was tested.

CURRENT APPROACHES

Skin irritancy of surfactants within the EEC plays a critical role because of the EEC's requirements for classification. As a result, the European Society of Contact Dermatitis has established guidelines for standardized testing of sodium lauryl sulfate (10) and noted the contribution of (high) purity (99% sodium dodecyl sulfate) to irritancy. The paper of Tupker *et al.* (10) notes the lower irritancy from the 96.5% pharmacopeial grade. This meticulous approach to chemical and procedural protocols—histopathology, TEWL, colorimetry (of erythema), clinical scoring, exposure conditions (repetitive occlusive tests or immersion testing), age, race, sex, testing site, and environmental conditions—was lacking in most earlier studies of other surfactants, including quaternaries. The Tupker *et al.* paper identifies sodium lauryl sulfate as ". . . a model irritant, suited for precise testing. . ." Thus it comes as no surprise that the four-hour closed patch test recommended by Basketter and colleagues (11–13) utilizes sodium lauryl sulfate (20%)

as the standard irritant to eliminate intersubject and other variations. Some conclusions from these investigations follow:

1. At moderate doses, up to 5% sodium dodecyl sulfate, irritant responses are more prevalent at 7.0°C than at 16.5°C (outside average daily maximum temperature).
2. The skin of Chinese subjects is more readily irritated than that of British subjects. Surprisingly, German volunteers were found to be more sensitive than Chinese subjects.
3. The pattern of irritancy of Skin Type VI individuals did not differ materially from that of the total British group of volunteers.
4. The likelihood of eliciting irritancy increased with the contact time (1, 2, 3, or 4 hours) of the patch.

York *et al.* (14) wished to confirm the European Union's classifications (R-34 causes burns, R-38 is irritating to skin, and NC is not classified) without an animal test and through use of human volunteers. This research led to the now widely adopted four-hour closed patch test, although the method development started only a few years ago [as reviewed by Basketter *et al.* (13)]. The patch test procedure is conventional (Hill Top Chambers), and the customary scoring is used (Fregert, S., *Manual of Contact Dermatitis,* Munksgaard, Copenhagen, 1981). Since severe reactions are undesirable, test materials are applied progressively for 15 and 30 minutes and for 1, 2, 3, and 4 hours. Any reaction during these periods is considered positive, and the panelist is not exposed further. After the maximum four-hour patch period, the site is examined at 24, 48, and 72 hours. The interpretation requires comparison to the standard irritant (20% sodium lauryl sulfate). Table V presents some of the data obtained (13,15). The data show only minor interlaboratory variation and do not suggest that quaternaries at cosmetic-use concentrations are particularly irritating.

This program was not designed to compare the irritancy of different surfactant groups. Instead, the plan was to find a relatively harmless way of designing a predictive non-animal skin irritation test. The test itself meets reproducibility criteria and should be a useful alternative to the Draize rabbit skin test. During the test development, some additional data of interest were reported (12). Atopics and non-atopics were tested via the four-hour patch test procedure, with the results recorded in Table VI. Atopics and

Table V
Irritancy Ratings Based on 4-Hour Patch Test

Chemical	Tested concentration	No. of positive reactions[a]	Classification[b]
Benzalkonium chloride	7.5%	4/27; 15/29	R38
Cocotrimonium chloride	35%	8/32; 12/57	NC
Sodium dodecyl sulfate	1%	21/100; 15/100; 16/31	NC
Sodium dodecyl sulfate	10%	79/100; 53/100; 42/64; 29/31	R38
Sodium dodecyl sulfate	20%	354/530	R38
Polysorbate 80	98%	1/25; 1/29	NC
Dodecyl betaine	20%	30/32	R38
$C_{12\text{-}15}$ pareth-5 phosphate	?	1/30	NC

[a] Results from different laboratories are included: No. of reactors/No. of subjects.
[b] Classification based on Annex I of the EC Dangerous Substances Directive: R-38 = irritancy to skin (significant irritation); NC = nonclassified (minor irritation).

Table VI
Response of Atopics and Non-Atopics to 4-Hour Patch Test Procedure

Substance	Positive response	
	Atopics	Non-atopics
Cocotrimonium chloride (35%)	3/29	5/29
Sodium dodecyl sulfate (20%)	16/30	10/28
Hydrochloric acid (10%)	5/29	5/29

non-atopics evidently did not react differently, and again the quaternary did not appear to be especially irritating.

Skin irritation by quaternaries in normal cosmetic use is difficult to assess because documentation is available for only a few isolated substances. There is little justification to judge human skin irritation on the basis of the classic Draize eye test. Animal and human patch tests of quaternaries rely almost exclusively on acute (single) applications, whereas it is known that in the case of dilute anionic surfactants, irritation responses are observed only after multiple exposure. As noted above, erythema—the most commonly recorded end point—is a late event. Its relationship to skin irritation (scaling, tautness) is undocumented. The assumption that a substance that under acute and strenuous test conditions elicits eythema will also cause skin irritation under less stringent conditions may not be valid since the mechanisms causing these phenomena may not be the same. Thus, the formulator is rarely able to prejudge the non-irritancy of a surfactant and must rely on safety/irritation tests of finished formulations.

