

The different decomposition properties of diazolidinyl urea in cosmetics and patch test materials

Takahiro Doi, Keiji Kajimura and Shuzo Taguchi

Department of Food, Drugs and Environment, Osaka Prefectural Institute of Public Health, Osaka 537-0025, Japan

doi:10.1111/j.1600-0536.2010.01862.x

Summary

Background. Diazolidinyl urea is a formaldehyde-releasing compound that releases formaldehyde through its decomposition. However, there have been few reports about the decomposition properties of diazolidinyl urea in cosmetics and patch test materials.

Objectives. The aim of this study was to show how diazolidinyl urea decomposes in cosmetics and patch test vehicles, and to determine which cosmetic compounds should be evaluated in patch test studies of diazolidinyl urea.

Method. We fractionated diazolidinyl urea-dissolving buffers or diazolidinyl urea-containing cosmetics with high-performance liquid chromatography–photodiode array detector (HPLC-PDA), and characterized them in liquid chromatography–mass spectrometry (LC-MS) and ^1H -nuclear magnetic resonance studies. Diazolidinyl urea-containing cosmetics and diazolidinyl urea patch test materials were also analysed with HPLC-PDA and LC-MS.

Results. Diazolidinyl urea was decomposed to (4-hydroxymethyl-2,5-dioxo-imidazolidine-4-yl)-urea (HU) and (3,4-bis-hydroxymethyl-2,5-dioxo-imidazolidine-4-yl)-urea (3,4-BHU) in most of the cosmetic samples tested. The peak patterns of the patch test materials analysed with the HPLC-PDA were different from those of the cosmetic samples.

Conclusions. The diazolidinyl urea-derived decomposition products differed between the cosmetics and patch test preparations. To test the contact sensitivity of the diazolidinyl urea present in cosmetics, patch tests with HU and 3,4-BHU in petrolatum should be performed.

Key words: cosmetic; diazolidinyl urea; formaldehyde-releaser; patch test vehicle; skin sensitization.

A wide variety of antimicrobial preservatives, such as formaldehyde and formaldehyde-releasers, are used in cosmetics (1–3). Diazolidinyl urea is one of the most commonly used preservatives in cosmetics, and is known to release formaldehyde through its decomposition. The

released formaldehyde concentrations of diazolidinyl urea-containing cosmetics and factors that affect formaldehyde release have been studied previously (4, 5). However, few data are available on the decomposition properties of diazolidinyl urea in cosmetics, and analytical methods for measuring diazolidinyl urea were only developed recently (6, 7). In a previous study, (4-hydroxymethyl-2,5-dioxo-imidazolidine-4-yl)-urea (HU) was reported to be the only end degradation product formed in basic conditions (6). Three molecules of formaldehyde are released from diazolidinyl urea to give HU, and the pH conditions of actual cosmetics range from acidic to basic (5), so diazolidinyl urea decomposition

Correspondence: Takahiro Doi, Department of Food, Drugs and Environment, Osaka Prefectural Institute of Public Health, 1-3-69 Nakamichi, Higashinari-ku, Osaka 537-0025, Japan. Tel: +81 6 6972 1321; Fax: +81 6 6972 2393. E-mail: tdoi@iph.pref.osaka.jp

Conflicts of interest: The authors have no conflict of interests to disclose.

Accepted for publication 5 December 2010

compounds other than HU are suspected to exist in cosmetics containing diazolidinyl urea.

Several patch test studies have reported on the contact sensitivities of diazolidinyl urea and other formaldehyde-releasers (8–15). According to the results of a previous study, the rates of positive reactions to diazolidinyl urea, imidazolidinyl urea and DMDM hydantoin in patch test studies differ with the vehicle used, that is, water or petrolatum (pet.) (8). This study showed that pet. vehicles are more sensitive than aqueous (aq.) vehicles for detecting allergic reactions to diazolidinyl urea. It was also reported that patients who show allergic reactions to diazolidinyl urea in aqueous vehicles are more likely to be formaldehyde-sensitive than those who show allergic reactions to diazolidinyl urea in pet. vehicles. In previous reports, the patch test cross-reactivity between formaldehyde-releasers was studied (12, 14, 16). As a result, it was found that the cross-reactive ratio between diazolidinyl urea and imidazolidinyl urea is higher than those for other substances. To understand the reason for these differences between water and pet. vehicles, and the mechanism of the cross-reaction between diazolidinyl urea and imidazolidinyl urea, it is important to know the chemical properties of diazolidinyl urea in patch test materials. Recently, a report on the formaldehyde concentration in formaldehyde-releaser patch test preparations was published (17), but there are no previous reports referring to diazolidinyl urea decomposition in patch test vehicles.

In this study, we show the diazolidinyl urea decomposition properties in buffers, cosmetics, and patch test materials. Furthermore, suspected diazolidinyl urea decomposition compounds were isolated by high-performance liquid chromatography (HPLC), and the isolates were characterized by liquid chromatography–electrospray ionization–mass spectrometry (LC-ESI-MS) and ^1H -nuclear magnetic resonance (^1H -NMR) analysis. Elucidation of diazolidinyl urea decomposition in cosmetics and patch test vehicles is thought to be key to understanding contact dermatitis caused by diazolidinyl urea in cosmetics and the cross-reactivity between diazolidinyl urea, other formaldehyde-releasers, and formaldehyde itself.

Materials and Methods

Chemicals and reagents

Liquid chromatography–mass spectrometry (LC-MS)-grade acetonitrile, HPLC-grade tetrahydrofuran and special-grade acetic acid, aqueous ammonia solution and ammonium acetate were purchased from Wako Pure

Chemical Industries (Osaka, Japan). Diazolidinyl urea (>95%) was obtained from Sigma (St Louis, MO, USA).

Instrumentation and chromatographic conditions

An LC-10AD liquid chromatography system (Shimadzu, Kyoto, Japan) equipped with a photodiode array detector (PDA) was used. The HPLC separation was performed as described previously (7), at 30°C on a COSMOSIL HILIC column [5 μm particle size, 4.6 mm internal diameter (ID) $\times$ 250 mm] (Nacalai tesque, Kyoto, Japan) under isocratic conditions. The mobile phase consisted of acetonitrile and water in a 90:10 ratio, and the flow rate was 1 ml/min. The volume of the injected sample was 10 or 50 μl .

