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# Contact allergy to formaldehyde

## Diagnosis and clinical relevance

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DEPT. OF OCCUPATIONAL AND ENVIRONMENTAL DERMATOLOGY | LUND UNIVERSITY



# Contact allergy to formaldehyde

Diagnosis and clinical relevance

Inese Dubnika Hauksson



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## AKADEMISK AVHANDLING

Som med vederbörligt tillstånd av Medicinska Fakulteten vid Lunds Universitet för avläggande av doktorsexamen i medicinsk vetenskap, offentlig kommer att försvaras i Lilla Aulan, Medicinskt Forskningscentrum, Jan Waldenströms gata 5, Skånes Universitetssjukhus Malmö, onsdagen den 28 maj, kl.9.00.

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<b>Title and subtitle:</b> Contact allergy to formaldehyde. Diagnosis and clinical relevance		
<b>Abstract:</b> Preservatives are biologically active substances mainly used in water-based products to prevent the growth of microorganisms. Most people are exposed to them on a daily basis. Formaldehyde is one of the oldest and most commonly used preservatives. However, it is a well-known contact sensitizer in dermatitis patients.  The aims of this work were: i) to investigate the prevalence of contact allergy to formaldehyde using the baseline patch test series; ii) to determine the optimal patch test concentration and dose for formaldehyde; iii) to study the clinical relevance of contact allergy to formaldehyde detected by formaldehyde 2.0% (0.60 mg/cm <sup>2</sup> ) but not by formaldehyde 1.0% (0.30 mg/cm <sup>2</sup> ); iv) to study the effects of low concentrations of formaldehyde on irritant contact dermatitis in formaldehyde-allergic patients; v) to semi-quantify the formaldehyde content in skin care products used by patients with suspected allergic contact dermatitis, and compare this with the declaration of contents; vi) to determine whether formaldehyde-allergic patients are more exposed to formaldehyde in skin care products than dermatitis patients without contact allergy to formaldehyde; vii) to investigate the patterns of concomitant contact allergy to formaldehyde and formaldehyde releasers.  The findings were as follows: i) patch testing with 15 µl formaldehyde 2.0% (0.60 mg/cm <sup>2</sup> ) using a micropipette detects significantly more reacting individuals than 1.0% (0.30 mg/cm <sup>2</sup> ), without a high frequency of irritant reactions. ii) individuals who react to formaldehyde 2.0% (0.60 mg/cm <sup>2</sup> ) but not to 1.0% (0.30 mg/cm <sup>2</sup> ) have a significant risk of developing an eczematous reaction when exposed to concentrations of formaldehyde allowed by the EU Cosmetic Directive. iii) daily exposure to low concentrations of formaldehyde is sufficient to exacerbate existing dermatitis in patients with contact allergy to formaldehyde. iv) to assess exposure and clinical relevance in formaldehyde-allergic patients, the patients' skin care products should be analysed, especially when the labelling of the products does not include formaldehyde or formaldehyde-releasing preservatives.		
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# Contact allergy to formaldehyde

Diagnosis and clinical relevance

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# THESIS AT A GLANCE

## Paper I

Routine diagnostic patch testing with formaldehyde 2.0% aq (0.60 mg/cm<sup>2</sup>) may be an advantage compared to 1.0%.

### Objective

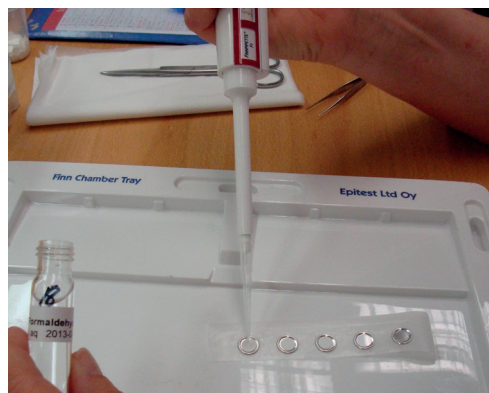
To compare the results of simultaneous patch tests with formaldehyde 1.0% and 2.0% aq in the baseline series in consecutively patch-tested dermatitis patients.

### Method

Formaldehyde 2.0% (0.60 mg/cm<sup>2</sup>) was included in our baseline series. 1397 patients were patch-tested with formaldehyde 1.0% and 2.0% between 1 January 2006 and 31 December 2007.

### Main findings/conclusions

Patch testing with 15µl formaldehyde 2.0% aq (0.60 mg/cm<sup>2</sup>) in 8mm diam. Finn Chambers using a micropipette detected twice as many reacting individuals than 1.0% (0.30 mg/cm<sup>2</sup>), without a high frequency of irritant reactions.



## Paper II

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

### Objective

To study the clinical relevance of contact allergy to formaldehyde detected by 2.0% aq (0.60 mg/cm<sup>2</sup>) but not by 1.0%.

### Method

17 individuals positive to formaldehyde 2.0% but negative to 1.0%, and a control group of 19 individuals negative to formaldehyde, performed a ROAT with moisturisers containing formaldehyde 2000 ppm during a period of 4 weeks.

### Main findings/conclusions

9 of 17 formaldehyde-allergic individuals reacted with an allergic contact dermatitis in response to the moisturiser containing formaldehyde. The results demonstrate that allergy to formaldehyde 2.0% may be clinically relevant.



## Paper III

Skin products containing low concentrations of formaldehyde, detected by the chromotropic acid method, can not be safely used by formaldehyde-allergic individuals.

### Objective

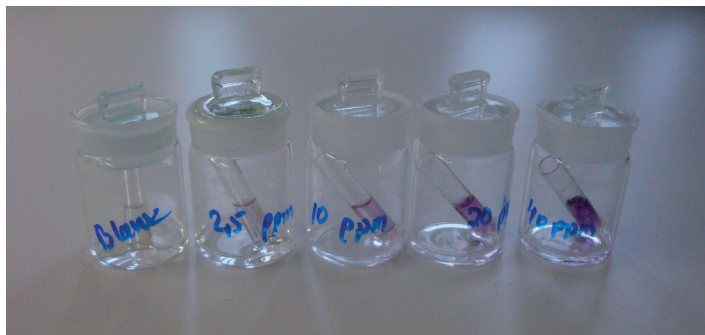
To study the effects of exposure to low concentrations of formaldehyde on irritant contact dermatitis in formaldehyde-allergic individuals.

### Method

15 formaldehyde-allergic individuals and a control group of 13 individuals without contact allergy to formaldehyde performed a ROAT with moisturisers containing 3 different concentrations of formaldehyde and a control moisturiser during 4 weeks on areas of experimentally induced SLS dermatitis.

### Main findings/conclusions

In 9 of 15 formaldehyde allergics the SLS dermatitis worsened on areas to which moisturisers containing different concentrations of formaldehyde were applied. No reactions were seen in the controls. This study demonstrates that exposure to low levels of formaldehyde exacerbates existing dermatitis.



## Paper IV

Are formaldehyde-allergic patients more exposed to formaldehyde in skin care products than dermatitis patients without contact allergy to formaldehyde?

### Objective

To survey the presence of formaldehyde in skin care products brought in by patients investigated due to suspected allergic contact dermatitis and to try to investigate whether formaldehyde-allergic patients are more exposed to formaldehyde than dermatitis patients without contact allergy to formaldehyde.

### Method

287 skin care products from 10 formaldehyde-allergic patients and 30 patients without contact allergy to formaldehyde were investigated using the chromotropic acid method.

### Main findings/conclusions

Formaldehyde was found in 20% of the products. Formaldehyde-allergic patients had statistically more “leave-on” products with >40 ppm formaldehyde than patients without contact allergy to formaldehyde. Inadequate labelling with regard to formaldehyde was found in 65% of the “leave on” products.



# LIST OF PUBLICATIONS

This thesis is based on the following papers, referred to in the text by their Roman numerals.

**I. Routine diagnostic patch testing with formaldehyde 2.0% (0.60 mg/cm<sup>2</sup>) may be an advantage compared to 1.0%**

Hauksson I, Pontén A, Gruvberger B, Isaksson M and Bruze M  
Acta Dermato-Venereologica 2010; 90: 480-484

**II. Clinically relevant contact allergy to formaldehyde 2.0% may be missed by testing with formaldehyde 1.0%**

Hauksson I, Pontén A, Gruvberger B, Isaksson M and Bruze M  
British Journal of Dermatology 2011; 164: 568-572

**III. Skin care products containing low concentrations of formaldehyde detected by the chromotropic acid method can not be safely used by formaldehyde-allergic individuals**

Hauksson I, Pontén A, Gruvberger B, Isaksson M, Engfeldt M and Bruze M  
Submitted for publication in the British Journal of Dermatology

**IV. Are formaldehyde-allergic patients more exposed to formaldehyde in skin care products than patients without contact allergy to formaldehyde?**

Hauksson I, Pontén A, Isaksson M, Hamada H, Engfeldt M and Bruze M  
In manuscript

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# ABBREVIATIONS

aq	aqua
CAS	Chemical Abstract Service
ESCD	European Society of Contact Dermatitis
EECDRG	European Environmental and Contact Dermatitis Research Group
GPMT	Guinea Pig Maximisation Test
HPLC	High Performance Liquid Chromatography
ICDRG	International Contact Dermatitis Research Group
ppm	parts per million
ROAT	Repeated open application test
SLS	Sodium lauryl sulphate





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# 1 INTRODUCTION

## 1.1 Preservatives

Preservatives are added to products to prevent decomposition. They are mostly used in water-based products to prevent the growth of microorganisms such as moulds, fungi, algae and bacteria, which can degrade the product, possibly leading to a change in activity, discoloration or malodour. Some microorganisms are pathogenic and may thus endanger the health of the consumer. Thus, preservatives are often a necessary component in product formulation as they improve the safety and usability of the product.

Preservatives are widely used in household and industrial products, and most people are exposed to one or more preservatives on a daily basis. Ideally, such substances should not sensitise the user. However, many preservatives are biologically reactive substances, and as such have allergenic potential. After fragrances, preservatives are the most frequent cause of allergic contact dermatitis caused by cosmetics (1). Formaldehyde is a well-known and widely used preservative, and contact allergy to formaldehyde and formaldehyde-releasing preservatives is common.

## 1.2 Formaldehyde

### 1.2.1 Historical background

Formaldehyde was first reported as a chemical substance in 1859 by the Russian chemist A.M. Butlerov, when he attempted to synthesize methylene glycol (2). However, formaldehyde was not conclusively identified until 1868, when A.W. Hofmann at the University of Berlin set out to clearly establish both the structure and identity of formaldehyde. Hofmann passed a mixture of methanol and air over a heated platinum spiral and then identified formaldehyde as the product formed. This method laid the foundation for the modern formaldehyde manufacturing process, i.e., the oxidation of methanol with air using a metal catalyst.



Commercial production of formaldehyde began in Germany in the 1880s. Initially, formaldehyde was mainly used as an embalming agent or medical preservative. Rapid developments in science and technology led to a wide variety of applications of formaldehyde. Formaldehyde became an important chemical in 1907, when Baekeland used a phenol formaldehyde resin in the formulation of the first completely synthetic plastic, popularly known as Bakelite (3). The first Swedish factory for the production of formaldehyde was built in Perstorp in 1905. Formaldehyde is manufactured in the form of an aqueous solution, usually containing 37% (36-50%) formaldehyde. In this form it is called formalin. Commercial applications of formaldehyde are still continuing to grow and, in 2010, over 7 million tons of formaldehyde was produced in Europe.(3) The global production exceeds 20 million tons per year.

### 1.2.2 The chemistry of formaldehyde

Formaldehyde is a naturally occurring organic compound composed of carbon, hydrogen and oxygen with the formula  $\text{CHOH}$  or  $\text{CH}_2\text{O}$ , a molecular weight of 30.03 g/mol and Chemical Abstract Service (CAS) number 50-00-0.

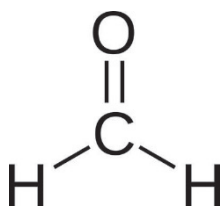


Figure 1. The structure of formaldehyde.

Formaldehyde is the simplest aldehyde, with the systematic name *methanal*. Other names are *methyl aldehyde* and *methylene oxide*. Formaldehyde is a colourless gas with a specific pungent odour. It is relatively stable at 80-100°C, but polymerises slowly at room temperature. It is soluble in water, alcohols, ether and other polar solvents. It is common to add 10-15% methanol to formalin to prevent polymerisation. Formaldehyde possesses a broad range of biological activity due to its reactive carbonyl group.

### 1.2.3 Properties, uses and exposure

Formaldehyde occurs naturally in the world around us (5), and is present everywhere at different concentrations, even in outer space. However, it does not accumulate in the atmosphere because it is quickly broken down by photo-oxidation. All forms of life including bacteria, plants, fish, animals and humans, produce low levels of formaldehyde. Formaldehyde is also a part of normal metabolism. The human body produces formaldehyde from serine, glycine and other amino acids or substances containing methoxy groups. The concentration of formaldehyde in the blood in humans is 2-3 mg/l (about 0.1 mmol/l) (5). Formaldehyde does not accumulate in our bodies, but is quickly metabolised to formic acid, which is slowly excreted in the urine (2).

Since formaldehyde has many useful chemical properties, it is a key building block in a wide range of applications, many of which are listed in Table 1.

Table 1. Some of the most common sources and uses of formaldehyde

Formaldehyde resins and plastics (urea- formaldehyde; phenol-formaldehyde; melamine-formaldehyde; polyacetal)	Paper industry
Paints and explosives	Dry cleaning materials, textiles
Mineral wool	Protective gloves
Chipboard	Tissue fixative and embalming agent
Polishes	Disinfectants
Glues	Cleaning products
Agricultural use	Medication, vaccines
Metalworking fluids	Personal care products
Photographic paper and solutions	Smoke from tobacco, automobile exhaust

It is difficult to estimate our exposure to formaldehyde since it is found in a large number of products, including cosmetics, household products, textiles and even protective gloves (6). Apart from specific occupational exposure, the most frequent source of exposure is personal care products.


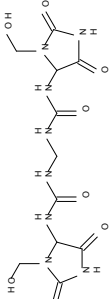
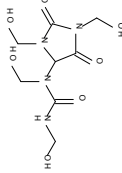
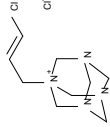
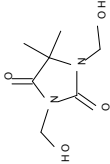
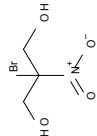
Formalin, the aqueous solution of formaldehyde, has extremely good bactericide, fungicide, virucide and sporicide properties, which make it useful as a preservative (7). For example, 20-550 ppm formaldehyde is effective against Gram-negative bacteria, 250 ppm is effective against Gram-positive bacteria, 90-750 ppm against yeast, 8000 ppm against fungal spores and 4000 ppm against mycobacteria (8). Formaldehyde is also used as antibacterial medication, e.g. in the prophylaxis and treatment of urinary tract infections (methenamine hippurate, Hiprex®). The use of free formaldehyde has decreased as a result of its reputation as an irritant, a sensitiser and a potential carcinogen, and formaldehyde-releasing agents are now used instead (9).

