

These articles have been accepted for publication in the *British Journal of Dermatology* and are currently being edited and typeset. Readers should note that articles published below have been fully refereed, but have not been through the copy-editing and proof correction process. Wiley-Blackwell and the British Association of Dermatologists cannot be held responsible for errors or consequences arising from the use of information contained in these articles; nor do the views and opinions expressed necessarily reflect those of Wiley-Blackwell or the British Association of Dermatologists

Accepted Date : 12-Nov-2010

Article type : Original Article

Clinically relevant contact allergy to formaldehyde may be missed by testing with formaldehyde 1.0%.

Key words, MeSH Terms:

Allergens

Dermatitis, Allergic Contact

Cosmetics

Formaldehyde

Patch tests

Preservatives

Key words, not MeSH: baseline series, double blind, concentration, relevance, repeated open application test, ROAT

I.Hauksson, A.Pontén, B.Gruvberger, M.Isaksson, M.Bruze

Department of Occupational and Environmental Dermatology, Lund University,
Skane University Hospital, Malmö, Sweden.

Corresponding author: Inese Hauksson, Department of Occupational and Environmental
Dermatology, Skane University Hospital, SE-20502 Malmö, Sweden.

E-mail: Inese.Dubnika_Hauksson@med.lu.se

Abstract

Background: It has been found that patch testing with 15µl formaldehyde 2.0% aq detects twice more allergies than testing with 1.0%. The clinical relevance of positive patch test reactions is often difficult to determine. Use tests are simple to do and help to evaluate the significance of patch test results.

Objectives: To study the clinical relevance of contact allergy to formaldehyde detected by 2.0% (0.60 mg/cm²) but not by 1.0%.

Patients/Methods: 18 patients positive to formaldehyde 2.0% but negative to 1.0% and a control group of 19 dermatitis patients without allergy to parabens, formaldehyde and formaldehyde releasers were included in the study. Formaldehyde 2000 ppm, the maximum concentration permitted in leave-on cosmetics according to the EU Cosmetics Directive, was added to a batch of a moisturiser preserved with parabens. The same batch without formaldehyde served as control. The study was double-blinded and randomised. The patients were provided with both moisturisers and instructed to apply one of them twice a day on a marked-out 5×5 cm area on the inside of one upper arm and the other moisturiser on the other arm. Reading of the test sites was done once a week for a maximum of 4 weeks.

Results: In the control group there were no allergic reactions to any of the moisturisers. 9 of the 17 formaldehyde-allergic patients reacted with an allergic reaction to the moisturiser which contained formaldehyde ($p < 0.001$). No positive reactions were observed to the moisturiser without formaldehyde.

Conclusions: Our results demonstrate that contact allergy to formaldehyde 2.0% may be clinically relevant.

Introduction

To establish contact allergy to formaldehyde, a defined dose per area is important. In a recent study we could show that doubling the currently recommended dose detected twice as many cases without causing a substantial number of irritant reactions¹. However, even if more contact allergy to an allergen is detected by a certain dose of the allergen in question, it is still important to establish the clinical relevance of these further cases found by the optimised test dose. For formaldehyde, the individual exposure is difficult to know, since formaldehyde is a ubiquitous allergen which, besides being present as a preservative in skin care products such as moisturizers and soaps, can be found in many other products in contact with the skin. Examples of other exposures are metalworking fluids, household products, textiles, and protective gloves²⁻⁵. Still, if the clinical importance of a found contact allergy is to be studied, a model which resembles everyday exposure is needed. To this end, variants of the Repeated Open Application Test (ROAT) have been used on several occasions⁶⁻¹³. In this study a controlled, double-blind randomized ROAT was used to study the clinical relevance of contact allergy to formaldehyde detected by 2.0% (0.60 mg/cm²) but not by 1.0%.

Materials and Methods

Patients

18 patients (mean age 44.1 years, range 23 - 64, 4 males, 14 females), who reacted positively to 2.0% (0.60 mg/cm²) formaldehyde but not to 1.0% when simultaneously patch-tested in the baseline series of our department were enrolled¹. Exclusion criteria were contact allergy to parabens and formaldehyde releasers. 19 matched controls (mean age 48.1 years, range 18 - 66, 7 males, 12 females) without contact allergy to formaldehyde (1.0% and 2.0%), formaldehyde releasers, or parabens were also enrolled among patients that were consecutively patch tested at our department.