The LC-MS experiments were performed with a Prominence UFLC liquid chromatography system and an LCMS-2020 quadrupole mass spectrometer (Shimadzu, Kyoto, Japan). A COSMOSIL-HILIC column (5 μm particle size, 2.0 mm ID $\times$ 50 mm) (Nacalai tesque) was used. The mobile phase was composed of acetonitrile and 10 mmol/l ammonium acetate in a 95:5 ratio under isocratic conditions. The flow rate of the mobile phase was 0.2 ml/min, and the injection volume was 10 μl . The column temperature was 30°C. The LC-MS studies were performed in the negative mode. The instrument parameters were as follows: source temperature, 350°C; desolvation temperature, 250°C; capillary voltage, 5.0 kV; Q-array DC voltage, 20 V; desolvation voltage, 20 V; and desolvation gas flow, 900 l/hr. The mass range of the spectra was from m/z 80–to m/z 580.

Purification and ^1H -NMR analysis

Compounds A and B were isolated from 3% and 0.1% diazolidinyl urea dissolved in 0.2 mol/l aqueous ammonia solutions. After 2 hr of stirring, the solutions were freeze-dried and redissolved in 20 mmol/l ammonium acetate solution (pH adjusted to 4.0 with acetic acid) at a concentration of 20%. The solutions were then diluted 10 times with acetonitrile, filtered, and injected into an HPLC system with a COSMOSIL HILIC column (5 μm particle size, 10 mm ID $\times$ 250 mm) (Nacalai tesque) and a 250- μl injection volume. The collected compound A and B fractions were freeze-dried prior to ^1H -NMR analysis.

^1H -NMR analysis was performed with an AV400M digital NMR (Bruker Biospin K.K., Kanagawa, Japan) at the Hitachi Chemical Techno Service Co., Ltd (Ibaragi, Japan). The solvent used was dimethylsulfoxide, and the field was 400.13 MHz.

Cosmetic and patch test samples

The brand names and manufacturers of the commercial cosmetics tested are shown in Table 1. Patch

Table 1. The brand names and manufacturers of the analysed commercial cosmetics

Brand names	Manufacturers
Solarcaine Cool Aloe Burn Relief	Schering-Plough HealthCare Products, Inc., Memphis, TN
Bull Frog Superblock Lotion Sunblock SPF50 with Titanium Dioxide Power	Chattem, Inc., Chattanooga, TN
Vagisil Feminine Moisturizer	Combe Inc., White Plains, NY
Daily Moisture with SPF 15 Lotion	Pfizer Consumer Healthcare, Morris Plains, NJ
Smackers Moisturizing Lotion Strawberry	The Bonne Bell Company, Lakewood, OH
Smackers Starburst Moisturizing Conditioner Strawberry	
Smackers Starburst Moisturizing Conditioner Orange	
Mill Creek Deep Conditioning Biotin Gel	U.S.I.T.C., Las Vegas, NV
Mill Creek Biotene H-24 Natural Scalp Conditioning Shampoo	
Dep Sport Styling Gel Endurance 11 Intense Hold	Henckel Consumer Goods Inc., Irvine, CA
Dep Styling Gel Texture 9 Ultimate Hold	
Jason All Natural Hi Shine Styling Gel	Hain Celestial Group Inc., Culver City, CA
Jason Apricot Roll-on Deodorant	
Hempz Pure Herbal Extracts Styling Gel	Supre Inc., Dallas, TX
Biolage Sunsentials Protective Sun Gel	Matrix LLC, New York, NY
Aqua Glycolic Shampoo Body Cleanser	Merz Pharmaceuticals, Greensboro, NC
SHIKAI Moisturizing Shower Gel Apricot Rose	SHIKAI products, Santa Rosa, CA
SHIKAI Color Reflect Gold Shampoo	
SHIKAI Color Reflect Mist Go Conditioner	
SHIKAI Color Reflect Intensive Repair Conditioner	
Emerald Forest Botanical Shampoo	Natural Nectar Corporation, Corona, CA
Emerald Forest Botanical Conditioner	
Emerald Forest Botanical Revitalizing Conditioner	
Hydrating Shea Butter Shampoo	Desert Essence, Valencia, CA
Bain de Terre Lemongrass Volumizing Shampoo	Bain de terre, Darien, CT
ABBA Pure Color Protect Shampoo	ABBA Pure Performance Hair Care Inc., Jacksonville, FL
Bellance Keratin Conditioner	Bellance International Cosmetics, Beaverton, OR
Fruit of the Earth Aloe Vera Skin Care Lotion	Fruit of the Earth Inc., Fort Worth, TX
ULTRA MIDE 25	IVAX Research Inc., Miami, FL

test samples were purchased from Chemotechnique Diagnostics® (Vellinge, Sweden), Almirall Hermal® GmbH (Reinbek, Germany), and Brial® allergen GmbH (Münster, Germany).

Preparation of samples from cosmetics and patch test vehicles

Sample preparation for the cosmetics and buffers was performed as described previously (7). The samples (0.1 ml or 0.1 g) were mixed briefly with 0.15 ml of 20 mmol/l ammonium acetate (pH 4.0) and 2.25 ml of acetonitrile. The mixed solutions were then centrifuged at 2080 g for 10 min at room temperature, and the supernatant was filtered through 0.45-µm polyethersulfone filters (Dainipon Seiki, Kyoto, Japan) prior to HPLC analysis.

The patch sample preparations were slightly modified. The pet. patch test samples (0.1 g) were completely suspended in 300 µl of tetrahydrofuran, mixed with 700 µl of 20 mmol/l ammonium acetate solution (pH adjusted to 4.0 with acetic acid), and centrifuged at 2080 g for 10 min at room temperature. The water layer of the centrifuged samples was transferred to a

new 15-ml tube and diluted 10 times with acetonitrile. For the preparation of the water-based patch test samples (0.1 ml), 200 µl of 20 mmol/l ammonium acetate solution (pH adjusted to 4.0 with acetic acid) were added instead of 300 µl of tetrahydrofuran in the first step.

