

Experimental elicitation of contact allergy from a diazolidinyl urea-preserved cream in relation to anatomical region, exposure time and concentration

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The elicitation potential of the cosmetic preservative diazolidinyl urea was studied in formaldehyde- and diazolidinyl urea-sensitized volunteer patients using a stepwise controlled exposure design. The test product was a facial moisturizer, preserved with varying concentrations of diazolidinyl urea, ranging from 0.05% to 0.6%. A repeated open application-like exposure test was performed on volunteers and a control group with the test product containing increasing preservative concentrations, on arm, neck and face, sequentially, for 2 weeks or until dermatitis developed. The preservative action in the cream at different test concentrations was tested in microbial challenge tests and was found effective at all concentrations tested. The study established a non-eliciting concentration of diazolidinyl urea of 0.05% in formaldehyde-sensitive patients and showed that the skin reactivity depends on the anatomical region, increasing from the upper arm to neck and, possibly, to the face. The study design, beginning on the upper arm and moving on to the neck and face seems to be relevant for the study of reactions to cosmetic products. A clear dose-response relationship was seen regarding preservative concentration in the product.

Key words: anatomical region; contact dermatitis; diazolidinyl urea; formaldehyde allergy; Germall II; ROAT. © Blackwell Munksgaard, 2005.

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Effective preservatives are needed to prevent microbial contamination of cosmetic products which may arise from raw materials, manufacturing processes and consumer use and which may lead to product degradation and constitute a risk to consumer health (1, 2). The formaldehyde releaser, diazolidinyl urea, provides effective broad spectrum inhibition of Gram-negative and Gram-positive bacteria, moulds and yeasts in the concentration range of 0.1%–0.5% (3). It has been used in both leave-on and rinse-off products for over 20 years, often in combination with parabens, because it only has a limited effect against fungi. It is believed that the antimicrobial effect of diazolidinyl urea is achieved, among others, through the activity of formaldehyde released from the parent molecule in an aqueous (aq.) environment.

Available data suggest that contact allergy to products preserved with diazolidinyl urea can be attributed to diazolidinyl urea itself, formaldehyde or both.

In a large group of patch-tested patients, 0.5%–1% reacted to diazolidinyl urea and 2%–3% to formaldehyde (4). Most patients reacting to diazolidinyl urea also react to formaldehyde (5, 6). However, there are also studies that indicate the opposite (7).

From earlier studies, it is known that formaldehyde may elicit an allergic reaction during repeated occlusive exposure down to 30 p.p.m. but not during a non-occluded exposure (8).

As the current understanding of diazolidinyl urea and formaldehyde allergy is mainly based on patch test studies, it was decided to perform an experimental use test exposure study with a cream preserved with diazolidinyl urea in diazolidinyl urea- and formaldehyde-allergic patients. We developed a dose-escalating design, including 4 diazolidinyl urea concentrations, and exposure to 3 different anatomical regions (upper arm, neck and face), each of 2-week duration.

Materials and methods

Test subjects

150 unselected eczema patients, males and females, 18–65 years of age, who previously had given at least a + patch test reaction to formaldehyde 1% aq. or diazolidinyl urea 2% petrolatum (pet.) were reviewed. 108 patients were contacted for inclusion in the study, and 65 patients agreed to be patch tested. All formaldehyde-positive patients, except those who showed a positive patch test response within 1 week preceding the study, were reconfirmed by a retest. Diazolidinyl urea-allergic subjects were either reconfirmed patients, who formerly had tested positive for diazolidinyl urea, or newly diagnosed patients. 10 healthy volunteers who had given a negative patch test to formaldehyde 1% aq. and diazolidinyl urea 2% pet. were included as controls. Pregnant or lactating women and subjects who had a current acute or widespread eczema at any site were excluded from the study. The local Ethics Committees approved the study, and all participants signed an informed consent. The study was performed at the dermatological clinics in Gentofte and Odense.