#### 1.2.4 Formaldehyde releasers

Formaldehyde releasers, or donors, can be divided into substances that release formaldehyde as a result of decomposition, and chemicals that are synthesized from formaldehyde and which can still contain and release free formaldehyde. In 2009, de Groot et al. published an inventory of the formaldehyde releasers in use at the time. They identified thirty-five chemicals as formaldehyde releasers, some of which are more common in industry, while others are used almost exclusively in skin care products (10). The characteristics of formaldehyde and five formaldehyde-releasing preservatives commonly used in cosmetics are given in Table 2.

Urea-formaldehyde resin and melamine-formaldehyde resin are chemical finishes used in textiles to make them wrinkle-resistant. In the 1950s and 1960s many cases of allergic contact dermatitis resulting from formaldehyde in textiles were reported. Chemical finishes releasing much less formaldehyde have been used since then, and the amount of free formaldehyde in most garments nowadays is probably too low to elicit allergic contact dermatitis (11-14).

Table 2. Formaldehyde and formaldehyde-releasing preservatives.

Formaldehyde/ formaldehyde releaser (INCI name)	Chemical Abstracts name	Examples of Trade name	CAS number	Maximally allowed conc. in cosmetics in EU	Structure
Formaldehyde	Methanal	-	50-00-0	0.2%	
Imidazolidinyl urea	<i>N,N'</i> -Methylenebis[ <i>N</i> -(3-(hydroxymethyl)-2,5-dioxo-4-imidazolidinyl)urea]	Germall 115	39236-46-9	0.6%	
Diazolidinyl urea	<i>N</i> -(1,3-Bis(hydroxymethyl)-2,5-dioxo-4-imidazolidinyl)- <i>N,N'</i> -bis(hydroxymethyl)urea	Germall II	78491-02-8	0.5%	
Quaternium-15	1-(3-Chloro-2-propenyl)-3,5,7-triaza-1-azoniatricyclo[3.3.1.1 <sup>3,7</sup> ]decane chloride	Dowicil 200	4080-31-3	0.2%	
DMDM hydantoin	1,3-Bis(hydroxymethyl)-5,5-dimethyl-2,4-imidazolidinedione	Glydant	6440-58-0	0.6%	
2-Bromo-2-nitropropane-1,3-diol	2-Bromo-2-nitro-1,3-propanediol	Bronopol	52-51-7	0.1%	

INCI, International Nomenclature of Cosmetic Ingredients.

### 1.3 Contact allergy and allergic contact dermatitis

Contact allergy is known as delayed hypersensitivity or a “type IV allergy”. Allergic contact dermatitis is the clinical manifestation of contact allergy. It develops when an individual with contact allergy to a substance is exposed to that substance, the allergen, at a concentration exceeding that individual’s threshold (16).

More than 4000 substances are recognized as causing contact allergy (17). Most contact allergens are small, chemically reactive molecules, usually with a molecular weight below 500 (18), and with high lipophilicity ( $\log P > 1$ ). Since these molecules are too small to act as antigens themselves, contact sensitisers are generally referred to as haptens (incomplete antigens). Haptens, such as formaldehyde, are thought to initiate contact allergy by binding covalently to skin proteins to produce an antigen complex.

Contact allergy and allergic contact dermatitis have two phases. The first is the sensitisation phase, when the immunological memory of the contact sensitiser is established, and the second is the elicitation phase, which begins when the individual is re-exposed to the sensitiser, and results in the clinical manifestation of allergic contact dermatitis (16). The sensitisation phase requires at least 4 days to several weeks; whereas the elicitation phase usually takes 1-2 days, but for some substances it can take up to 2-3 weeks (16, 19-21).

In the sensitisation phase, the antigen in the epidermis is taken up by antigen presenting cells called Langerhans cells, which migrate from the epidermis to the regional lymph nodes, where they are presented to uncommitted T-cells that become activated. Activated T-cells release cytokines, leading to the proliferation and differentiation of the T-cells into hapten-specific memory T-cells that are released into the blood circulation (16). Upon re-exposure to the hapten, i.e. the elicitation phase, Langerhans cells present the antigen to the memory T-cells circulating in the body. These allergen-specific T-cells become activated and initiate a cascade of inflammatory events in the exposed area of the skin, leading to an eczematous reaction (22). Since these allergen-specific T-cells are circulating in the blood, eczema can appear anywhere on the body where the skin is exposed to the allergen. Interestingly, formaldehyde was one of the contact allergens used to clarify the role of the Langerhans cells in the development of contact dermatitis, and microscopic studies provided evidence that it is a contact allergen (2).

Once established, contact allergy is usually lifelong, although the degree of sensitivity can change over time. It is important to differentiate between contact allergy and allergic contact dermatitis. Thus, if a sensitised individual avoids contact with the allergen in question or substances chemically related to the allergen, he or she will not develop allergic contact dermatitis.



## 1.4 Patch testing

The method used to establish contact allergy is patch testing. Jadassohn introduced the method in 1895, and it was described in detail by Bloch in 1929 (23). Allergens are applied to intact skin under controlled conditions. An eczematous reaction occurring on the test site indicates contact allergy. The patch test method has been, and continues to be, developed and standardised with regard to allergens, vehicles, concentrations, doses and scoring of the patch test reactions (24-27).

The suspected allergens are either dissolved or evenly distributed at appropriate concentrations in a “vehicle” and then applied to the skin in small test chambers that are fixed to the skin on the patient’s back with adhesive tape and left in place for 48 hours, as shown in Figure 2. It is recommended that patch tests be read on two occasions (25) (4 and 7 days after application), and it has been shown that these paired readings are the most accurate (28). The overall incidence of reactions to allergens in the baseline series appearing between day 4 and day 7 has been reported to be 7.2% and 8.2% (28, 29). At the Department of Occupational and Environmental Dermatology in Malmö, routine readings are performed on day 3/4 and day 7.

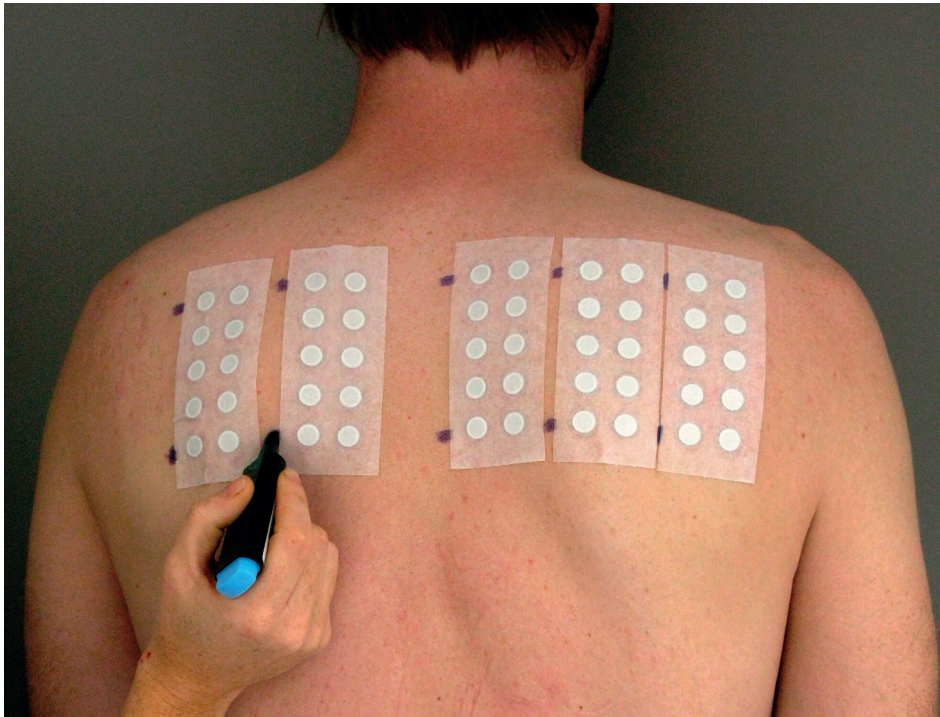


Figure 2. Patch testing

Readings are performed in accordance with the International Contact Dermatitis Research Group's (ICDRG) guidelines, and reactions are scored as (+) = doubtful, + = weak positive, ++ = strong positive or +++ = extreme positive (30). Additional gradings can also be used. The minimum criteria for a *positive patch test reaction* are redness and infiltration covering the test area.

*False-positive reactions* are positive patch test reactions in the absence of contact allergy, with morphology undistinguishable from a contact allergic reaction. The cause of a false-positive reaction is irritancy. To exclude false-positive reactions, patch testing with dilution series and/or patch testing of controls can be performed.

*False-negative reactions* are negative patch test reactions in the presence of contact allergy. Common causes of false-negative reactions are too low patch test concentrations, systemic treatment with corticosteroids during patch testing or that the reading was made too early (25). It has also been shown that the stability of patch test preparations can be affected by storage time and temperature (31, 32). If these preparations are used, the concentrations may be much lower than intended, possibly leading to false-negative reactions.

*Late patch test reactions* are reactions that are not visible at the test site on day 7 but appear later (30). Some allergens such as acrylates and corticosteroids are known to cause late reactions. It has also been demonstrated that patch test reactions in already sensitised individuals can appear after 10-14 days (19-21, 33, 34). However, late reactions may also indicate sensitisation caused by the patch test.

*Patch test sensitisation* is an adverse effect of patch testing. If a positive patch test reaction occurs 10 - 20 days after patch testing on the area of a previously negative patch test, and a positive reaction appears on days 2-4 when re-testing is performed, active sensitisation is the most probable explanation of the late reaction. To elucidate this, patch testing with serial dilutions of the allergen in question is recommended (27), since some sensitised individuals may react to lower concentrations of the allergen in question later than day 7 (20, 21). Patch test sensitisation is generally considered to be extremely infrequent, especially for the chemicals in the baseline series (35).

## 1.5 Sensitising potential of formaldehyde

Animal studies are used to establish whether a chemical or substance causes sensitisation. Contradictory results regarding formaldehyde have been reported from guinea pig maximisation tests (GPMT) ranging from sensitisation of none of the animals to all of them following dermal challenge (2, 36-39). One explanation of this wide variation in results is that toxic doses of the sensitiser during the induction phase in the GPMT may influence the skin reactivity during challenge (40). In

one publication presenting data from animals (the GPMT and the local lymph node assay) and humans (Buehler test) formaldehyde was considered to be a strong sensitiser (41). Formaldehyde was found to be the strongest allergen when the allergenicity of 10 aldehydes was evaluated using the local lymph node assay (42).

## 1.6 Contact allergy to formaldehyde

The general aim of the work presented in this thesis was to investigate contact allergy to formaldehyde and allergic contact dermatitis caused by formaldehyde.

Formaldehyde has long been known to be a prominent contact allergen, and is described in all the textbooks on contact allergy. Patch testing with formaldehyde began already in 1929 (43). The first baseline series proposed by Bonnevie in 1939 contained formaldehyde (44). The ICDRG recommended the inclusion of formaldehyde in the baseline series in the 1960s, and it has been included in almost all screening patch test series since then (45). The optimal patch test concentration has been discussed as a result of false-positive and false-negative reactions.

### 1.6.1 The prevalence of contact allergy to formaldehyde

While formaldehyde has been included in almost all baseline series and has been used at different concentrations in patch tests, the results of testing vary considerably. In the 1960s, the prevalence of contact allergy to formaldehyde was found to exceed 20% (45). The prevalence of contact allergy to formaldehyde has generally been found to be higher in the USA than in Europe over recent decades, being 8-9% in the USA and 2-3% in European countries (10). The reason for this could be that the population in the USA is more exposed to cosmetics, or that the cosmetics in the USA contain formaldehyde-releasing preservatives more frequently or at higher concentrations than in the European countries. In 1988-89, the prevalence of contact allergy to 1.0% formaldehyde among consecutively patch-tested patients in Denmark was 2.3% and in Europe 2.6% (46). Contact allergy to formaldehyde 1% was found in about 2.5% of patients tested with the European baseline series during the period 1991-2000 (47). The European Environmental and Contact Dermatitis Research Group (EECDRG) reported an incidence of contact allergy to 1.0% formaldehyde of 2.5% between the years 2001 and 2008 (48).

Formaldehyde allergy is more common in women than in men, probably because women are more exposed to formaldehyde and formaldehyde-releasing preservatives in cosmetics and household products. Patients with contact allergy to formaldehyde often have hand eczema with or without facial dermatitis (49). Formaldehyde is a significant contact allergen in women with hand eczema (50).

Formaldehyde belongs to the “basic contact allergens” and has always been included in the baseline series at the Department of Occupational and Environmental Dermatology in Malmö. Before this research started (prior to 2006), the patch test concentration of formaldehyde used in the baseline series of patch tests was 1.0% (0.30 mg/cm<sup>2</sup>), and a higher concentration of 2.0% (0.60 mg/cm<sup>2</sup>) was used in patients showing doubtful reactions to 1.0%, and when there was a strong suspicion of contact allergy to formaldehyde. The prevalence of contact allergy to a 1.0% aqueous solution of formaldehyde in the baseline series among consecutively patch-tested dermatitis patients at our department during the period 1995 - 2005 is shown in Figure 3. The overall frequency of contact allergy to formaldehyde 1.0% (0.30 mg/cm<sup>2</sup>) during this period was 1.6% for men (range 0.8 - 3.0) and 2.7% for women (range 1.0 - 4.1) giving an average of 2.1%.

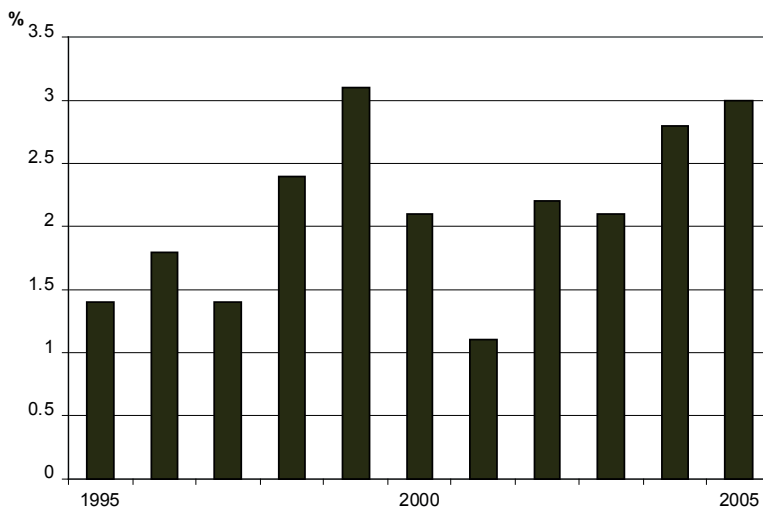


Figure 3. Percentage of individuals with contact allergy to formaldehyde 1.0% aqua (0.30 mg/cm<sup>2</sup>) in the baseline patch test series among consecutively patch-tested dermatitis patients at the Department of Occupational and Environmental Dermatology, Malmö University Hospital, Malmö, 1995-2005.