Results

HPLC analysis of diazolidinyl urea decomposition in buffers

A possible diazolidinyl urea decomposition pathway is shown in Fig. 1. Diazolidinyl urea (compound **1**) is thought to release formaldehyde to give HU (compound **8**), and six candidates (compounds **2–7**) for intermediate decomposition compounds are shown. To study how diazolidinyl urea is converted to HU, diazolidinyl urea solution dissolved in 20 mmol/l ammonium acetate (pH adjusted to 4.0, 6.5, or 9.0) was incubated at 25°C for 2 months and analysed with HPLC-PDA in a time-dependent manner. The representative chromatograms produced by HPLC-PDA analysis are shown in Fig. 2a. The ultraviolet (UV) chromatogram of diazolidinyl urea

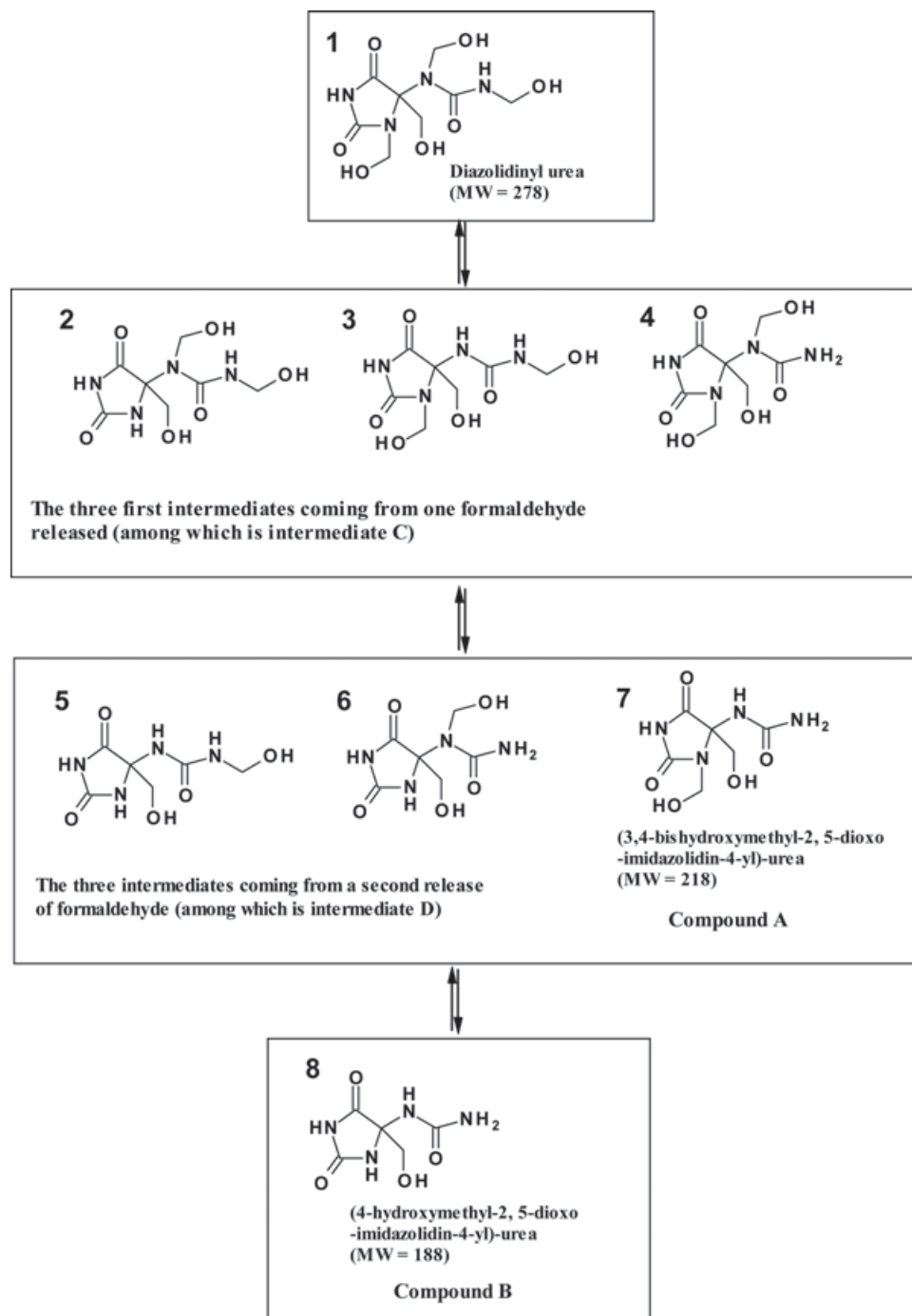


Fig. 1. Possible decomposition pathway of diazolidinyl urea to (4-hydroxymethyl-2,5-dioxo-imidazolidine-4-yl)-urea.

dissolved in buffers showed at least four peaks, which are thought to be derived from diazolidinyl urea and its decomposition compounds. These peaks were arrow-headed, and were named peak A to peak D for descriptive purposes.

Time-dependent changes in the peak area ratios of each peak are shown in Fig. 2b. At a concentration of 0.3%, diazolidinyl urea decomposition seemed to reach equilibrium on day 1 at pH 9.0 and on day 5 at pH 6.5. However, diazolidinyl urea still continued to decompose