Test material

According to the EU Cosmetics Directive¹⁴, the highest permitted concentration in leave-on products is 2000 ppm (0.2% w/w). Formaldehyde at this concentration was added to half the batch of a moisturizer (aqueous cream) preserved with parabens (0.1% methylparaben + 0.2% propylparaben) while the other half was left unaltered. Both moisturisers with and without formaldehyde were manufactured by the pharmacy at the Skane University Hospital in Malmö, Sweden and delivered in aluminium tubes. Pairs of tubes were assembled, one with formaldehyde and one without formaldehyde. Thereafter, within each pair randomisation was

made implying one tube with red tape and one tube with blue tape. The content of formaldehyde in the moisturizers with and without added formaldehyde was analysed at our laboratory with high-performance liquid chromatography (HPLC) after the study, detection limit was $<0.05\mu\text{g/g}$.¹⁵

Repeated Open Application Test

The patients received a pair of tubes with moisturisers each, one marked with red tape and the other with blue tape. At the first visit the dermatologist, who saw the patients throughout the study, marked out a 5×5 cm area on the patient's both upper inner arms and applied the moisturiser marked with red on the left arm and the moisturiser marked with blue on the right arm. They were shown the amount of moisturiser to apply evenly twice a day over the area, approximately 0.1 g. The treated areas were investigated for signs of dermatitis by a dermatologist once a week. A protocol was used, in which the presence of erythema, papules/infiltration, and vesicles were recorded as well as the proportion of the test area involved. The overall clinical impression was also recorded, as well as the number of applications done prior to each reading. The minimum criteria for regarding the reaction as a positive ROAT were erythematous infiltration with or without papules and/or vesicles covering at least 25% of the treated area. The strength of the reaction in the ROAT was classified as mild, moderate, or strong¹⁶. The patients ceased to apply both moisturisers directly after the ROAT became positive on any of the arms. The study went on for a maximum of 4 weeks. When a doubtful ROAT appeared, the patients were encouraged to continue the applications until the reaction became positive or for four weeks in total. The location of the patient's own dermatitis was also recorded and afterwards information about the patients' occupation and history of atopic dermatitis was retrieved from the patients' case records.

8 of the 18 patients with formaldehyde sensitivity were patch tested with both moisturisers "as is" used in the ROAT.

Statistical calculations

The number of reactions to the formaldehyde-containing moisturiser in the formaldehyde-allergic patients and the controls was compared using the Fisher's exact test. The number of reactions in the formaldehyde-allergic patients using moisturisers with and without formaldehyde was compared with McNemar's test. A p-value <0.05 was considered significant.

Results

One of the 18 formaldehyde-allergic patients discontinued her participation in the study after 24 applications due to itching, but no visible reaction was noted. Nine of the 17 formaldehyde-allergic patients completing the study, reacted positively to the moisturiser containing formaldehyde (Table 1, Fig.1 a,b,c). No one among the controls reacted positively to the formaldehyde-containing moisturiser. No reactions to the moisturiser without formaldehyde were recorded in any of the groups. Within the group of formaldehyde-positive patients 9/17 reacted to the formaldehyde-containing moisturiser vs. 0/17 who reacted to the moisturiser without formaldehyde ($p < 0.001$, McNemar's test). The difference between the formaldehyde-allergic patients and the controls was statistically significant ($p < 0.001$, Fisher's exact test)). The mean number of applications before a recorded positive ROAT was 37, range 9 - 56. Among the 5 patients included in the study with moderate (++) reactions to formaldehyde 2.0%, 3 did not react at all in the ROAT, while 2 had the lowest number of applications among the reacting patients before developing a positive reaction in the ROAT. One formaldehyde allergic participant had petechia on the test area. This reaction appeared after 45 applications of the formaldehyde-containing moisturiser. This patient completed the study without developing an eczematous reaction on the test area. One of the controls had a reaction with dryness and scaling, thus not a positive ROAT according to the criteria. This scaling appeared after 56 applications of the formaldehyde-containing moisturiser and thus did not develop into an eczematous reaction on the test area (Table 1). None of the 8 formaldehyde sensitive patients reacted to any of the moisturisers patch tested as is.