Patch test materials

Formaldehyde 1% aq., diazolidinyl urea (Germall II) 2% pet., diazolidinyl urea used in the test cream formulation 1% aq., paraben mix 16% pet., ethylenediaminetetraacetic acid (EDTA) 1% pet., test cream without diazolidinyl urea and rubber from finger cots were used for patch testing. Formaldehyde, paraben mix, EDTA and diazolidinyl urea were obtained from Hermal Chemie (Reinbek, Germany).

Patch testing

Patch testing was performed with 15 µl of the aqueous solutions. The test cream without diazolidinyl urea and the finger cot rubber were applied under patch in quantities suitable for patch testing. The patches were applied for 2 days to the upper back using Finn Chambers (Epitest OY, Helsinki, Finland) and Scanpor tape (Norges plaster A/S, Oslo, Norway). Readings were performed at 2 and 3–4 days. The International Contact Dermatitis Research Group (ICDRG) recommendations were followed (9).

Test cream

The test cream was a typical marketed facial moisturizer formulation containing some twenty usual ingredients, such as emulsifiers, moisturizers

and silicone and acrylate polymers. The only difference between the test cream and the marketed formulations was the concentrations of the diazolidinyl urea.

The 4 versions of the test cream, containing 0.05%, 0.15%, 0.30% and 0.60% diazolidinyl urea, respectively, were formulated.

Analytical quantification of formaldehyde in the test cream

Each version of the test cream was analysed for the total formaldehyde content using the European Union Working Party method L 108/92 (10).

Generally, the method is that of determination by pentane-2,4-dione colourimetry in which formaldehyde reacts with pentane-2,4-dione in the presence of ammonium acetate to form 3,5-diacetyl-1,4-dichlorolutidine. The latter is extracted with butan-1-ol, and the absorbance of the extract is measured at 410 nm.

The European Union Working Party on Methods and Chemical Analysis of Cosmetic Products developed, validated and published, in 1990, this method intended for determination of free and combined formaldehyde in the presence of formaldehyde releasers, among them diazolidinyl urea (10).

Recently, the method of analysis of diazolidinyl urea and other formaldehyde releasers was the subject of the Scientific Committee on Consumer Products and Non-Food Products intended for Consumers (SCCNFP) Opinions (11). The Opinions refer to the observations that diazolidinyl urea degrades spontaneously in aqueous/polar solvents. Intensive investigations, using several analytical techniques, failed to verify the identity of the decomposition products under experimental conditions.

It has not been established whether all the molecules present in the solution of diazolidinyl urea are decomposition products of the parent molecule or the parent molecule itself is a mixture of isomers. The Opinions concluded that it was not possible to develop reliable method(s) of analysis of diazolidinyl urea, and therefore, the compliance of cosmetic products, with respect to the content of diazolidinyl urea, should be ascertained by measuring the total formaldehyde content in the cosmetic product. More recent studies on characterization of diazolidinyl urea (12) reconfirmed that it consists of a complex mixture of numerous allantoin-formaldehyde condensation products and that different equilibria may exist between different structures, depending on the formula matrix.

Preservative efficacy testing (challenge test)

The efficacy of preservation of the cream samples was assayed using a challenge-test method derived from the European Pharmacopoeia method (13).

Organisms. The micro-organisms used in this study were all strains from American Type Culture Collection (ATCC). They were the following: *Escherichia coli* (ATCC 8739), *Pseudomonas aeruginosa* (ATCC 19429), *Staphylococcus aureus* (6538), *Enterococcus faecalis* (ATCC 33186), *Candida albicans* (ATCC 10231) and *Aspergillus niger* (ATCC 6275).

Inocula preparation. Bacteria were cultured on Tryptic soy agar (AES, Combourg, France) for 24 ± 1 hr at $35 \pm 2^\circ\text{C}$. *Candida albicans* was grown on Sabouraud agar (AES) for 24 ± 1 hr at $35 \pm 2^\circ\text{C}$ and *A. niger* on malt extract agar (AES) for 5 days at $35 \pm 2^\circ\text{C}$.

For microbial inocula, the cells were harvested into Tryptone salt broth (Biomérieux, Lyon, France) by gentle agitation and adjusted to yield suspensions of approximately 10^8 colony-forming units (CFUs)/ml or 10^8 spores/ml using turbidimetry absorbance correlated to an aerobic plate count. The diluent used for harvesting *A. niger* contained 0.03% (v/v) of Tween-80 (Sigma-Aldrich, Milan, Italy).