### 1.6.2 Formaldehyde contact allergy in the occupational setting

Formaldehyde is an important occupational contact allergen. The first case of occupational contact dermatitis caused by formaldehyde was documented in 1905, when Galewsky described eczema among physicians and medical staff in Germany (51). Formaldehyde-releasing preservatives are also widely used in water-containing products such as metalworking fluids, cosmetic products and detergents (10). Occupational sensitisation to formaldehyde has also been reported among hairdressers, medical staff, embalmers, masseurs and others using protective creams, detergents and liquid soaps. However, occupational sensitisation is most common among industrial workers exposed to metalworking fluids. The frequency of contact allergy to formaldehyde among patients who are suspected of having occupational dermatitis can be up to 2-3 times higher than in general dermatological patients (52). Occupational allergic contact dermatitis caused by formaldehyde is most often seen on the hands and forearms. Only limited data of clinical relevance on contact allergy to formaldehyde releasers used in metalworking fluids are available, and more studies are required in this area (53).

## 1.7 Other skin effects

Type I allergy to formaldehyde is less common than type IV allergy, but was described as early as 1921. Urticarial reactions have been elicited by various kinds of exposure to formaldehyde, including formaldehyde vapour, direct contact with the skin and mucous membranes, and systemic exposure through vaccination, dialysis and medication. Formaldehyde can elicit both immunological and non-immunological urticaria. Specific IgE antibodies have been detected in cases of immunologically mediated urticaria. IgE-mediated urticaria (54, 55) and anaphylaxis (56) due to formaldehyde in root-canal disinfectants have been reported.

Contact urticaria appearing on healthy skin following repeated applications, and after a single application to diseased skin, have also been described (57).

Systemic allergic contact dermatitis thought to be caused by formaldehyde in aspartame has been described (58).

Although formaldehyde does not absorb light in the UV range (290-400 nm) and is thus not recognised as a photosensitiser, immediate sunburn-like reactions together with a positive photo-patch test to formaldehyde have been reported (59).

Animal studies on rabbits have shown that 37% formaldehyde produces severe skin irritation under an occlusive dressing (60), and high concentrations of formaldehyde can also produce chemical burns.

## 1.8 Other health aspects

As mentioned above, formaldehyde is present all around us at different concentrations. We still do not know if it is necessary to life, and have insufficient knowledge about its health effects.

### 1.8.1 Irritation

Formaldehyde vapour causes irritation of the mucous membranes (61). Contact with the eyes causes irritation, burning, itching, redness and tearing. The threshold for slight eye irritation is between 0.05 ppm and 1.0 ppm (62, 63). Studies on human volunteers showed no objective signs of irritation at exposures to concentrations up to 0.4 ppm, with peaks of 0.8 ppm (64). Average indoor levels of formaldehyde in Europe are usually between 0.02 ppm and 0.035 ppm (65). Irritation of the upper airways is the most common respiratory effect, and can occur over a wide range of concentrations, but is most frequent above 1 ppm. Symptoms of upper airway irritation include dry throat, itching and burning sensations in the nose, and nasal congestion. Air concentrations above 5 ppm readily cause lower airway irritation characterised by coughing, tightness of the chest and wheezing. Concentrations above 50 ppm can cause severe pulmonary reactions such as oedema and bronchial irritation, which can result in death. The concentration of formaldehyde in air that is immediately dangerous to health and life is 100 ppm (63).

### 1.8.2 Carcinogenic effects

The first report on the carcinogenicity of formaldehyde appeared in 1980, in which chronic inhalation of high concentrations of formaldehyde was shown to induce a high incidence of nasal squamous cell carcinoma in rats (4). This finding raised the question of whether formaldehyde exposure could also cause cancer in humans, and was followed by several human cancer studies. The relationship between formaldehyde exposure and carcinogenicity in humans has been evaluated in a large number of epidemiological studies. Increased numbers of nasopharyngeal carcinomas and leukaemia have been found in humans exposed to formaldehyde (4, 66) resulting in formaldehyde being considered a human carcinogen. According to the International Agency for Research on Cancer, formaldehyde is genotoxic and probably a human carcinogen, belonging to group 1 (B1). The amount of data for both humans and animals is, however, insufficient for the definite classification of formaldehyde as carcinogenic (62).

Recommended safe levels of indoor formaldehyde in vapour in Sweden are 0.5 - 1.0 ppm (67).

### 1.8.3 Other effects

An association has been reported between formaldehyde exposure and asthma (68, 69). Formaldehyde has also been suggested to be a possible cause of occupational asthma (70).

A possible connection between migraine and formaldehyde in the artificial sweetener aspartame has also been reported (71).

Recent studies in animals showed that systemic/mutagenic effects were not induced by formaldehyde (72).

## 1.9 Regulations governing formaldehyde

Formaldehyde is manufactured and used within the EU in accordance with European regulations such as REACH (the Registration, Evaluation, Authorisation and Restriction of Chemicals), CLG/GHS (the Classification, Labelling and Packaging of Substances and Mixtures), the Biocides Directive and other European and national health and safety legislation (73).

European regulations on cosmetics regulate the contents of formaldehyde, para-formaldehyde and formaldehyde releasers in cosmetic products (15). According to Regulation No. 1223/2009, the maximum allowed concentration of free formaldehyde in cosmetic products is 0.2% or 2000 ppm (0.1% or 1000 ppm in products for oral hygiene). All products containing formaldehyde or substances that release formaldehyde must be declared with the warning text “Contains formaldehyde” if the concentration of free formaldehyde exceeds 0.05%. Formaldehyde releasers must always be declared, i.e., there is no minimal concentration that does not have to be declared. The maximum permissible concentrations of formaldehyde releasers are given in Table 2.





## 2 AIMS

The general aim of the work described in this thesis was to investigate contact allergy to formaldehyde and its clinical relevance. More specifically, the purposes of the studies were:

- to investigate the prevalence of contact allergy to formaldehyde among dermatitis patients consecutively patch-tested with a baseline patch test series,
- to determine the optimal patch test concentration and dose for formaldehyde,
- to study the clinical relevance of contact allergy to formaldehyde detected by 2.0% (0.60 mg/cm<sup>2</sup>) but not by 1.0% (0.30 mg/cm<sup>2</sup>) formaldehyde,
- to study the effects of low concentrations of formaldehyde on irritant contact dermatitis in formaldehyde-allergic patients,
- to determine the presence of formaldehyde in skin care products used by patients suspected of having allergic contact dermatitis and compare it to the declaration of contents,
- to determine whether formaldehyde-allergic patients are more exposed to formaldehyde in skin care products than dermatitis patients without contact allergy to formaldehyde, and
- to investigate the patterns of concomitant contact allergies to formaldehyde and formaldehyde releasers among the dermatitis patients consecutively patch-tested with a baseline patch test series.



# 3 MATERIALS AND METHODS

Detailed descriptions of the materials and methods are given in the individual papers. This section presents an overview of the methods used. The first study, described in Paper I, involved comparative patch testing. The second and third studies, described in Papers II and III, were clinical experimental studies, and the final study was a clinical study based on laboratory analysis.

## 3.1 Study populations

All the patients studied in this work were investigated at the Department of Occupational and Environmental Dermatology, Skåne University Hospital, Malmö, Sweden. They included patients investigated due to suspected occupational or environmental skin disease, as well as dermatitis patients referred from the Department of Dermatology, Skåne University Hospital, Malmö, Sweden. All the patients underwent patch testing with our baseline series.

### 3.1.1 Patch testing with formaldehyde 1.0% and 2.0%

In this study (Paper I) the outcome of simultaneous testing with formaldehyde 1.0% (0.30 mg/cm<sup>2</sup>) and 2.0% (0.60 mg/cm<sup>2</sup>) was investigated. In total, 1397 dermatitis patients, 519 males (37%) and 878 females (63%) were consecutively patch-tested between 1 January 2006 and 31 December 2007.

### 3.1.2 Repeated open application tests

#### *ROAT on healthy skin*

Repeated open application tests (ROAT) were performed on healthy skin (Paper II) in individuals who had reacted positively to 2.0% formaldehyde but not to 1.0%, when these preparations were simultaneously patch-tested in the baseline series in Study I. Eighteen formaldehyde-allergic individuals (4 males and 14 females) participated in the study. Nineteen age- and gender-matched individuals (7 males and 12 females) who were also consecutively patch-tested with the baseline series

during the same period and who did not react to formaldehyde were served as controls. Exclusion criteria in both groups were contact allergy to formaldehyde-releasers and formaldehyde-based resins in the baseline series, and parabens. The formaldehyde-releasing preservatives and resins included in the baseline series during the period of this work are given in Table 4.

### *ROAT on experimental dermatitis*

In this study (Paper III), ROATs were performed on experimentally induced dermatitis in a test group consisting of 15 individuals (5 males and 10 females) with contact allergy to 1.0% and/or 2.0% formaldehyde in our baseline series. The control group consisted of 12 age- and gender-matched individuals (4 males and 8 females) without contact allergy to formaldehyde. All individuals were chosen from the dermatitis patients who were consecutively patch-tested between 1 January 2006 and 31 December 2011. Exclusion criteria for both groups were contact allergy to formaldehyde releasers and formaldehyde-based resins in the baseline series used at our department between 1 January 2006 and 31 December 2011 (Table 4), treatment with oral corticosteroids and/or ongoing dermatitis at or near the skin sites to be used for ROAT and patch testing. Table 3 gives information on the individuals included in these studies.

Table 3. Characteristics of the individuals participating in the ROAT studies described in Papers II and III.

		No. of participants	Men/women	Mean age (range) (years)	Reactivity to formaldehyde	
					1.0%	2.0%
Paper II	Test group	17	4/14	44 (23 - 64)	neg	pos
	Control group	19	7/12	48 (18 - 66)	neg	neg
Paper III	Test group	15	5/10	44 (25 - 69)	pos (11)*	pos (14)*
	Control group	13	4/9	48 (31 - 69)	neg	neg

\* These patients were patch-tested 2006 – 2011 and were positive to formaldehyde 1.0% and/or 2.0%. Before performing the ROAT on experimental dermatitis, the patients were patch-tested with dilutions of formaldehyde to determine their present patch test reactivity.

### 3.1.3 Determination of formaldehyde in skin care products

Individuals consecutively patch-tested at the department during a 4-month period (between 1 October 2012 and 31 January 2013) received written information before coming to the department instructing them to bring the skin care products that they were using, or had recently used, to the clinic before the first patch test reading (Paper IV). The information specified which kind of products they should bring, and that the products should be brought to the clinic in their original packaging.

Samples were taken from all the products and the packages and ingredient labelling were photographed. The individuals participating in the study were divided into two groups: formaldehyde-allergic individuals, and individuals without contact allergy to formaldehyde. In total, 154 individuals brought 996 products. When an individual was found to be positive to formaldehyde and/or formaldehyde releasers, his or her products were analysed with the chromotropic acid method to determine the amount of formaldehyde. For each formaldehyde-allergic individual, 3 age- and gender-matched individuals without contact allergy to formaldehyde were randomly chosen from the patients patch-tested during the same time ( $\pm 1$  month), and products brought by these individuals were analysed at the same time. Eighty-one products brought in by 10 individuals (10 females) found to be positive to formaldehyde 1.0% and/or 2.0% (in the baseline patch test series and 206 products from 30 individuals (30 females) without contact allergy to formaldehyde or the formaldehyde releasers were analysed, giving a total of 287 products analysed. The design of the study is illustrated in Figure 4.

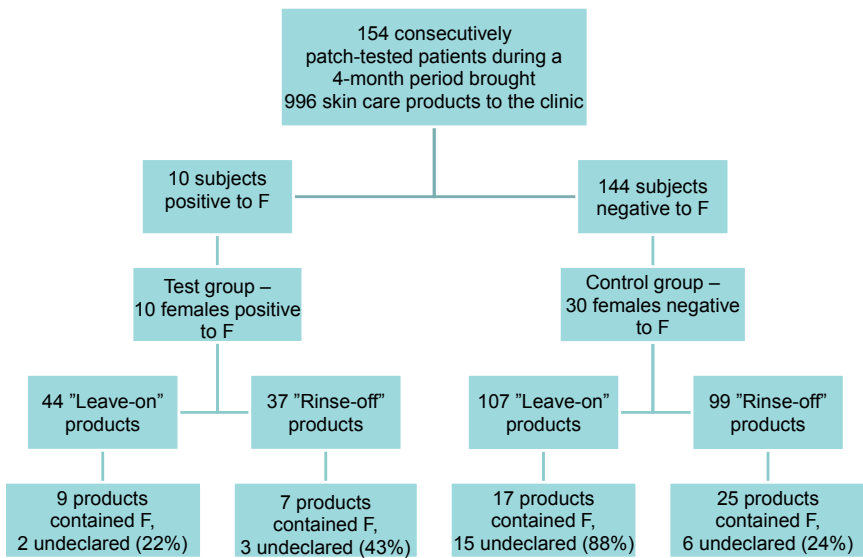


Figure 4. Flowchart of Study IV (F denotes formaldehyde).

## 3.2 Patch testing

### 3.2.1 Patch test preparations

Our baseline series is based on the European baseline series and supplemented with test preparations representing metals, preservatives, plastics, corticosteroids and textile dyes. All patch test preparations in the baseline series except formaldehyde were supplied by Chemotechnique Diagnostics (Vellinge, Sweden). Formaldehyde (37% (w/v) in aqua) was bought from Acros Organics (Geel, Belgium) and prepared for patch testing at our department.

Table 4. Formaldehyde-releasing preservatives and resins used in our baseline series during the period of the present research. Concentrations of test substances in aqua (Aq.) are given in w/v and in petrolatum (Pet.) in w/w.

Test preparations	Concentration	Vehicle	Years covered
Formaldehyde	2.0% (w/v)	Aq.	2006 - 2013
Formaldehyde	1.0% (w/v)	Aq.	2006 - 2013
Formaldehyde	3.0% (w/v)	Aq.	2007 *
Formaldehyde	0.32% (w/v)	Aq.	2007
Formaldehyde	0.10% (w/v)	Aq.	2007
Formaldehyde	0.032% (w/v)	Aq.	2007
Quaternium-15	1.0% (w/w)	Pet.	2006 - 2013
Quaternium-15	1.0% (w/v)	Aq.	2009 - 2013
Imidazolidinyl urea	2.0% (w/v)	Aq.	2006 - 2013
Imidazolidinyl urea	2.0% (w/w)	Pet.	2009 - 2013
Diazolidinyl urea	2.0% (w/v)	Aq.	2006 - 2013
Diazolidinyl urea	2.0% (w/w)	Pet.	2009 - 2013
DMDM hydantoin	2.0% (w/v)	Aq.	2011 - 2013
DMDM hydantoin	2.0% (w/w)	Pet.	2011 - 2013
4-tert-Butylphenol-formaldehyde resin	1.0% (w/w)	Pet.	2006 - 2013
Phenol-formaldehyde resin	1.0% (w/w)	Pet.	2006 - 2013
Tosylamide/formaldehyde resin	10.0% (w/w)	Pet.	2006 - 2013

\*27 individuals were patch-tested.

Formaldehyde in aqua at a concentration of 2.0% (w/v) was included in the baseline series from 1 January 2006. Additional concentrations of formaldehyde were included in our baseline series from 1 January 2007 to 31 December 2007 (Table 4). During a short period (about 2 weeks) formaldehyde 3.0% (w/v) aqua was included. Formaldehyde-releasing preservatives and formaldehyde-based resins included in our baseline series during the period of this research are also included in Table 4.

