

Benzalkonium Chloride: A Known Irritant and Novel Allergen

Ashley B. Wentworth, MD,* James A. Yiannias, MD,† Mark D.P. Davis, MD,‡ and Jill M. Killian, BS§

Background: Benzalkonium chloride (BAK), a detergent and preservative found in health care and household products, is an established irritant, yet BAK is seldom considered to cause allergic contact dermatitis. We have, however, observed positive patch test reactions more often than is typically reported. From 2001 through 2005 and 2006 through 2010, BAK was among the top 10 most frequent allergens in our standard series.

Objective: The aim of this study was to review the Mayo Clinic experience from 2000 to 2012 with patch testing to BAK.

Methods: An electronic patch test database was used to acquire results of patients who underwent patch testing for BAK 0.1% aqueous after it was introduced to the standard series in 2000 until 2012. Previous reports (1998–2000, 2001–2005, 2006–2010) from our institution were also reviewed.

Conclusions: Our study showed BAK to be an allergen of increasing importance. From 1998 through 2000, 2001 through 2005, and 2006 through 2010, the rate of allergic patch test results to BAK increased. More than half of the reactions in each period studied were graded as macular erythema, with at least one third of all reactions deemed to be relevant. Irritancy rates were consistently low.

Benzalkonium chloride (BAK) is a quaternary ammonium cationic detergent and preservative found in health care, household, and personal care products, including cosmetics and ophthalmic solutions.¹ Because it is an established irritant, BAK^{2–5} has been used to study irritant contact dermatitis.⁶ Numerous reports have also associated BAK with allergic reactions, including contact dermatitis and conjunctivitis.^{7–20} Sensitization has been reported particularly among health care workers,^{12,21,22} as BAK is used as a preoperative disinfectant and in the care of burns, cutaneous infections, ulcers, and wounds.¹ Benzalkonium chloride is also found in many products commonly encountered outside of health care, and a search of SkinSAFE (formerly known as CARD, the Contact Allergen Replacement Database) revealed that of 11,979 products listed, 2007 (17%) contain BAK or one of its cross-reactors (eg, other quaternary ammonium compounds such as benzethonium chloride [BEC]).²³ Even with this evidence, the potential for BAK to cause allergic contact dermatitis (ACD) remains under debate,^{24–27} as positive patch test results have been postulated to be misinterpretations of irritant responses.²⁴

Few reports highlight the significance of BAK as an allergen; however, in our experience of patch testing with the standard series, BAK was one of the top 10 most frequent allergens: number 9 in 2006 through 2010²⁸ and up from number 10 in 2001 through 2005.²⁹ For this reason, we retrospectively reviewed our database to determine the prevalence and relevance of this emerging allergen. Herein, we report results of patch testing this novel allergen from its introduction to the standard series in 2000 until 2012. We also compared the allergenicity of BAK from our previously published reports (1998–2000,³⁰ 2001–2005,²⁹ and 2006–2010²⁸).

METHODS

The study was approved by the Mayo Clinic Institutional Review Board. After approval, an electronic patch test database (Sybase, Sybase Inc) was used to acquire the results of patients tested to BAK 0.1% aqueous (aq) in the standard series, from 2000 to 2012. Data from patients who did not approve use of their records for research purposes were excluded. To extract information that had been reported previously, we reviewed publications^{28–30} from this institution that described BAK patch test results for the study period. All information not previously reported came from the electronic patch test database and included the overall rate of positive and irritant patch test results to BAK from 2000 to 2012, the reaction strength of positive patch test results from 2000 and 2001 through 2005, the relevance of positive patch test results from 2000, and the irritancy rate from 2000. We also compared the rate of positive and irritant reactions to BAK from 1998 through 2000 with the rate from 2006 through 2010.

Patch testing was performed as described previously.^{28–30} Reactions were read as negative, macular erythema, weak, strong, extreme, or

From the *Mayo Clinic College of Medicine, Mayo Clinic, Rochester, MN; †Department of Dermatology, Mayo Clinic, Scottsdale, AZ; Divisions of ‡Clinical Dermatology, and §Biomedical Statistics and Informatics, Mayo Clinic, Rochester, MN.

Dr Wentworth is now with Department of Dermatology, Mayo Clinic, Rochester, MN.

Address reprint requests to: James A. Yiannias, MD, Department of Dermatology, Mayo Clinic, 13400 E Shea Blvd, Scottsdale, AZ 85259.