Inoculation. The formulations (samples of 30 g) were placed in sterile glass containers and separately inoculated with bacterial and fungal suspensions to give a final level of approximately 10^6 CFU/ml. After a contact time of 7 days at $22 \pm 2^\circ\text{C}$, the samples (1.0 g) were removed and placed into 9.0 ml of neutralizing medium LT100 broth (Biomérieux). Cell viability was determined by the plate count method on Agar LT100 (Biomérieux), and CFUs were counted after a 2-day incubation at $35 \pm 2^\circ\text{C}$ for bacteria and a 5-day incubation at $35 \pm 2^\circ\text{C}$ for fungi.

Study design

Phase 1. The formaldehyde-allergic patients were divided into 3 (non-randomised) groups of 10 and exposed to the test cream with 0.15%, 0.30% and 0.60% diazolidinyl urea, respectively. Additional 10 patients sensitized to diazolidinyl urea and some of them, simultaneously to formaldehyde (7/10), were exposed to the test cream containing 0.15% diazolidinyl urea.

Controls were exposed to the test cream with 0.60% diazolidinyl urea.

The study proceeded in a stepwise fashion:

For ethical reasons, the product containing the lowest concentration of diazolidinyl urea was tested first in the full sequence (upper arm, neck and face), before the higher concentration group began the treatment. Each anatomical area was exposed for 2 weeks. If no reaction occurred on the upper arm after 2 weeks, the cream application continued on the neck for another 2 weeks, and on the face, in a similar fashion, for further 2 weeks.

If a positive reaction was observed at any time before the end of the 6-week period, the patient was examined and withdrawn from the study.

Decision whether to move to the next concentration group or not was taken by the investigators at the end of each test sequence according to the frequency and the level of the reactions observed in the patients.

Phase II. At the end of the test sequence described above, a decision was taken to continue the study by exposing 10 formaldehyde-allergic patients to the test cream with 0.05% diazolidinyl urea. The patients were recruited from among those already tested with 0.15% and 0.3% diazolidinyl urea. The time lapse from the participation in the Phase I of the study was between 23 and 33 months (mean 28.3 months). These patients have already had a positive patch test twice and were therefore considered formaldehyde allergic. In these patients, with the exception of the face area, the repeated open application test (ROAT) was carried out on the side of the body (arm and neck) not previously exposed to the test cream.

ROAT

ROAT was carried out by the patient, who applied the test material twice daily to the 5×5 cm of the healthy skin of the flexor aspect of the mid upper arm.

The area was defined by marking the skin with an ink pen.

Thereafter, an area of approximately 5×5 cm on the side of the neck was used.

The size of the area was only approximate, as the square was not marked permanently on the skin, but only indicated to the patient at the beginning of the study.

On the face, the patient was instructed to apply the cream to the whole area, except around the mouth and eyes.

Approximately 0.1 ml of the test cream was applied twice daily for the maximum period of 2 weeks in each area. Tubes of cream were weighed by a technician before and after use. Test sites

Overall clinical impression	Negative	Doubtful	Weakly positive	Moderately positive	Strongly positive
Involved area of application	0 % (0)	1–24% (1)	25–49% (2)	50–89% (3)	90–100% (4)
Erythema	Involvement	None (0)	Spotty (1)		Homogenous (2)
	Strength	Weak (1)	Medium (2)		Strong (3)
Papules/ infiltration	None (0)	Few: <10 (1)	Some: 10–15 (2)	Many: >25 (3)	Homogenous infiltration (4)
Vesicles	None (0)	Few: <10 (1)	Some: 10–15 (2)	Many: >25 (3)	Confluent (4)
Sensory manifesta- tions	Negative (0)			Positive (1)	
Total score	18				

Fig. 1. Grading scale for reading of ROAT: morphology and strength of reactions.

were evaluated every second week, and a new tube of test cream was issued.

Skin grading of the ROAT

Readings of the use test area were quantified according to a reading scale (14), slightly modified to also take into account any subjective reactions (15). The maximum numerical score was 18. The grading scale is shown in Fig. 1.