All the patients described in Paper III were additionally patch-tested with dilution series of formaldehyde, DMDM hydantoin, sodium lauryl sulphate (SLS), and DM hydantoin. Patch tests were carried out using serial dilutions of formaldehyde and DMDM hydantoin in aqua in 10 steps. The dilution series of DMDM hydantoin in petrolatum was prepared in 5 steps. DM hydantoin was used for patch testing in aqua and petrolatum at concentrations equimolar to 2.0% DMDM hydantoin. SLS was also diluted in aqua in 5 steps. All patients were also patch-tested with the moisturisers “as is” used in this ROAT study (Paper III).

### 3.2.2 Patch testing technique

The allergens were applied to Finn Chambers 8 mm in diameter (EpiTest Ltd Oy, Tuusula, Finland) mounted on Scanpor tape (Norgeplaster A/S, Vennesla, Norway). Fifteen  $\mu\text{l}$  of the aqueous solutions were applied with a micropipette to filter paper discs in the test chambers (26). For the test preparations in petrolatum, the amount of 20 mg was used (24). These were then applied to the upper part of the back, as shown in Figure 2, and left in place for 48 hours. The patch test reactions were read on day 3/4 and day 7 by a dermatologist, and scored according to the ICDRG guidelines (30).

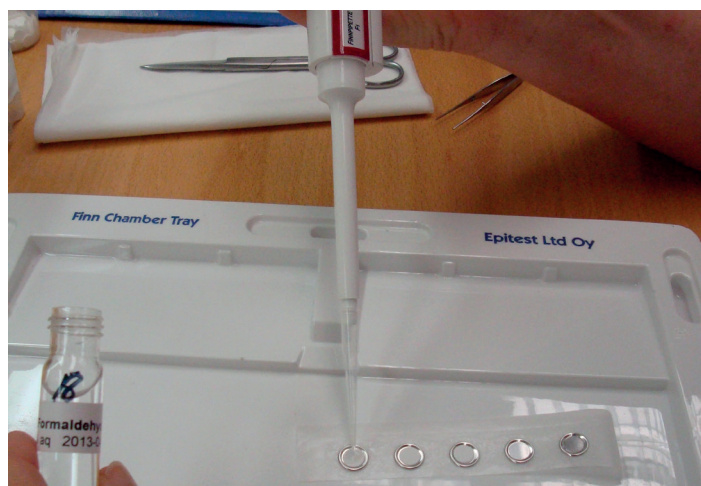


Figure 5. Application of the patch test solutions with a micropipette.

### 3.3 ROAT studies

It can sometimes be difficult to determine the clinical relevance of positive patch test reactions. Use tests are simple to perform and help to evaluate the importance of a detected contact allergy. The ROAT is a provocation test that simulates the mode and frequency of the ordinary application of the suspected agent, and helps to verify the clinical significance of positive patch test results. The ROAT can be used in cases when an individual has a contact allergy to a sensitiser present in a product, but where patch testing with this product is negative, or when it is strongly suspected that allergic contact dermatitis is caused by a certain product. The ROAT can also be used in scientifically designed clinical studies when groups of hypersensitive patients undergo ROATs under controlled conditions (74). Two controlled, double-blinded and randomised ROAT studies are included in this thesis (Papers II and III).

#### 3.3.1 ROAT on healthy skin

The clinical relevance of contact allergy to formaldehyde detected by 2.0% (0.60 mg/cm<sup>2</sup>) but not by 1.0% (0.30 mg/cm<sup>2</sup>) formaldehyde was investigated in this study (Paper II). The ROAT was performed on healthy skin. An aqueous moisturising cream (Cetylanum 9 g, Parafinum liquidum 6 g, Vaselinum album 15 g and Aqua purificata ad 100 g) preserved with parabens (0.1% methylparaben and 0.2% propylparaben) was manufactured at the pharmacy of Skåne University Hospital in Malmö, Sweden. The highest permitted concentration of formaldehyde in cosmetics (2000 ppm) was added to half the batch of aqueous moisturising cream and the other half was left unaltered. Each individual was given a pair of tubes marked with red or blue tape, together with instructions on how to apply the moisturiser. The ROAT was performed on the inside of the upper arms, on 5 x 5 cm areas, twice a day. The maximum study period was 4 weeks. Reading was performed by a dermatologist once a week. The reaction was defined as positive when erythematous infiltration with possible papules and/or vesicles appeared on at least 25% of the treated area. The strength of the reaction was classified as mild, moderate, or strong (75). When a test site showed a positive reaction, the ROAT in this area was terminated but continued in the other area.



### 3.3.2 ROAT on experimental dermatitis

The effects of exposure to low concentrations of formaldehyde in formaldehyde-allergic individuals were investigated on SLS-induced irritant contact dermatitis (Paper III). The study design is illustrated in Figure 6.

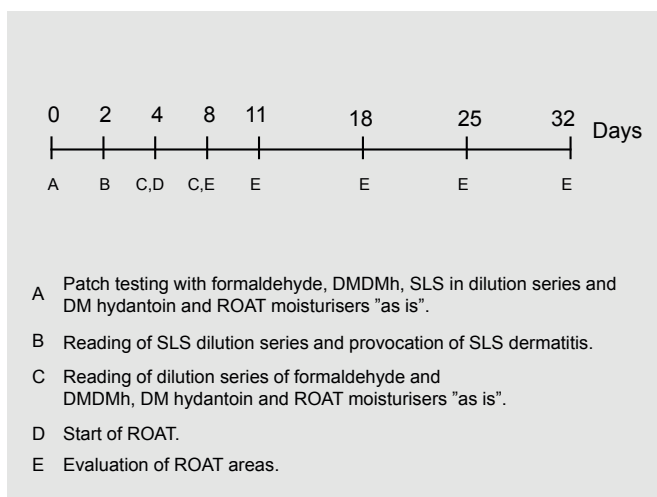


Figure 6. Design of the ROAT study on experimental dermatitis. ROAT, Repeated open application test; SLS, sodium lauryl sulphate; DMDMh, DMDM hydantoin.

An aqueous moisturising cream preserved with phenoxyethanol was manufactured by the pharmacy of Skåne University Hospital in Malmö. Since formaldehyde is a gas it is technically difficult to incorporate small amounts into different cosmetic formulations. Thus, DMDM hydantoin was used as a formaldehyde releaser and was added at our laboratory at concentrations of 0.6, 0.33 and 0.06%, giving moisturisers with high (H), medium (M) and low (L) contents of formaldehyde. Moisturiser without formaldehyde is called the control moisturiser (0). The moisturisers were filled in plastic syringes by our laboratory staff.

Based on the results of patch testing with SLS, the lowest concentration resulting in a positive reaction (erythema and infiltration, according to the ICDRG criteria) was chosen to provoke 4 areas of irritant contact dermatitis on the inside of the upper arms, as described above. Each individual was given 4 syringes of moisturisers, marked with different colours, and detailed instructions on how to apply each moisturiser to a 3 x 3 cm area, twice a day. The maximum study period was 4 weeks. When a positive response was seen, individuals ceased application to this area, but continued application to the other areas. Reading was performed by a dermatologist

twice during the first week, and then once a week for the remaining three weeks. The ROAT reaction was defined as positive when: (i) the SLS dermatitis healed and dermatitis reappeared showing erythematous infiltration with possible papules and/or vesicles covering at least 25% of the treated area; (ii) the SLS dermatitis did not heal, but deteriorated with the addition of papules and vesicles; (iii) delayed healing was seen in the areas treated with the moisturisers containing formaldehyde (H, M, L) compared to the area treated with the control moisturiser (Figure 7).

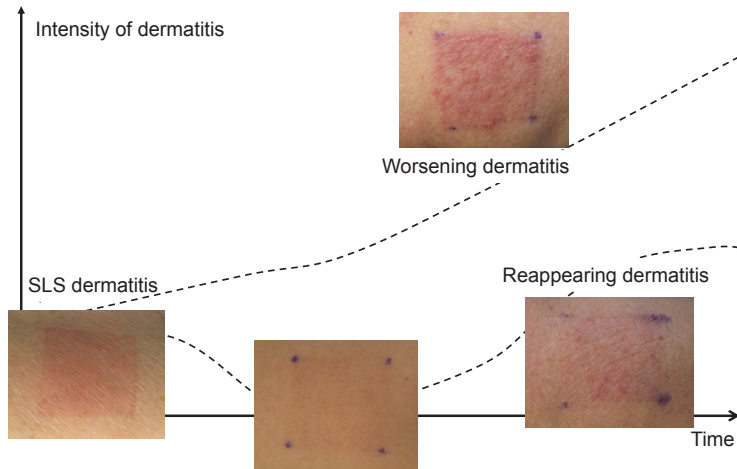


Figure 7. Examples of possible outcomes of the exposure of damaged skin (SLS dermatitis) to formaldehyde using the ROAT.

## 3.4 Chemicals

The main chemicals used in these studies are listed in Table 5.

Table 5. Main chemicals with manufacturers/suppliers

Chemical	Study	Manufacturer/supplier
Ammonium acetate	III	J.T. Baker, Deventer, Holland
Anhydrous disodium hydrogen phosphate	III	Janssen Chimica, Geel, Belgium
Acetic acid	III	Merck, Darmstadt, Germany
Chromotropic acid	III, IV	Merck, Darmstadt, Germany
Dichloromethane	III	Prolab, Leuven, Belgium
2,4-Pentadione	III	ICN Biomedicals Inc., Auror, Ohio, USA
DM hydantoin	III	Acros Organics, Geel, Belgium
Formaldehyde	I, II, III, IV	Acros Organics, Geel, Belgium
Orthophosphoric acid	III	Acros Organics, Geel, Belgium
Sodium chloride	III	Acros Organics, Geel, Belgium
Sodium hydroxide	III	Acros Organics, Geel, Belgium
Sodium lauryl sulphate	III	Acros Organics, Geel, Belgium
Sulphuric acid	III, IV	Sigma-Aldrich Chemie, GmbH, Stenheim, Germany

## 3.5 Chemical investigations

### 3.5.1 The chromotropic acid method

This method was first described in 1959 by Blohm (76). The chromotropic acid method is a semi-quantitative method based on the chemical reaction between chromotropic acid and free formaldehyde evaporating from the sample, giving a purple reaction product. A rough estimate of the formaldehyde concentration in a sample can then be made by comparing the intensity of the colour of the sample with those of standards in the range 2.5 - 40 ppm (77). Various standards are shown in Figure 8. The possibility of determining higher concentrations in standard solutions, 60 and 80 ppm was investigated (unpublished data), showing that it was possible to distinguish between 40 and/or 60 or 80 ppm, and that there was good concordance between evaluators.



Figure 8. Standard solutions of formaldehyde used for comparison in the chromotropic acid method.

Other aldehydes and ketones can also react with chromotropic acid, affecting the colour of the sample. When these substances are present, it is not possible to determine whether the sample releases formaldehyde, or its concentration. The chromotropic acid method is described in detail in Paper III, where it was used to select the concentrations of free formaldehyde in ROAT moisturisers and to analyse these moisturisers before and after the study. The release of formaldehyde from the personal care products (shower cream and liquid soap) provided by our department to be used by the individuals during the second ROAT study (Paper III), and syringes with and without moisturisers were also analysed with the chromotropic acid method. The release of formaldehyde from the skin care products in Study IV was analysed with the chromotropic acid method.

### 3.5.2 High performance liquid chromatography

High performance liquid chromatography (HPLC) is a method used to separate chemical components, based on their physico-chemical properties, by passing the sample through a stationary phase in a mobile phase. HPLC was used in the ROAT study on healthy skin (Paper II) to analyse the amount of formaldehyde in the moisturisers with and without added formaldehyde, after the study. It was also used in the ROAT study on experimental dermatitis (Paper III) to determine the DMDM hydantoin content in the ROAT moisturisers, by analysing its degradation product DM hydantoin. The HPLC system and the linear gradient for used for the elution of the solvents are described in Paper III. The detection limit for formaldehyde (Paper II) was  $< 0.05 \mu\text{g/g}$  and for DM hydantoin (Paper III) was  $< 40 \mu\text{g/g}$ .

## 3.6 Data recording

The data obtained from the various studies were recorded in the database registration system Daluk, in which age, gender and contact allergies are recorded (78). These data were used to obtain information on the simultaneous patch testing with formaldehyde 1.0% and 2.0% aqua (Paper I), and to select the patients for the ROAT studies (Papers II and III).

## 3.7 Ethics

The studies described in Papers II and III were approved by the Ethics Committee of the Faculty of Medicine, Lund University, and conducted in accordance with the ethical standards specified in the Declaration of Helsinki and the ICH guidelines on Good Clinical Practice. All patients gave their informed written consent to participate in the study.

## 3.8 Statistical calculations

### 3.8.1 Study I

McNemar's test was used to compare the number of positive reactions to formaldehyde 2.0% and 1.0%. Fisher's two-sided exact test was used to compare the rate of contact allergy in males and females, as well as the association between the separate formaldehyde-releasing preservatives and formaldehyde-based resins. A p-value <0.05 was considered to indicate statistically significant differences.

### 3.8.2 Study II

Fisher's two-sided exact test was used to compare the number of reactions to the formaldehyde-containing moisturiser in the formaldehyde-allergic patients and the controls. The number of reactions in the formaldehyde-allergic patients using moisturisers with and without formaldehyde was compared with McNemar's test. The differences were considered significant when  $p < 0.05$ .

### 3.8.3 Study III

Fisher's two-sided exact test was used to compare the number of positive ROAT reactors to the various formaldehyde-releasing moisturisers in the formaldehyde-allergic individuals and the controls. Within the group of formaldehyde-allergic individuals the number of positive ROAT reactors to each of the 3 formaldehyde-containing moisturisers was compared individually with the number of positive ROAT reactors to the moisturiser without formaldehyde using the two-sided McNemar test. A possible dose-response relationship regarding the number of positive reactors to the 4 moisturisers with different concentrations of formaldehyde (H, M, L and 0) was investigated with the Page test. The Mann-Whitney test for 2 independent variables was used to investigate a possible association between the intensity of reactivity to formaldehyde 2.0% and the number of positive ROAT reactors to the moisturiser with the highest concentration of formaldehyde (H). The number of days until healing of the irritant dermatitis on the site to which the moisturiser without formaldehyde had been applied was compared using the log-rank test. The differences were considered statistically significant when  $p < 0.05$ .

### 3.8.4 Study IV

Fischer's two-sided exact test was used to compare the number of formaldehyde-allergic individuals using "leave-on" products containing  $>40$  ppm formaldehyde with individuals without contact allergy to formaldehyde using the same type of products. It was also used to compare the number of products containing formaldehyde between "leave-on" and "rinse-off" groups. The number of products in which formaldehyde was not declared in the labelling in the "leave-on" and "rinse-off" groups was also compared using Fischer's two-sided exact test. The number of products brought in by the formaldehyde-allergic individuals and controls was investigated with the Mann-Whitney two-sided test. The differences were considered significant when  $p < 0.05$ .