E-mail: yiannias.j@mayo.edu.

The authors have no funding or conflicts of interest to declare.

DOI: 10.1097/DER.0000000000000160

© 2016 American Contact Dermatitis Society. All Rights Reserved.

irritant. In general, weak reactions were defined by nonvesicular erythema, infiltration, or papules, or a combination; strong reactions were defined as edematous, vesicular, or both; and extreme reactions were defined as spreading, bullous, or ulcerative or a combination. Relevancy was determined on the basis of history of contact with the allergen in question. Designations included the following:

- Irritant
- Allergic, not relevant (no history consistent with potential for allergen contact)
- Allergic, questionably relevant (existing contact with substances that may contain the allergen)
- Allergic, formerly relevant (previous contact with likely allergen-containing product)
- Allergic, relevant (use test or patch test of a substance containing the allergen is positive, or allergen is confirmed in a product the patient is using).

Positive results included those defined as weak, strong, or extreme at the final patch test reading as well as those featuring macular erythema only—if the result was deemed relevant after the exclusion of irritant reactions.

Statistical Analyses

Statistical analyses were completed with SAS software, version 9.3 (SAS Institute, Inc, Cary, NC). Results were compared using a χ^2 test. Statistical significance was defined as $P < 0.05$.

RESULTS

Since 2000, BAK (0.1% aq) has been included in Mayo Clinic's standard series. Between 2000 and 2012, this allergen was tested on 8448 unique patients. The rate of positive patch test results to BAK was 8.8%, and the irritant rate was 1.3%.

From 1998 through 2000, BAK had a positive patch test rate of 5.5%³⁰ and an irritancy rate of 1.5% (1324 patients tested). From 2001 through 2005, the allergenicity rate was 8.3%, and the irritancy rate was 1.6% (3854 patients tested).²⁹ From 2006 through 2010, BAK had a positive patch test rate of 8.8% and an irritancy rate of 0.7% (3115 patients tested).²⁸

We compared data from 2006 through 2010 with data from 2001 through 2005 and did not find a significant difference in the rate of allergenicity to BAK.²⁸ However, there was a significant increase ($P < 0.05$) in the rate of allergic patch test results from 1998 through 2000 (5.5%) and 2001 through 2005 (8.3%).²⁹ The difference between the rate of allergenicity from 1998 through 2000 (5.5%) and 2006 through 2010 (8.8%) was also statistically significant ($P = 0.005$).

In 2000, the reaction strength was graded as macular erythema in 55.6% of reactions, as weak in 38.9%, as strong in 2.8%, and as extreme in 2.8%. From 2001 through 2005, 55.7% of positive tests were graded as macular erythema, 40.9% as weak, 3.1% as strong, and 0.3% as extreme. From 2006 through 2010, 57.9% of positive reactions to BAK were graded as macular erythema, 36.4% as weak, 5.0% as strong, and 0.8% as extreme.²⁸

Of the allergic reactions from 2000, 33.3% were categorized as relevant, 58.3% as questionably relevant, 2.8% as not relevant, and 5.6% as unknown. From 2001 through 2005, 39.0% of positive reactions to BAK were categorized as relevant, 56.0% as questionably relevant, 1.6% as formerly relevant, and 1.9% as not relevant.²⁹ During 2006 through 2010, 34.1% were classified as relevant, 62.5% as questionably relevant, 1.1% as formerly relevant, and 1.5% as not relevant.²⁸

DISCUSSION

Our findings revealed an increasingly high rate of positive reactions to BAK. The rate of allergenicity was 5.5% from 1998 through 2000, 8.3% from 2001 through 2005, and 8.8% from 2006 through 2010. In each period, reactions were most commonly graded as macular erythema, and the irritancy rates were low. For each period, approximately one third of the reactions were categorized as relevant. From 2000 to 2012, the overall rate of BAK allergenicity was 8.8%, and the overall rate of irritancy was 1.3%. This experience shows that BAK, an established irritant, is also an important allergen and, thus, is a useful addition to the standard series.

The determination of relevance was a limitation of the study. Categorizing relevance is a decision based in part on clinical judgment, which may differ among physicians. In addition, given that many patients evaluated at our institution are not local and schedule follow-up with their home physicians, relevance was often determined at the final patch test reading, rather than after the patient had avoided the suspected allergen.