The overall positive reactions were also evaluated qualitatively, as weak, moderate or strong, based on the clinical signs, erythema, papules and vesicles. Reaction sites were photographed for documentation.

Results

Patch testing

65 formaldehyde and/or diazolidinyl urea-allergic patients were patch tested. 38 patients could reproduce their formaldehyde allergy (58%) on retest, and 5 were newly diagnosed patients. 5 of the 7 diazolidinyl urea-allergic patients could reproduce their allergy, and 2 were newly diagnosed patients.

All the control subjects were negative.

The results of the patch test are summarized in Table 1.

Analytical results

The formaldehyde analysis results are summarized in Table 2.

Preservative efficacy testing (challenge test) results

The microbial challenge test results are summarized in Table 3.

The 4 versions of the test cream (0.05%, 0.15%, 0.30% and 0.60% diazolidinyl urea) reduced both the bacterial and the fungal inoculums by a factor of 10^5 within 7 days with the exception of the formulation at 0.05%, which reduced the *E. faecalis* inoculum by a factor of 10^3 (99.9%) within 7 days. These results are in compliance with the Cosmetic Toiletry and Fragrance Association (CTFA) recommendations regarding the determination of preservative adequacy in cosmetic formulations (16). The recommended criteria are the following: at least a 99.9% reduction of vegetative bacteria within 7 days and at least a 90% reduction of yeasts and molds within 7 days.

ROAT results

The overall results from the ROAT with the test creams are summarized in Table 4. The total number of positive reactions in 40 tested patients was 25 with 12 weak reactions, 12 moderate and 1 strong.

1 patient (formaldehyde-sensitive, diazolidinyl urea group 0.05%) developed an irritant eczematous reaction on the face at the end of the ROAT on the arm. This patient did not perform the ROAT on the face. The reactions were judged not allergic in nature and not related to the ROAT.

Representative examples of positive reactions are shown in Fig. 2.

Table 1. Patch test results of the 65 patients tested with formaldehyde 1% aqueous (aq.), diazolidinyl urea 2% petrolatum (pet.) (commercial patch) or diazolidinyl urea 1% aq. (test product) for the inclusion in the study

Patch test results	Formaldehyde 1% aq.(n)*	Diazolidinyl urea 2% pet. (commercial patch) (n)*	Diazolidinyl urea 1% aq. (test product) (n)*
+++	3 (0)	0	0
++	16 (4)	3 (0)	2 (0)
+	24 (1)	8 (6)	8 (3)
Negative or doubtful	22 (19)	54 (18)	55 (21)

*The number of subjects excluded from either the formaldehyde-sensitive or the diazolidinyl urea-sensitive group.

Table 2. Diazolidinyl urea and formaldehyde content of the test creams

Diazolidinyl urea (%)	0.05	0.15	0.3	0.6
Total formaldehyde (p.p.m)	130	370	730	1500

The results of the ROAT, in relation to the tested concentrations and anatomical sites, are summarized in Table 5.

The number of positive reactions to ROAT, expressed as percent of the tested subjects, is summarized in Table 6.

For the formaldehyde-sensitive patients, the below-threshold concentrations were 0.3% diazolidinyl urea on the arm and 0.05% on the neck and face.

Only 1 diazolidinyl urea-sensitive individual did not react to 0.15% diazolidinyl urea in any of the anatomical regions.

The average exposure to diazolidinyl urea, in $\mu\text{g}/\text{cm}^2$ /application, is summarized in Table 7.

There was no correlation between the strengths of the patch test reactions and the ROAT reactions.

The number of ROAT days necessary to elicit a positive reaction depended on the anatomical site and on the diazolidinyl urea percentage concentration. The results are presented as a Kaplan–Meier plot in Fig. 3.

The number of positive ROAT reactions, time to onset and anatomical site could be correlated to the diazolidinyl urea percentage concentration in the cream but not to the allergen exposure in $\mu\text{g}/\text{cm}^2$.

