Patch testing with 2.0% (0.60 mg/cm^2) formaldehyde instead of 1.0% (0.30 mg/cm^2) detects significantly more contact allergy

Ann Pontén[1], Kristiina Aalto-Korte[2], Tove Agner[3], Klaus E. Andersen[4], Ana M. Giménez-Arnau[5], Margarida Gonçalo[6], An Goossens[7], Jeanne D. Johansen[8], Christophe J. Le Coz[9], Howard I. Maibach[10], Thomas Rustemeyer[11], Ian R. White[12] and Magnus Bruze[1]

[1]Department of Occupational and Environmental Dermatology, Skåne University Hospital, Lund University, 205 02 Malmö, Sweden, [2]Finnish Institute of Occupational Health, Health and Work Ability, Control of Hypersensitivity Diseases, Topeliuksenkatu 41 a A, FI-00250 Helsinki, Finland, [3]Department of Dermatology, University of Copenhagen, Bispebjerg Hospital, 2400 Copenhagen, Denmark, [4]Department of Dermatology and Allergy Centre, Odense University Hospital, University of Southern Denmark, DK-5000 Odense C, Denmark, [5]Department of Dermatology, Hospital del Mar and Univeristat Autònoma of Barcelona, 08003 Spain, [6]Department of Dermatology, Coimbra University Hospital, 3000-075 Coimbra, Portugal, [7]Department of Dermatology, University Hospital, Katholieke Universiteit Leuven, 3000 Leuven, Belgium, [8]Department of Dermato-Allergology, National Allergy Research Centre, Copenhagen University Hospital Gentofte, DK-2900 Gentofte, Denmark, [9]Cabinet de Dermatologie and Laboratoire de Dermatochimie, 4 rue Blaise Pascal, 67070 Strasbourg Cedex, France, [10]Department of Dermatology, School of Medicine, University of California San Francisco, San Francisco, CA 94143-0989, USA, [11]Department of Dermatology, VU University Medical Centre, De Boelelaan 1117, 1081 HV Amsterdam, The Netherlands, and [12]St John's Institute of Dermatology, St Thomas' Hospital, London SE1 7EH, UK

doi:10.1111/j.1600-0536.2012.02169.x

Summary

Background. The currently used patch test concentration for formaldehyde is 1.0% (wt/vol) in water. However, clinical experience and previous studies suggest that 1.0% might be insufficient for detecting an optimized number of clinically relevant cases of contact allergy to formaldehyde.

Objectives. To validate earlier patch test results for comparison of 1% (wt/vol) and 2% (wt/vol) formaldehyde in water, and to investigate co-reactivity with quaternium-15.

Materials and methods. In 12 dermatology clinics, 3591 patients were routinely patch tested simultaneously with 2.0% (wt/vol) (0.60 mg/cm^2) and 1.0% (wt/vol) (0.30 mg/cm^2) formaldehyde. Micropipettes were used for delivering the exact dosage of the allergen.

Results. Significantly more patients reacted to 2.0% formaldehyde than to 1.0% (3.4% versus 1.8%, $p < 0.001$). Overall, there were no sex differences between those reacting positively to 2.0% and 1.0%. Of 25 quaternium-15-positive patients, 4 (0.1%) reacted positively without reacting to formaldehyde.

Conclusion. On the basis of the results of this multicentre study, as well as of previous studies, it can be suggested that 2.0% (wt/vol) in water formaldehyde should be used in routine patch testing in the baseline series.

Key words: contact allergy; dose; false-negative; formaldehyde; micropipette; patch test.

The currently used patch test concentration for formaldehyde is 1.0% (wt/vol) in water. Clinical experience and a previous study suggest that 1.0% might be insufficient for detecting an optimized number of clinically relevant cases of contact allergy to formaldehyde. Furthermore, patch test reactions to formaldehyde have been regarded as difficult to judge with respect to false-positive reactions, that is, irritant reactions, and patch test concentrations were therefore gradually reduced from 4–5% to 1% (1, 2). When different patch test concentrations are compared, it is important to ascertain that the dose is the same in all patch tested patients. This means that, when a certain patch test system is used, and the concentration of the test substance in the patch test preparation is known, the amount of the patch test preparation must be the same in the tested patients. For liquids, a micropipette

Correspondence: Ann Pontén, Department of Occupational and Environmental Dermatology, Skåne University Hospital, Lund University, 205 02 Malmö, Sweden. Tel: +46 4033 7833; Fax: +46 4033 6213. E-mail: ann.ponten@med.lu.se

Conflicts of interest: The authors have declared no conflicts.