HPLC analysis of one of the used formaldehyde-containing moisturizers showed a content of 0.2% (w/w) formaldehyde. One of the used control moisturizers was also analyzed and contained 0.0013% formaldehyde¹⁵.

Discussion

The ROAT is a provocation test aiming at mimicking everyday exposure. It can be used for establishing the clinical relevance of a suspected reaction to a skin care product in patch-test-negative patients and for investigating the clinical relevance of contact allergy detected with different patch test doses. For unknown allergens, however, a positive ROAT in an individual patient cannot differentiate between an allergic and an irritant reaction. The clinical relevance of contact allergy to formaldehyde, formaldehyde releasers, other preservatives and allergens

in skin care products has previously been studied using ROAT^{7-11,17,18}. The advantage of the present study is the precise dose used for the diagnosis of contact allergy to formaldehyde, which was the base of the inclusion criterion for the ROAT¹. Certainly, it can be as difficult to assess the clinical relevance of any contact allergy to formaldehyde and not only for patients reacting to 2.0% but not to 1.0%. The aim of the present study was, however, to investigate whether any clinically relevant cases were found by routinely patch testing with 2.0% instead of 1.0%.

Unexpectedly and to our surprise, the ROAT turned out to be positive. We chose the maximal allowed concentration of formaldehyde in the moisturiser for several reasons. First, we wanted to optimise the ROAT by using the highest possible concentration allowed in a leave-on product. Second, as the ROAT would be performed on healthy skin we expected that the possibility of a positive ROAT would be minimal or fairly low, and had planned to perform a ROAT on damaged skin had this study been negative. As mentioned, the results in the present study are based on the application of the moisturiser on healthy skin, but moisturisers are bound to often be applied to skin with an impaired barrier, which most probably further increases the risk of developing eczema in an allergic patient. In a study of preservatives in moisturisers on the Swedish market, 1% of the moisturisers contained formaldehyde, the concentrations ranging from 0.02 – 0.09%¹⁵. It can be mentioned that according to the Cosmetics Directive, products which contain more than 0.05% formaldehyde must be labelled with respect to their content of formaldehyde besides indicating other ingredients¹⁴. There may be additive exposures of formaldehyde from different skin care products and from occupational exposure, such as metal working fluids.

Formaldehyde is volatile and therefore most probably partly evaporate when applied on the skin and thereby the dose per area most probably is lower than the calculated/accumulated dose. Despite this, a significant number of exposed formaldehyde-allergic individuals reacted. If, for example, the application area had been occluded similarly as by gloves, the number of reacting individuals as well as the degree of reactivity would probably have been greater. The minute amount of formaldehyde in one of the analysed moisturisers without formaldehyde can be explained by traces of formaldehyde most likely emanating from the raw material¹⁵. From a clinical point of view, the finding that applications twice daily for approximately 3 weeks or more was needed to elicit a positive ROAT in 5/9 patients is important (Table 1). The association over time between the offending product and the dermatitis might thus be difficult to discern. Within this limited study, there was no obvious association between the reactivity in the previous patch test and the number of applications needed to elicit a positive

ROAT. The predictive value of the strength of the patch test reaction to 2.0% therefore seems to be limited. A model for investigating the dose-response relationship between patch test results in dilution series and ROAT has been developed and in that study a relation was found for both nickel and methyldibromo glutaronitrile. The authors concluded that when patch test data are used to determine safe levels of allergen exposure, a negative patch test in a dilution series is not a safe dose, since the ROAT can elicit a reaction in an allergic patient¹⁹. For an investigated volatile compound (hydroxyisohexyl 3-cyclohexene carboxaldehyde, HICC), the same study found that the relation between patch test results and ROAT was different compared to the non-volatile compounds¹⁹. In our study, it is noteworthy that most of the patients who reacted positively to the ROAT had weak reactions to the patch test with 2.0% formaldehyde. The contact allergy in these patients would not have been found if patch tested with the currently used and recommended test dose. Individuals with a history of atopic dermatitis are prone to develop irritant contact dermatitis. There were, however, not more individuals with atopic dermatitis among the ROAT-reacting patients than among the individuals that did not react. Therefore, it is highly unlikely that the eczematous reactions to the ROAT as well as the patch test with 2.0% formaldehyde represent irritant reactions in individuals with a history of atopic dermatitis. Several patients, both with and without a positive ROAT, had occupations with possible occupational formaldehyde exposure, such as in metal-working. With regard to their occupation, the ROAT might be individually important for the evaluation of the clinical relevance of their contact allergy to formaldehyde.