A Kaplan–Meier plot could not be constructed when exposure to diazolidinyl urea was expressed in $\mu\text{g}/\text{cm}^2$ of skin. A considerable inter-patient variability in the quantity of cream used made it impossible to group the volunteers into discrete clusters in terms of $\mu\text{g}/\text{cm}^2$. This was true for all areas on which ROAT was carried out, and it is

Table 3. Antimicrobial effectiveness of diazolidinyl urea in a cream formulation: log CFU/g after 7 days

	Concentration diazolidinyl urea (v/v) (%)			
	0.05	0.0715	0.100	0.300
<i>Escherichia coli</i>	1.0	1.0	1.0	1.0
<i>Pseudomonas aeruginosa</i>	1.0	1.0	1.0	1.0
<i>Staphylococcus aureus</i>	1.0	1.0	1.0	1.0
<i>Enterococcus faecalis</i>	2.0	1.0	1.0	1.0
<i>Candida albicans</i>	1.0	1.0	1.0	1.0
<i>Aspergillus niger</i>	1.0	1.0	1.0	1.0

illustrated in Fig. 4 for the neck, where the greatest number of allergic reactions was recorded. Figure 4 shows exposure in $\mu\text{g}/\text{cm}^2$ as a continuum with rising tendency, but not as distinct groups.

None of the patients in the control group developed a positive reaction to the ROAT (Fig. 3).

Discussion

The 6-week study covering 3 anatomical regions established a non-eliciting diazolidinyl urea concentration in formaldehyde-allergic (but not diazolidinyl urea-allergic) individuals. None of the 10 formaldehyde-allergic patients exposed to the cream preserved with 0.05% diazolidinyl urea, as well as the control group, showed an allergic reaction. The 0.05% diazolidinyl urea concentration corresponded to 130 p.p.m. formaldehyde in the tested formulation.

Study design

The test design is a further development of the traditional ROAT in that it used the anatomical areas relevant to the studied cosmetic product (neck and face for the facial moisturizer). To assure safety of the volunteers, exposure began

Table 4. Outcome of repeated open application test (ROAT) in formaldehyde- and diazolidinyl urea-sensitive individuals exposed to a diazolidinyl urea-preserved cream at 4 concentrations and on 3 anatomical regions

	Diazolidinyl urea concentration (%)				
	Formaldehyde sensitive†				Diazolidinyl urea sensitive†
	0.05	0.15	0.3	0.6	0.15
Tested	10	10	10	10	10
Weak reactions	0	0	4 (6)	3 (6–7)	5 (3–7)
Moderate reactions	0	2 (10)	3 (8–13)	3 (11–12)	4 (9–13)
Strong reactions	0	0	0	1 (16)	0
Total number of reactions	0	2	7	7	9
Average skin grading for the ROAT* (maximum 18)	0	10	7.9 (6–13)	10 (6–16)	7.7 (3–13)

*Among the volunteers who reacted.

†DATA in brackets represent ROAT scores including range, where appropriate.



Fig. 2. (a) A weak-positive reaction on the arm in a formaldehyde-sensitive patient after 14 days of repeated open application test (ROAT) with test cream with 0.6% diazolidinyl urea. (b) A moderate-positive reaction on the neck in a formaldehyde-sensitive patient after 7 days of ROAT with test cream with 0.3% diazolidinyl urea. (c) A strong-positive reaction on the neck in a formaldehyde-sensitive patient after 4 days of ROAT with test cream with 0.6% diazolidinyl urea. (d) A moderate-positive reaction on the face in a formaldehyde-sensitive patient after 12 days of ROAT with test cream with 0.15% diazolidinyl urea.

Table 5. Positive reactions to ROAT in relation to the anatomical region and the allergen concentration

	Diazolidinyl urea (%)	Arm (%)	Neck (%)	Face (%)	Total
Formaldehyde sensitive	0.05	0	0	0	0
	0.15	0	1 (10)	1 (11)	2
	0.3	0	6 (60)	1 (25)	7
	0.6	1	4 (67)	2 (40)	7
Diazolidinyl urea sensitive	0.15	6 (60)	3 (75)	0	9
Total		7	14	4	

on the upper arm, the traditional anatomical site for a ROAT.