# 4 RESULTS

The results of the 4 studies are described in detail in the corresponding papers. The results will be compared and commented on briefly in this section.

## 4.1 Patch testing with formaldehyde 1.0% and 2.0%

A total of 1397 patients underwent patch testing with at least the baseline series between 1 January 2006 and 31 December 2007. The results of the patch testing are given in Table 6. In all, 68 (4.9%) patients reacted positively to formaldehyde: 37 reacted only to 2.0%, 29 reacted to both concentrations and 2 patients reacted only to 1.0%. Significantly more patients were diagnosed as having contact allergy to formaldehyde with 2.0% than 1.0% ( $p < 0.001$ , McNemar's test). Small numbers of irritant reactions to formaldehyde 1.0% and 2.0% were found (0.3% and 0.1%, respectively), and a high number to 3.0% (29.6%).

Table 6. Reactions to 15 µl formaldehyde of different concentrations (w/v %) when patch-tested in Finn Chambers (ø8 mm).

Formaldehyde w/v % ( mg/cm <sup>2</sup> )	Contact allergy reactions					Other reactions				Total tested
	+++ <sup>1</sup>	++	+	Tot	% <sup>2</sup>	? <sup>3</sup>	% <sup>2</sup>	IR <sup>4</sup>	% <sup>2</sup>	N
3.0 (0.90)										
All	2	0	0	2	7.4	1	3.7	8	29.6	27
Men	0	0	0	0	0					0
Women	2	0	0	2	7.4					27
2.0 (0.60)										
All	11	22	33	66	4.7	48	3.4	5	0.3	1397
Men	2	5	7	14	2.7					519
Women	9	17	26	52	5.9					878
1.0 (0.30)										
All	3	18	10	31 <sup>5</sup>	2.2	21 <sup>6</sup>	1.5	1	0.1	1397
Men	0	5	2	7	1.3					519
Women	3	13	8	24	2.7					878
0.32 (0.09)										
All	1	4	6	11	1.5	2	0.3	0	0	738
Men	0	0	2	2	0.8					264
Women	1	4	4	9	1.9					474
0.10 (0.03)										
All	1	2	1	4	0.5	1	0.1	0	0	738
Men	0	0	0	0	0					264
Women	1	2	1	4	0.5					474
0.032 (0.009)										
All	0	0	0	1	0.1	0	0	0	0	738
Men	0	0	0	0	0					264
Women	0	1	0	1	0.1					474

<sup>1</sup>The strongest observed reaction on day (D) 3/4 and/or D7 is given. <sup>2</sup>Proportion (%) of all tested patients. <sup>3</sup>Doubtful reaction without any positive reaction to other simultaneously tested formaldehyde patch test preparations in the baseline series. <sup>4</sup>Irritant reaction. <sup>5</sup>2 patients reacted to 1.0% without reacting to 2.0%. <sup>6</sup>Another 17 patients had doubtful reactions to 1.0%, but reacted positively to 2.0%

Twenty-eight positive reactions were found to the different formaldehyde releasers in our baseline series (Table 7). The association between contact allergy to formaldehyde, independent of patch test concentration, and all 3 formaldehyde releasers was statistically significant ( $p < 0.05$ , Fisher's two-sided exact test). No significant association was found between contact allergy to formaldehyde and to formaldehyde-based resins.



Table 7. Positive reactions to 15 µl formaldehyde 2.0% and 1.0% aqua (aq.) in 1397 patients with simultaneous contact allergy to formaldehyde releasers and formaldehyde-based resins.

	Positive reactions		Simultaneous reactions to formaldehyde			
	N <sup>1</sup>	% <sup>2</sup>	2.0%		1.0%	
			N <sup>3</sup>	% <sup>4</sup>	N <sup>3</sup>	% <sup>4</sup>
Formaldehyde releasers						
Quaternium-15, 1.0% pet.	10	0.7	9	90	7	70
Diazolidinyl urea, 2.0% aq.	10	0.7	5	50	4	40
Imidazolidinyl urea, 2.0% aq.	8	0.6	3	38	2	25
Formaldehyde-based resins						
4-tert-Butylphenol-formaldehyde resin, 1.0% pet.	13	0.9	0	0	0	0
Phenol-formaldehyde resin, 1.0% pet.	16	1.1	0	0	0	0
Tosylamide-formaldehyde resin, 10.0% pet.	2	0.1	1	50	0	0

<sup>1</sup>Total number of positive reactions. <sup>2</sup>Proportion (%) of positive reactions among all those tested. <sup>3</sup>Number of simultaneous positive reactions to a formaldehyde releaser or a formaldehyde-based resin. <sup>4</sup>Proportion (%) of formaldehyde-allergic patients among those positive to a formaldehyde releaser or a formaldehyde-based resin.

## 4.2 Results of the ROAT studies

### 4.2.1 ROAT on healthy skin

Nine of the 17 formaldehyde-allergic individuals completing the study showed an allergic reaction to the moisturiser that contained formaldehyde (Figure 9), while none of these 17 reacted to the control moisturiser ( $p < 0.001$ , McNemar's test). No allergic or other reactions to either of the moisturisers were observed in the control group. The difference between the formaldehyde-allergic individuals and control group was statistically significant ( $p < 0.001$ , Fisher's two-sided exact test).



Figure 9. Positive reaction to the moisturiser containing formaldehyde in the ROAT study on healthy skin.

The results of the patch tests using formaldehyde 2.0%, the number of applications, and the results of the ROAT are given in Table 8. No association was found between the patch test reactivity and reactivity in the ROAT. Among the 5 individuals showing 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 individuals before developing a positive reaction in the ROAT. The mean number of applications before a positive ROAT reaction was recorded was 37, range 9 - 6.

Table 8. Results of the Repeated open application test (ROAT) in 17 patients (nos 1 - 17) showing a positive (Pos.) reaction in patch testing to 2.0% (0.60 mg/cm<sup>2</sup>) formaldehyde, but not to 1.0%, and 19 controls (nos 18 - 36) without contact allergy to formaldehyde.

Patient no.	Sex	Age (years)	Previous patch test reaction to formaldehyde 2.0% aq.	Outcome of ROAT	No. of applications <sup>1</sup>	Atopic dermatitis <sup>2</sup>
1	F	23	++	Pos. moderate	13	No
2	M	52	+	Pos. strong	55	No
3	F	33	+	Pos. weak	29	Yes
4	F	43	+	Pos. moderate	29	No
5	M	60	++	Pos. strong	9	No
6	F	56	+	Pos. weak	45	No
7	M	41	+	Pos. moderate	40	No
8	F	42	+	Pos. strong	56	No
9	F	32	+	Pos. weak	56	No
10	F	61	+	Neg.	56	No
11	M	60	++	Neg.	56	No
12	F	24	+	Neg.	56	Yes
13	F	37	++	Neg.	56	No
14	F	38	++	Neg.	56	Yes
15	F	64	+	Neg.	56	No
16	F	29	+	Neg.	56	No
17	F	53	+	Neg.	56	Unknown
18-36 <sup>3</sup>	7 M, 12 F	18 - 66	-	Neg.	56	Various

F, female; M, male; neg., negative. Weak positive reaction (+): erythema and infiltration covering at least 25% of area. Moderate positive reaction (++) : erythema and infiltration, more than 10 papules on the area. Strong positive reaction (+++) : erythema, infiltration, papules and/or vesicles. <sup>1</sup>Until a positive reaction to the ROAT appeared or the study was terminated. <sup>2</sup>Debut of dermatitis on body and eyelids at the age of 16 years or younger. <sup>3</sup>Matched controls (dermatitis patients consecutively patch-tested at our department) without contact allergy to formaldehyde, formaldehyde releasers or parabens.

#### 4.2.2 ROAT on experimental dermatitis

##### *Patch test results*

Table 9 presents the results of the patch test. In the test group (individuals who reacted positively to formaldehyde 1.0% and/or 2.0% aqua in our baseline series between January 2006 and December 2011), 9/15 individuals showed a positive reaction to at least one concentration in the dilution series of formaldehyde. The 3 individuals with contact allergy to DMDM hydantoin showed +++ reactions to formaldehyde 2.0%. Six of the 9 ROAT-positive individuals showed no reaction to DMDM hydantoin at patch testing, and 2 of the 9 ROAT-positive individuals showed no reaction to formaldehyde at patch testing. One individual showed a + reaction to moisturiser H and a doubtful reaction to moisturiser M. This individual showed no reaction in the ROAT. No positive patch test reactions were observed in the control group.

Table 9. Patch test reactions in the dilution series of formaldehyde (F), DMDM hydantoin (DMDMh), DM hydantoin (DMh), and Repeated Open Application Test (ROAT) moisturisers containing high (H), medium (M), and low (L) levels of F and the control (0) moisturiser in individuals with (nos 1 - 15) and without (nos 16 - 27) hypersensitivity to F. Outcome of ROAT with moisturisers H, M, L and 0.

Substance	Conc. %	Vehicle	Individual no.															
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16 - 27
F	2.0	Aq.	+++1	+++	+++	+++	++(+)	+	+++	-	-	+	+	(+)	(+)	-	-	-
			+++	+	+++	+	+++	-	-	-	-	-	-	-	-	-	-	-
	0.63		+++	++	+++	+++	-	-	++	-	-	-	-	-	-	-	-	-
			++	-	-	++	-	-	-	-	-	-	-	-	-	-	-	-
	0.063 - 0.0002		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Previous reaction <sup>1</sup> in patch test	Year		2009	2007	2006	2009	2009	2009	2009	2009	2011	2008	2010	2007	2010	2008	2006-2011	
	2.0		++	+++	+++	++	+++	++	++	+	+	++	++	++	++	++	-	
	1.0		-	++	++	-	+++	-	++	-	+	+	+	+	+	+	-	
DMDMh	2.0		(+)	-	-	(+)	-	-	++	-	-	-	-	-	-	-	-	-
	0.63 - 0.00063		-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-
DMDMh	2.0		++	(+)	-	-	-	-	(+)	-	-	-	-	-	-	-	-	-
	1.36 - 0.02		-	-	-	3	-	-	-	-	-	-	-	-	-	-	-	-
DMh	1.36		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	As is		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
ROAT moisturisers H, M, L, 0																		
			Pos. H M L	Pos. H M L	Pos. H M(L)	Pos. H M(L)	Pos. H M(L)	Pos. H M	Pos. H(M)	Pos. H	Pos. H (M)	Pos. H (H)	Pos. H (M)	Pos. H (H)	-	-	-	-
Outcome of ROAT																		

Aq, aqua; pet, petrolatum; veh, vehicle; (+), doubtful reaction; pos, positive. <sup>1</sup>The strongest recorded reaction on D3/4 and/or D7 is given. <sup>2</sup>The patient had doubtful reactions to DMDMh 0.063 and 0.00063. <sup>3</sup>The patient had a doubtful reaction to DMDMh 0.63 pet.

### *ROAT results*

All positive reactions to the ROAT involved a significant deterioration of the initial SLS-induced dermatitis, i.e. the SLS induced dermatitis did not heal, but was exacerbated, leading to the development of papules and vesicles. Table 10 lists the outcome of the ROATs in all 27 individuals. Figure 10 shows the positive ROAT reaction to moisturisers H and M in individual no. 2. The difference between the formaldehyde-allergic individuals and the controls was statistically significant for moisturisers H and M ( $p=0.0011$  and  $p=0.020$ , respectively, Fisher's two-sided exact test). However, no significant difference was found for moisturiser L ( $p > 0.3$ ). No differences in the healing time of the SLS dermatitis were seen between individuals with and without contact allergy to formaldehyde when using the moisturiser without formaldehyde (Table 10).



Figure 10. Positive reaction to the Repeated open application tests using moisturisers containing a high (left) and medium (right) levels of formaldehyde, after 7 days, in individual no. 2.

Table 10. Results of the Repeated open application tests (ROATs) in individuals with (nos 1-15) and without (nos 16-27) contact allergy to formaldehyde (F) using moisturisers containing high (H), medium (M) and low (L) levels of F, and a control moisturiser (0) on experimental SLS dermatitis

Individual no.	Atopic dermatitis	F level in moisturiser	No. of days of treatment	No. of days until dermatitis healed	Outcome of ROAT
1 <sup>1</sup>	Yes	H	14		Pos.
		M	7		Pos.
		L	14		Pos.
		0			
2	Unknown	H	7	21	Pos.
		M	7		Pos.
		L	21		Pos.
		0	28		Neg.
3	No	H	4	14	Pos.
		M	4		Pos.
		L	28		(Pos). <sup>2</sup>
		0	28		Neg.
4	No	H	4	7	Pos.
		M	4		Pos.
		L	28		(Pos). <sup>2</sup>
		0	28		Neg.
5	Yes	H	7	4	Pos.
		M	14		Pos.
		L	28		Neg.
		0	28		Neg.
6	Yes	H	28	4	Pos.
		M	28		Pos.
		L	28		Neg.
		0	28		Neg.
7	Yes	H	21	14	Pos.
		M	28		(Pos). <sup>2</sup>
		L	28		Neg.
		0	28		Neg.
8	Yes	H	21	21	Pos.
		M	28		Neg.
		L	28		Neg.
		0	28		Neg.
9	No	H	21	4	Pos.
		M	28		(Pos). <sup>2</sup>
		L	28		Neg.
		0	28		Neg.
10	Yes	H	28	14	(Pos). <sup>2</sup>
		M	28		Neg.
		L	28		Neg.
		0	28		Neg.
11	No	H	28	21	(Pos). <sup>2</sup>
		M	28		Neg.
		L	28		Neg.
		0	28		Neg.
12	Yes	H	28	14	Neg.
		M	28		Neg.
		L	28		Neg.
		0	28		Neg.
13	Unknown	H	28	7	Neg.
		M	28		Neg.
		L	28		Neg.
		0	28		Neg.
14	Unknown	H	28	4	Neg.
		M	28		Neg.
		L	28		Neg.
		0	28		Neg.
15	Yes	H	28	4	Neg.
		M	28		Neg.
		L	28		Neg.
		0	28		Neg.
6 controls	Yes	H, M, L, 0	28	4 - 21	Neg.
6 controls	No	H, M, L, 0	28	7 - 28	Neg.

<sup>1</sup>The patient misunderstood the instructions and suffered extremely strong reactions and was treated with 30 mg prednisolone after 14 days. <sup>2</sup>Doubtful reaction, the dermatitis was not healed, but did not fulfil the criteria for a positive reaction.

In the group with formaldehyde allergy, a dose-response relationship was seen concerning the number of positive ROAT reactions to the 4 moisturisers with different concentrations of formaldehyde ( $p < 0.001$ ), as shown in Figure 11. Two of the 9 ROAT-positive individuals reacted to all 3 formaldehyde-releasing moisturisers, 4/9 reacted to moisturisers H and M, and 3/9 reacted only to moisturiser H, also shown in Figure 11.