Accepted for publication 11 August 2012

ensures the exact dosage (3). In a recent study, 2.0% and 1.0% were compared by the use of parallel patch testing with 15 µl of formaldehyde 2.0% and 1.0% in Finn Chambers® in the baseline series. It was concluded that patch testing with 2.0% detected significantly more patients than patch testing 1.0% (4). Furthermore, a significant number of patients who reacted to 2.0% but not to 1.0% developed dermatitis in a repeated open application test (5). The present multicentre study evaluated the earlier findings, with the use of a micropipette to ascertain an exact dose of the contact allergen, with respect to an optimized patch test concentration. The relationship between positive reactions to formaldehyde and those to the formaldehyde-releaser quaternium-15, which is currently part of the European baseline series, was also investigated (6).

Materials and Methods

Aqueous solutions with formaldehyde 2.0% (wt/vol) and 1.0% (wt/vol) were prepared from formaldehyde 37% (wt/wt) aqua obtained from Acros Organics (Geel, Belgium). All patch test solutions were manufactured at the laboratory in the Department of Occupational and Environmental Dermatology in Malmö, Sweden, and distributed to the participating clinics every 10th week, that is, five times. Glass containers (13 ml) with Teflon caps were filled with the solutions, and distributed to the participating clinics: 11 dermatology clinics in Europe, and one in the United States. All participating clinics used micropipettes to obtain the correct test dose (0.60 and 0.30 mg/cm^2, respectively); 15 µl for Finn Chambers® and 20 µl for van der Bend® chambers (3). For quaternium-15 in petrolatum, 20 mg is recommended for Finn Chambers® (7). The patch test preparations of quaternium-15 1% pet. were obtained from Chemotechnique Diagnostics AB (Vellinge, Sweden). Patch testing and reading days were in accordance with the routines of the participating clinics. The reading days differed, and for reporting the results they were classified as follows: reading days 2–4, reading 1; and reading days 5–8, reading 2. This means that, even if there have been two to three readings, data in the compilation from all clinics might show up as missing reading 2. Evaluations of the morphology of the patch test results were performed according to the recommendations of the International Contact Dermatitis Research Group (8). The patch testing took place between 1 July 2009 and 31 January 2010.

High-performance liquid chromatography was used for analyses of formaldehyde content prior to the distribution of the patch test solutions (9).

Results

The results from 3591 (66.7% females) routinely patch tested patients in the 12 participating clinics are shown in Table 1. In this multicentre study 122 patients (3.4%) were diagnosed with contact allergy to formaldehyde by patch testing with 2.0%, and 66 patients (1.8%) were diagnosed by patch testing with 1.0%. Only 4 (0.1%) patients reacted positively to 1.0% without reacting positively to 2.0%. The proportions of cases reacting to formaldehyde varied between the participating clinics. The lowest proportion among patients reacting positively

Table 1. Positive reactions to formaldehyde 2.0% (wt/vol) and 1.0% (wt/vol) when tested simultaneously in 3591 patients in 12 dermatology clinics

			2.0% positive (%)[a]			1.0% positive (%)[a]			
	Total tested	Females	All	Females	Males	All	Females	Males	2.0%/1.0% [b]
Amsterdam, The Netherlands	684	461 (67.4)	19 (2.8)	13 (2.8)	6 (2.7)	8 (1.2)	6 (1.3)	2 (0.9)	2.4
Barcelona, Spain	269	188 (69.9)	7 (2.6)	5 (2.7)	2 (2.5)	6 (2.2)	4 (2.1)	2 (2.5)	1.2
Coimbra, Portugal	149	114 (76.5)	5 (3.4)	5 (4.4)	0 (0)	1 (0.7)	1 (0.9)	0 (0)	5.0
Copenhagen (Bispebjerg), Denmark	203	142 (70.0)	11(5.4)	6 (4.2)	5 (8.2)	5 (2.5)	2 (1.4)	3 (4.9)	2.2
Copenhagen (Gentofte), Denmark	382	260 (68.0)	13 (3.4)	7 (2.7)	6 (4.9)	9 (2.4)	6 (2.3)	3 (2.5)	1.4
Helsinki, Finland	59	30 (50.8)	7 (11.9)	4 (13.3)	3 (10.3)	5 (8.5)	3 (10)	2 (6.9)	1.4
Leuven, Belgium	228	159 (69.7)	9 (3.9)	5 (3.1)	4 (5.8)	8 (3.5)	7 (4.4)	1 (1.4)	1.1
London, United Kingdom	845	540 (63.9)	24 (2.8)	19 (3.5)	5 (1.6)	7 (0.8)	5 (0.9)	2 (0.7)	3.4
Malmö, Sweden	346	233 (67.3)	16 (4.6)	12 (5.2)	4 (3.5)	11 (2.9)	8 (3.4)	3 (2.7)	1.5
Odense, Denmark	202	128 (63.4)	7 (3.4)	6 (4.7)	1 (1.4)	3 (1.5)	2 (1.6)	1 (1.4)	2.3
San Francisco, United States	150	85 (56.7)	2 (1.3)	1 (1.2)	1 (1.5)	1 (0.7)	1 (1.2)	0 (0)	2.0
Strasbourg, France	74	54 (73.0)	2 (2.7)	1 (1.9)	1 (5.0)	2 (2.7)	1 (1.9)	1 (5.0)	1.0
Total	3591	2394 (66.7)	122 (3.4)	84 (3.5)	38 (3.2)	66 (1.8)	46 (1.9)	20 (1.7)	1.8