From a clinical viewpoint, the dose-escalating methodology employed, both in reference to the concentration and the anatomical regions, represents an important advancement in the field of experimental elicitation studies, as neither severe allergic reactions, particularly on the face, nor spread or generalized flare of dermatitis were seen among the 40 diazolidinyl urea- and formaldehyde-allergic patients.

The more severe allergic reactions seen in real life may reflect a higher preservative concentration and product use on a larger skin area. Also, the relationship between the development of dermatitis and the product use may not be immediately obvious to the consumer who may continue to use the product after the onset of dermatitis.

A model with an initial ROAT on the upper arm and subsequently on the neck and face may well be helpful in the clinic to clarify and evaluate allergic reactions due to cosmetic products, used mostly on the face rather than the arms.

Formaldehyde-sensitive patients

With an increasing concentration of diazolidinyl urea from 0.15% to 0.6% (corresponding to formaldehyde concentration of 370 p.p.m. –1500 p.p.m., respectively), a dose-dependent increase in patients' reactivity was seen.

Table 6. The number of positive reactions to ROAT as percentage of tested subjects

	Formaldehyde sensitive		Diazolidinyl urea (%)	
	Tested	Reacted	Tested	Reacted
Arm	40	1 (2.5%)	10	6 (60%)
Neck	39	11 (28%)	4	3 (75%)
Face	28	4 (14%)	1	0

The dose-response was not only quantitative in terms of an increasing incidence in the number of reactors but also qualitative: the only reaction on the arm and the only reaction graded as 'strong' were recorded in the highest diazolidinyl urea exposure group (0.6%).

The reactivity depended upon the anatomical region as indicated by the number of patients who did not react on the arm but reacted on the neck or face.

The neck was clearly a more sensitive site than the upper arm: 28% of the tested patients reacted compared with 2.5%, respectively. 4 of the 28 patients tested (14%) reacted on the face, which would suggest that the face is less sensitive than the neck.

Alternative explanation could be a lower theoretical exposure on the face (Table 7). However, the overall number of subjects tested is too small to allow a definitive conclusion.

Diazolidinyl urea-sensitive patients

Patients allergic either to diazolidinyl urea only or to both formaldehyde and diazolidinyl urea comprised a separate group. Their sensitivity was greater than that of the formaldehyde-sensitive patients. The ROAT scores in the diazolidinyl urea-sensitive patients exposed to 0.15% diazolidinyl urea were comparable with those of formaldehyde-sensitive patients exposed to 0.3% diazolidinyl urea. No elicitation threshold was established in this group of patients under the study conditions (at 0.15%, the lowest tested diazolidinyl urea concentration) and only 1/10 completed the whole test sequence, with 6 of the patients reacting on the upper arm and 3 on the neck.

A possible explanation may be that these patients were sensitized to more than 1 allergen.

Table 7. Average calculated exposure to diazolidinyl urea in formaldehyde-sensitive patients.

Diazolidinyl urea concentration (%)	Average exposure ($\mu\text{g}/\text{cm}^2/\text{use}$)		
	Arm	Neck	Face
0.05	2.88 (1.63)	2.32 (1.34)	0.19 (0.09)
0.15	8.56 (3.77)	7.47 (4.08)	0.74 (0.21)
0.3	9.85 (7.02)	12.8 (6.16)	1.23 (0.45)
0.6	15.85 (9.91)	14.64 (5.40)	2.31 (0.76)

Standard error marked in brackets.

Group means joined by a vertical line are not statistically and significantly different [Duncan test ($\alpha = 0.05$) on log-transformed data].

Note: Exposure area on the arm is accurate (25 cm^2), on the neck – approximate (25 cm^2) and on the face – unverified (500 cm^2).