The potential allergenicity of BAK is an important topic for discussion, given its use in many household and health care settings.¹ Nearly 20% of products listed in SkinSAFE (accessible for patients or providers through Web or smartphone applications) contain BAK or a cross-reactor.²³

Cosmetics are a considerable source of contact dermatitis. A recent study of pooled data from patients patch tested for suspected contact dermatitis revealed a 9.8% rate of positive reactions to cosmetics.³¹ Preservatives such as BAK and fragrances have been reported among the top 2 most common categories of substances in cosmetics that cause allergic reactions in patients with contact dermatitis due to cosmetics.^{32,33} Therefore, BAK has been recommended for consideration in patch testing of patients with suspected cosmetic contact dermatitis.^{31,34}

Benzalkonium chloride is found in a variety of products other than cosmetics that also cause ACD, including antifungal solutions,¹⁷ antiseptic bath oil,¹⁹ detergents,^{16,20} ethanol,¹⁴ medicated adhesive tape,¹² ophthalmic solutions,¹³ plaster of Paris,^{9,15} and topical cream.¹⁸ Patients with allergic conjunctivitis from BAK-containing ophthalmic preparations have also had positive patch test reactions to BAK.^{7–10} Table 1 summarizes the findings of several studies at other institutions^{25–27,35–37} that reported results of patch testing with BAK; many of those studies found it to be a potential, if infrequent, allergen. The authors of 1 of these studies also noted that when patients who had positive patch test results avoided BAK, their clinical signs and symptoms improved.³⁵

TABLE 1. Studies of BAK Allergenicity and Irritancy

Study	Objective	Methods	Results	Conclusions
Benjamin et al, 2012 ³⁵	To distinguish between irritant and allergic patch test results of 2 quaternary ammonium compounds	Eight patients with rash after disinfectant contact or previous positive BAK patch test results and 2 patients receiving routine patch testing were patch tested to BEC and BAK, with results evaluated by visual inspection and in vivo RCM.	Two patients had relevant, allergic reactions to BAK 0.15% aq by visual inspection and RCM. One patient with a history of BAK exposure (no known contact with BEC) had positive reactions to both BEC and BAK.	Quaternary ammonium compounds can produce relevant allergic reactions. BAK and BEC can cross-react. RCM aids in differentiating irritant from allergic results.
Dao et al, 2012 ²⁵	To establish prevalence of sensitization to BAK and BEC and to compare patch test concentrations and vehicles	In the process of routine patch testing, 142 patients thought to have ACD were also tested to BAK and BEC in various concentrations and vehicles.	Twenty-nine patients (20%) had reactions to BAK and/or BEC that were morphologically irritant, doubtful, or 1+. Of these patients, 14 were ultimately judged to have definite or possible ACD to BAK and/or BEC. Nine patients (6.3%) had possible irritant reactions. Testing with BAK 0.15% aq and 0.15% pet revealed 2 times as many patients with possible ACD to BAK than would have been found if only 0.1% aq had been used	BAK and BEC sensitization is rare but possible. Detection of BAK allergenicity may be increased by the addition of BAK in pet to a series, along with increasing the concentration from 0.1% aq to 0.15% aq.
Lee et al, 2012 ²⁶	To determine prevalence of allergies to preservatives	A total of 584 patients suspected of having contact dermatitis to cosmetics underwent patch testing to preservative allergens, including BAK 0.1% aq	Seventy-one patients (12.1%) had a positive reaction to BAK. Thirty patients (5.1%) had a questionable/irritant reaction. BAK RI was 0.4, and PR was 89%. Of 30 control patients who received irritancy patch testing with allergens of differing concentrations, 7 had irritant readings to BAK 0.1%.	BAK had the highest rate of allergic reactions among the preservatives tested. BAK had a low RI and a high PR. Positive patch test reactions to BAK may be misinterpretations of irritant responses, so results should be carefully assessed.