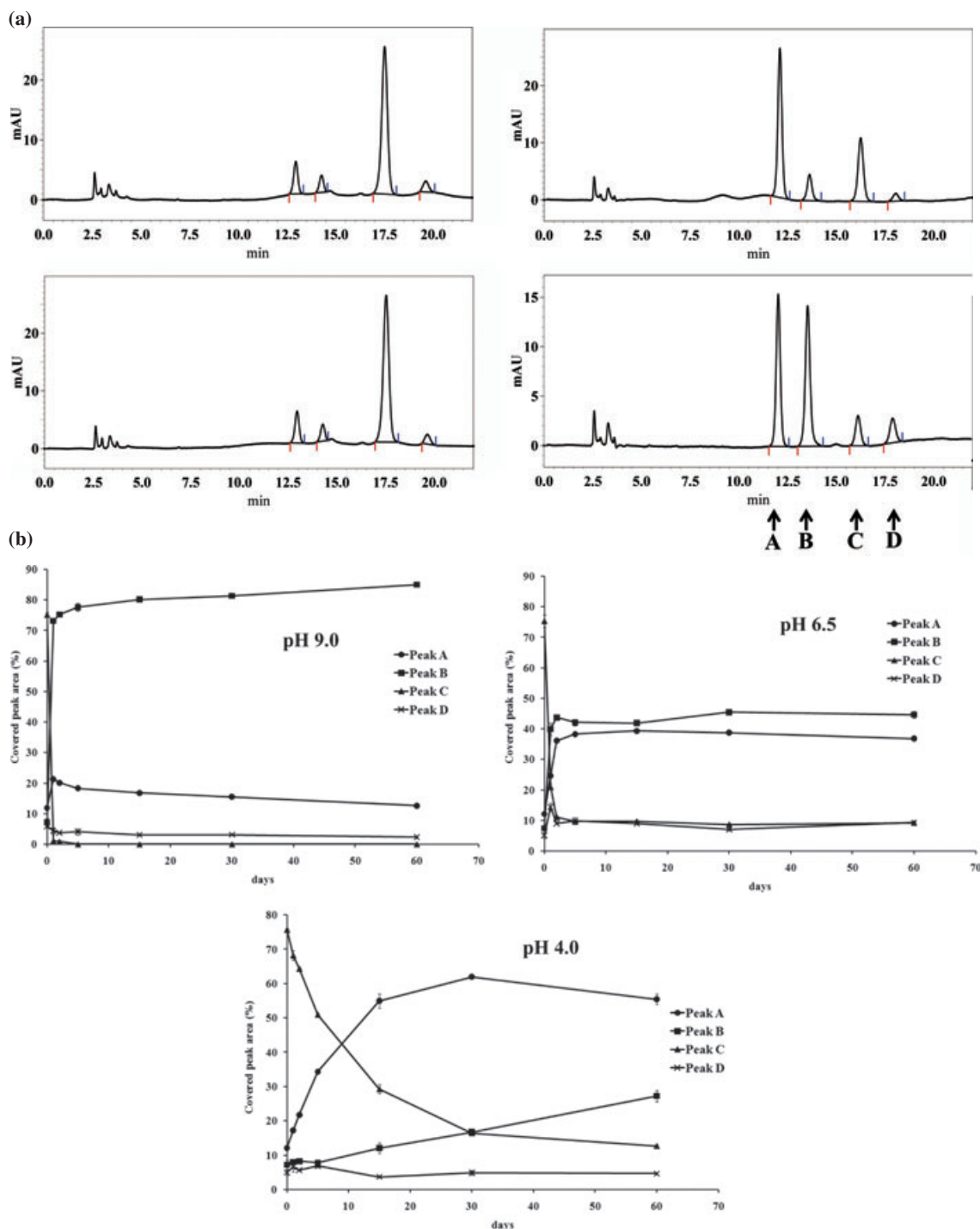


Fig. 2. Decomposition of diazolidinyl urea dissolved in buffers. The diazolidinyl urea-derived peaks are arrow-headed and labelled. (a) HPLC chromatogram (wavelength = 210 nm) for 0.3% diazolidinyl urea in 20 mmol/l ammonium acetate; pH 4.0 at day 0 (upper left); pH 4.0 at day 15 (upper right); pH 6.5 at day 0 (bottom left); pH 6.5 at day 15 (bottom right). (b) Time-dependent changes in the ratio of peak area to total peak area. Each value represents the mean $\pm$ standard deviation ($n=3$); circles, peak A; squares, peak B; triangles, peak C; crosses, peak D. Diazolidinyl urea solutions in 20 mmol/l ammonium acetate (pH value adjusted to 4.0, 6.5 or 9.0 with acetic acid or aqueous ammonia) were prepared at a concentration of 0.3% and incubated at 25°C for 60 days. Sampling was performed on days 1, 2, 5, 15, 30, and 60. For the HPLC–PDA conditions, see Materials and Methods.