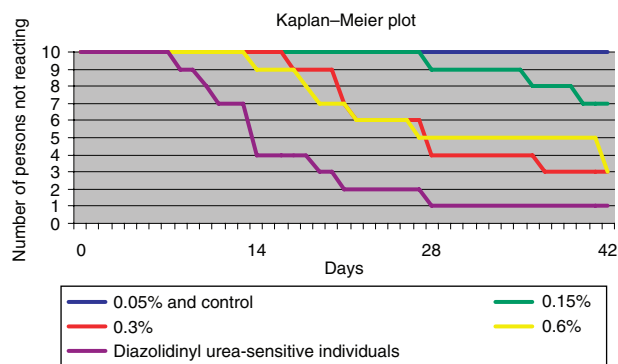


Fig. 3. The Kaplan–Meier plot shows the number of patients not reacting at a given date dependent on dose of diazolidinyl urea in the test cream. The day 0–14 represents the arm, the day 15–28 the neck and the day 29–42 the face. 1 patient in the 0.05% group did not perform the ROAT in the face.

7 of the 10 were sensitized to both formaldehyde and diazolidinyl urea (4 of these reacted on the arm). Diazolidinyl urea itself has been reported to exist in several forms, which may have different immunogenic properties (12). It is known that the threshold for a patch test response is lowered by simultaneous presence of more than 1 allergen. This combination effect can be either additive (17) or synergistic (18, 19).

Re-exposure to the allergen

The patients in the lowest concentration group (0.05%) were re-exposed to their allergen after a 2- to 2.5-year time lapse. All 10 of them were tested in the Phase I of the study on the 3

anatomical regions with the cream containing either 0.15% or 0.3% diazolidinyl urea. 9 of these patients completed the Phase I without adverse reactions, and 1 patient reacted on the face.

Re-exposure to the allergen in the Phase II of the study, even on the face, did not provoke dermatitis. This suggests that a genuine no-effect level may have been found in these 10 patients, as increased reactivity has been reported at the previous dermatitis sites, albeit after 8 months time lapse (20).

Skin grading

The clinical reactions were graded according to the grading scale proposed by Johansen et al. (14), further developed and used in practice by Krasteva et al. (15). The scale contains an overall clinical assessment as well as a numerical evaluation of the individual skin changes. The grading was performed by 2 investigators (CZ and KEA) and later validated on the basis of photographs taken by the authors.

Of the maximum 18 points, weak reactions scored 3–7, moderate 8–13 and the only strong reaction observed in this study scored 16 points (0.6% diazolidinyl urea on the neck).

The outcome of the ROAT is summarized in Table 4 and illustrated in Fig. 2. with pictures of the weak, moderate and strong reactions. The clinical details of the ROAT from the present study will be presented in a separate publication.

A positive ROAT in our study typically starts with follicular papules in the test area and eventually develops into papular vesicles (21). After day's redness, a homogenous infiltration develops

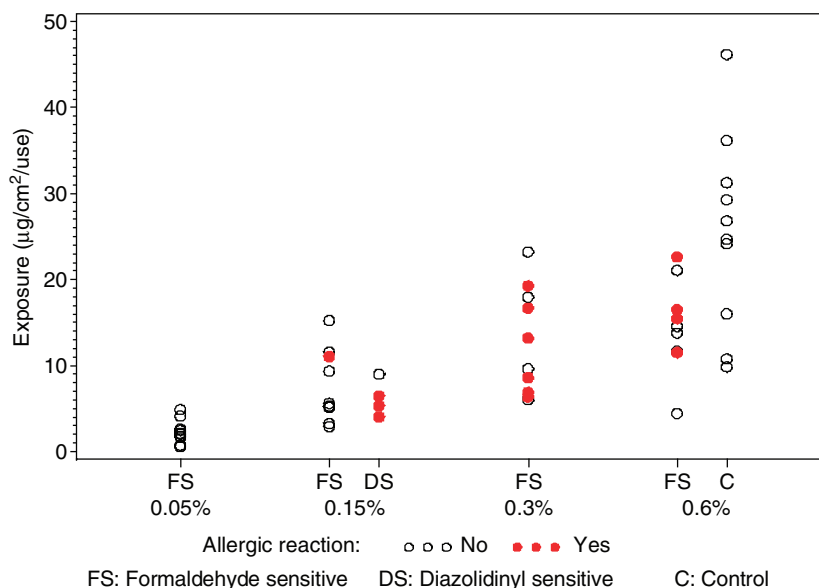


Fig. 4. Exposure to diazolidinyl urea (µg/cm²) of patients in different diazolidinyl urea % concentration groups (neck).

eventually. Typically, the provocation will be stopped at this point. This sequence of the development of the clinical lesions during the ROAT reaction also explains why the patch test reading scale is unsuitable for the ROAT and why a special reading scale is necessary to evaluate its results (14, 15). The present study is the first ROAT investigation beyond the arms. The types of reactions seen on the neck are similar to those seen on the arms. The type of reactions on the face was more polymorphous and unpredictable with redness, oedema and papules. Some of the reactions had clinical similarities with early rosacea (Fig. 2d).