Uter et al, 2008 ²⁷	To analyze patch test results of BAK 0.1% pet and to evaluate the risk of BAK contact allergy in specific occupations	A total of 42,898 patients were patch tested to BAK 0.1% in ≥1 of 3 different series (disinfectants, ophthalmics, and topical drugs).	Of the patients, 320 (0.75%) had + positive reactions to BAK, whereas 41 (0.09%) had ++ or +++ reactions.	Many + allergic reactions to BAK may be false positives given their low reproducibility and only slight differences between potential exposure groups. These reactions should be viewed with caution and confirmed with testing for validation. BAK can lead to contact sensitization after exposure to certain substances (ie, disinfectants). Thus, this irritant may act as a contact allergen, although incidence is rare.
Boyvat et al, 2005 ³⁶	To determine the frequency of sensitization to common preservatives, including BAK 0.1% aq	A total of 308 patients with contact dermatitis were patch tested with the standard series and additional preservatives.	Reproducibility of + reactions was low in those tested for BAK allergenicity with ≥2 series. Positive results were found more frequently in those tested with the disinfectant series. Testing with this series led to a greater than 6-fold higher proportion of ++ or +++ reactions. Variation in BAK test results among various occupational subgroups was not significant.	BAK was one of the top 2 most frequent sensitizers among the preservatives tested.
Fuchs et al, 1993 ³⁷	To discover the significance of BAK in causing ACD	A total of 2146 patients were patch tested to BAK.	Five patients (1.6%) had positive reactions to BAK. Five patients (1.6%) had irritant reactions. Allergic reactions to BAK occurred in 225 patients. Twelve of these cases had clinical relevance. There were 258 irritant reactions.	BAK is a potential but weak allergen. Reactions graded as “doubtful” may be irritant. The repeated open application test may be used to determine clinical relevance in questionable cases.

1+ or +, weak positive reaction (generally defined as featuring erythema, infiltration, or papules or a combination); ++, strong positive reaction (erythema, infiltration, papules, or vesicles or combination); ++++, extreme positive reaction (bullous)³⁸; RCM, reflectance confocal microscopy.

Comparison of these studies reveals the potential for influence of the vehicle on patch test results. In patients with suspected preservative or disinfectant exposure, very low rates (0.09%–0.75%) of positive reactions to BAK occurred when the patients were tested with 0.1% petrolatum (pet).²⁷ Higher positive reaction rates occurred in patients tested with BAK 0.1% aq: 1.6% in a study examining patients with contact dermatitis³⁶ and 12.1% in another study of patients with potential contact dermatitis to cosmetics.²⁶ However, in a report comparing various concentrations and vehicles of BAK, a greater number of positive reactions was found with the use of 0.15% aq and 0.15% pet rather than solely 0.1% aq, although this may be secondary to the increase in concentration.²⁵ Further investigation regarding the optimal vehicle to minimize irritancy but capture allergenicity is needed.

The authors of a review article²⁴ concluded that BAK is most properly categorized as a skin irritant with rare allergenicity. After reviewing case reports of allergic contact sensitivity to BAK, they suggested that the misinterpretation of patch test results could lead to a higher frequency of false-positive results and, accordingly, to a higher frequency of skin allergy diagnoses. Such conclusions are supported by studies that calculate 2 measures to assess the quality of substances tested as allergens: positivity ratio (PR) and reaction index (RI).^{39–41} The PR is found by dividing the number of a substance's weak (+) reactions by the total number (+, ++, or +++) of positive reactions.⁴⁰ A high PR correlates with substances producing many weak reactions that may represent false positives.⁴¹ The RI is calculated by subtracting a substance's questionable and irritant reactions from the total number of allergic reactions and dividing by the sum of all reactions.⁴⁰ Higher indices therefore indicate a greater number of true allergic reactions.⁴¹ When the North American Contact Dermatitis Group examined patch test results from 1994 to 2006, they found that BAK 0.1% aq had a high PR (75.2%) and low RI (0.02) and thus met their criteria for a "problematic preparation," defined as one with a PR higher than 55% and an RI less than or equal to 0.46. Benzalkonium chloride was subsequently removed from the North American Contact Dermatitis Group standard series.

Nevertheless, BAK has been reported as a frequent sensitizer for those persons working in health care,^{21,22,42} which is not surprising given that it is found in products used to disinfect the skin and surgical instruments.^{1,3} In one study that sought to determine the most frequent allergens causing ACD among health care workers, those working in health care had a 7.5% rate of relevant reaction to BAK, whereas non-health care workers had a rate of relevant reaction of 2.3%.²² Among the 31 health care workers identified as having occupationally related ACD, BAK was a relevant occupational allergen in 9.7%.^{22,43} In a report that described sources of occupationally related dermatoses in nurses, sensitization to BAK 0.1% was present in 23.8% of 223 nurses studied.²¹ Another study found BAK sensitization to be slightly more frequent in female health care workers (2.0%) than in a control group (1.6%).⁴²