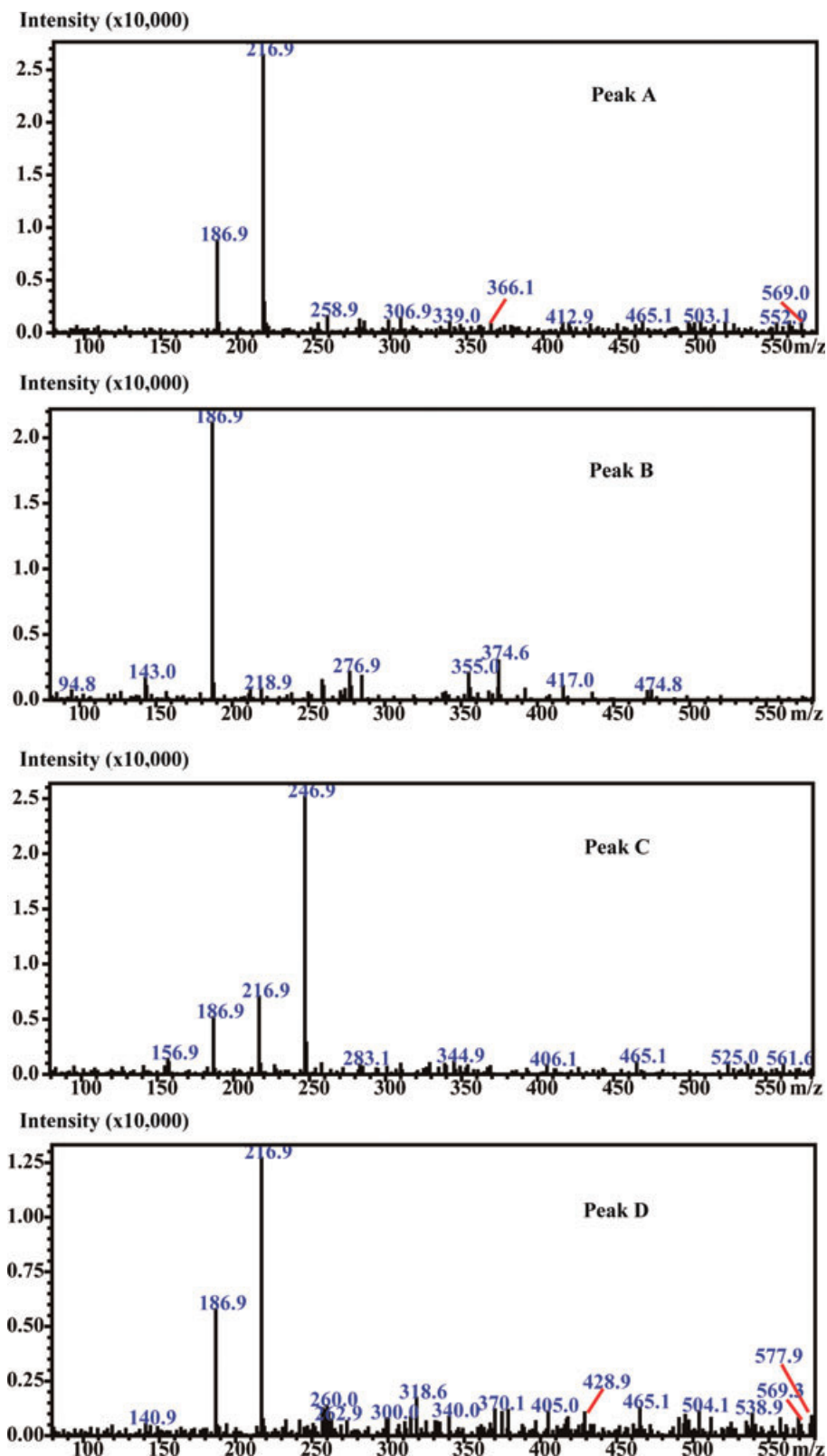


Fig. 3. Mass spectra of each peak isolated from 0.3% diazolidinyl urea in 20 mmol/l ammonium acetate (pH adjusted to 9.0 with aqueous ammonia) produced by HPLC analysis.

until day 60 at pH 4.0. This result showed that compound C decomposed to compound B via compound A.

LC-MS analysis of diazolidinyl urea decomposition in buffers

After 30 days of incubation at 25°C, diazolidinyl urea solution (0.3%, pH 6.5) was fractionated with HPLC-PDA, and the isolated peaks were analysed by LC-ESI-MS for further characterization (Fig. 3).

In the electrospray ionization (ESI) spectra, ions were observed at m/z 217 (parent ions) and m/z 187 (elimination of a formaldehyde unit) in the negative mode analysis of peak A and peak D. In the mass spectrum of peak C, ions were observed at m/z 247, m/z 217 (elimination of a formaldehyde unit) and m/z 187 (elimination of two formaldehyde units) in the negative mode analysis. These data suggest that the molecular weights of compound A and D, compound B and compound C were 218, 188, and 248, respectively. All isolated peaks except for peak B were converted to compound B after mixing with an equivalent volume of 20 mmol/l ammonium acetate (pH adjusted to 6.5) and incubation at 25°C for 2 weeks (data not shown). From these results, compound B was suspected to be the final degradation compound (HU, compound 8).

Diazolidinyl urea decomposition in cosmetics

To elucidate the decomposition properties of diazolidinyl urea in cosmetics, purchased cosmetic samples were analysed with HPLC-PDA and LC-MS. Typical HPLC chromatograms of diazolidinyl urea-containing cosmetics are shown in Fig. 4a. As with the diazolidinyl urea in the buffers, we observed four peaks in the HPLC chromatograms of most of the cosmetics analysed. Compounds A and B covered approximately 90% of the total peak area on the average of all samples (Fig. 4b).

To identify the peaks derived from cosmetics containing diazolidinyl urea, the peaks were isolated from ABBA Pure Color Protect Shampoo and analysed by LC-ESI-MS (data not shown). In the ESI spectra, ions were observed at m/z 217 (parent ions) and m/z 187 (elimination of a formaldehyde unit) in the negative mode analysis of peak A and peak D. In the mass spectrum of peak B, signals were observed at m/z 187 in the negative mode analysis. In the mass spectrum of peak C, ions were observed at m/z 247, m/z 217 (elimination of a formaldehyde unit) and m/z 187 (elimination of two formaldehyde units) in the negative mode analysis. These data suggest that the compounds isolated from the cosmetic samples were identical to those isolated from the diazolidinyl urea dissolved in the buffers.

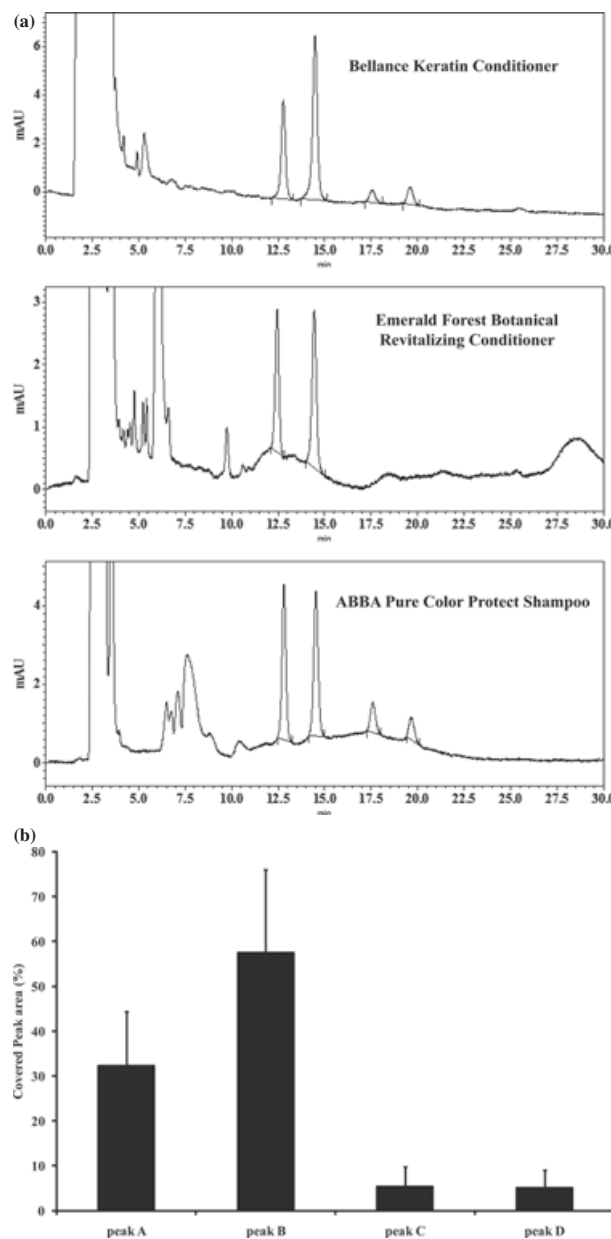


Fig. 4. HPLC analysis of purchased cosmetic samples. (a) The typical UV chromatogram (wavelength = 210 nm) of the cosmetic samples. (b) Mean ratio of peak area to total peak area of the cosmetic samples. Each value represents the mean $\pm$ standard deviation ($n=3$).