[a]For all positives, a reaction on an early (days 2–4) and/or a late (days 5–8) reading is given. Both the number of reacting patients and the proportion (%) among those tested are given. [b]The ratios between all positive reactions to 2.0% and 1.0%.

to 1.0% formaldehyde was 0.7%, and the highest was 8.5%. For formaldehyde 2.0%, the range was 1.3–11.9%. The ratios between cases found when patch testing with 2.0% and 1.0% thus ranged from 5 to 1.

Of the 2.0% of positives, 68.9% were female, and of the 1.0% of positives, 69.7% were female; thus, there was no sex difference. Regarding the participating clinics, the relative proportions of formaldehyde-positive males and females varied (Table 1). The total number of reported reacting individuals was 258. The number of irritant and doubtful reactions reported by the participating clinics varied. The proportions of irritant reactions thus ranged between 0% for both 1.0% and 2.0%, and 21% for 2.0%, and 8% for 1.0%. Regardless of reading day, among 39 doubtful reactions to 1%, recorded when two readings were performed, 17 were positive to 2%, that is, 14% (17/122) of all the 2% of positives, and 22 were doubtful or negative (Fig.1). Approximately 10% of all cases were diagnosed on a second late reading (as defined above) by 2.0%, and 5% were diagnosed on a second reading by 1.0%. The proportion of negative, irritant or doubtful reactions not evaluated on a second reading was ~ 10%.

In all, 25 cases of contact allergy to quaternium-15 were reported, but only 4 cases had contact allergy to this substance without reacting positively to formaldehyde (Table 2).

The analyses of the content of formaldehyde in the patch test solutions (detection limit 0.00005%) were performed for three different batches with ranges of 0.99–1.16% and 2.03–2.14%, respectively.

Discussion

As stated above, previous data suggested that 2.0% formaldehyde detects significantly more contact allergies than 1.0% (4). In the present multicentre study, the variation between the participating clinics in the proportions of cases found among the patch tested patients was 10-fold, regardless of patch test concentration. Furthermore, the proportions of additionally found cases with contact allergy to formaldehyde when 2.0% and 1.0% were compared showed a five-fold variation. Regardless of these variations, the overall result shows that significantly more cases are found when patch testing with 2.0% than when patch testing with 1.0% (3.4% versus 1.8%; $p < 0.001$, McNemar's test).

From the present data, it is difficult to evaluate the disadvantages of 2.0% as compared with 1.0% with respect to irritant reactions, as they varied greatly between the participating clinics. Whether the variation depends on differences in evaluation of the morphology of a test reaction or possibly other reasons is not known. However,

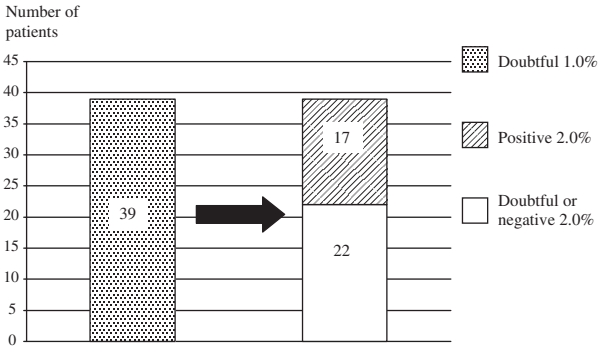

Fig. 1. The number of doubtful reactions to 1.0% (wt/vol) formaldehyde in patients who were read both at days 2–4 and 5–8 that were diagnosed as positive and doubtful/negative to 2.0% (wt/vol) formaldehyde.