The allergenicity of BAK is also important because of its potential to cross-react with other structurally related compounds.^{1,5} Benzethonium chloride and BAK are both quaternary ammonium salts with shared absorption and penetration properties, which can

lead to a potential for cross-reaction.²⁵ A report of allergic reactions to feminine hygiene sprays included 2 patients who had allergic contact reactions to BEC; these 2 patients also had positive patch test results to BAK.⁴⁴ In a study that focused on differentiating allergic and irritant quaternary ammonium patch test reactions, 1 of 8 patients with the potential for ACD due to BAK (but no BEC exposure) had a cross-reaction between BAK and BEC.³⁵ Of 8 patients with reactions to BEC in another study, 6 also had coreactions to BAK.²⁵ Such findings have led some authors²⁵ to propose BEC testing when there is suspicion for sensitivity to BEC or BAK.

Given the higher rate of positive patch test reactions among patients working in health care professions, BAK should be considered in the evaluation of occupational contact dermatitis. Aside from routine patch testing, there are several additional steps to inform diagnosis of occupational contact dermatitis, including the provocative use test, the repeat open application test, and serial dilutions of the substance in question.⁴⁵ These tests assist in confirming results and informing determination of relevance. In one study, 3 nurses with positive reactions to BAK were willing to undergo further testing, but only 1 had a positive use test result.⁴⁶ The other patients' positive reactions to BAK were not designated allergic. Importantly, the nurse with a positive use test result improved with avoidance of BAK. These results emphasize the importance of further investigation in determining whether a reaction is relevant. Repeat open application test has also been used to judge relevancy when patch testing BAK.³⁷

The concentration of BAK that is often tested is 0.1% aq or pet.^{10,18,21,22,26,27,36,42,47} However, concentration strength can cause irritant reactions,¹⁶ which has led to recommendations for using a lower concentration of BAK, for example, 0.01%⁵ or 0.05%²⁶ in ACD testing. If tested in studies as an irritant or nonsensitizer, concentrations are often higher. Several studies of irritant contact dermatitis have used BAK 0.5%,^{48–50} although concentrations in other reports on irritants in patients with eczema ranged from 0.1% to 2%.^{51,52} When used as an irritant or nonsensitizer in sensitization studies, concentrations range from 0.2% for in vitro human epidermis testing⁵³ to 2% for murine lymph node testing.⁵⁴ It is therefore not surprising that in a predictive test for irritancy, BAK 7.5% and 10% were found to be irritating during a human 4-hour patch test.⁵⁵

In summary, the allergenicity of BAK 0.1% aq is increasing. Because of its growing importance as an allergen, BAK is a valuable addition to the standard series. The optimal concentrations for patch testing remain to be determined by future studies.

REFERENCES

1. Rietschel RL, Fowler JE, Fisher AA. *Fisher's Contact Dermatitis*. 6th ed. Lewiston, NY: BC Decker; 2008:862.
2. Ling TC, Hight AS. Irritant reactions to an antiseptic bath emollient. *J Dermatolog Treat* 2000;11(4):263–267.
3. Rycroft RJG, Menne T, Frosch PJ, et al, eds. *Textbook of Contact Dermatitis*. 3rd ed. Berlin, Germany: Springer; 2001:1114.
4. Saw NK, Hindmarsh JR. Acute irritant reaction to an antiseptic bath emollient. *Postgrad Med J* 2005;81(952):131–132.