¹H-NMR analysis of diazolidinyl urea decomposition products

The purification of compounds A and B is described in Materials and Methods.

¹H-NMR chemical shifts are expressed relative to tetramethylsilane (0.0 ppm).

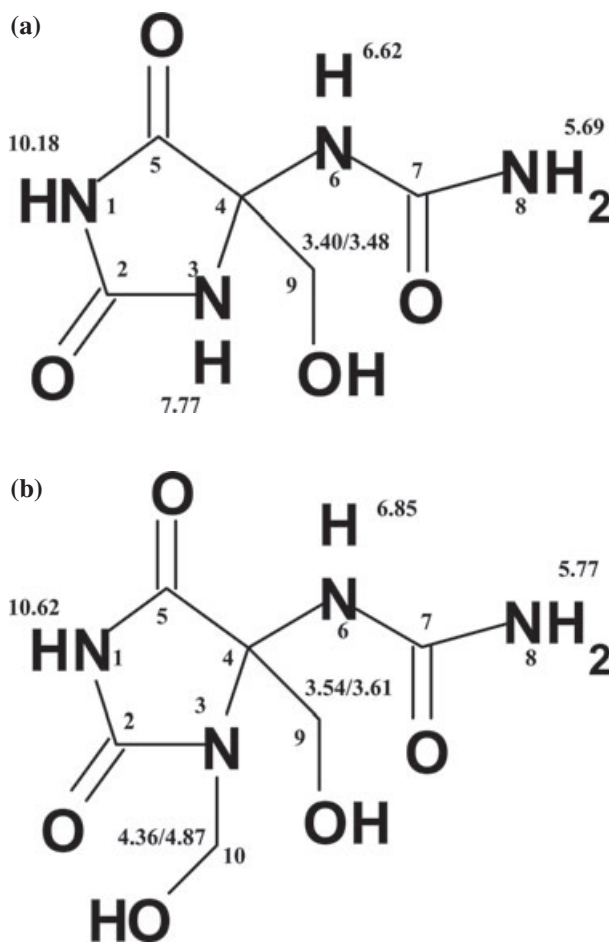


Fig. 5. Nuclear magnetic resonance assignments of 4-hydroxymethyl-2,5-dioxo-imidazolidine-4-yl)-urea (a) and (3,4-bis-hydroxymethyl-2,5-dioxo-imidazolidine-4-yl)-urea (b).

Compound A: δ 3.54/3.61 [H-C(9), both d ($J = 11.0$ Hz), 2H], 4.36/4.87 [H-C(10), both d ($J = 11.2$ Hz), 2H], 5.77 [H₂-N(8), s, 2H], 6.85 [H-N(6), s, H], and 10.62 [H-N(1), s, H].

Compound B: δ 3.40/3.48 [H-C(9), both d ($J = 10.6$ Hz), 2H], 5.69 [H₂-N(8), s, 2H], 6.62 [H-N(6), s, H], 7.77 [H-N(3), s, H], and 10.18 [H-N(1), s, H].

The signals of compound B were in agreement with the published data for HU (Fig. 5a)(6). The ¹H-NMR data of compound A were similar to those of compound B, except for the lack of a proton at the N(3) position and the presence of presumed methylene protons at the C(10) position. From the results of mass spectrometry analysis and ¹H-NMR analysis, compound A was supposed to be (3,4-bis-hydroxymethyl-2,5-dioxo-imidazolidine-4-yl)-urea (3,4-BHU, compound 7) (Fig. 5b).

Analysis of diazolidinyl urea in patch test materials

Commercial and prepared patch test materials were analysed with HPLC-PDA. The peak area ratios of each peak in the UV chromatogram at a wavelength of 210 nm are shown in Figs 6 and 7. In every analysis of the purchased patches, compound C covered approximately 75% of the total peak area. The peak patterns of the prepared patch test substances analysed immediately after preparation were very similar to those of the purchased patches (Figs 6 and 7).

As the patch test materials are usually applied to patients' skin for 48 hr, we evaluated the stability of diazolidinyl urea in prepared patches at 37°C for 48 hr (Fig. 7). The pet. patches showed no changes in their UV chromatograms, but the water patches showed the decomposition of diazolidinyl urea-derived compounds after 48 hr. In the water patches at 48 hr, compounds A and C were the major components and covered approximately 80% of the total peak area.

Discussion

The present study demonstrated that there were at least four diazolidinyl urea decomposition compounds – with molecular weights of 188, 218, and 248 – in samples prepared from buffers, cosmetics, and patch test materials. The compositions of these degradation compounds were different between the cosmetics and pet. patches. Our results also suggest that diazolidinyl urea is mostly present as HU (compound 8) and 3,4-BHU (compound 7) in cosmetics.

In our previous study, we could hardly find a peak with the same retention time as the diazolidinyl urea standard solution in an HPLC-PDA analysis of cosmetic samples (7). As formaldehyde was detected in the cosmetics, we regarded diazolidinyl urea decomposition as the reason why the peaks disappeared. Therefore, in this study, to understand the degradation properties of diazolidinyl urea in various conditions, we first evaluated diazolidinyl urea decomposition in buffers. The buffer studies showed that an increase in pH and a decrease in concentration promote diazolidinyl urea decomposition (Fig. 3 and data not shown). These data are in agreement with previous studies of formaldehyde release (4, 18). We showed that, in the acidic buffer, diazolidinyl urea decomposed to 3,4-BHU via compound C (molecular weight of 248, possibly compound 3 or 4) and some of the 3,4-BHU degraded to HU after incubation at 25°C for 60 days.