Table 2. Positive reactions to quaternium-15 1% pet. in relation to formaldehyde 2.0% (wt/vol) and 1.0% (wt/vol) in water when tested simultaneously in 3591 patients in 12 dermatology clinics

	Quaternium-15 1% pet.	Positive to formaldehyde 1.0% (wt/vol) aqua	Positive to formaldehyde 2.0% (wt/vol) aqua	Negative to formaldehyde
Amsterdam, The Netherlands	6	4	4	2
Barcelona, Spain	1	1	1	0
Coimbra, Portugal	2	1	1	0
Copenhagen (Bispebjerg) Denmark	4	2	2	2
Copenhagen (Gentofte) Denmark	2	2	2	0
Helsinki, Finland	0	0	0	0
Leuven, Belgium	1	1	1	0
London, United Kingdom	2	1	2	0
Malmö, Sweden	3	3	3	0
Odense, Denmark	2	2	2	0
San Francisco, United States	0	0	0	0
Strasbourg, France	2	2	2	0
Total	25	19	20	4

if it is taken for granted that the protocol was followed by the participating clinics, the former explanation is the more probable. If so, the variation implies that standardization is warranted not only for the dose of the patch test but also for the morphology of irritant, doubtful and weak reactions. The results also show, as is the case for many other contact allergens, the importance of a second late reading for formaldehyde (10–13).

Formaldehyde is a ubiquitous allergen. When dermatitis caused or aggravated by exposure to formaldehyde is to be prevented, the diagnosis of contact allergy to formaldehyde must be as correct as possible. It has been shown that a significant number of persons who react to 2.0% but not to 1.0% develop dermatitis when exposed to a moisturizer containing formaldehyde (5). On the basis of these data and the results of this multicentre study, it can be suggested that 2.0% (wt/vol) formaldehyde in water should be used in routine patch testing in the baseline series.

References

1 Epstein E, Maibach H I. Formaldehyde allergy. Incidence and patch test problems. *Arch Dermatol* 1966: **94**: 186–190.

2 Trattner A, Johansen J D, Menné T. Formaldehyde concentration in diagnostic patch testing: comparison of 1% with 2%. *Contact Dermatitis* 1998: **38**: 9–13.

3 Frick-Engfeldt M, Gruvberger B, Isaksson M, Hauksson I, Pontén A, Bruze M. Comparison of three different techniques for application of water solutions to Finn Chambers®. *Contact Dermatitis* 2010: **63**: 284–288.

4 Hauksson I, Pontén A, Gruvberger B, Isaksson M, Bruze M. Routine diagnostic patch-testing with formaldehyde 2.0% (0.6 mg/cm^2) may be an advantage compared to 1.0%. *Acta Derm Venereol* 2010: **90**: 480–484.

5 Hauksson I, Pontén A, Gruvberger B, Isaksson M, Bruze M. Clinically relevant contact allergy to formaldehyde may be missed by testing with formaldehyde 1.0%. *Br J Dermatol* 2011: **164**: 568–572.

6 Bruze M, Andersen K E, Goossens A, ESCD, EECDRG. Recommendation to include fragrance mix 2 and hydroxyisohexyl 3-cyclohexene carboxaldehyde (Lyral) in the European baseline patch test series. *Contact Dermatitis* 2008: **58**: 129–133.

7 Bruze M, Isaksson M, Gruvberger B, Frick-Engfeldt M. Recommendation of appropriate amounts of petrolatum preparation to be applied at patch testing. *Contact Dermatitis* 2007: **56**: 281–285.

8 Wilkinson D S, Fregert S, Magnusson B et al. Terminology of contact dermatitis. *Acta Derm Venereol* 1970: **50**: 287–292.

9 Benassi C A, Semenzato A, Bettero A. High-performance liquid chromatographic determination of free formaldehyde in cosmetics. *J Chromatogr* 1989: **464**: 387–393.

10 Bruze M, Hedman H, Björkner B, Möller H. The development and course of test reactions to gold sodium thiosulfate. *Contact Dermatitis* 1995: **33**: 386–391.

11 Isaksson M, Bruze M. Late patch-test reactions to budesonide need not be a sign of sensitization induced by the test procedure. *Am J Contact Dermat* 2003: **14**: 154–156.

12 Isaksson M, Lindberg M, Sundberg K et al. The development and course of patch-test reactions to 2-hydroxyethyl methacrylate and ethyleneglycol dimethacrylate. *Contact Dermatitis* 2005: **53**: 292–297.

13 Frick-Engfeldt M, Isaksson M, Zimerson E, Bruze M. How to optimize patch testing with diphenylmethane diisocyanate. *Contact Dermatitis* 2007: **57:** 138–151.