When a certain topical product is suspected to cause dermatitis in a patient with contact allergy to an ingredient present the product in question, it might be patch tested *as is*. Had the formaldehyde-containing moisturiser used in our study for the ROAT been tested *as is* in Finn Chambers, the calculated dose per area of formaldehyde would have been 0.06 mg/cm². This is ten times lower than the dose per area of the initial patch test (0.60 mg/cm²), making it less probable that the studied patients should react when patch tested *as is* with our formaldehyde-containing moisturiser.

Conclusion

In individuals who react to 2.0% formaldehyde but not to 1.0%, there is a significant risk to develop an eczematous reaction when exposed to a moisturizer containing formaldehyde in accordance with the European Cosmetics Directive. These patients would not have been diagnosed with the currently recommended patch test concentration, and consequently their skin disease might have been misdiagnosed as irritant contact dermatitis or endogenous

dermatitis instead of allergic contact dermatitis. A correct diagnosis is of major importance for the management and prevention of the dermatitis in question.

Based on the results of the present study it is suggested that the routinely used patch test dose of formaldehyde should be changed from 1.0% to 2.0% and that the patch technique should be optimized by routinely using micropipettes when applying the formaldehyde solution to the patch test chamber^{1,20}.

- 1 Hauksson I, Ponten A, Gruvberger B *et al.* Routine diagnostic patch-testing with formaldehyde 2.0% (0.6 mg/cm²) may be an advantage compared to 1.0%. *Acta Derm Venereol*; **90**: 480-4.
- 2 Henriks-Eckerman ML, Suuronen K, Jolanki R. Analysis of allergens in metalworking fluids. *Contact Dermatitis* 2008; **59**: 261-7.
- 3 Ponten A. Formaldehyde in reusable protective gloves. *Contact Dermatitis* 2006; **54**: 268-71.
- 4 Flyvholm MA. Contact allergens in registered cleaning agents for industrial and household use. *Br J Ind Med* 1993; **50**: 1043-50.
- 5 Donovan J, Skotnicki-Grant S. Allergic contact dermatitis from formaldehyde textile resins in surgical uniforms and nonwoven textile masks. *Dermatitis* 2007; **18**: 40-4.
- 6 Schnuch A, Uter W, Dickel H *et al.* Quantitative patch and repeated open application testing in hydroxyisohexyl 3-cyclohexene carboxaldehyde sensitive-patients. *Contact Dermatitis* 2009; **61**: 152-62.
- 7 Fischer LA, Menne T, Avnstorp C *et al.* Hydroxyisohexyl 3-cyclohexene carboxaldehyde allergy: relationship between patch test and repeated open application test thresholds. *Br J Dermatol* 2009; **161**: 560-7.
- 8 Zaghi D, Maibach HI. Quantitative relationships between patch test reactivity and use test reactivity: an overview. *Cutan Ocul Toxicol* 2008; **27**: 241-8.
- 9 Fischer LA, Johansen JD, Menne T. Methylidibromoglutaronitrile allergy: relationship between patch test and repeated open application test thresholds. *Br J Dermatol* 2008; **159**: 1138-43.
- 10 Isaksson M, Gruvberger B, Bruze M. Repeated open application tests with methylidibromoglutaronitrile in dermatitis patients with and without hypersensitivity to methylidibromoglutaronitrile. *Dermatitis* 2007; **18**: 203-7.
- 11 Zachariae C, Hall B, Cupferman S *et al.* ROAT: morphology of ROAT on arm, neck and face in formaldehyde and diazolidinyl urea sensitive individuals. *Contact Dermatitis* 2006; **54**: 21-4.
- 12 Gruvberger B, Andersen KE, Brandao FM *et al.* Repeated open application test with methylidibromo glutaronitrile, a multicentre study within the EEC DRG. *Contact Dermatitis* 2005; **52**: 19-23.
- 13 Bruze M, Johansen JD, Andersen KE *et al.* Deodorants: an experimental provocation study with isoeugenol. *Contact Dermatitis* 2005; **52**: 260-7.
- 14 A Clarification on the Formaldehyde and para-Formaldehyde Entry in Direktive 76/768/EEC on cosmetic products. In, Vol. SCCNFP/587/02: The Scientific Committee on Cosmetic Products and Non-Food Products intended for Consumers. 2002.
- 15 Gruvberger B, Bruze M, Tammela M. Preservatives in moisturizers on the Swedish market. *Acta Derm Venereol* 1998; **78**: 52-6.