5. Johansen JD, Frosch PJ, Lepoittevin JP, eds. *Contact Dermatitis*. 5th ed. Berlin, Germany: Springer; 2011:1252.
6. Willis CM, Young E, Brandon DR, et al. Immunopathological and ultrastructural findings in human allergic and irritant contact dermatitis. *Br J Dermatol* 1986;115(3):305–316.
7. Fisher AA, Stillman MA. Allergic contact sensitivity to benzalkonium chloride: cutaneous, ophthalmic, and general medical implications. *Arch Dermatol* 1972;106:169–171.
8. Afzelius H, Thulin H. Allergic reactions to benzalkonium chloride. *Contact Dermatitis* 1979;5(1):60.
9. Lovell CR, Staniforth P. Contact allergy to benzalkonium chloride in plaster of Paris. *Contact Dermatitis* 1981;7(6):343–344.
10. Fisher AA. Allergic reactions to contact lens solutions. *Cutis* 1985;36(3):209–211.
11. Hatinen A, Terasvirta M, Fraki JE. Contact allergy to components in topical ophthalmologic preparations. *Acta Ophthalmol (Copenh)* 1985;63(4):424–426.
12. Fisher AA. Allergic contact dermatitis and conjunctivitis from benzalkonium chloride. *Cutis* 1987;39:381–383.
13. Cox NH. Allergy to benzalkonium chloride simulating dermatomyositis. *Contact Dermatitis* 1994;31(1):50.
14. Ortiz-Frutos FJ, Argila D, Rivera R, et al. Allergic contact dermatitis from benzalkonium chloride used as a denaturant of ethanol. *Contact Dermatitis* 1996;35(5):302.
15. Stanford D, Georgouras K. Allergic contact dermatitis from benzalkonium chloride in plaster of Paris. *Contact Dermatitis* 1996;35(6):371–372.
16. Kanerva L, Jolanki R, Estlander T. Occupational allergic contact dermatitis from benzalkonium chloride. *Contact Dermatitis* 2000;42(6):357–358.
17. Park HJ, Kang HA, Lee JY, et al. Allergic contact dermatitis from benzalkonium chloride in an antifungal solution. *Contact Dermatitis* 2000;42(5):306–307.
18. Chowdhury MM, Statham BN. Allergic contact dermatitis from dibutyl phthalate and benzalkonium chloride in Timodine cream. *Contact Dermatitis* 2002;46(1):57.
19. Hann S, Hughes TM, Stone NM. Flexural allergic contact dermatitis to benzalkonium chloride in antiseptic bath oil. *Br J Dermatol* 2007;157(4):795–798.
20. Cusano F, Luciano S. Contact allergy to benzalkonium chloride and glutaraldehyde in a dental nurse. *Contact Dermatitis* 1993;28(2):127.
21. Kiec-Swierczynska M, Krecisz B. Occupational skin diseases among the nurses in the region of Łódź. *Int J Occup Med Environ Health* 2000;13(3):179–184.
22. Suneja T, Belsito DV. Occupational dermatoses in health care workers evaluated for suspected allergic contact dermatitis. *Contact Dermatitis* 2008;58(5):285–290.
23. SkinSAFE. HER Inc; 2015. Available at: www.skinsafeproducts.com. Accessed July 8, 2015.
24. Basketter DA, Marriott M, Gilmour NJ, et al. Strong irritants masquerading as skin allergens: the case of benzalkonium chloride. *Contact Dermatitis* 2004;50(4):213–217.
25. Dao H Jr, Fricker C, Nedorost ST. Sensitization prevalence for benzalkonium chloride and benzethonium chloride. *Dermatitis* 2012;23(4):162–166.
26. Lee SS, Hong DK, Jeong NJ, et al. Multicenter study of preservative sensitivity in patients with suspected cosmetic contact dermatitis in Korea. *J Dermatol* 2012;39(8):677–681.
27. Uter W, Lessmann H, Geier J, et al. Is the irritant benzalkonium chloride a contact allergen? A contribution to the ongoing debate from a clinical perspective. *Contact Dermatitis* 2008;58(6):359–363.
28. Wentworth AB, Yiannias JA, Keeling JH, et al. Trends in patch-test results and allergen changes in the standard series: a Mayo Clinic 5-year retrospective review (January 1, 2006, to December 31, 2010). *J Am Acad Dermatol* 2014;70(2):269–275.e4.
29. Davis MD, Scalf LA, Yiannias JA, et al. Changing trends and allergens in the patch test standard series: a Mayo Clinic 5-year retrospective review, January 1, 2001, through December 31, 2005. *Arch Dermatol* 2008;144(1):67–72.
30. Wetter DA, Davis MD, Yiannias JA, et al. Patch test results from the Mayo Clinic Contact Dermatitis Group, 1998–2000. *J Am Acad Dermatol* 2005;53(3):416–421.
31. Biebl KA, Warshaw EM. Allergic contact dermatitis to cosmetics. *Dermatol Clin* 2006;24(2):215–232, vii.