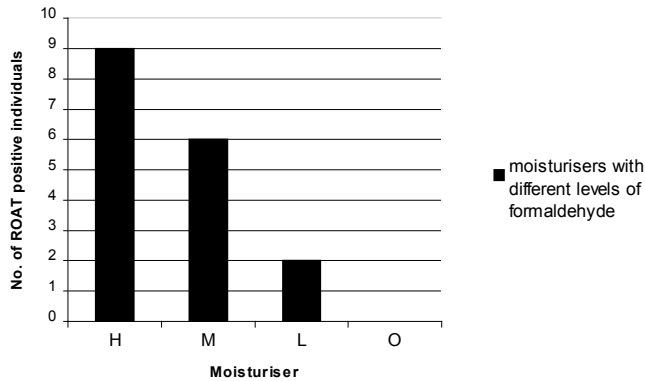


Figure 11. The number of patients showing positive reactions to the ROAT using moisturisers containing high (H), medium (M) or low (L) levels of formaldehyde and the control (O) moisturiser among the 15 formaldehyde-allergic individuals.

All individuals showing a ++ and or a +++ patch test reactivity for formaldehyde reacted positively in the ROAT, as can be seen in Figures 12 and 13.

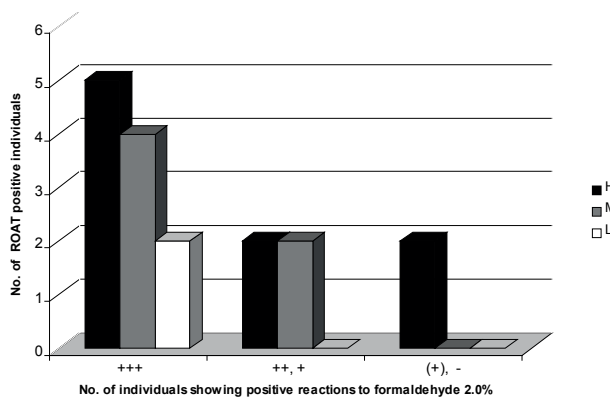


Figure 12. Relationship between the patch test reactivity to formaldehyde 2.0% and positive reactions to ROAT using moisturisers containing high (H), medium (M) or low (L) levels of formaldehyde in the group of 15 formaldehyde-allergic individuals.

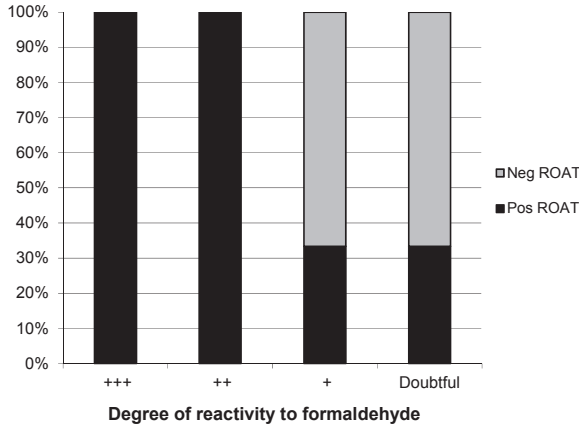


Figure 13. Relationship between the patch test reactivity to formaldehyde 2.0% and reactions in ROAT.

A significant association was also found between the intensity of the patch test reaction to formaldehyde 2.0% (+++; ++ and +; doubtful and negative) and the number of positive ROAT reactors to moisturiser H ( $p=0.04$ , Mann-Whitney test).

Within the group of formaldehyde-allergic individuals, 9/15 reacted to moisturiser H, while none reacted to the control moisturiser ( $p<0.001$ ). The corresponding comparisons for moisturisers M and L yielded  $p$ -values of 0.031 and  $>0.3$ , respectively. No reactions to the control moisturiser were recorded in either of the groups.

Three individuals with contact allergy to DMDM hydantoin had simultaneous +++ reactions to formaldehyde 2.0%, leading to a statistically significant difference between those with a +++ reaction and those with a lower degree of reactivity in the group of formaldehyde-allergics (3 of 5 versus 0 of 10;  $p=0.022$ , Fisher's two-sided exact test).

### 4.3 Demonstration of formaldehyde in skin care products

During a 4-month period, 154 consecutively tested patients (37 males and 117 females) brought 996 skin care products and cosmetics to the clinicure. In this study (Paper IV), 287 products were investigated using the chromotropic acid method. In total, 151 "leave-on" and 136 "rinse-off" products were investigated. The results of the chromotropic acid analyses are summarised in Table 11. Formaldehyde was found in 58 of the 287 products (20.5%): 26/151 (17.2%) of the "leave-on" products contained formaldehyde, and neither formaldehyde nor formaldehyde



releasers were declared in the labelling of 17/26 (65.4%) of these. Among the “rinse-off” products 32/136 (23.5%) contained formaldehyde, and 9/32 (28.0%) of these were not labelled as containing formaldehyde or formaldehyde-releasing preservatives. The overall percentage of discoloured products was 16.4%, and there was no significant difference between the products brought in by the test group and the control group (18.5% in the test group and 15.5% in the control group). Discolouration can occur when other ketones apart from formaldehyde are present in the product. In these cases, the chromotropic acid method can neither confirm nor rule out the presence of formaldehyde. To do this, more advanced methods, such as HPLC, are needed. However, no HPLC analysis was performed in this study, and the discoloured products were evaluated as not containing formaldehyde.

The difference between the number of formaldehyde-allergic individuals using “leave-on” products with >40 ppm formaldehyde and the number of controls using such products was statistically significant (5/10 versus 4/30,  $p=0.029$ , Fisher’s two-sided exact test). The number of products not declared to contain formaldehyde among the “leave-on” products and “rinse-off” products was also significantly significant (17/26, 65.4% vs. 9/32, 28.0%) ( $p=0.0013$ , Fisher’s two-sided exact test). There was no statistical difference between the number of products containing formaldehyde in the “leave-on” (17/151) and “rinse-off” (9/136) products, independent of group.

Table 11. Occurrence and amount of formaldehyde (F) determined using the chromotropic acid method in “leave-on” (L) and “rinse-off” (R) products brought in by 40 patch-tested dermatitis patients (10 individuals allergic to F and formaldehyde releasers (FR) in our baseline series, and 30 matched control individuals negative to F and FR).

No.	Age	No. of prod./L prod	Reactivity to F <sup>1</sup>	Reactivity to FR <sup>1</sup>				Concentration of F in products (ppm)											
				D	DU	Q15	IU	2.5		10.0		20.0		≥40.0					
								L	R	L	R	L	R	L	R				
Test group																			
1	45	13/5	++	+	++	+	-	-	-	-	-	-	-	-	-	-			
2	46	9/3	++	-	-	-	-	-	-	-	-	-	-	-	-	-			
3	29	8/6	+	-	++	-	-	-	-	-	-	-	-	-	-	-			
4	42	6/3	++	-	-	-	-	-	-	-	-	-	-	-	-	-			
5	55	12/7	+++	+++	-	-	-	-	-	-	-	-	-	-	-	-			
6	54	4/0	++	-	++	-	-	-	-	-	-	-	-	-	-	-			
7	18	3/0	+	-	-	-	-	-	-	-	-	-	-	-	-	-			
8	14	9/7	+	-	-	-	-	-	-	-	-	-	-	-	-	-			
9	36	9/8	+++	+	++	-	-	-	-	-	-	-	-	-	-	-			
10	33	8/5	+++	-	++	-	-	-	-	-	-	-	-	-	-	-			
Control group																			
11	46	8/4	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
12	48	8/5	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
13	42	5/5	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
14	46	6/3	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
15	45	8/4	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
16	50	7/4	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
17	32	8/4	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
18	26	5/1	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
19	30	6/4	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
20	45	4/2	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
21	45	12/8	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
22	44	16/6	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
23	55	7/2	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
24	56	4/2	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
25	56	3/0	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
26	57	6/3	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
27	49	3/3	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
28	48	4/4	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
29	19	11/8	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
30	20	7/2	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
31	23	7/5	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
32	16	4/2	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
33	21	14/4	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
34	22	7/4	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
35	33	11/2	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
36	35	5/3	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
37	35	4/4	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
38	39	3/2	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
39	30	7/3	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
40	31	6/4	-	-	-	-	-	-	-	-	-	-	-	-	-	-			

D, DMDM hydantoin; DU, diazolidinyl urea; IU, imidazolidinyl urea; Q15, quaternium-15; B, bronopol; <sup>1</sup>The strongest recorded reaction on D3/4 or D7 is given; <sup>2</sup>No F or FR was declared in the labelling of the product. <sup>3</sup>The product was not labelled.

Figure 14 shows the number of products with and without formaldehyde and the distribution between “leave-on” and “rinse-off” products containing formaldehyde. There was no statistically significant difference ( $p=0.148$ , Mann-Whitney two-sided test) between the number of products brought in by the formaldehyde-allergic individuals (3-13, mean 8.1) and the controls (3-16, mean 6.9).

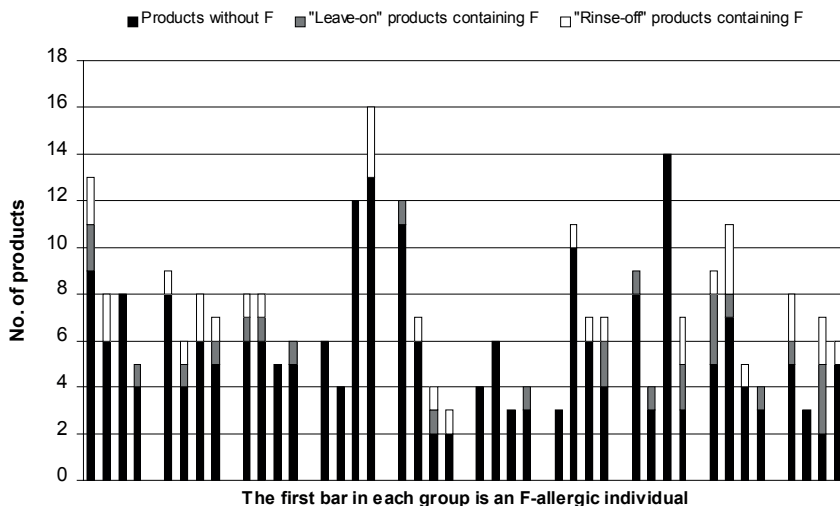


Figure 14. Number of products with and without formaldehyde (F) brought in by the 10 formaldehyde-allergic individuals and 30 age- and gender-matched controls without contact allergy to F.

The formaldehyde releasers found on the labelling of the investigated skin care products are also given in Table 11. DMDM hydantoin was the most common formaldehyde releaser declared in the products (20/30; 67%). Imidazolidinyl urea was declared in 7/30 (23%) and quaternium-15 in 3/30 (10%) products. Five of 30 products (17%) contained both DMDM hydantoin and imidazolidinyl urea, according to the labelling.

# 5. GENERAL DISCUSSION

## 5.1 Patch testing with formaldehyde 1.0% and 2.0%

### 5.1.1 Contact allergy to formaldehyde

The elicitation of a positive patch test reaction depends, among other things, on the vehicle used and the allergen dose, which is determined by the concentration and volume/amount of test preparation applied per unit area (27). There is a risk of false-negative patch test reactions if the dose is too low, while too high a dose can cause irritant reactions or induce patch test sensitisation. Doubtful reactions may be the expression of both irritant reactions and weak contact-allergic reactions. Substances that may cause irritant reactions must be carefully evaluated.

Formaldehyde has been regarded as a problematic patch test substance. Patch testing with formaldehyde started in 1929 with concentrations in the range 1 - 5% aqua. The high prevalence of contact allergy and poor reproducibility of allergic reactions to formaldehyde led to the suspicion that irritant reactions were being misinterpreted as positive contact-allergic reactions (45). The recommended standard patch test concentration was thus reduced from 4% to 2% in the 1980s. When many clinics changed from using the AI-test to Finn Chambers, the ICDRG anticipated an increase in irritant reactions to formaldehyde 2% and, therefore, reduced the recommended patch test concentration to 1% (43). At our department, patch testing with formaldehyde 2.0% has been used for several decades in cases of doubtful reactions to 1.0%.

To establish contact allergy to formaldehyde, it is important to use a defined dose per unit area. Different patch test techniques are used for patch testing using solutions. It is common practice in many clinics use the so-called drop technique, in which the solution is applied by squeezing the plastic bottle containing the solution until a drop is released. A study performed at our department showed that the amount released, and thus the dose, could vary by a factor of 4 when using this technique (26). The same study showed a considerably smaller variation in dose when the micropipette technique was used. The micropipette technique has been

used routinely at our department for patch testing using solutions since the 1980s (79). According to our findings, 15  $\mu\text{l}$  is the optimal volume for Finn Chambers 8 mm in diameter (80).

The comparative study described in Paper I shows that patch testing with 15  $\mu\text{l}$  2.0% formaldehyde (w/v) using a micropipette detects twice as many reacting individuals as 1.0%, without a high frequency of irritant reactions. To the best of our knowledge, there is only one previously published study in which simultaneous testing with 1.0% and 2.0% formaldehyde has been compared (43). No statistically significant difference was found in that study between these two concentrations. The same patch test system as in the present study was used, but neither the amount of solution nor the technique used to apply the solution were stated. We assume that the micropipette technique was not used. In the present study, the number of irritant reactions was low: 0.4% and 0.1% to formaldehyde 2.0% and 1.0% respectively. The corresponding values in the study mentioned above were 3.9% and 2.1% (43). The difference in the results could be explained by differences in the application of the substance, leading to different doses. The high number of irritant reactions to formaldehyde 3.0% aqua (0.90 mg/cm<sup>2</sup>) (29.6%) reported previously (81) indicates that the test concentration should not be increased above that presently recommended, i.e. 2.0% (0.60 mg/cm<sup>2</sup>). There were more doubtful reactions to 1.0% formaldehyde than to 2.0% (31/38 and 48/66, respectively). However, 17/38 (45%) of the patients showing doubtful reactions to 1.0% showed positive reactions to 2.0% formaldehyde, supporting the hypothesis that formaldehyde 1.0% aqua (0.30 mg/cm<sup>2</sup>) may be too low concentration for patch testing.

The results of this comparative study (Paper I) show that patch testing with 15  $\mu\text{l}$  formaldehyde 2.0% aqua (0.60 mg/cm<sup>2</sup>) detects significantly more reacting individuals than 1.0% aqua (0.30 mg/cm<sup>2</sup>). When micropipettes are used, 2.0% does not lead to a high frequency of irritant reactions.

### 5.1.2 Contact allergy to formaldehyde releasers

The use of free formaldehyde has decreased since the 1960s, and formaldehyde-releasing preservatives are now used instead. The prevalence of contact allergy to these preservatives is slightly lower than that of formaldehyde. (82). These preservatives have the ability to replace utilized formaldehyde by releasing very small amounts over time. When the released formaldehyde has been consumed, the formaldehyde releaser supplies more. In this way, the level of free formaldehyde in the product remains low, but sufficient to prevent bacterial growth. The amount of formaldehyde released depends on the preservative used and its concentration, and the pH, temperature and amount of water in the product. These substances have antibacterial and antifungal properties in addition to their formaldehyde-releasing action (83, 84). Contact allergy to formaldehyde releasers may be due to the

preservative itself or to the formaldehyde they release. It has been shown that there is a clear relationship between positive patch test reactions to formaldehyde releasers and formaldehyde contact allergy (85). Individuals allergic to formaldehyde-releasing preservatives are often allergic to formaldehyde, but most formaldehyde-allergic individuals do not react to formaldehyde-releasing preservatives. Individuals allergic to formaldehyde are recommended to avoid all formaldehyde-releasing preservatives. The relationship between contact allergy to formaldehyde and to formaldehyde releasers has been reviewed extensively (10, 13, 14, 53, 82, 85-90).