HPLC-PDA and LC-MS studies suggested that the detected decomposition compounds had taken one or more molecules of formaldehyde from diazolidinyl urea. To clarify whether compound B was HU, we tried

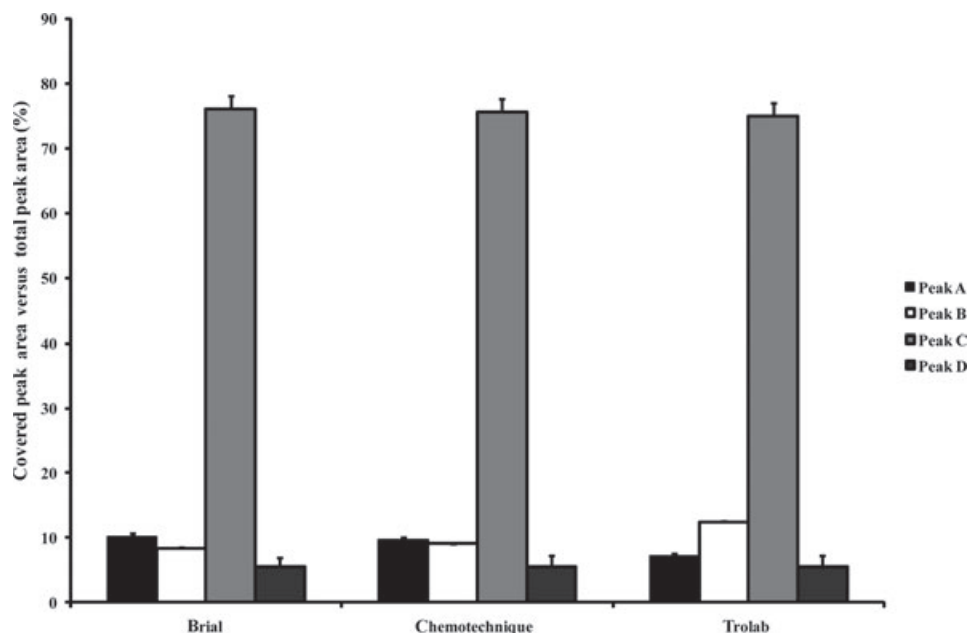


Fig. 6. Ratios of peak area to total peak area in the UV chromatograms (wavelength = 210 nm) of the purchased patch test materials. Each value represents the mean $\pm$ standard deviation ($n=3$); black, peak A; white, peak B; light grey, peak C; dark grey, peak D).

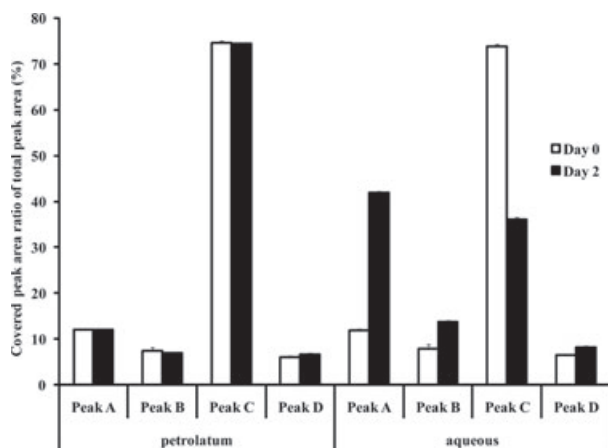


Fig. 7. Ratios of peak area to total peak areas in the UV chromatograms (wavelength = 210 nm) of the prepared patch test materials. Each value represents the mean $\pm$ standard deviation ($n=3$; open bar, day 0; closed bar, 48 hr after incubation at 37°C).

to completely degrade diazolidinyl urea, as described previously (6). However, we did not observe the complete conversion of diazolidinyl urea to compound B after 2 hr of stirring in 0.2 mol/l NH_3 at a concentration of 30 mg/ml. In the chromatogram of this solution produced by HPLC-PDA, peak A covered 50%, and peaks B and C covered approximately 20% of the total peak area (data not shown). As the diazolidinyl urea concentration had

decreased to 1 mg/ml, we were able to confirm that most of the diazolidinyl urea had been converted to compound B. A subsequent ^1H -NMR study showed that compound B was HU. In addition to HU, we showed that 3,4-BHU was the second most common diazolidinyl urea decomposition compound found in the cosmetics by purification and an ^1H -NMR study of compound A. The molecular weights of the minor compounds C and D were suspected to be 248 and 218 from LC-MS studies. However, as compounds C and D partially decompose during the purification process and lyophilization, we have not been able to isolate and identify them yet.

Immediately after preparation, every sample—in buffers, water, and pet.—showed similar chromatograms, in which compound C covered approximately 75% of the total peak area. In our previous report, we regarded the major chromatographic peak as diazolidinyl urea itself. However, in this LC-MS study, the compound was shown to be a degraded product, which had taken one molecule of formaldehyde from diazolidinyl urea. A recent ^{13}C -NMR study found no free formaldehyde in pet.-based patch test materials containing diazolidinyl urea, and 0.29% wt/wt of free formaldehyde was detected in 2.0% diazolidinyl urea aqueous solution (17). Therefore, diazolidinyl urea is suspected to exist as it does in pet. patches, and one or more molecules of formaldehyde were considered to be released when the pet./tetrahydrofuran solutions were mixed with 20 mmol/l ammonium acetate solution (pH adjusted

to 4.0 with acetic acid) in the sample preparation of our HPLC analysis method. The rapid formaldehyde release of diazolidinyl urea dissolved in water and buffer, as well as in the samples prepared from pet. patches, appears to result from contact with the aqueous solvent. It is expected that the development of a method for diazolidinyl urea analysis in non-aqueous conditions will uncover the trigger of diazolidinyl urea decomposition.