- 16 Johansen JD, Bruze M, Andersen KE *et al.* The repeated open application test: suggestions for a scale of evaluation. *Contact Dermatitis* 1998; **39**: 95-6.
- 17 Fischer LA, Johansen JD, Menne T. Nickel allergy: relationship between patch test and repeated open application test thresholds. *Br J Dermatol* 2007; **157**: 723-9.
- 18 Isaksson M, Gruvberger B, Goon AT *et al.* Can an imidazolidinyl urea-preserved corticosteroid cream be safely used in individuals hypersensitive to formaldehyde? *Contact Dermatitis* 2006; **54**: 29-34.
- 19 Fischer LA, Voelund A, Andersen KE *et al.* The dose-response relationship between the patch test and ROAT and the potential use for regulatory purposes. *Contact Dermatitis* 2009; **61**: 201-8.
- 20 Frick-Engfeldt M, Gruvberger B, Isaksson M *et al.* Comparison of three different techniques for application of water solutions to Finn Chambers(R). *Contact Dermatitis*; **63**: 284-8.







Legend.

Fig.1 a – c: positive ROAT after 8, 13 and 55 applications, in 3 different formaldehyde sensitive patients, respectively.

Table 1. Repeated open application test (ROAT) in 17 patients (no 1-17) with a positive (pos) patch test to 2.0% (0.60 mg/cm²) formaldehyde but not to 1.0% and 19 controls (no 18 – 36) without formaldehyde allergy.

No of pat.	Sex ^{a)}	Age	2.0% formaldehyde ^{b)}	ROAT with formaldehyde	ROAT without formaldehyde	No of applications ^{c)}	Location of dermatitis	Atopic dermatitis	Occupation
1	F	23	++	pos moderate	neg	13	hands	no ^{d)}	hairdresser apprentice
2	M	52	+	pos strong	neg	55	hands, arms	no	metal worker
3	F	33	+	pos weak	neg	29	hands	yes	restaurant worker
4	F	43	+	pos moderate	neg	29	legs, arms	no	office worker
5	M	60	++	pos strong	neg	9	hands, feet	no	office worker
6	F	56	+	pos weak	neg	45	hands, trunk	no	office worker
7	M	41	+	pos moderate	neg	40	hands	no	plumber
8	F	42	+	pos strong	neg	56	hands	no	shop worker
9	F	32	+	pos weak	neg	56	hands, arms	no	cleaner
10	F	61	+	neg	neg	56	face	no	retired teacher
11	M	60	++	neg	neg	56	hands	no	metal worker
12	F	24	+	neg	neg	56	trunk, limbs, hands	yes	shop worker
13	F	37	++	neg	neg	56	lips	no	office worker
14	F	38	++	neg	neg	56	hands	yes	assistant nurse
15	F	64	+	neg	neg	56	lips	no	nurse
16	F	29	+	neg	neg	56	hands	no	nurse
17	F	53	+	neg ^{e)}	neg	56	trunk, face	unknown	shop-owner
18-36 ^{f)}	7 M 12 F	18-36	neg	neg ^{g)}	neg	56	various	various	various

^{a)} F, female; M, male. ^{b)} Strength of the patch reaction to formaldehyde 2.0% prior to the inclusion in the ROAT study (++, moderate reaction; +, weak reaction). ^{c)} Until a positive ROAT appeared or the study was terminated. ^{d)} At the age of 16 years debut of dermatitis on body and eyelids.

^{e)} The patient had petechia. ^{f)} Matched controls (dermatitis patients consecutively patch tested at our department) without contact allergy to formaldehyde, formaldehyde releasers and parabens. ^{g)} One patient had dryness and scaling.