There were two unexpected results in this study

Effective microbial preservation was achieved using 1/6 of the diazolidinyl urea concentration typically used in facial moisturizers, and no correlation was found between the number of reactors/reaction site and the dose per unit area of the allergen, expressed as μg allergen/ cm^2/use .

Antimicrobial preservation. Formaldehyde releasers constitute a valuable class of antimicrobial agents used in cosmetic products. However, their use is normally avoided in cosmetics intended for the more sensitive segment of the consumer population. The present study suggests that diazolidinyl urea may be effectively employed at the concentrations where the risk of elicitation, and hence induction, is minimal.

It must be borne in mind, however, that the antimicrobial efficacy of the diazolidinyl urea will depend on the formula matrix in which it is used and it must be verified each time the product is reformulated.

Nevertheless, as with all preservatives, the recommended practice should be always to use the minimum effective concentration.

Dose–response

A clear dose–response was recorded in relation to the allergen concentration (percentage in the cream) but not to the quantity of the allergen per unit area of the skin. This was most likely due to the variability observed in the amount of cream used by the patients and the uncertainty concerning the actual surface area exposed to the cream.

The exposure area of 25 cm^2 on the upper arm, as well as the neck, could be assumed with some confidence. The facial area of 500 cm^2 , used for the calculations, is only an approximation (22).

The available evidence, based on controlled laboratory studies, points to the dose per unit area (rather than the total dose of the allergen) as the key factor in induction of contact allergy (23). The present study has demonstrated the impracticality of expressing this dose in terms of $\mu\text{g}/\text{cm}^2$, rather than as percentage concentration, in real-life product use studies. Where both the quantity and the area of exposure are subject to considerable inter-individual variability, the only measurable and controlled parameter is the allergen percentage concentration.

Furthermore, human studies have never been systematically performed to compare the effect of total amount, $\mu\text{g}/\text{cm}^2$ or simply percentage concentration, on elicitation of contact dermatitis. Such a study still needs to be carried out with the product applied to different, well-controlled, skin areas. The present study is not suitable to evaluate such an idea, as the individual panellists used different amounts of cream on different skin areas.

Conclusions

The study established a non-eliciting concentration of diazolidinyl urea of 0.05% in formaldehyde-sensitive patients. The cream containing the allergen was well tolerated during the 6 weeks' twice daily exposure, of which 2 weeks were on the face.

Patients sensitive to diazolidinyl urea were more sensitive to the preservative than the formaldehyde-sensitive patients: 9 of the 10 reacted to 0.15% diazolidinyl urea. Diazolidinyl urea exhibited a significant wide spectrum antimicrobial activity at the concentration of 0.05% in the test cream. Nevertheless, it should be noted that the nature of the formulation in which diazolidinyl urea is incorporated as a preservative could have considerable effect on its efficacy.

Our results suggest that the skin reactivity may depend on the anatomical region, increasing from the upper arm to neck and, possibly, to the face.

Positive ROAT reactions on the upper arm and neck have similar symptoms and typically start with follicular papules. Reactions on the face were more polymorphic and unpredictable with redness oedema and papules. Some of these reactions had clinical similarities to early rosacea.

The study design, beginning on the upper arm and moving upon to the neck and face, seems to be a safe design to study reactions to cosmetic products.

In our study, elicitation of allergy correlated with the concentration (%) of the allergen but

not with the amount per surface area ($\mu\text{g}/\text{cm}^2$) applied. This observation was a result of the design of the study in which the product use by the patients reflected real-life variability in the habits of cosmetic product use.

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