The results of patch tests using the baseline series were evaluated regarding concomitant contact allergy to formaldehyde 1.0% and 2.0%, formaldehyde releasers and formaldehyde-based resins (Paper I). The prevalence of formaldehyde allergy in patients showing positive responses to formaldehyde-releasing preservatives showed considerable overlap, as has been reported in other studies (52, 82). More simultaneous reactions to formaldehyde releasers were found with 2.0% formaldehyde than with 1.0% formaldehyde, but the number of patients was too small to draw any definite conclusions. The results showed a similar distribution of concomitant contact allergy between formaldehyde 1.0% and 2.0% and quaternium-15 (70-90%) to that in other studies (52, 82, 86). Concerning simultaneous reactions to formaldehyde 1.0% and 2.0% in patients allergic to diazolidinyl urea and/or imidazolidinyl urea, our results (25-50%) were higher than in some previous reports but lower than others (52, 82, 86, 91).

The ESCD recommends a sensitiser be included in the baseline series when the contact allergy rates in a baseline series exceed 0.5 - 1.0% (27). Quaternium-15 is currently the only formaldehyde releaser included in the European baseline series for patch testing (92). Considering the fact that individuals allergic to quaternium-15 have a higher prevalence of contact allergy to formaldehyde than other individuals allergic to formaldehyde-releasing preservatives, the presence of quaternium-15 in the baseline series should be re-evaluated.

It has been shown that formaldehyde allergy in patients allergic to formaldehyde releasers is positively associated with the amount of formaldehyde released from formaldehyde-releasing preservatives (86). Quaternium-15, diazolidinyl urea and DMDM hydantoin release high amounts of free formaldehyde, which explains the high prevalence of concomitant patch test reactions to formaldehyde (93). Imidazolidinyl urea releases less, and bronopol the lowest amount of formaldehyde among the formaldehyde releasers discussed in this thesis, and concomitant patch test reactions to formaldehyde and imidazolidinyl urea are, therefore, generally lower (82). The results obtained in the present work regarding concomitant patch test reactivity to different formaldehyde releasers and formaldehyde at our department during 2002-2013 is shown in Figure 15 and generally confirm these findings.

The formaldehyde content in patch test preparations was studied by Emeis et al.

using  $^{13}\text{C}$  nuclear magnetic resonance spectroscopy, and they found that most aqueous solutions of formaldehyde releasers contained free formaldehyde (94). Patch test preparations in petrolatum do not contain free formaldehyde, however, formaldehyde release starts upon contact with water, i.e. in the patch test situation. Some allergens such as DMDM hydantoin, imidazolidinyl urea and diazolidinyl urea, have been shown to release even more formaldehyde in petrolatum than in aqueous solutions, and more allergies are detected when using petrolatum as the vehicle than when using water (91). However, when free formaldehyde was analysed in patch test preparations used at our department, using the chromotropic acid method, patch test preparations in petrolatum were found to release considerably lower amounts of formaldehyde than aqueous preparations.

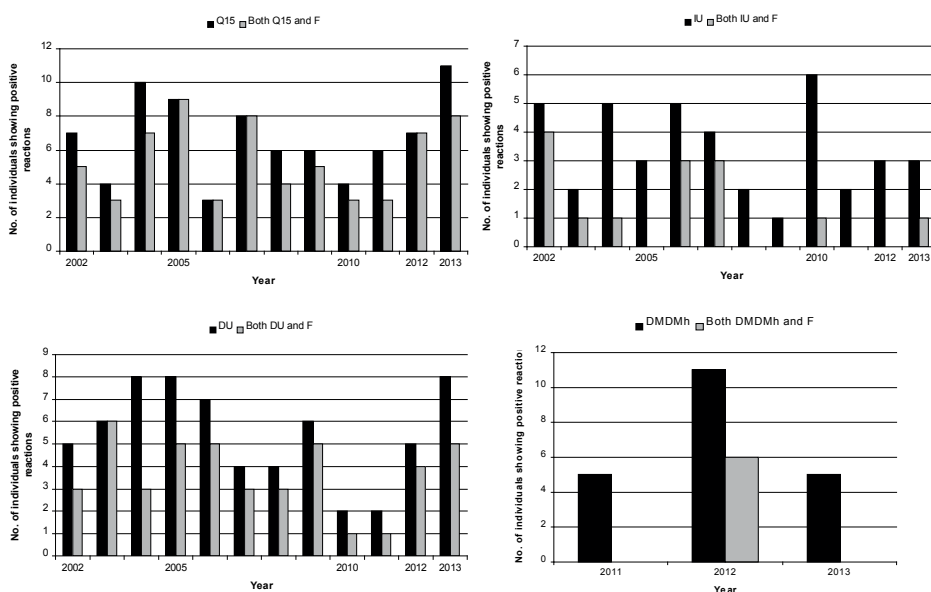


Figure 15. Number of individuals showing contact allergy to quaternium-15 (Q15), diazolidinyl urea (DU), imidazolidinyl urea (IU), DMDM hydantoin (DMDMh) and formaldehyde (F) in the baseline patch test series among consecutively patch-tested dermatitis patients from 2002 to 2013 at the Department of Occupational and Environmental Dermatology, Malmö University Hospital, Malmö, Sweden. Both 1.0% and 2.0% formaldehyde have been included in our baseline patch test series since 2006.

Most preservatives releasing formaldehyde are tested in aqueous solutions. It has been discussed whether petrolatum or water is the more sensitive vehicle for the detection of contact allergy to these substances. The North American Contact Dermatitis Group has patch-tested with different formaldehyde releasers in both vehicles for more than 10 years. Initially, they found that the number of patients reacting to

imidazolidinyl urea was approximately the same with both vehicles, but that each vehicle formulation missed about half of the cases that the other vehicle formulation detected (95). However, retrospective analysis of patch testing with imidazolidinyl urea, diazolidinyl urea and DMDM hydantoin in water and petrolatum (91) showed that significantly more cases were identified when petrolatum was used as the vehicle. Simultaneous patch testing with formaldehyde-releasing preservatives in petrolatum and water in the baseline series at our department during 2009-2013 showed that different vehicle formulations complement each other and that the question of which vehicle detects more contact allergies is still open to debate (Figure 16).

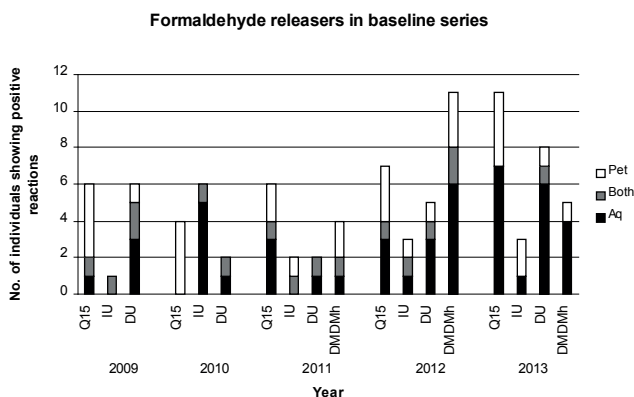


Figure 16. Number of individuals with contact allergy to quaternium-15 (Q15), imidazolidinyl urea (IU), diazolidinyl urea (DU) and DMDM hydantoin (DMDMh) in aqua (aq.) and petrolatum (pet.) in the baseline patch test series among consecutively patch-tested dermatitis patients at the Department of Occupational and Environmental Dermatology, Malmö University Hospital, Malmö, Sweden, 2009-2013.

## 5.2 ROAT studies

Use tests have been developed to evaluate the clinical significance of patch test results. The ROAT has been used since 1986 (96) with different allergens (97), in different anatomical regions (98, 99) and on normal and damaged skin (100). The clinical relevance of formaldehyde, formaldehyde releasers and other preservatives has been studied previously using the ROAT (74, 98, 101-103). In individual cases, a positive reaction to the ROAT shows that the tested product can cause eczema, but provides no information on the nature of the reaction, i.e. the eczematous response may be allergic or irritant, although the latter is rare in skin care products used by a large number of consumers. When the ROAT is performed under controlled



conditions using many patients sensitised to the allergen in question and control patients not sensitised to the particular allergen, a positive outcome provides much more information on the clinical relevance of patch test results and the nature of the ROAT reactions (74).

### 5.2.1 ROAT on healthy skin

The main conclusion presented in Paper I is that patch testing with 15 µl formaldehyde 2.0% in aqua (0.60 mg/cm<sup>2</sup>) detects significantly more reacting individuals than 1.0% aqua (0.30 mg/cm<sup>2</sup>). When micropipettes are used, patch testing with 2.0% does not lead to a high frequency of irritant reactions. The results presented in this study (Paper II) showed that contact allergic reactions to formaldehyde 2.0% (0.60 mg/cm<sup>2</sup>) but not to 1.0% (0.30 mg/cm<sup>2</sup>) in the same individual can be clinically relevant.

To optimise the ROAT, it was performed with the highest concentration of free formaldehyde allowed in cosmetic products according to the EU Cosmetics Directive (2000 ppm) (15). The responses to the ROAT were positive despite the fact that most of the ROAT-positive individuals showed weak reactions to the patch test with 2.0% formaldehyde (7/9 had a + reaction and 2/9 a ++ reaction). No association was found between the patch test reactivity and the number of applications needed to elicit a positive ROAT reaction in this limited study. The ROAT reaction was graded as mild, moderate or strong, but theoretically mild reactions could have become moderate or strong if the applications had been continued. For 3/9 patients, 3 weeks or more was needed to elicit a positive ROAT reaction, showing that ROAT studies should be performed at least for 3-4 weeks.

Higher concentrations than those used in skin care products are usually required to demonstrate a contact allergy to most preservatives. It has been shown that the dose per unit area, per application, required to elicit a positive reaction in the ROAT is lower than the dose per unit area required to elicit a positive reaction in the patch test (104). If the formaldehyde-containing moisturiser used in the ROATs in the present study (Paper II) had been tested *as is* in Finn Chambers, the calculated dose of formaldehyde per unit area would have been 0.06 mg/cm<sup>2</sup>. This is ten times lower than the dose per unit area in the patch test using formaldehyde 2.0% (0.60 mg/cm<sup>2</sup>), and patch testing with moisturisers containing this concentration of formaldehyde would probably give a negative result. This, in combination with the fact that for 5/9 patients 3 weeks or more were needed to elicit a positive ROAT reaction is very important from the clinical point of view.

In summary, this study shows that individuals, who react to 2.0% formaldehyde but not to 1.0%, have a significant risk of developing an eczematous reaction on healthy skin when exposed to a moisturiser containing formaldehyde in accordance

with the EU Cosmetics Directive. These patients would not have been properly diagnosed with the patch test concentration that was recommended and used when this research project was initiated (1.0%), and their skin disease would probably have been misdiagnosed as irritant contact dermatitis or endogenous dermatitis instead of allergic contact dermatitis. Based on the results of this ROAT study and other comparative studies (105, 106), the ESCD and the ECDRG have recommended that the patch test concentration of formaldehyde in the European baseline series be changed from 1.0% (0.30 mg/cm<sup>2</sup>) to 2.0% (0.60 mg/cm<sup>2</sup>) (81). It is also recommended that the patch technique be optimized by routinely using micropipettes to apply the formaldehyde solution to the patch test chamber.

### 5.2.2 ROAT on experimental dermatitis

In the ROAT study on healthy skin discussed above (Paper II), it was shown that the presently allowed concentration of formaldehyde in cosmetics and household products in the EU and the USA can induce allergic contact dermatitis on healthy skin in individuals with contact allergy to formaldehyde. The EU Cosmetic Directive states that formaldehyde-releasing preservatives must be declared in the labelling on cosmetics and household products, and that products must be labelled with the warning “Contains formaldehyde” when the concentration of free formaldehyde is > 500 ppm (15). Free formaldehyde is no longer used in skin care products. Formaldehyde-allergic individuals are advised to avoid products containing formaldehyde releasers, as stated in product labelling and material safety data sheets. When the skin care products of formaldehyde-allergic patients attending our department are routinely analysed with the chromotropic acid method, formaldehyde is often found at concentrations around 2.5 - 40 ppm (107). Approximately 50% of these products include information in the labelling that they contain formaldehyde-releasing preservatives. Undeclared formaldehyde may be present in finished products at low concentrations due to the release from chemicals such as emulsifiers and surfactants (108). Another source of formaldehyde “contamination” may be the material used for packaging (e.g., melamine- or carbamide-formaldehyde resin) (109). The question is, whether the exposure to these low levels is clinically relevant in formaldehyde-allergic individuals? It has been shown that levels of 200 - 300 ppm of free formaldehyde in cosmetic products are capable of inducing dermatitis in normal skin (10). Exposure to products with formaldehyde at concentrations around 2.5 - 40 ppm may be not relevant on healthy skin. However, many formaldehyde-allergic individuals have dermatitis and a damaged skin barrier, and the daily application of products containing low concentrations of formaldehyde may exacerbate their condition or prevent the healing of existing dermatitis. To the best of our knowledge, no studies on the effects of exposure of damaged skin to low concentrations of formaldehyde have been published previously.

The result of applying a moisturiser to skin already affected by irritant dermatitis may vary. In individuals who are not allergic to any of the substances in the moisturiser the eczema may heal; the healing time varying between individuals. In individuals allergic to a substance in the moisturiser, the eczema may heal but the healing time may be prolonged, the eczema may become worse, or it may heal and then reappear, as illustrated in Figure 7. In the study on the exposure of experimental dermatitis (Paper III), all the patients showing positive reactions to the ROAT exhibited exacerbation of the initial SLS dermatitis.

In several cases (4/9), reactions appeared after 3 weeks, similar to the results of the ROAT on healthy skin (Paper II). This has already been pointed out (110, 111), and is very important from a clinical point of view, since the physician or the patient does not usually associate the appearance of dermatitis with a product that the patient has been using for a relatively long period. The study was terminated after 28 days, and it is possible that at least 6 positive reactions in the ROAT would have appeared if application had been continued for a longer period.

A model has been developed for the investigation of the time- and dose-response relationship in the elicitation of allergic contact dermatitis (104), showing a relationship for non-volatile compounds (nickel and methylidibromo glutaronitrile), but it was noticed that for volatile compounds the ROAT could be influenced by evaporation. Formaldehyde is volatile and, therefore, the dose per unit area is probably lower than the intended dose, which may affect the result of similar studies. In the present ROAT study (Paper III), all the patients were patch-tested with serial dilutions of formaldehyde before the study to determine their current patch test reactivity. As expected, there was an individual variation in patch test reactivity to formaldehyde. The threshold concentration for formaldehyde in two individuals was 0.2% aqua, and both showed a positive ROAT reaction to moisturisers H and M within a week, demonstrating a time and dose-response relationship. This relationship has also been demonstrated in other ROAT studies with volatile compounds performed at our department (99, 112). Furthermore, a dose-response relationship was found in the group with formaldehyde allergy concerning the number of positive ROAT reactions to the 4 moisturisers with different concentrations of formaldehyde.