Only a few quaternaries have undergone a rigid irritancy review: (a) Dihydrogenated tallow dimonium chloride is graded as a mild irritant in rabbit patch tests. At a concentration of 7.5% it reached a mean primary irritation index of 0.26 out of a maximum of 8, after 15 alternating 24-hour patch tests. (b) Benzethonium and methylbenzethonium chlorides produced no irritancy in repeated human patch tests at concentrations below 0.5%. (c) Benzalkonium chloride, by contrast, appears to be much more irritating. Although repeated patch testing at 0.1% caused no irritant responses, tests at 0.5% or more produced a much higher response rate. The past use of benzalkonium chloride for creating of test protocols is an unfortunate choice. This substance and related compounds are toxic to microbiota and probably also to mammalian epidermal cells. It is not surprising that investigators consistently find this material irritating in closed patch tests (16).

Additional details about the irritant characteristics of these three compounds can be found in the reports of the Cosmetic Ingredient Review [*J. Am. Coll. Toxicol.* 1(2), 71, 1982; *ibid.* 4(5), 65, 1985; and *ibid.* 8(4), 589, 1989].

ASSESSMENT OF SURFACTANT IRRITANCY

The cosmetic use of surfactants varies from product to product. Thus, a compound's irritancy potential should be expected to depend on how it is used. The irritancy of a surfactant may be quite high if it is allowed to remain in prolonged contact with the

skin, while removal of the offending substance may reduce potential irritancy to negligible.

Even prolonged contact of a surfactant with the skin might not elicit irritancy effects; adverse reactions might depend on permeation to lower strata of the skin. For example, an oil-soluble surfactant may exhibit adverse effects that depend on the stratum corneum lipid levels. A surfactant that can blend into or mix with barrier lipids might have long-term effects on the barrier competency of the skin.

Penetration into or through skin is a function of the surfactant's molecular weight and shape. Unless the stratum corneum is damaged prior to or during the surfactant's application, high-molecular-weight compounds should remain on the surface.

Blends of surfactants exhibit features that might differ radically from the effects of the individual components. Formulators must proceed cautiously in the blending of surfactants since the results might be unpredictable and might depend on the formation of mixed micellar species.

In light of older as well as more recent data, the irritancy of diverse types of surfactants on intact human skin should not be judged on the basis of their ionization. Other characteristics and usage are likely to exert a far greater impact:

Lower skin irritancy	Higher skin irritancy
High molecular weight	Low molecular weight
Water solubility	Lipid solubility
Rinse-off product	Leave-on product
Little or no skin permeation	High skin permeation

Rational analysis, based on some of the above concepts, suggests that the water-insoluble stearalkonium chloride, e.g., left on the skin is less likely to cause irritation than the lipid-soluble (and penetration-enhancing) laureth-4.

The mechanisms of skin irritancy manifestations such as scaling and tautness remain obscure. The literature includes many suggestions for explaining skin irritancy after (repeated) mild exposure to dilute surfactant solutions. Particularly noteworthy are the reviews by Rhein (17), Imokawa (18), Abraham (19), and Rawlings *et al.* (20). Despite much effort, no definitive evidence has been published that can explain the phenomena of skin irritancy by surfactant exposure. Any attempt to explain the phenomena caused by different surfactants by a single mechanism is likely to fail. Instead, it is more likely that irritant responses in skin can be caused by several mechanisms, either alone or in concert, and might depend on the nature of the surfactant.

CONCLUSIONS

1. Skin irritancy by surfactants is related to the fate of the topically applied substance. Permeation into the epidermis is a primary requisite. Permeation through the epidermis is likely to elicit toxic responses, especially in the case of quaternaries. Photodecomposition of surfactants remaining on the skin might cause responses that cannot be assessed by patch testing (21). The chemical structures of quaternaries and their general stability make them useful in cosmetic products.
2. The tendency of quaternaries to bond to negatively charged sites blocks their ten-

dency to permeate unless high concentrations are employed.
3. Formulators should not reject the use of quaternaries in hair or skin care products because of earlier and probably unjustified generalizations about irritancy.
4. It must be recognized that the proof of non-irritancy and safety of every surfactant-containing product must be assessed before marketing.

APPENDIX*

"MR. LATVEN: We have investigated between 45 and 50 surface-active agents following instillation in the eye and found essentially the same result.

However, we have got to add one thing, namely, that the nonionics can be just as irritating as the cationics in specific instances, as you have already pointed out. The incidence, however, is less. We find around 25 per cent of all nonionics are irritating in consideration of corneal opacity and around 62 per cent of anionics fall into the opacification class, where it is 100 per cent with the cationics.

As you have also pointed out, the important question is whether or not the final formulas produce corneal opacity. That raises a very important question, namely: When one investigates a final formulation and obtains results such as that, only one out of nine or ten animals shows opacification. How can one interpret it? I must admit complete ignorance.

DR. DRAIZE: Occasionally one serious reaction only is obtained in a group of test subjects. Such a single reaction is deemed significant, since in the general population an occasional sensitive individual may be encountered, and from a standpoint of overall safety such an individual may not be overlooked.

MR. LATVEN: I wonder if I could ask another question on your interpretation, namely, the insidious character of a number of these surface-active agents is the fact that they don't produce pain on instillation. . . ."

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* Discussion after the Draize and Kelley presentation (4). From page 4 of the *Proceedings of the Scientific Section of the Toilet Goods Association,* 17, May 1952.

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