HPLC-PDA and LC-MS studies of cosmetic samples indicate that most diazolidinyl urea-derived compounds are present as decomposition compounds. On average, more than 90% of diazolidinyl urea decomposes to HU and 3,4-BHU in cosmetics. Compounds C and D were not detected in 9 of 29 samples (data not shown). We also measured time-dependent changes of diazolidinyl urea decomposition with 0.5% diazolidinyl urea solutions prepared with diazolidinyl urea-free lotion and conditioner. In both of these samples, more than 80% of diazolidinyl urea was converted to compound A or B after incubation at 25°C for 30 days (data not shown). In contrast, in the UV chromatogram of the sample solutions prepared from pet. patch test substances, the estimated ratio of HU and 3,4-BHU was less than 20% in all cases. Although the effect of moisture conditions on the skin were ignored in our analysis, 48 hr of incubation at 37°C did not change

the chromatograms of diazolidinyl urea pet. patches. Further incubation at 37°C until day 15 made no difference to the HPLC result for the diazolidinyl urea pet. sample, and diazolidinyl urea was also stable after incubation at 25°C for 30 days when it was dissolved in dimethylsulfoxide (data not shown). These data are in support of diazolidinyl urea stability in non-aqueous conditions, such as in pet.. With the use of diazolidinyl urea pet. patches, positive reactions for HU and 3,4-BHU can be discriminated because of their low content in the patches. In the water vehicles, the proportion of 3,4-BHU was increased from 10% to nearly 40% after 48 hr of incubation at 37°C. With water vehicles, because diazolidinyl urea decomposition progresses after application to the skin, if the patch test shows a positive reaction it is difficult to identify the causative compounds. Taken together, these findings show that, in the case of contact dermatitis caused by cosmetics containing diazolidinyl urea, patch testing for HU and 3,4-BHU with pet. as the vehicle is useful for the identification of the source compound.

Acknowledgements

This study was partly supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

References

- de Groot A C, Veenstra M. Formaldehyde-releasers in cosmetics in the USA and in Europe. *Contact Dermatitis* 2010; **62**: 221–224.
- de Groot A C, White I R, Flyvholm M A, Lensen G, Coenraads P J. Formaldehyde-releasers in cosmetics: relationship to formaldehyde contact allergy. Part 1. Characterization, frequency and relevance of sensitization, and frequency of use in cosmetics. *Contact Dermatitis* 2010; **62**: 2–17.
- de Groot A C, Flyvholm M A, Lensen G, Menné T, Coenraads P J. Formaldehyde-releasers: relationship to formaldehyde contact allergy. Contact allergy to formaldehyde and inventory of formaldehyde-releasers. *Contact Dermatitis* 2009; **61**: 63–85.
- Rosen M, McFarland A G. Free formaldehyde in anionic shampoo. *J Soc Cosmet Chem* 1984; **35**: 157–169.
- Doi T, Kajimura K, Taguchi S. Survey of formaldehyde (FA) concentration in cosmetics containing FA-donor preservatives. *J Health Sci* 2010; **56**: 116–122.
- Lehmann S V, Hoeck U, Breinholdt J, Olsen C E, Kreilgaard B. Characterization and chemistry of imidazolidinyl urea and diazolidinyl urea. *Contact Dermatitis* 2006; **54**: 50–58.
- Doi T, Kajimura K, Takatori S, Fukui N, Taguchi S, Iwagami S. Simultaneous measurement of diazolidinyl urea, urea, and allantoin in cosmetic samples by hydrophilic interaction chromatography. *J Chromatogr B Analyt Technol Biomed Life Sci* 2009; **877**: 1005–1010.
- Rietschel R L, Warshaw E M, Sasseville D et al. Sensitivity of petrolatum and aqueous vehicles for detecting allergy to imidazolidinylurea, diazolidinylurea, and DMDM hydantoin: a retrospective analysis from the North American Contact Dermatitis Group. *Dermatitis* 2007; **18**: 155–162.
- Jong C T, Statham B N, Green C M et al. Contact sensitivity to preservatives in the UK, 2004–2005: results of multicentre study. *Contact Dermatitis* 2007; **57**: 165–168.
- Warshaw E M, Buchholz H J, Belsito D V et al. Allergic patch test reactions associated with cosmetics: retrospective analysis of cross-sectional data from the North American Contact Dermatitis Group, 2001–2004. *J Am Acad Dermatol* 2009; **60**: 23–38.
- Zug K A, Warshaw E M, Fowler J F Jr et al. Patch-test results of the North American Contact Dermatitis Group 2005–2006. *Dermatitis* 2009; **20**: 149–160.
- Statham B N, Smith E V, Bodger O G et al. Concomitant contact allergy to methylchloroisothiazolinone/methylisothiazolinone and formaldehyde-releasing preservatives. *Contact Dermatitis* 2010; **62**: 56–57.
- Thyssen J P, Engkilde K, Lundov M D, Carlsen B C, Menné T, Johansen J D. Temporal trends of preservative allergy in Denmark (1985–2008). *Contact Dermatitis* 2010; **62**: 102–108.
- Lundov M D, Johansen J D, Carlsen B C, Engkilde K, Menné T, Thyssen J P. Formaldehyde exposure and patterns of concomitant contact allergy to formaldehyde and formaldehyde-releasers. *Contact Dermatitis* 2010; **63**: 31–36.
- Garcia-Gavin J, Gonzalez-Vilas D, Fernandez-Redondo V, Toribo J. Allergic

- contact dermatitis in a girl due to several cosmetics containing diazolidinyl-urea or imidazolidinyl-urea. *Contact Dermatitis* 2010; **63**: 49–50.
- 16 de Groot A, White I R, Flyvholm M A, Lensen G, Coenraads P J. Formaldehyde-releasers in cosmetics: relationship to formaldehyde contact allergy. Part 2. Patch test relationship to formaldehyde contact allergy, experimental provocation tests, amount of formaldehyde released, and assessment of risk to consumers allergic to formaldehyde. *Contact Dermatitis* 2010; **62**: 18–31.
- 17 Emeis D, de Groot A C, Brinkmann J. Determination of formaldehyde in formaldehyde-releaser patch test preparations. *Contact Dermatitis* 2010; **63**: 57–62.
- 18 Emeis D, Anker W, Wittern K P. Quantitative ¹³C NMR spectroscopic studies on the equilibrium of formaldehyde with its releasing cosmetic preservatives. *Anal Chem* 2007; **79**: 2096–2100.