32. Tomar J, Jain VK, Aggarwal K, et al. Contact allergies to cosmetics: testing with 52 cosmetic ingredients and personal products. *J Dermatol* 2005;32(12):951–955.
33. Hamilton T, de Gannes GC. Allergic contact dermatitis to preservatives and fragrances in cosmetics. *Skin Therapy Lett* 2011;16(4):1–4.
34. Wetter DA, Yiannias JA, Prakash AV, et al. Results of patch testing to personal care product allergens in a standard series and a supplemental cosmetic series: an analysis of 945 patients from the Mayo Clinic Contact Dermatitis Group, 2000–2007. *J Am Acad Dermatol* 2010;63(5):789–798.
35. Benjamin B, Chris F, Salvador G, et al. Visual and confocal microscopic interpretation of patch tests to benzethonium chloride and benzalkonium chloride. *Skin Res Technol* 2012;18(3):272–277.
36. Boyvat A, Akyol A, Gürgey E. Contact sensitivity to preservatives in Turkey. *Contact Dermatitis* 2005;52(6):329–332.
37. Fuchs T, Meinert A, Aberer W, et al. Benzalkonium chloride: a relevant contact allergen or irritant? Results of a multicenter study of the German Contact Allergy Group [in German]. *Hautarzt* 1993;44(11):699–702. German.
38. Wilkinson DS, Fregert S, Magnusson B, et al. Terminology of contact dermatitis. *Acta Derm Venereol* 1970;50(4):287–292.
39. Brasch J, Henseler T. The reaction index: a parameter to assess the quality of patch test preparations. *Contact Dermatitis* 1992;27(3):203–204.
40. Geier J, Uter W, Lessmann H, et al. The positivity ratio: another parameter to assess the diagnostic quality of a patch test preparation. *Contact Dermatitis* 2003;48(5):280–282.
41. Warshaw EM, Nelsen DD, Sasseville D, et al. Positivity ratio and reaction index: patch-test quality-control metrics applied to the North American Contact Dermatitis Group database. *Dermatitis* 2010;21(2):91–97.
42. Schnuch A, Uter W, Geier J, et al. Contact allergies in healthcare workers: results from the IVDK. *Acta Derm Venereol* 1998;78(5):358–363.
43. Nettis E, Colanardi MC, Soccio AL, et al. Occupational irritant and allergic contact dermatitis among healthcare workers. *Contact Dermatitis* 2002;46(2):101–107.
44. Fisher AA. Allergic reaction to feminine hygiene sprays. *Arch Dermatol* 1973;108:801–802.
45. Marrakchi S, Maibach HI. What is occupational contact dermatitis? An operational definition. *Dermatol Clin* 1994;12(3):477–484.
46. Klein GF, Sepp N, Fritsch P. Allergic reactions to benzalkonium chloride? Do the use test! *Contact Dermatitis* 1991;25(4):269–270.
47. Schnuch A, Geier J, Uter W, et al. Patch testing with preservatives, antimicrobials and industrial biocides: results from a multicentre study. *Br J Dermatol* 1998;138(3):467–476.
48. Willis CM, Stephens JM, Wilkinson JD. Experimentally-induced irritant contact dermatitis: determination of optimum irritant concentrations. *Contact Dermatitis* 1988;18(1):20–24.
49. Willis CM, Stephens CJ, Wilkinson JD. Epidermal damage induced by irritants in man: a light and electron microscopic study. *J Invest Dermatol* 1989;93(5):695–699.

50. Willis CM, Stephens CJ, Wilkinson JD. Differential patterns of epidermal leukocyte infiltration in patch test reactions to structurally unrelated chemical irritants. *J Invest Dermatol* 1993;101(3):364–370.
51. Bjornberg A. Skin reactions to primary irritants and predisposition to eczema. *Br J Dermatol* 1974;91(4):425–427.
52. Bjornberg A. Increased skin reactivity to primary irritants provoked by hand eczema. *Arch Dermatol Forsch* 1974;249(4):389–400.
53. Saito K, Nukada Y, Takenouchi O, et al. Development of a new in vitro skin sensitization assay (Epidermal Sensitization Assay; EpiSensA) using reconstructed human epidermis. *Toxicol In Vitro* 2013;27(8):2213–2224.
54. Gerberick GF, Cruse LW, Miller CM, et al. Selective modulation of B-cell activation markers CD86 and I-Ak on murine draining lymph node cells following allergen or irritant treatment. *Toxicol Appl Pharmacol* 1999;159:142–151.
55. York M, Griffiths HA, Whittle E, et al. Evaluation of a human patch test for the identification and classification of skin irritation potential. *Contact Dermatitis* 1996;34(3):204–212.