It has been found that strong patch test reactions to formaldehyde are correlated with positive reactions to the formaldehyde releaser quaternium-15 in women (113). Interestingly, in the present study, the 3 individuals with contact allergy to DMDM hydantoin, independent of vehicle, also showed a simultaneous +++ reaction to formaldehyde 2.0%, which indicates that the contact allergy to DMDM hydantoin was directed towards formaldehyde, and that the formaldehyde released by DMDM hydantoin was too low to elicit a positive patch test reaction in others than those showing a +++ reaction.

Different methods can be used to provoke experimental dermatitis (114). The reason for choosing SLS was that it can be used to provoke dermatitis in all individuals. SLS has often been used to provoke experimental dermatitis in clinical studies, and the recommended concentrations of SLS to elicit irritant dermatitis are 0.25% (0.0625 mg/cm<sup>2</sup>), 0.5% (0.125 mg/cm<sup>2</sup>), 1.0% (0.25 mg/cm<sup>2</sup>) and 2.0% (0.50 mg/cm<sup>2</sup>) (115). In our experience, 2.0% SLS is not always sufficient to provoke irritant dermatitis and a higher concentration of 3.0% (0.75 mg/cm<sup>2</sup>) was included. Individuals with atopic dermatitis have an altered skin barrier, and it has been suggested that they develop irritant contact dermatitis more easily than individuals with no history of atopic dermatitis (116). However, no such relationship was seen in the present study.

The results presented in Paper III demonstrate that exposure to a low concentration of formaldehyde is sufficient to exacerbate existing dermatitis. Since such low levels are not declared in the product labelling or material safety data sheets, chemical analysis is necessary to ensure optimal management of formaldehyde-allergic patients, in order to allow healing of the dermatitis and to prevent the dermatitis from becoming chronic.

### 5.3 Demonstration of formaldehyde in skin care products

The results presented in Paper IV confirmed that formaldehyde-releasing preservatives are widely used in skin care products and cosmetics. Formaldehyde was found in 58/276 products (20.5%): in 26 “leave-on” products (17.2%) and in 32 “rinse-off” products (23.5%). No formaldehyde releasers were declared in the labelling of 17/26 (65.4%) “leave-on” or 9/32 “rinse-off” products (28.0%). Studies in Denmark and Sweden in the 1990s showed that formaldehyde was present in approximately 30% of cosmetic products (117, 118). Similar results were reported in the USA: approximately 20% of cosmetics and personal care products contained formaldehyde releasers: 17% “leave-on” products and 27% “rinse-off” products (88). Two unpublished studies on preservatives in skin care products and cosmetics in Israel and the United Arab Emirates reported even higher contents of formaldehyde in products, especially in “rinse-off” products (119, 120). In a pilot study at our department skin care products obtained from consecutively tested patients for a period of 3 months were analysed with the chromotropic acid spot test (145 “leave-on” products and 96 “rinse-off” products) (121). In the 241 products analysed, formaldehyde was found in 20 (13.8%) “leave-on” products and 23 (24.0%) “rinse-off” products (121).

Spot tests can be used to demonstrate both organic and inorganic compounds. Two spot tests are frequently used to detect formaldehyde in various types of products: the chromotropic acid method and the acetylacetone method (77). At our department we prefer to use the chromotropic acid method. The results of this spot test cannot always be evaluated due to discoloration, and HPLC method, based on derivatization with hydrazine, must be used (122). In this study, we decided to determine the formaldehyde release only with the chromotropic acid method, and not HPLC.

Formaldehyde-allergic individuals are advised to avoid products containing formaldehyde and formaldehyde releasers based on the information given in product labelling and material safety data sheets. However, undeclared formaldehyde may be present in the products due to addition of formaldehyde in the raw material or release from other chemicals. Surfactants, used as emulsifiers in cleaning products and toiletries, can produce formaldehyde due to auto-oxidation, and this process increases under certain conditions, e.g., heat and high relative humidity (108, 123). It has been shown that auto-oxidation of surfactants can produce levels of formaldehyde above threshold concentrations and possibly even above the limit requiring warnings on labels (500 ppm) (15). Another source of formaldehyde contamination may be material used in packaging such as melamine or carbamide-formaldehyde resin (109).

In the present study (Paper IV), 58/287 (20.5%) of the products contained formaldehyde, and 17/26 (65.4%) of the “leave-on” products and 9/32 of the “rinse-off” products (28.0%) had no declaration of formaldehyde releasers in the labelling. Interestingly, more “rinse-off” products were stated to contain a formaldehyde releaser than “leave-on” products (23/32 versus 7/26). In a pilot study conducted at our department, formaldehyde or formaldehyde releasers were not declared in the labelling of 14/20 (70%) of the “leave-on” products and 11/23 (47.8%) of the “rinse-off” products found to contain these substances (121).

Contact allergy to formaldehyde is almost impossible to suspect. Allergic contact dermatitis caused by formaldehyde can often be chronic since it is difficult to avoid formaldehyde-containing products. Exposure to low concentrations of formaldehyde around 10 - 20 ppm, such as those found in the products in the present work and other studies, may not be sufficient to induce sensitisation or to cause allergic contact dermatitis on intact skin in a formaldehyde-allergic individual, however, exposure to such products can maintain or aggravate existing dermatitis (124).

The results of the present investigation do not provide any conclusive information on whether dermatitis patients with formaldehyde allergy are more exposed to formaldehyde than those without formaldehyde allergy, although the allergic ones were more exposed to “leave-on” products releasing >40 ppm (5/10 versus 4/30). Knowledge on the relative importance of “leave-on” and “rinse-off” products,

exposure patterns, and the significance of aggregate exposure is still inadequate. Furthermore, possible occupational exposure to formaldehyde from other products or industrial processes was not considered in this study.

In summary, the study presented in Paper IV shows that formaldehyde releasers are widely used in skin care products and that the information provided on the packaging is often inadequate. To assess exposure and clinical relevance in formaldehyde-allergic individuals, their skin care products and occupational products to which they are exposed *not* stated to contain formaldehyde or formaldehyde-releasing preservatives should be analysed, especially “leave-on” products, as these remain on the skin for longer time.

## 6. SUMMARY AND CONCLUDING REMARKS

The aim of the work presented in this thesis was to improve the diagnosis of contact allergy and allergic contact dermatitis caused by formaldehyde. The most important findings are given below.

- Patch testing with formaldehyde 2.0% aqua (0.60 mg/cm<sup>2</sup>) detects significantly more contact allergies than 1.0% aqua (0.30 mg/cm<sup>2</sup>). When comparisons are made between the results obtained with different concentrations, it is important that the dose per unit area is standardised. Using micropipettes in the patch test technique with 2.0% aqua formaldehyde does not lead to a high frequency of irritant reactions.
- The results of performing the ROAT on healthy skin demonstrate that individuals who react to 2.0% formaldehyde but not to 1.0%, have a significant risk of exhibiting an eczematous reaction when exposed to a moisturiser containing levels of formaldehyde in accordance with the EU Cosmetics Directive (2000 ppm).
- Formaldehyde is a ubiquitous contact allergen, present in many of the products in daily use. Approximately 20% of skin care products contain formaldehyde. Formaldehyde-release corresponding to around 2.5 - 40 ppm is common in these products, despite the fact that no formaldehyde or formaldehyde-releasing preservatives are declared in the labelling.
- The results of the ROATs performed on experimental dermatitis demonstrate that exposure to moisturisers with formaldehyde concentrations of 2.5 - 40 ppm is sufficient to exacerbate existing dermatitis.
- In both ROAT studies, 3 weeks or more were needed to elicit a positive reaction in 50% of the individuals. This is a very important finding from the clinical point of view, since the physician or the patient does not normally associate dermatitis with a product that has been used for an extended period.

Further comparative studies have been performed to confirm the finding that formaldehyde 2.0% aqua (0.60 mg/cm<sup>2</sup>) should be used as the routine test concentration in baseline series (105, 106). Based on the results of these studies, and the ROATs on healthy skin presented in Paper II, the ESCD and EECDRG have recommended that the formaldehyde concentration in the European baseline series

should be increased to 2.0% (0.60 mg/cm<sup>2</sup>), and that 15µl of the solution should be administered using a micropipette (81).

Analyses of skin care products used by formaldehyde-allergic individuals have been routinely carried out at our department, using the chromotropic acid method, for several decades. However, to the best of our knowledge, such analyses are rarely performed at other clinics performing patch tests. Since exposure to low concentrations of formaldehyde has been shown to be clinically relevant, at least in patients with dermatitis, it is important that the patients' skin care products and sources of occupational exposure be analysed, especially when formaldehyde or formaldehyde releasers are not declared in the product labelling. This will facilitate optimal management of formaldehyde-allergic patients, ensuring healing of their dermatitis and the prevention of a chronic condition.



# POPULAR SCIENTIFIC SUMMARY IN SWEDISH

Konserveringsmedel är biologiskt aktiva ämnen som tillsätts vattenbaserade produkter, t ex hygienartiklar och skärvätskor, för att förhindra växt av mikroorganismer, vilket gör att produkterna kan förvaras längre. Konserveringsmedel är också kända kontaktallergen.

Syftet med avhandlingen har varit att förbättra diagnostiken av allergiska kontakteksem orsakade av formaldehyd, att undersöka klinisk relevans av en funnen kontaktallergi och även att utreda hur ofta formaldehyd finns i hudnära produkter som vi använder i vardagen.

Formaldehyd är ett av de äldsta och tidigare mest använda konserveringsmedlen. Numera finns det andra konserveringsmedel som tillsätts vattenbaserade produkter och som avger formaldehyd, så kallade formaldehydavgivare. Formaldehyd är ett välkänt kontaktallergen och har varit inkluderat i basserier för lapptestning sedan 1930-talet. Andelen formaldehydallergiska bland testade patienter utgör 2-3% i Europa och 8-9% i USA. Kontaktallergi mot formaldehyd är problematisk eftersom det är svårt att misstänka formaldehydallergi från patientens anamnes. Samtidigt är formaldehyd mycket vanlig i miljön och används både privat och i arbetslivet, vilket innebär att det är svårt att undvika kontakt med detta ämne. Det är därför viktigt med förbättrad diagnostik samt utvärdering av klinisk relevans av en funnen formaldehydallergi.

Testmetoden som används för att upptäcka en kontaktallergi kallas lapptest. Testningen innebär att en viss mängd av det misstänkta ämnet späds i ett spädmedel, appliceras i testkammare och sätts fast på ryggen. Testen är positiv om det på testplatsen utvecklas ett eksem. Koncentrationen av testpreparationen anpassas så att den inte irriterar huden och att man varken underdiagnosticerar allergi eller gör att patienten kan bli allergisk för det testade ämnet.

Den traditionella testkoncentrationen för formaldehyd i världen har varit 1.0% men den kliniska erfarenheten har antytt att den kan vara för låg. I det första delarbetet finns testresultat från lapptestning av 1397 personer som under 2006-2007 utreddes vid Yrkes- och miljödermatologiska avdelningen i Malmö. Dessa personer testades samtidigt med formaldehyd 1.0% och 2.0% i vatten. Nästan dubbelt så många individer reagerade för formaldehyd 2.0% jämfört med 1.0%. Till skillnad från

tidigare utförda studier, hade färre individer än förväntat irritationsreaktioner mot formaldehyd 2.0% vilket vi förklarar med förbättrad testmetodik då vi kontrollerar dosen genom att använda mikropipett vid applicering av testberedningar.

I det andra delarbetet försökte vi svara på frågan om den påvisade kontaktallergin för formaldehyd 2.0% är kliniskt relevant, dvs. om kontaktallergiska utvecklar eksem vid formaldehydexponering. För att kunna undersöka detta utförde vi en klinisk experimentell studie, s.k. användartest (Repeated open application test, ROAT). Försökspersonerna använde en kräm som innehöll maximalt tillåten koncentration av formaldehyd enligt EUs kosmetikalagstiftning. Testgruppen bestod av individer som reagerat för formaldehyd 2.0% men inte för 1.0% (dvs. individer som hade formaldehydallergi men som vi aldrig hade upptäckt om vi inte hade testat med 2.0%). Statistiskt signifikant fler individer utvecklade positiv ROAT och slutsatsen som framgick från studien var att ”svag” formaldehydallergi har klinisk relevans och det är relevant att testa rutinmässigt med formaldehyd 2.0%.

Nästan alla individer som utreds för kontaktallergi har eksem och därmed också skadad hudbarriär. I det andra delarbetet visade vi att formaldehydallergiska individer med frisk hud får eksem vid upprepad exponering för höga men fortfarande tillåtna halter av formaldehyd i mjukgörande krämer. Kemiska analyser av patienternas hygienartiklar på vårt laboratorium har visat att de ofta innehåller låga halter av formaldehyd och att det inte alltid är deklarerat på produkten att de innehåller formaldehyd eller formaldehydavgivande konserveringsmedel. Hur påverkar låga doser av formaldehyd en formaldehydallergisk individ som redan har skadad hud? Denna frågeställning studerades i tredje delarbetet. Resultaten visade att låga halter av formaldehyd som ofta finns i produkter som vi använder dagligen, kan försämra eller underhålla ett redan existerande eksem.

I delarbete fyra undersöktes hur ofta hudvårdsprodukter som vi använder dagligen innehåller formaldehyd och om individer som har kontaktallergi för formaldehyd utsätts för detta konserveringsmedel mer jämfört med individer som inte är allergiska för formaldehyd. Cirka 20% av alla analyserade produkter innehöll formaldehyd och ofta var det inte deklarerat på produkten. Individer som hade kontaktallergi för formaldehyd använde statistiskt signifikant mer så kallade ”leave-on” produkter som innehöll formaldehyd jämfört med dem som inte hade formaldehydallergi. Resultaten tyder på att de produkter som används av formaldehydallergiska individer bör undersökas för att kunna bedöma den kliniska relevansen av allergin, speciellt produkter som inte sköljs av huden och om formaldehyd eller formaldehydavgivande konserveringsmedel inte är deklarerade på förpackningen.

De resultat som vi kommit fram till har praktisk betydelse i den kliniska vardagen vid lapptestning. För att bekräfta resultatet av vår första studie utfördes rutinmässig lapptestning med formaldehyd 1.0% och 2.0% på 12 kliniker i Europa och i Sverige med liknande resultat. Eftersom vi också har visat att den kontaktallergi

för formaldehyd som endast fångas med 2.0% och ej med 1.0% är kliniskt relevant, har detta medfört att ESCD och EECDRG rekommenderat att ändra testkoncentrationen för formaldehyd i Europeiska basserien från 1.0% till 2.0% (0.60 mg/cm<sup>2</sup>). Våra ROAT studier har visat att det ofta kan ta upp till flera veckor innan man utvecklar eksem då man exponeras för ett ämne som man är allergisk för och som finns i produkten som man använder. Det har stor praktisk betydelse därför att man ofta inte misstänker en produkt som har använts länge och om kemisk analys inte utförs finns det en risk att eksemet inte läker trots adekvat behandling.



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